*

1   1   2   1   1   1†

1   /   210095

2   210008

W_tS

$W_{nt}S$ W_t W_{nt} 4

CMC- Na   PCR- RFLP

W_tS $W_{nt}S$ W_t W_{nt}4   OTUs   23 26 20 19

11

Streptomycetaceae *Flavobacterium* *Sphingobium*

Pseudomonas *Phyllobacterium* *Paenibacillus* Promicromonosporaceae *Sphingobacterium*

pH

PCR- RFLP

Q938.1   A

30% 50% [1]

[5]

[2]

[3]

[6-7]

[4]

[8]

Ulrich

[9]   537

Saha[10]

54

16S rDNA

10　T-RFLP　　　Yang[11]

35

RFLP

[12]

[13]

[14-16]

PCR-RFLP

1

1.1

2006

$35°00'N$

$114°32'E$

13.9　　　　　　　　615 mm

-

4　　　1　　　4

表 1

Table 1　Design of the experiment

Treatment code	Experimental treatments
W_tS	
$W_{nt}S$	
W_t	
W_{nt}	

14 m× 6.5 m　2013　12

0　10 cm

4　　-20

17

1.2

E. coli DH5　　　　　　Taq

pMD-19T　　　　*Hha* I　*Afa* I

Takara　　　　CMC-Na　PCR

1.3

10

g　　　　　　90 ml　　　　　　120 r min^{-1}

20 min　　　　10^{-2}　10^{-4}

CMC-Na　　30　　4　6 d

CMC-Na

1.4　　　　　　　　　　**PCR-RFLP**

PCR　　　　　　　　　16S

rDNA　　　　　　1 500 bp

27f　5′-AGAGTTTGATCCTGGCTCAG-3′

1492r　5′-GGTTACCTTGTTACGACTT-3′

25 μl　PCR　　　　10× Buffer

2.5 μl　dNTP　25 mmol L^{-1}　2.5 μl　Mg^{2+}　25

mmol L^{-1}　2.5 μl　　27f　25 mmol L^{-1}　0.3 μl

1492r　25 mmol L^{-1}　0.3 μl　Taq

5U μl^{-1}　0.3 μl　10%　80 2.0 μl　DNA

ddH$_2$O　25 μl　　　　　94　　5 min

94　　30 s　52　　30 s　72　　90 s　30

72　　15 min　10　　10 min

1%　　　　　　PCR

DNA　　　　TaKaRa Biotech-

nology　　　　　　16S rDNA

1%

Hha I　*Afa* I

5 μl　　ddH$_2$O 2.8 μl

Afa I　10 U μl^{-1}　0.1 μl　*Hha* I　10 U μl^{-1}

0.1 μl　　　10× T　0.5 μl　0.1% BSA 0.5 μl

PCR　1.0 μl　37　　　　　　　8%

Operational Taxonomic Unit　OTU　4

16S rDNA　　　　　　Shannon-Wiener　　　　　　　　　　　　　　84%
Simpson　Evenness　Richness
　　　　　18　　　　　　　　　　　　　　　　　　116%

1.5　　　　　　　　　　　　　　　　　　　　　　　36%
　　　　　1　2
　　　　　　　　NCBI　National Center　15%
Biotechnology Information　　　BLAST
　Genbank　　　　　　　　　　　　　　　**2.2**
MEGA 5.0

　　　　　　　　　　　　　　　4　　　　　　　　　　425
　　　　　　　　　　　　　　Hha I　*Afa* I　　　　　33　OTU
2　　　　　　　　　　　　　4　　　　　　　　　　　　2　4

2.1　　　　　　　　　　　　　　　91.67%　95.28%

　　　　　1　　　　　　　　　　　　　　　2
W$_t$S　W$_{nt}$S　W$_t$　W$_{nt}$　　　　　　2　　　　4
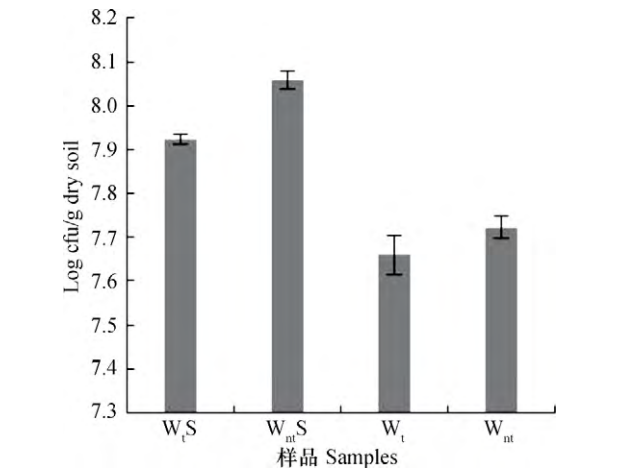
1
Fig. 1　Counts of cellulose degrading bacteria in the soil samples
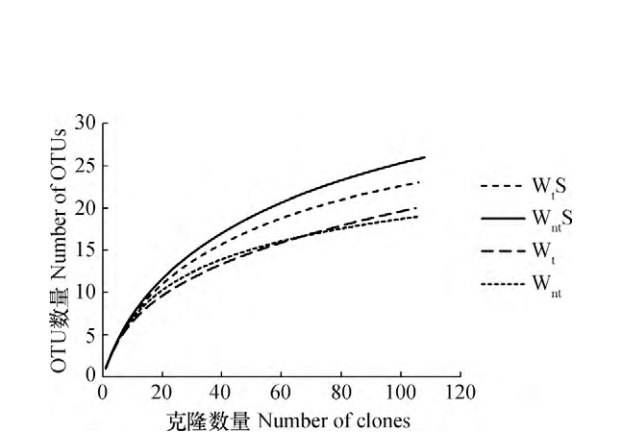
2
Fig. 2　Gene library abundance curves of the cellulose decomposing bacteria CDB in four types of soils

2

Table 2　Community diversity structural indices of the bacteria gene libraries in the four soil samples

Library	OTU Type of OTU	Coverage C %	Shannon-Wiener index	Simpson index	Evenness	Richness
W$_t$S	23	93.40	2.67	0.905	0.573	4.72
W$_{nt}$S	26	91.67	2.79	0.914	0.596	5.34
W$_t$	20	92.38	2.44	0.881	0.524	4.08
W$_{nt}$	19	95.28	2.53	0.898	0.543	3.86

OTU　　　　　　　　　OTU

3　　W$_t$S　W$_{nt}$S　W$_t$　W$_{nt}$

OTU 23　　26　　20　　19

　　OTU　　7

　8　OTU　　　　　　　3

OTU

3　　4

Fig. 3　Venn diagram showing relationships between the genetic cleavage types of soil cellulose degrading bacteria in four soils OTU

OTU

Note　The digits in the figure mean numbers of OTU the percentages belowpercentages of clones in each corresponding library and the English letters types of OTU

2.3

33　OTU　　　　　　　　NCBI

BLAST

4　　　　　Mega

5　　　　　　　　　　16S rDNA

80%　99% 33　OTU　　4　　7

11　　15　　20

Sphingobium　Stretomycetaceae

4
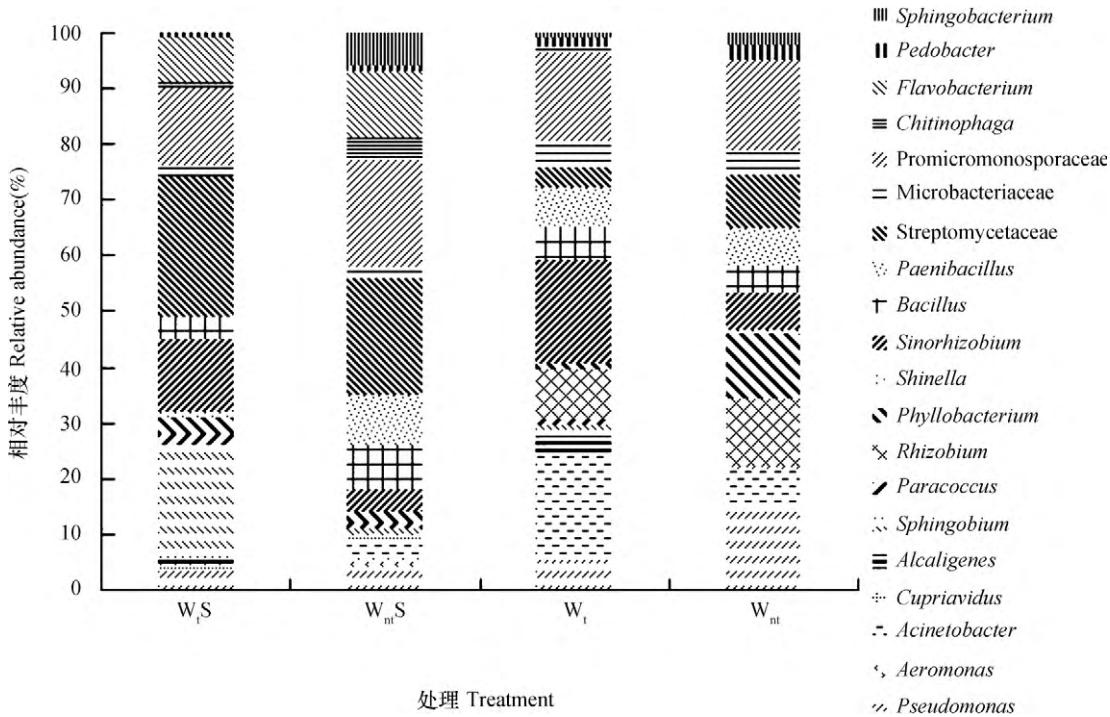
Fig. 4　Percentage of each group of CDB contained in the gene libraries
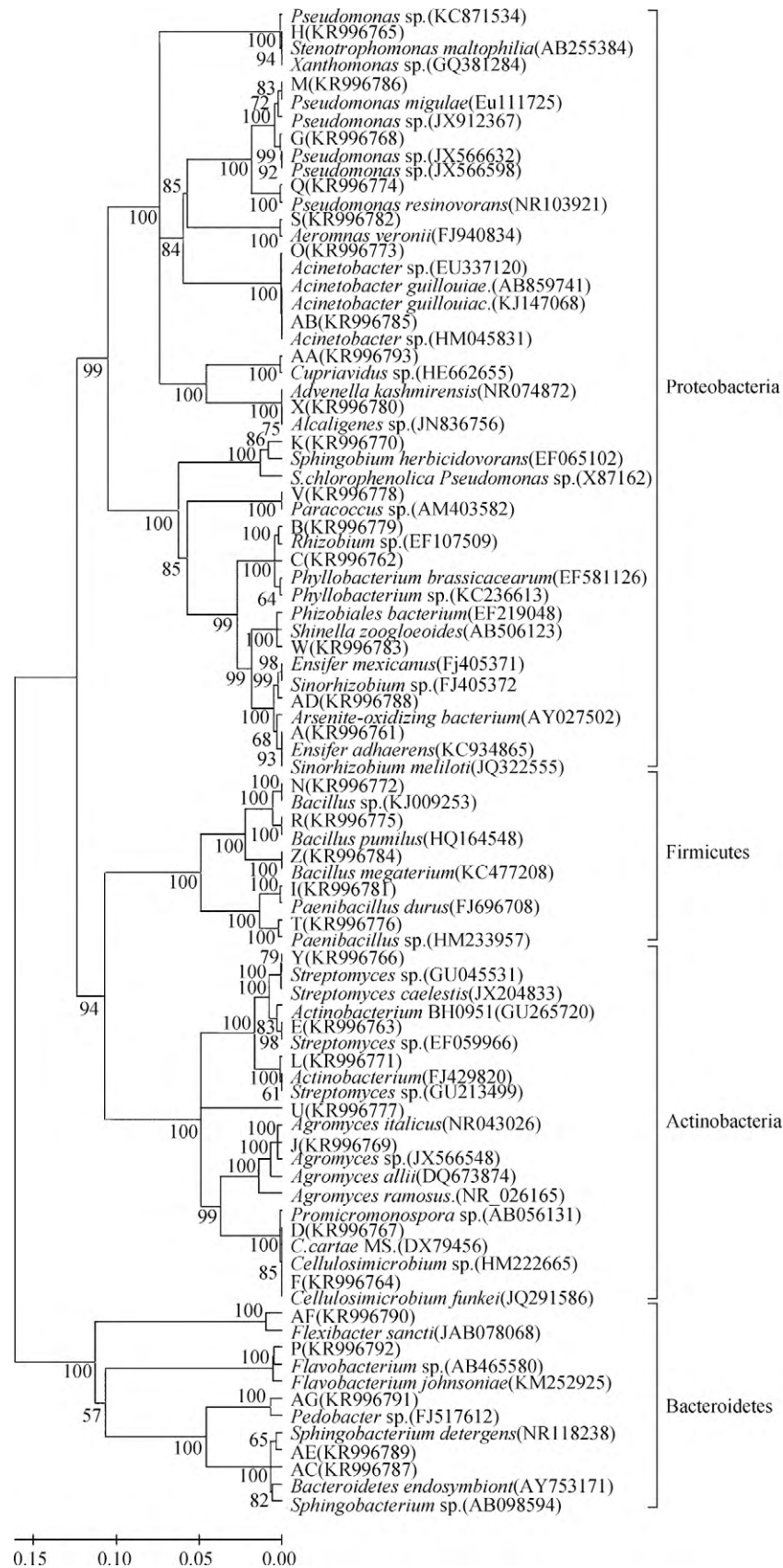
Fig. 5 Phylogenetic tree based on 16S rDNA sequences of the CDB in the four soils

Sinorhizobium *Promicromonsporaceae*
67.92%

Stretomycetaceae *Promicromonsporaceae*
Flavobacterium 48.15%
Sinorhizobium
Promicromonosporaceae *Acinetobacter*
54.29%

Rhizobium *Phyllobacterium* *Pseudomonas*
Promicromonsporaceae 63.21%

Streptomycetaceae *Flavobacterium*
Sphingobium

Flavobacterium

Pseudomonas *Phyllobacterium*
Paenibacillus Promicromonosporaceae
Sphingobacterium

Proteobacteria
Firmicutes Actinobacteria Bacteroidetes 4
Proteobacteria 15
OTU 11
Acinetobacter *Sphingobium* *Sinorhizobium*
Promicromonosporaceae Streptomycetaceae

2.4

6 4

4

pH

C/N

C/N

[18-20]

图 6
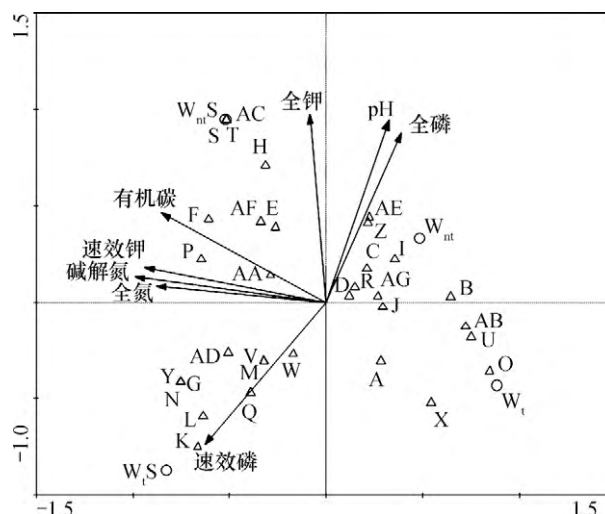
Fig. 6 Canonical correspondence analysis of environmental factors and CDB community structure

3

[21] [22]

/

[23]

[24-26]

[27]

0 10 cm
[28-29]

4

OTU
OTU

OTU

Proteobacteria Firmicutes

Actinobacteria Bacteroidetes

45%

11% 34% 10%

Talia [30] CMC

52 Proteobacteria

Actinobacteria Firmicutes 90%

6% 4%

16S rDNA

4

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Effects of Tillage and Straw Mulching on Diversity of Cellulose-decomposing Bacteria in Fluvo-aquic Soil

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Abstract   Objective   No-tillage and straw mulching are two important techniques in soil building. It is   therefore   of great significance to understand effects of the two on diversity of the cellulose-decomposing bacteria   CDB   community in the soil. To this end   analyses were done of effects of long-term no-tillage and straw mulching on diversity of CDB   microbial community structure   and richness and dominance of CDB   so as to provide a theoretical basis for the study and demo-extrapolation of the technique of conservation tillage.   Method   A long-term experiment   designed to have four tillage treatments   i.e. tillage with mulching W_tS no-tillage with mulching $W_{nt}S$ tillage without mulching W_t and no-tillage without mulching W_{nt} was conducted at the State Experimental Station for Agro-Ecology in Fengqiu   Henan Province. Soil samples were collected from the plots of the four treatments   incubated in CMC-Na Congo red medium for counting of CDB and analyzed with PCR-RFLP for effects of no-tillage and straw mulching on CDB community.   Result   The results of plate counting show that the population of CDB was 8.40×10^7 W_tS 11.4×10^7 $W_{nt}S$ 4.57×10^7 W_t and 5.27×10^7 W_{nt} indicating that both No-tillage and straw mulching increased the number of CDB to a varying extent. Out of the soil samples collected from the four treatment plots   a total of 425 strains of CDB were isolated   4 CDB gene libraries built based on treatment   and a total of 33 OTUs obtained. The number of OTUs of CDB in the treatments range from 19 to 26   among which 7 were found common in all the 4 treatments. 8 particular in the treatments with straw mulching soil and 3 in the treatments of no-tillage   which indicates that the practices of straw mulching and no-tillage did have some effects on CDB community structure in the soil. The 4 clone libraries varied sharply in CDB diversity index   which was the highest in Treatment $W_{nt}S$ and higher in the two treatments with straw mulching than in the two without straw mulching   which indicates that compared with tillage   no-tillage is more conducive to diversity of the CDB in the soil. Phylogenic analysis shows that the CDB in the soils varied in the range of 80%   99% in 16S rDNA homology. The 33 OTUs could be sorted into 4 phyla   7 classes   11 orders   15 families and 20 genera. Proteobacteria is the dominant phylum   composed of 15 OTUs   which could be sorted into 11 genera. In Treatment W_tS *Sphingobium*   Streptomycetaceae   *Sinorhizobium* and Promicromonsporaceae are the dominant groups   accounting for 67.92%   in Treatment $W_{nt}S$ Streptomycetaceae   Promicromonosporaceae and *Flavobacterium* are   accounting for 48.15%   in Treatment W_t *Sinorhizobium*   Promicromonosporaceae and *Acinetobacter* are   accounting for 54.29%   and in Treatment W_{nt} *Rhizobium*   *Phyllobacterium*   *Pseudomonas* and Promicromonosporaceae are   accounting for 63.21%. The treatments with straw mulching are significantly higher than the treatments without straw mulching in relative abundance of Streptomycetaceae   *Flavobacterium* and *Sphingobium*   which indicates that straw mulching is conducive to growth of these bacteria   which play an important role

in straw decomposition. And the treatments of no-tillage are apparently higher than the treatments of tillage in relative abundance of *Pseudomonas* *Phyllobacterium* *Paenibacillus* *Promicromonosporaceae* and *Sphingobacterium* which indicates that no-tillage is conductive to growth of these bacteria. Canonical correspondence analysis shows that great changes have taken place in CDB community structure in all the four treatments that pH TP organic carbon and TK is significantly and positively related to no-tillage and that TN analyzable N readily available K readily available P and organic carbon is significantly and positively related to straw mulching. Conclusion It is quite clear that no-tillage and straw mulching can significantly raise the number and diversity of cellulose-decomposing bacteria in the soil and alter cellulose-decomposing bacteria community structure. Conducted as key techniques for sustainable agriculture to improve soil quality by increasing the number and variety of microorganisms no-tillage and straw mulching or incorporation do have some theoretical significance and enormous potential for extrapolation.

Key words No-tillage Straw mulching Cellulose-decomposing bacteria Diversity PCR-RFLP