Journal of Agricultural and Crop Research
Vol. 9(5), pp. 112-120, May 2021
doi: 10.33495/jacr_v9i5.21.123
ISSN: 2384-731X
Research Paper

Effect of *Hvsusiba2* transgenic rice on soil bacterial community and functional gene in paddy field in Fujian Province China

SONG Yana* • SU Jun • WU Mingji • WANG Feng

Institute of Biological Technology, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China.

*Corresponding author: syana@sina.com

Accepted 19th April, 2021.

Abstract. A field experiment involving *Hvsusiba2* transgenic rice (SUSIBA) and its wild type Nipponbare rice (NIPP) has been on-going in Fujian Province China since 2017. The composition of bacterial communities and functional genes in paddy soil were investigated at the full heading stage of rice in the third year of the experiment. The results showed that the bacterial communities or microbial functional structures in the paddy soil with SUSIBA clearly separated from those of NIPP. According to ADONIS and ANOSIM analysis, separations of bacterial communities or microbial functional structures between SUSIBA and NIPP were significant ($P < 0.05$). The relative abundance of Acidobacteria significantly ($P < 0.05$) decreased in the paddy soil with SUSIBA, while increased in Proteobacteria. Otherwise, the planting of line SUSIBA markedly ($P < 0.05$) reduced the intensity of functional genes such as carbon cycling, organic remediation, virulence and other, especially genes of carbon fixation and degradation. In summary, the planting of *Hvsusiba2* transgenic rice changed the composition of bacterial communities and intensity of functional genes in paddy soil at heading stage of the third year.

Keywords: *Hvsusiba2* transgenic rice, bacterial community composition, microbial functional gene, Illumina MiSeq, GeoChip.

INTRODUCTION

In 2018, twenty-six countries planted 191.7 million hectares of biotech crops according to the published of ISAAA (International Service for the Acquisition of Agri-biotech Applications) (James, 2018). Planting transgenic crop has brought many economic benefits but also raised some concern over the potential impact on the environment, such the soil microbial community (Lu *et al*., 2017). According to previous studies, releasing many transgenic plants affect soil microbial communities such as diversity, abundance of indigenous soil bacteria and fungi in Bt (Bacillus-thuringiensis) transgenic cotton (Donegan *et al.*, 1995) and Bt transgenic maize (Castaldini *et al.*, 2005) soil.

Hvsusiba2, a transcriptional factor in barley (*Hordeum vulgare* L.), acts as a sugar-inducible genes regulator and mediates carbon partitions between aboveground parts and roots (Sun *et al.*, 2003; Sun *et al.*, 2005). The synthetic *Hvsusiba2* gene was built in the downstream of the specific promoter SBEIIb for starch synthesis, and transformed into rice by the Agrobacterium method (Su *et al.*, 2015). The *Hvsusiba2* transgenic rice with high-starch in grains because of the advantage to expression of SBEIIb in the grain regulated the distribution of carbohydrate in rice plant, and then could reduce the methane emissions of the paddy (Su *et al.*, 2015).

Paddy field is the main agricultural emission source of the second largest greenhouse gas CH_4, and it was estimated that it can emit 50 to 60 Tg of methane into the

atmosphere every year (Yvon *et al.*, 2014). Developing rice varieties with low methane emission and high yield is one of the key measures to reduce greenhouse effect and realize low carbon production of rice. The *Hvsusiba2* transgenic rice just meets the dual requirements of low methane emission and high yield, and becomes an ideal rice material which is helpful to meet the urgent needs of global sustainable food production (Bodelier, 2015). Therefore, it is necessary to further develop and utilize *Hvsusiba2* transgenic rice, however the safety of genetic modified crops should be evaluated firstly, and the impact on the soil microbial communities is one of the important contents in evaluating the ecological safety of genetically modified crops.

The *Hvsusiba2* transgenic rice promotes carbon transport to plant aboveground and reduces the transportation of carbon to underground part, which would reduce the accumulation of carbon in rhizosphere and soil (Su *et al.*, 2015). Therefore planting *Hvsusiba2* transgenic rice may affect the survival of soil microorganism, such as to change the diversity of microbial communities and functional structures in the paddy soil. Diversity and function of the microbial communities are directly relevant the soil material energy cycle, and the stability and sustainable of soil ecosystem. It is worth attention on the influence of planting *Hvsusiba2* transgenic rice on soil microbial communities.

At present, high throughput sequencing of microbial has been used, such as, the study that found the abundance in soil microbial of rotation cropping was far higher than that of continuous cropping by 16S rRNA-based Illumina MiSeq sequencing (Zhang *et al.*, 2014). In addition, a high-throughput gene chip technology of GeoChip was developed for detecting microbial functional groups. GeoChip has been used in research on environmental microbial functional structures, such as global climate change, oil field, heavy metal pollution, acidic mining waste water, sediment, human intestinal (Yang *et al.*, 2018; Xue *et al.*, 2016; Xue *et al.*, 2018; Li *et al.*, 2017; Kuang *et al.*, 2016; Wu *et al.*, 2018; Chen *et al.*, 2014; Li *et al.*, 2014).

In this study, 16S rRNA-based Illumina MiSeq and GeoChip were used to reveal the effect of planting *Hvsusiba2* transgenic rice on bacterial communities and functional groups of microbial in the paddy soil.

MATERIALS AND METHODS

Field experiment and sampling

A field experiment has been performed at Shoushan (26°11′N, 119°16′E) located in Fujian Province China since 2017. The experiment was approved by the Ministry of Agriculture and Rural Affairs of the People's Republic of China. Two rice varieties were planted at the experimental site. The varieties were *Hvsusiba2* transgenic rice (*Oryza sativa* L. ssp. Japonica) SUSIBA2-77 (SUSIBA) and a corresponding wild type non-*Hvsusiba2* rice Nipponbare (NIPP). A double T-DNA vector pCDMARUBb-Hyg of the synthetic *Hvsusiba2* gene was constructed and transformed to rice Nipponbare by the Agrobacterium method. The non-marked (with the hygromycin gene removed) *Hvsusiba2* gene homozygous rice strain SUSIBA2-77 was obtained through the detection and selection of multi-generation self-crossing.

The soil at the experiment site contained total N 1.19 g kg^{-1}, P 0.25 g kg^{-1} and K 18.26 g kg^{-1}, organic C 19.50 g kg^{-1}, and pH 5.78. Each treatment was carried out in 35 m^2 per plot randomly distributed in the field, where replication was carried out four times. The field experiment has been carried out for three years, with the management of water and fertilizer according to the conventional methods in the area.

The soil adhering to the roots, at about 0-20 cm depth, was collected as the root zone soil in the third year of the experiment at heading stage of rice. In each field replicate, soil of five sites were excavated randomly and pooled to give one sample per field plot. Soil samples were stored at -70 ℃ until molecular analysis or air-dried for chemistry analysis, respectively.

Soil DNA extraction and molecular analysis

Soil DNA was extracted using the FastDNA®SPIN Kit for Soil (QBIOgene, USA) according to the manufacturer's instructions. The concentration of DNA samples was determined by a Nanodrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA), and were sent to MAGIGENE Co., Ltd (China) for analysis of high-throughput sequencing (16S rRNA-based Illumina MiSeq) and functional gene arrays (GeoChip 5.0 60K).

Illumina MiSeq sequencing of 16S rRNA

The V3-V4 region of 16S rRNA was amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCAG- 3') and 806R (5'-GGACTACHVGGGTWTCTAAT- 3') (Walters *et al.*, 2011). PCR reactions, containing 25 μL 2 × Premix Taq (Takara Biotechnology, Dalian Co. Ltd., China), 1 μl each primer (10 pmol·μL^{-1}) and 3 μL DNA (20 ng·μL^{-1}) template in a volume of 50 μL, was amplified by thermocycling: 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 52°C, and 30 s at 72°C; followed by 10 min at 72°C. PCR products were mixed and purified with EZNA Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) and sequenced on an Illumina Miseq platform (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China) (Schloss *et al.*, 2009; Caporaso *et al.*, 2010a).

Sequences analysis was performed by Usearch software

(V10，http://www.drive5.com/usearch/). Sequences with ≥ 97% similarity were assigned to the same OTU (operational taxonomic unit) (Caporaso *et al.*, 2010b). Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) analysis was performed by R software based on the weighted and unweighted UniFrac distance matrix (Lozupone and Knight, 2005). Analysis of ADONIS (non-parametric multivariate analysis of variance) and ANOSIM (analysis of similarities) was performed by R software based on the bray-curtis distance, the weighted and unweighted UniFrac distance to display the extent of differences between groups and whether the differences were significant.

GeoChip analysis

GeoChip 5.0 60K contains 57, 603 probes targeting sequences from 373 functional gene families involved carbon cycling, nitrogen metabolism, sulfur cycling, phosphorus cycling, metal homeostasis, organic remediation, secondary metabolism, virulence activity and other. The purified DNA was labeled with the Cy3 dye by random primers and the Klenow fragment of DNA polymerase I. Labeled DNA was purified with a QIA quick purification kit (Qiagen, Valencia, CA, USA) and then dried down in a Labconco Centrivap Concentrator (Labconco Corp., Kansas City, MO) at 50°C for 45 min. Dried DNA was diluted to the same concentration, then incubated at 95°C for 5 min and maintained at 42°C until hybridization. Subsequently, labeled DNA was placed onto the array, and then, the array preheated to 42°C on a hybridization station (MAUI, BioMicro Systems, Salt Lake City, UT, USA) for at least 5 min, samples were loaded onto the array surface and hybridized approximately 16 h. Finally, microarray was scanned by NimbleGen MS200 scanner (Roche, Madison, WI, USA) to obtain the optical signals.

ImaGene 6.0 (Biodiscovery Inc., El Segundo, CA, USA) software was used to convert optical signals to digital signals to obtain probe signal intensity. Dimensionality reduction analyses such as PCA (Principal Component Analysis), DCA (Detrended Correspondence Analysis), PCoA and NMDS were performed according to the probe intensity. Two non-parametric analysis of ADONIS and ANOSIM were performed by three kinds of distance matrix: Jaccard, Bray Curtis and Euelidean, and the gene intensity was compared by T-test.

Soil chemical properties and RDA analysis

Soil chemical properties including organic C, pH (1: 2.5 H_2O), alkali-hydrolyzable N, Olsen-P and NH_4OAc-K were analyzed by standard procedures (Bao, 2007), and as environmental factors for redundancy correspondence analysis (RDA) of Illumina MiSeq data or GeoChip data were calculated by R software. Statistical significance of differences in soil chemical properties between the two rice lines was assessed by analysis of variance (ANOVA) and LSD (least significant difference) using SPSS, version 17.0.

RESULTS

Bacterial community composition in the paddy soils

A total of 13,352 OTUs with an average of 1,669 ± 85 OTUs per soil replicate was identified, which based on the total of 549,261 pairs of clean reads obtained from Illumina MiSeq. There was a clear separation between the paddy soil replicates in bacterial community of SUSIBA and those of NIPP by NMDS or PCoA analysis based on the weight UniFrac distance, respectively (Figure 1A and B). For the analysis of NMDS or PCoA based on the unweighted UniFrac distance (Figure 1C and D) also showed a clear separation between SUSIBA and NIPP. The analysis of ADONIS and ANOSIM differences based on the distances of bray-curtis, unweighted and weighted UniFrac all suggested that the separation of bacterial communities in paddy soil for SUSIBA was statistically significant ($P < 0.05$) in comparison with NIPP (Table 1).

The most abundant phylum was Proteobacteria in the paddy soil of the two variety rice, which was followed by Acidobacteria and Chloroflexi, and then by Nitrospirae, Bacteroidetes, Planctomycetes, Verrucomicrobia, Firmicutes, Patescibacteria and Actinobacteria, and so on. These major bacterial phyla accounted for over 96% of total abundance in the bacterial communities in the paddy soil of both NIPP and SUSIBA. The relative abundance of Proteobacteria, Nitrospirae, Bacteroidetes or Patescibacteria was significantly higher ($P < 0.05$) in the paddy soil with SUSIBA compared with NIPP (Figure 2A), but the relative abundance of Acidobacteria was significantly lower ($P < 0.01$) in the paddy soil with SUSIBA than that of NIPP (Figure 2A). The most ten bacterial genus in the relatively abundance showed that three genus of *Candidatus_Solibacter*, *Candidatus_Koribacter* and *Bryobacter* all classify as Acidobacteria were significantly decreased ($P < 0.01$) in the paddy field with SUSIBA, while the relative abundance of *Methylocystis* ($P < 0.01$) and *Rhodomicrobium* ($P < 0.05$) all classify as Proteobacteria were markedly increased in the paddy field with SUSIBA (Figure 2B).

In addition, bacteria biomarkers in the paddy soil with NIPP or SUSIBA were shown by LDA Effect Size (LEfSe) (Figure 3), which suggested that Acidobacteria (including Acidobacteriia, Acidobacteria and Acidobacteriales) was significant enrichment of specie in the paddy soil with NIPP, while Proteobacteria was significant enrichment of

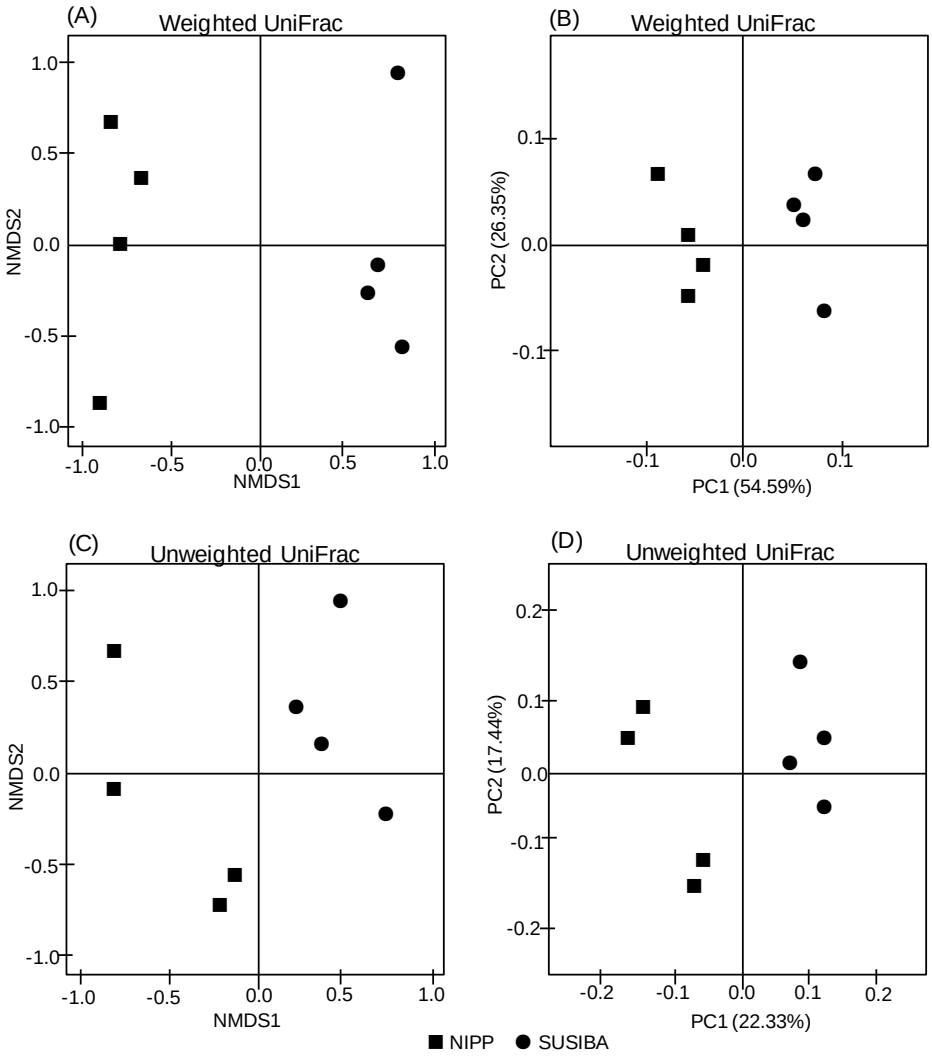

Figure 1. NMDS (non-metric multidimensional scaling) and PCoA (principal coordinate analysis) of bacterial communities in the paddy soil with different rice lines. A and B were NMDS and PCoA plots based on weighted UniFrac distance, C and D were NMDS and PCoA plots based on unweighted UniFrac distance. Square and dot indicated NIPP and SUSIBA, respectively. NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77.

specie in the paddy soil with SUSIBA (Figure 3). The enrichment of Proteobacteria or Acidobacteria in the paddy soil with SUSIBA or NIPP should have a degree impact on the difference in soil bacterial communities between SUSIBA and NIPP, respectively.

Microbial functional genes in the paddy soils

The microbial functional genes in the paddy soil were examined by GeoChip 5.0. A total of 315,312 gene probe was detected with an average of 39,414 gene probes per soil replicated. The Venn diagram showed that there were 34,084 overlap probes in soil replicates of SUSIBA and NIPP.

The dimensionality reduction analysis of microbial functional structures based on GeoChip data was shown in Figure 4. The plots of PCoA, PCA and DCA suggested that the microbial functional structure was separated based on rice variety (Fig. 4 B, 4 C, 4 D), although the four soil replicates of SUSIBA were not clearly separated from the four replicates of NIPP in the NMDS plot (Fig. 4 A). The analysis of ADONIS and ANOSIM differences based on Bray-curtis, Jaccard and Euclidean all suggested that the separation in the paddy soil microbial functional structures of SUSIBA from those of NIPP was statistically significant ($P < 0.05$) (Table 1).

Differences of microbial functional genes intensity in the paddy soil between planting NIPP and SUSIBA were analyzed by T-test. There were twenty functional genes

Table 1. ADONIS and ANOSIM difference in soil bacterial communities or functional genes between NIPP and SUSIBA based on Illumina MiSeq or GeoChip.

	Group	Method	ADONIS					ANOSIM	
			df	SumsOfSqs	MeanSqs	F. Model	R^2	*Pr* (>F)	*R*-value	*P*-value
Illumina MiSeq	SUSIBA.vs.NIPP [a)]	Bray-Curtis	1	0.0624	0.0624	4.1491	0.4088	0.031*	1.00	0.025*
	SUSIBA.vs.NIPP	Unweighted UniFrac	1	0.1172	0.1172	1.5497	0.2053	0.032*	1.00	0.031*
	SUSIBA.vs.NIPP	Weighted UniFrac	1	0.0273	0.0273	6.4578	0.5184	0.038*	1.00	0.028*
GeoChip	SUSIBA.vs.NIPP	Bray-Cutis	1	0.0019	0.0019	1.8496	0.2356	0.022*	0.23	0.022*
	SUSIBA.vs.NIPP	Jaccard	1	0.0044	0.0044	2.0949	0.2587	0.038*	0.33	0.026*
	SUSIBA.vs.NIPP	Euclidean	1	1, 950.87	1, 950.87	1.6672	0.2174	0.027*	0.33	0.027*

SumsOfSqs: total variance; MeanSqs: mean square error namely SumsOfSqs/df; F.Model: F test value.
*: $P < 0.05$.
a) : NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77.

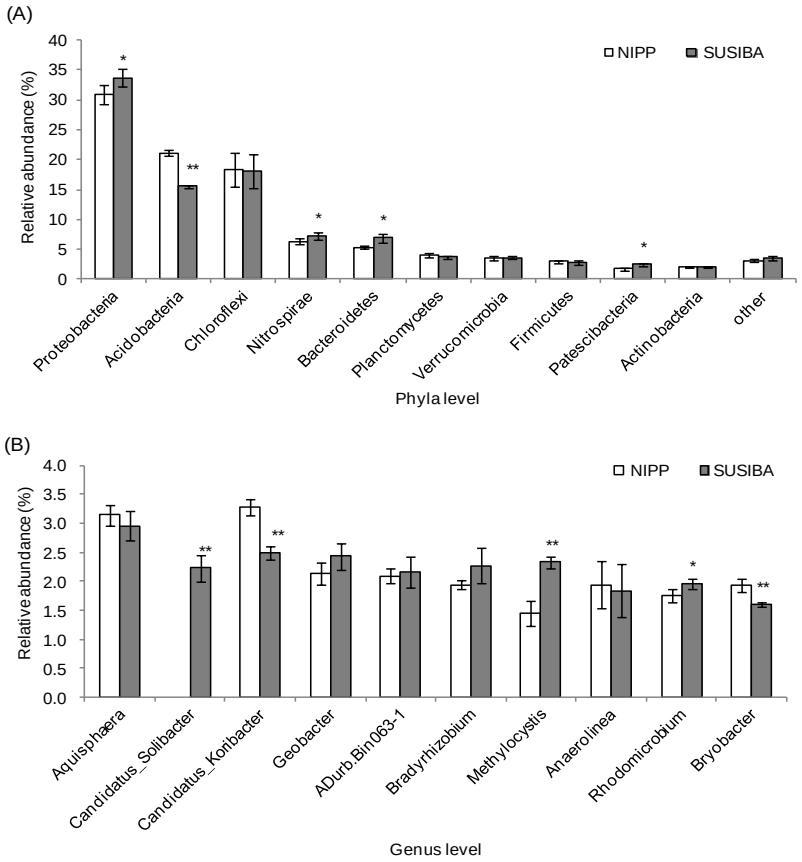

Figure 2. Relative abundance of bacterial at phyla (A) or genus (B) level in the paddy soil with different rice lines. NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77. *: $P < 0.05$, **: $P < 0.01$.

in the paddy soil were significant differences (P<0.05) in gene intensity between NIPP and SUSIBA, and all of its intensity in the paddy soil with SUSIBA were significantly lower (P<0.05) than those of NIPP (Table 2). More than 50% of the twenty genes classify as the gene category of carbon cycling, including eight genes of carbon fixation

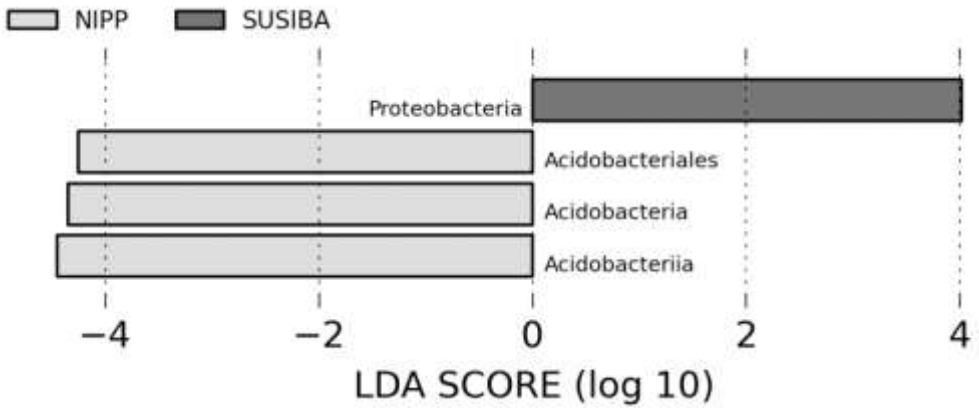

Figure 3. The LDA value distribution histogram of significant differences species in the paddy soil with different rice lines. NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77. The figure showed the significant differences species in abundance with the value of LDA is greater than two (default is set to 2) between NIPP and SUSIBA.

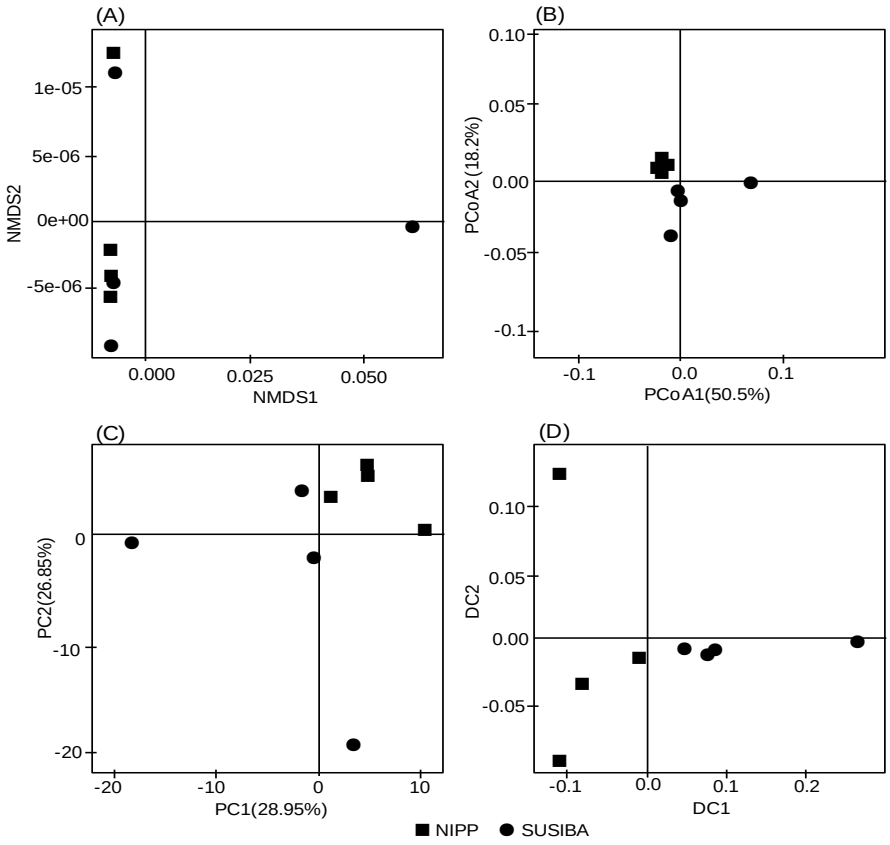

Figure 4. Dimensionality reduction analyses of microbial functional structures in the paddy soil with different rice lines. A, B, C and D were NMDS (Non-metric multidimensional scaling), PCoA (principal coordinate analysis), PCA (Principal component analysis) and DCA (detrended correspondence analysis) plots. Square and dot indicated NIPP and SUSIBA, respectively. NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77.

and three genes of carbon degradation. About 30% of the twenty genes classify as gene category of organic remediation, including four genes of aromatics remediation and each one gene of pesticides and herbicides remediation. On the other hand, there were two genes belonging to the other of phylogentic and one

Table 2. Analysis of differences in intensity of soil microbial functional genes between NIPP and SUSIBA by T-test.

Gene	Freedom	T-value	Significance ($P < 0.05$)	Gene category	Subcategory1	Subcategory2
sdhA	6	-4.7199	0.0033	Carbon cycling	Carbon fixation	3-hydroxypropionate bicycle
mch	6	-4.6061	0.0037	Carbon cycling	Carbon fixation	3-hydroxypropionate bicycle
mct	6	-3.9276	0.0077	Carbon cycling	Carbon fixation	3-hydroxypropionate bicycle
C_CoA_hydratase	6	-2.5293	0.0447	Carbon cycling	Carbon fixation	3-hydroxypropionate/4-hydroxybutyrate cycle
3HP_CoAs	6	-2.4957	0.0468	Carbon cycling	Carbon fixation	3-hydroxypropionate/4-hydroxybutyrate cycle
mdh	6	-2.4906	0.0471	Carbon cycling	Carbon fixation	Reductive tricarboxylic acid cycle
AcnA	6	-3.5921	0.0115	Carbon cycling	Carbon fixation	Reductive tricarboxylic acid cycle
ccmM	6	-3.7115	0.0100	Carbon cycling	Carbon fixation	Bacterial microcompartments
cdh	6	-3.4762	0.0132	Carbon cycling	Carbon degradation	Terpenes
Glucose_oxidase_fungi	6	-3.0451	0.0227	Carbon cycling	Carbon degradation	Glucose
hyaluronidase	6	-2.9470	0.0257	Carbon cycling	Carbon degradation	Hyaluronic acid
linc	6	-2.5981	0.0408	Organic remediation	Pesticides related compound	NA
trzn	6	-3.7903	0.0091	Organic remediation	Herbicides related compound	NA
dbdd	6	-3.4346	0.0139	Organic remediation	Aromatics	Heterocyclic aromatics
cumc	6	-2.8951	0.0275	Organic remediation	Aromatics	Other aromatics
tmoabe	6	-2.6406	0.0385	Organic remediation	Aromatics	BTEX and related aromatics
bphc	6	-2.4967	0.0467	Organic remediation	Aromatics	Polycyclic aromatics
EF1a_Oxymonads	6	-5.7763	0.0012	Other	Phylogenetic	NA
cox1_Viridiplantae	6	-2.9432	0.0258	Other	Phylogenetic	NA
fosx	6	-2.7684	0.0325	Virulence	Antibiotic resistance	Degradation

gene of antibiotic resistance degradation had significant differences ($P < 0.05$) in intensity between NIPP and SUSIBA.

Soil chemical properties in the paddy soils

In this study, soil chemical properties were measured and as environmental factors for redundancy correspondence analysis (RDA) of bacterial communities or microbial functional structures. Soil organic C and pH in the paddy field cultivated with *Hvsusiba2* transgenic rice SUSIBA were similar to those of its wild type rice NIPP (Table 3). Soil availability N and P were lower ($P < 0.05$) in the paddy field with SUSIBA than those of NIPP, while soil availability K increased in the paddy field with SUSIBA (Table 3). So, planting *Hvsusiba2* transgenic rice had effect on the efficiency of nitrogen, phosphorus and potassium in the paddy soil.

The results of RDA indicted that there were significant ($P = 0.022, 0.027$) correlation between availability P or availability K and bacterial communities in the paddy fields. At the same time, soil availability N, availability P and availability K had significant ($P = 0.001, 0.013, 0.012$) correlation with the microbial functional structures.

DISCUSSION

The release of transgenic crop varieties may alter soil microbial community through root exudates and plant residues, over both short and long term (Song *et al.*, 2014). In the experimental field site, *Hvsusiba2* transgenic rice SUSIBA2-77 (SUSIBA) and their wild type Nipponbare rice (NIPP) had been planted for three years. The results of the current study showed that here was a clear separation in soil bacterial community between SUSIBA and NIPP, by using NMDS or PCoA based on weighted and unweighted UniFrac distance. At the same time, the dimensionality

Table 3. Chemical characters in the paddy soil with different rice lines.

Rice line [b)]	Availability N (Alkali-hydrolyzable N)	Availability P (Olsen-P)	Availability K (NH_4OAc-K)	Organic C	pH (H_2O)
	mg·kg^{-1} D. W.[a)]	mg.kg^{-1} D. W.	mg.kg^{-1} D. W.	g·kg^{-1} D. W.	
NIPP	92.40 ± 5.94 [a]	34.03 ± 1.78 [a]	50.25 ± 4.72 [b]	22.55 ± 1.27 [a]	5.31 ± 0.01 [a]
SUSIBA	78.40 ± 7.92 [b]	25.63 ± 0.54 [b]	72.65 ± 4.19 [a]	24.50 ± 3.06 [a]	5.33 ± 0.05 [a]

[a)]: D.W.: dry weight. Values are mean ± SD (n = 4). Values in the same column followed by different letters are significantly different (P < 0.05).
[b)]: NIPP: Nipponbare rice, SUSIBA: *Hvsusiba2* transgenic rice SUSIBA2-77.

reduction analysis of GeoChip data also showed that the microbial functional structure in the paddy soil with SUSIBA clearly separated from that of NIPP. The above separation between SUSIBA and NIPP was certified statistically significant (P < 0.05) by the test of ADONIS or ANOSIM. Moreover, the relative abundance of Acidobacteria decreased in the paddy soil with SUSIBA while that of Proteobacteria increased. It was worthy attention that the enhancement in the relative abundance of Methylocystis in the paddy soil with SUSIBA compared with NIPP, what is more it may have a contribution to the low methane emissions of the paddy with *Hvsusiba2* transgenic rice.

Based on the GeoChip data, we found that planting of *Hvsusiba2* transgenic rice reduced some microbial functional genes intensity for carbon cycling, such as sdhA, mch and mct of 3-hydroxypropionate bicycle, C_CoA_hydratase and 3HP_CoAs3HP_CoAs of 3-hydroxypropionate/4-hydroxybutyrate cycle, mdh and AcnA of reductive tricarboxylic acid cycle. Carbon cycle is one of the most important biogeochemical cycles in ecosystems, and 3-hydroxypropionate bicycle, 3-hydroxypropionate/4-hydroxybutyrate cycle and reductive tricarboxylic acid cycle was three main pathways of microbial carbon fixation (Liu *et al.*, 2017; Yuan *et al.*, 2011). The probable decrease in soil microbial carbon fixation gene intensity may be an indication that CO_2 fixation potential in the paddy soil with *Hvsusiba2* transgenic rice could be lower compared to its wild type Nipponbare rice. At the same time, three microbial functional genes intensity of carbon degradation also decreased in the paddy soil with *Hvsusiba2* transgenic rice, including the degradation of terpenes, glucose and hyaluronic acid. Otherwise, the release of *Hvsusiba2* transgenic rice reduced the intensity of microbial organic remediation genes, including the remediation of pesticides, herbicides and aromatics.

In this study, we observed that the planting of *Hvsusiba2* transgenic rice make a change in bacterial community composition or microbial functional groups in the paddy soil in the season of rice heading at least for three years. Results of this study demonstrated that planting of *Hvsusiba2* transgenic rice could reduce some microbial functional genes intensity, especially microbial genes of carbon cycling. The reduction of microbial carbon fixation or degradation gene intensity means that the potential of carbon cycling in the paddy with *Hvsusiba2* transgenic rice will be affected. So a deep study is necessary on the abundance and activity of microbial functional genes of carbon cycling, and the influence on transformation and utilization of microbial carbon in paddy field with *Hvsusiba2* transgenic rice, by a long-term field experiment.

CONCLUSIONS

Results of this study demonstrated that *Hvsusiba2* transgenic rice could make a change in the composition of soil bacterial communities and functional gene groups at least under heading period of rice in the experiment for three years. At the same time, the planting of *Hvsusiba2* transgenic rice could reduce the intensity of microbial function genes on carbon cycling in the paddy soil.

ACKNOWLEDGEMENTS

The study was supported by the Natural Science Foundation of Fujian Province, China (Grant No: 2019J01108).

REFERENCES

Bao SD (2007). Soil Analysis in Agricultural Chemistry. Beijing: China Agricultural Press, p. 30-34, p. 56-58, p. 81-83, p. 106-108.

Bodelier PE (2015). Bypassing the methane cycle. Nature. 523:534-535.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a). PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 26:266-267.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010b). QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335-336.

Castaldini M, Turrini A. Sbrana C, Benedetti A, Marchionni M, Mocali S, Fabiani A, Landi S, Santomassimo F, Pietrangeli B, Nuti MP, Miclaus N, Giovannetti M (2005). Impact of Bt corn on rhizospheric and on beneficial mycorrhizal symbiosis and soil eubacterial communities iosis in experimental microcosms. Appl. Environ. Microbiol. 71:6719-6729.

Chen YF, **Qin N**, **Guo J**, **Qian GR, Fang AQ, Shi D, Xu M, Yang FL, He ZL, Van Nostrand JD, Yuan T, Deng Y, Zhou JZ, Li LJ (2014).** Functional gene arrays-based analysis of fecal microbiomes in patients with liver cirrhosis. BMC Genomics. 15:753-765.

Donegan KK, Palm CJ, Fieland VJ, Porteous LA, Ganio LM, Schaller DL, Bucao LQ, Seidler RJ (1995). Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the Bacillus-thuringiensis Var. Kurstaki endotoxin. Appl. Soil Ecol. 2:111-124.

James C (2018). Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. ISAAA Brief No. 54, ISAAA: Ithaca, NY.

Kuang JL, **Huang L**, **He ZL, Chen LX, Hua ZS, Jia P, Li SJ, Liu J, Li JT, Zhou JZ, Shu WS (2016).** Predicting taxonomic and functional structure of microbial communities in acid mine drainage. ISME J. 10:1527-1539.

Li P, **Jian, Z**, **Wang Y, Deng Y, Van Nostrand JD, Yuan T, Liu H, Wei D, Zhou JZ (2017).** Analysis of the functional gene structure and metabolic potential of microbial community in high arsenic groundwater. Water Res. 123:268-276.

Li Y, **He J**Z, **He ZL, Yuan Z, Yuan MT, Xu X, Sun FF, Liu CC, Li JY, Xie WB, Deng Y, Qin YJ, Van Nostrand JD, Xiao LY, Wu LY, Zhou JZ, Shi WY, Zhou XD (2014).** Phylogenetic and functional gene structure shifts of the oral microbiomes in periodontitis patients. ISME J. 8:1879-1891.

Liu YY, Wang S, Li SZ, Deng Y (2017). Advances in molecular ecology on microbial functional genes of carbon cycle. J. Microbiol. China, 44:1676-1689.

Lozupone C, Knight R (2005). UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71:8228-8235.

Lu GH, Zhu YL, Kong LR, Cheng J, Tang CY, Hua XM, Meng FF, Pang YJ, Yang RW, Qi JL, Yang YH (2017). Impact of a glyphosate-tolerant soybean line on the rhizobacteria, revealed by Illumina MiSeq. J. Microbiol. Biotech. 27:561-572.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75:7537-7541.

Song YN, Su J, Chen R, Lin Y, Wang F (2014). Diversity of microbial community in a paddy soil with cry1Ac/cpti transgenic rice. Pedosphere. 24:349-358.

Su J, Hu C, Yan X, Jin Y, Chen Z, Guan Q, Wang Y, Zhong D, Jansson C, Wang F, Schnürer A, Sun C (2015). Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice. Nature. 523:602-606.

Sun C, Höglund AS, Olsson H, Mangelsen E (2005). Antisense oligodeoxynucleotide inhibition as a potent strategy in plant biology: identification of SUSIBA2 as a transcriptional activator in plant sugar signaling. Plant J. 44:128-138.

Sun C, Palmqvist S, Olsson H, Borén M, Ahlandsberg S, Jansson C (2003). A novel WRKY transcription factor, susiba2, participates in sugar signaling in barley by binding to the sugar- responsive elements of the iso1 promoter. Plant Cell. 15:2076-2092.

Walters WA, Caporaso JG, Lauber CL, Berg-Lyons D, Fierer N, Knight R (2011). Primer Prospector: de novo design and taxonomic analysis of barcoded polymerase chain reaction primers. Bioinformatics. 27:1159-1161.

Wu XQ, **Wu L**Y, **Liu YN, Zhang P, Li QH, Zhou JZ, Hess NJ, Hazen TC, Yang WL, Chakraborty R (2018).** Microbial interactions with dissolved organic matter drive carbon dynamics and community succession. Front. Microbiol. 9:1-12.

Xue K, Yuan MM, Shi ZJ, Qin Y, Deng Y, Cheng LX, Wu LY, He ZL, Van Nostrand JD, Bracho R, Natali S, Schuur EAG, Lou CW, Konstantinidis KT, Wang Q, Cole JR, Tiedje JM, Luo YQ, Zhou JZ (2016). Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. Nature Clim. Change. 6:595-600.

Xue K, Zhou JZ, Van Nostrand JD, Mench M, Bes C, Giagnoni L, Renella G (2018). Functional activity and functional gene diversity of a Cu-contaminated soil remediated by aided phytostabilization using compost, dolomitic limestone and a mixed tree stand. Environ. Pollut. 242:229-238.

Yang JJ, Li G, Qian Y, Yang YF, Zhang F (2018). Microbial functional gene patterns related to soil greenhouse gas emissions in oil contaminated areas. Sci. Total Environ. 628-629:94-102.

Yuan HC, Qin HL, Liu SL, Nie SA, Wei WX, Wu JS (2011). Advances in research of molecular ecology of carbon fixation microorganism. Sci. Agric. Sinica. 44:2951-2958.

Yvon-durocher G, Allen A P, Bastviken D, Conrad R, Gudasz C, St-pierre A, Thanh-duc N, Giorgio P A (2014). Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. Nature. 507(7493):488-491.

Zhang F, Lin SY, Xu YJ (2014). The Effect of Continuous Cropping Rice on Diversity of Soil Bacteria Microbial in Jiangsu Province. J. Shandong Agri. Univ. (Nat. Sci. Edition) 45:161-165.