

# Pyrosequencing based analysis of soil bacterial communities under long-term soil tillage and crop residue management in semiarid environments

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## Introduction

Conservation tillage coupled with crop residue mulching has been used to reduce soil evaporation and erosion and enhance crop productivity and water use efficiency in arid and semiarid environments worldwide. However, little is known about the long-term effect of these farming practices on nutrient cycling and soil biological properties. The objective of this study was to investigate the response of soil bacterial community to soil tillage and residue management in a 15-year field experiment. The hypothesis was that soil microbial communities have been changed after long-term tillage and residue management.

## Materials & methods

### *Site description*

The Conservation Tillage experiment was established in 2000 at Loess Plateau Field Research Station (35°40'N, 107°40'E; elevation, 1298m) of the Lanzhou University at Qingyang city, Gansu province, in Northwest of China. The area is characterized by a semi-arid, monsoon climate, with cold winters and hot summers. Averaged annual temperature is 8.3°C. The mean annual rainfall is 560mm, it's mainly concentrated in the month of July-September. The soil is a Heilu soil (Entisol of US classification), with pH8~8.5, and soil organic carbon content is lower than 1.0 %. It is an infertile silt loam soil with 70% silt, and represents the major cropping soil of the region.

### *Experiment design and sample collection*

Treatments were arranged in 2 × 2 factorial in a randomized block design with 4 replications. Four treatments were: (1) conventional tillage (CT): soil was tilled and residue removed from field; (2) conventional tillage with residue retention (CTS): soil was tilled and residue retained; (3) no-tillage (NT): soil was not tilled and residue removed; (4) no tillage with residue retention (NTS): soil was not tilled and residue retained. Winter wheat (*Triticum aestivum* L.)-- soybean (*Glycine max* L.) --corn (*Zea mays* L.) were rotated in a 2-year rotation system. The soil samples were collected at each plot in winter wheat root zone when wheat was flowering in May 2014, sieved through 2-mm, and stored at -20 °C for molecular analysis of the taxonomic diversity of soil bacterial communities.

### Soil DNA extraction, PCR amplification and amplicons sequencing

Soil Microbial DNA was extracted using Soil DNA Kit by following the manufacturer's instructions. The V4-V5 region of the bacteria 16S ribosomal RNA gene were amplified by PCR using primers 515F 5'-barcode- GTGCCAGCMGCCGCGG)-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where barcode is an eight-base sequence unique to each sample (Beller *et al.* 2013). Amplicons were extracted and sequenced on an Illumina MiSeq platform according to the standard protocols.

### Statistical analysis

Mothur V.1.30.1 (Schloss *et al.*, 2011) and R software (Version 3.1.2) were used for data trimming and analysis.

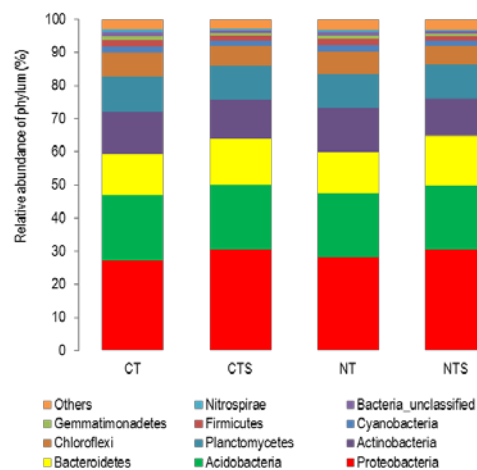
## Results

A total of 240,701 raw reads were obtained and analysed in this study. There were 34,372 different OTU<sub>97</sub> was found. Thirty-seven different phyla were detected in soil. Of all the sequences obtained, 99.1% could be identified in phyla level. Proteobacteria (29.1% of the total of the OTU<sub>97</sub>), Acidobacteria (19.4%), Bacteroidetes (13.4%), Actinobacteria (12.3%), Planctomycetes (10.4%), Chloroflexi (6.3%) are dominant bacterial phyla in this soil. The relative abundances of these six bacterial phyla contain 91% of the total of the OTU<sub>97</sub> that detected in soil bacteria phyla (**Fig.1**).

There were no significant differences among treatments in the relative abundances of OTU<sub>97</sub> in phyla level (**Fig.1**). But Proteobacteria, Chloroflexi, Firmicutes and Bacteria-unclassified were significantly different with soil stubble retention ( $P<0.05$ ). Gemmatimonadetes was the only phyla that was significantly affected by soil tillage and residue retention ( $P<0.05$ ).

Residue retention significantly decreased relative abundance of Chloroflexi ( $P<0.01$ ), Firmicutes and Gemmatimonadetes ( $P<0.05$ ), but obviously increased ( $P<0.05$ ) soil Proteobacteria's relative abundance of the OTU<sub>97</sub>.

Heat map was established on 76 different classes of bacteria (Y-axis) for each treatment (**Fig.2**). Four classes belonging to Proteobacteria phyla were found with Alphaproteobacteria accounting for 9.4%, Betaproteobacteria 8.5%, Gammaproteobacteria 8.0% and Deltaproteobacteria 3.2% of the total OTU<sub>97</sub>. They are possessed 29.1% of the relative abundance of the OTU<sub>97</sub> in the detective communities in class level (**Fig.2**).



**Fig. 1.** Relative abundances of phylogenetic groups in soils under different tillage and residue management in a long-term experiment at Qingyang (Gansu, China). CT, conventional tillage with residue removed; CTS, conventional tillage with residue retention; NT, no-tillage with residue removed; NTS, no-tillage with residue retention

No significant changes in richness, diversity and total abundance of bacteria were found between the treatments or residue/tillage factors in phyla and class level (data not shown).

## Conclusions

Tillage and residue management have an effect on the bacterial community using taxonomic and phylogenetic information. Crop residue retention had a more pronounced effect on the soil bacterial communities than tillage in phyla and class level.

Proteobacteria, Chloroflexi, Firmicutes and Bacteria-unclassified phyla were affected by crop stubble retention, while Gemmatimonadetes was affected by both tillage and residue management. These results indicate that bacterial communities respond to tillage and residue management and may play a major role in soil processes under different management systems.

Crop residue retention enhanced the predominant bacterial community of Proteobacteria most in soil, which could increase soil nutrient cycling.

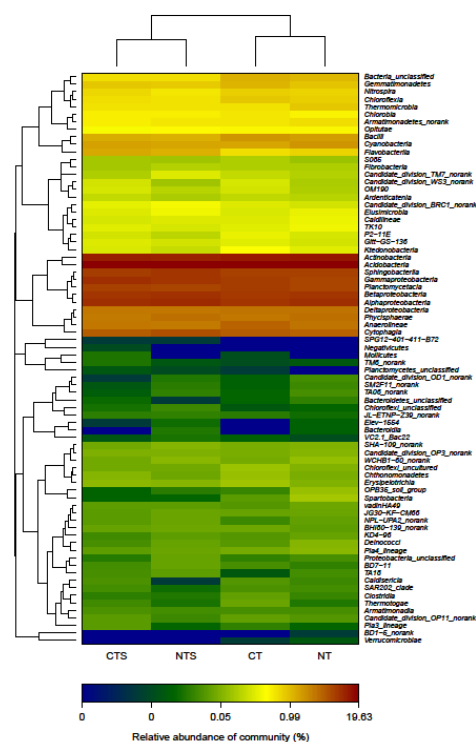
Bacterial community richness and diversity didn't show significant difference between treatments probably due to the ego defense response of bacterial community to those treatments.

## Acknowledgements

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## References

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**Fig. 2.** The heat map depicts the relative abundance of each bacterium (variables clustering on Y-axis) within each treatment (X-axis clustering). Clustering of the treatments was similarly based upon abundance of the soil bacterial classes in class level among treatments. The heat map colors represent the relative abundance of the bacterial classes with the legend indicate at the bottom of the figure. Treatments code as Fig.1.