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Changes in bacterial community of soil induced by long-term straw returning

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ABSTRACT: Straw returning is an effective way to improve soil quality. Whether the bacterial community development has been changed by long-term straw returning in non-calcareous fluro-acquic soil is not clear. In this study, the following five treatments were administered: soil without fertilizer (CK); wheat and corn straw returning (WC); wheat straw returning with 276 kg N ha^{-1} yr^{-1} (WN); manure, 60,000 kg ha^{-1} pig manure compost (M) and wheat and corn straw returning with 276 kg N ha^{-1} yr^{-1} (WCN). The high-throughput 16S rRNA sequencing technology was used to evaluate the bacterial communities. The results showed that the community was composed mostly of two dominant groups (*Proteobacteria* and *Acidobacteria*). Bacterial diversity increased after the application of straw and manure. Principal component analyses revealed that the soil bacterial community differed significantly between treatments. The WCN treatment showed relatively higher total soil N, available P, available K, and organic carbon and invertase, urease, cellulase activities and yield than the WC treatment. Our results suggested that application of N fertilizer to straw returning soil had significantly higher soil fertility and enzyme activity than straw returning alone, which resulted in a different bacterial community composition, *Stenotrophomonas*, *Pseudoxanthomonas*, and *Acinetobacter* which were the dominant genera in the WC treatment while *Candidatus*, *Koribacter* and *Granulicella* were the dominant genera in the WCN treatment. To summarize, wheat and maize straw returning with N fertilizer would be the optimum proposal for improving soil quality and yield in the future in non-calcareous fluro-acquic-wheat and maize cultivated soils in the North China Plain in China.
Keywords: long-term experiment, soil quality, soil bacterial composition

Introduction

Although under high-yielding systems, the use of higher inputs of inorganic fertilizers has been shown to increase crop yields (Cui et al., 2010), such application reduces soil quality, a feature which ensures the quality of the environment, promotes the foundation of both animal and human health, and increases greenhouse gas emissions (Dusenbury et al., 2008; Guo et al., 2010). Straw returning is an effective way to improve soil quality by increasing enzymatic activity in soil and to regulate soil microbial activity (Bastida et al., 2007; Cardinale et al., 2006). Soil microorganisms are the core of soil fertility while soil bacteria can account for about 70 % - 90 % of the total of soil microorganisms (Lazarev and Abrashin, 2000; Kennedy, 1999). Therefore, the study of fertilization application on soil bacteria communities is vital to the improvement of soil quality and has been attracting deserved attention in recent years (Zhong et al., 2010).

It is a challenging task to fully characterize soil bacteria communities. In recent years, high throughput sequencing technologies have been more frequently used to characterize soil microbial community structures (Marschner et al., 2003). However, the response of the soil bacterial community to fertilization has varied considerably from study to study and no clear trends have emerged. Börjesson et al. (2012) found that the soil bacterial community composition did not differ significantly between unfertilized soils and fertilized soils in a long-term winter wheat trial while Enwall et al. (2005), Kirchmann et al. (2013) and Navarrete et al. (2015) have found clear changes in soil bacteria communities after the application of organic and inorganic fertilizer amendments and straw retention. This difference may contribute to environmental and management related factors, such as climate, soil properties and plant cover (Böhme et al., 2005). Non-calcareous fluro-acquic soil is one of the highest in productivity in wheat and maize cultivated soils in the North China Plain (NCP), which produces about 34 % of total maize production in China (Chen et al., 2011). The present study was conducted to examine the response of soil chemical properties, enzyme activity, maize yield and soil bacterial community development using high-throughput 16S rRNA sequencing technology applied to different long-term straw returning amounts with N fertilizer, including soil without fertilizer (CK); wheat and corn straw returning (WC); wheat straw returning with 276 kg N ha^{-1} yr^{-1} (WN); manure (M), 60,000 kg ha^{-1}, pig manure compost (M) and wheat and corn straw returning with 276 kg N ha^{-1} yr^{-1} (WCN) in non-calcareous fluro-acquic soil.

Materials and Methods

Experimental design

The field experiment was conducted at the Experimental Station (36°10′ N, 120°31′ E, altitude: 30.5 m) in Yantai, Shandong Province, China. In order to illustrate the effect of long-term straw returning on soil quality and crop production, a long-term field experiment (incorporating application of manure/straw returning and a

control treatment) was initiated in 2009. The soil type at the study site is non-calcareous fluro-acquic soil. At the start of the experiment, the soil had a pH (H_2O) of 6.8, 5.0 g kg^{-1} organic matter, 0.6 g kg^{-1} total N, and 16.3 and 72.0 mg kg^{-1} of available P and K, respectively. The site has a temperate and monsoonal type climate with annual average temperature and precipitation of 11.2 °C and 779 mm, respectively.

The experiment had winter wheat and summer maize rotations with a completely randomized design with five treatments and three replicates for each treatment. Total area was 33.3 m^2 per plot per repetition. In this study, five treatments were selected as follows:

1) Soil without fertilizer (control check, CK).

2) 14,301 kg ha^{-1} wheat and corn straw returning (WC). Dry straw of maize and wheat had a 2 % average N content. The application of wheat and corn straw returning amount is calculated according to the same inorganic nitrogen application content.

3) Wheat straw returning with 276 kg N ha^{-1} (WN).

4) Manure, 60,000 kg ha^{-1}, pig manure compost (M).

5) Wheat and corn straw returning with 276 kg N ha^{-1} (WCN).

The manure compost had 50 g kg^{-1} organic matter; 3.0 and 2.0 g kg^{-1} total N and P, respectively, and about 50 % water content. Manure, P and K were applied as basal fertilizers. 8 % of the N was applied as a basal dressing and 18 % and 25 % top-dressed on the wheat crop at the reviving growth stage and jointing stage, respectively. 25 % of the N fertilizer was applied at jointing stage and the remaining N fertilizer was applied at the bell stage on the maize crop. Urea was used as an N fertilizer.

Soil sampling

Soil samples were collected across the field at a depth of 0-20 cm after maize harvesting in Sept 2014. Five soil cores were collected and pooled together as a composite sample from each of the three replicates for each treatment. Composite samples were sieved through a 2 mm screen and homogenized prior to analysis. One portion of the composite soil was stored for molecular analysis, while another portion was used for enzyme activity.

Soil chemical analysis

Total soil N was measured using a vario MACRO cube element analyzer (Elementar Analysensysteme Gesellschaft mit beschrankter Haftung, Hanau, Kinzig, Germany). Available P was analyzed by the Olsen method (Olsen and Sommers, 1982) and available K was determined by ammonium displacement of the exchangeable cations. Soil organic carbon was determined by dichromate oxidation. Soil pH was measured with a compound electrode using a soil to water ratio of 1:2.5.

Soil enzyme activity characterization

Soil invertase, urease, and cellulase activities were estimated according to Sun et al. (2014a). Invertase and cellulase activities were estimated colorimetrically by determining the reduction of 3.5-dinitrosalicylic acid from reducing sugars after the soil was incubated with a buffered sucrose and sodium carboxymethylcellulose solution and toluene at 37 °C for 24 h and 72 h, respectively. Soil invertase activities are indicated as the number of milligrams of glucose in 1 g dry soil at 37 °C for 24 h. Soil cellulase activities is indicated as the number of milligrams of glucose in 10 g dry soil at 37 °C for 72 h. Soil urease activity was detected using improved sodium phenate and sodium hypochlorite colorimetry and indicated as the number of milligrams of NH_3-N in 1 g dry soil at 37 °C for 24 h (Li et al., 2008).

Isolation of DNA from soil and high-throughput sequencing

There was little difference between replicates in other indicators, such as the invertase, urease and cellulase activity of soil, maize yield and so on. Three replicate samples were randomly pooled together as a composite sample for one treatment and only used for molecular analysis. Total metagenome DNA from samples was extracted using the CTAB/SDS method (Hess et al., 2011). DNA concentration and purity were monitored on 1 % agarose gels. According to the concentration, DNA was diluted to 1 ng μL^{-1} using sterile water.

16S rDNA bacterial sequencing and data processing

PCR amplifications were conducted with the 515f/806r primer set that amplifies the V4 region of the 16S rDNA gene (Caporaso et al., 2011) using the specific primer with the barcode. All PCR reactions were carried out in 30 µL reactions with 15 µL of Polymerase Chain Reaction (PCR) Master Mix; 0.2 µM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s culminating with 72 °C for 5 min (Caporaso et al., 2011). The same volume of 1X loading buffer (contained SYB green) was mixed with PCR products and electrophoresis was operated on 2 % agarose gel for detection. Samples with a bright main strip between 400-450 bp were chosen for further experiments. The PCR products were mixed in equidensity ratios. Then, the mixture of PCR products was purified with a Gel Extraction Kit. Sequencing libraries were generated using New England Biolabs (NEB) following the manufacturer's recommendations and index codes were added. Library quality was assessed by the Fluorometer and Agilent Bioanalyzer 2100 system. Finally, the library

was sequenced on an Illumina MiSeq platform and 250 base pair paired-end reads were generated.

FLASH was used to merge the pairs of reads from the original DNA fragments (Magoč and Salzberg, 2011). Sequencing reads were assigned to each sample according to the unique barcode of each sample. Sequences were analyzed with the QIIME software package (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010) and UPARSE pipeline (Edgar, 2013), in addition to custom Perl scripts to analyze alpha and beta diversity. We retained reads with more than 75 % base quality.

Next, the UPARSE pipeline was used to identify operational taxonomic units (OTUs) by making an OTU table. In order to eliminate the experiment error and artificial statistical error, the sequence numbers (average 58287) of the samples were set at the same depth level (at 97 % similarity), especially for the comparable in the comparative analysis. A homogenization method is used to set a threshold (typically the sample with the lowest sequence number), then the sequence numbers which were randomly extracted in the threshold set from the samples, were further analyzed, that is, the effective tags (average 47829) of the samples minus the number of unique tags. In this study, sequences were assigned to OTUs with 97 % similarity. The protocol described in Caporaso et al. (2010) was used to determine the composition and diversity of the bacterial communities under different treatments. We selected a representative sequence for each OTU and used the ribosomal database project classifier (Caporaso et al., 2011) to assign taxonomic data to each representative sequence (40,000). In order to compute Alpha divesity, we rarified the OTU table and calculated three metrics: Chao1 metric for estimating the richness, the observed OTUs′ metric which was simply the count of unique OTUs found in the sample, and a Shannon index. Rarefaction curves and rank abundance curves were generated from these three metrics.

Statistical analysis of sequencing data

Analysis of variance was performed using SPSS Statistics 18, significance was accepted at $p < 0.05$ (International Business Machine, Armonk, New York, USA) for soil chemical and enzyme activities analysis and yield. Uparse Software (Uparse v. 7.0.1001, http://drive5.com/uparse/) (Edgar, 2013) was used to analyze the community richness index, community diversity index, data preprocessing, and unit-based operational taxonomic. The histogram was created using Microsoft Excel 2010. Canoco 4.5 was used for principal component analysis (PCA) of the relative abundance of bacterial genera.

Results

Soil chemical properties, enzyme activity and maize yield

Compared with CK treatment, long-term straw returning (WN, WC, and WCN) and manure (M) treatments significantly increased total soil N, available P, available K, and organic carbon, especially long-term straw returning with N fertilizer or manure, applied alone (Table 1). Soil pH values were not affected by long-term straw returning and fertilizer treatments.

Significant differences in maize yield were found under different treatments: M > WCN > WN > WC > CK (Table 2). Compared with the CK treatment, maize yield was significantly increased 8 %, 116 %, 161 %, and 154 % in WC, WN, M and WCN, respectively. Corresponding significantly improved effects were found in soil invertase, urease, and cellulase activity in three different straw returning and organic fertilization treatments (Table 2). The improved trends were similar in the three soil enzyme activities: lowest in WC treatment, highest in WCN treatment, with WN and M treatment in the middle.

Table 1 – Soil pH and nutrient concentrations of different treatments.

Treatments	Total N	Available P	Available K	Total organic carbon	pH
	g kg^{-1}	mg kg^{-1}		g kg^{-1}	1:2.5 g/v
CK	0.56 e	16.43 e	30.41 e	4.97 e	6.8
WC	1.06 d	28.88 d	31.43 d	6.24 d	6.7
WN	1.27 bc	48.31 c	100.08 c	8.69 c	6.8
M	1.40 a	70.72 a	130.49 a	12.74 a	6.8
WCN	1.30 b	64.24 b	117.32 b	10.96 b	6.8

Three replicates of soil were used for each treatment. Analysis of variance was performed using SPSS Statistics 18; Numbers followed by different letters indicate significant differences between different treatments ($p < 0.05$); Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

Table 2 – The invertase, urease and cellulase activity of soil and maize yield of different treatments.

Treatments	Invertase (glucose, mg 1 g^{-1}, 24 h, 37 °C)	Urease (NH_3-N, mg g^{-1}, 24 h, 37 °C)	Cellulase (glucose, mg 10 g^{-1}, 72 h, 37 °C)	Yield (kg ha^{-1})
CK	3.88 e	24.11 e	9.15 e	3,678 e
WC	6.23 d	25.88 d	11.15 d	3,960 d
WN	7.24 c	28.56 c	21.51 c	7,950 c
M	8.19 b	32.16 b	28.24 b	9,600 a
WCN	8.31 a	41.37 a	31.47 a	9,322 b

Three replicates of soil were used for each treatment; Analysis of variance was performed using SPSS Statistics 18; Numbers followed by different letters indicate significant differences among different treatments ($p < 0.05$); Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

Richness

More than 40,000 valid reads were obtained for each treatment through a sequence optimization process, and the soil bacterial community richness index was calculated as shown in Table 3. In the WC, WN, M and WCN samples, more than 400, 600, 400 and 700 additional OTUs were observed compared with the CK treatment (Figure 1). The Lower Shannon and Chao1 indices in CK treatment indicated that straw returning and organic fertilization treatments improved diversity within the bacterial community. However, no difference was found between different treatments in the Rank Abundance Curve (Figure 2), indicating approximately equally distributed OTU abundance among community members.

Taxonomic coverage

All of the sequences were classified into 10 phyla levels by the mothur program. The straw returning and manure treatments did not affect the type of top 10 phylum variety in the bacterial community, while the distribution of each phylum or group did vary (Figure 3). In all samples, Proteobacteria, and Acidobacteria were the two most dominant phyla, accounting for more than 50 % of the reads. Significantly more unclassified species were detected in the WCN treatments, which were in accordance with their higher diversity indices. Compared with the CK treatment (3 % in *Planctomycetes*, 3 % in *Verrucomicrobia*, 4 % in *Chloroflexi*, 6 % in *Gemmatimonadetes*, 5 % in *Nitrospirae*,), straw returning (WN, WC and WCN) and manure (M) treatments increased the percentage of *Planctomycetes* (WC: 3 %, WN: 3 %, M: 2 %, WCN: 4 %), *Verrucomicrobia* (WC: 2 %, WN: 4 %, M: 3 %, WCN: 4 %), and *Chloroflexi* (WC: 1 %, WN: 2 %, M: 1 %, WCN: 2 %), but reduced the percentage of *Gemmatimonadetes* (WC: 1 %, WN: 2 %, M: 2 %, WCN: 2 %), and *Nitrospirae* (WC: 2 %, WN: 2 %, M: 3 %, WCN: 3 %).

To further compare the microbiota between the different treatments, we performed principal component analysis (PCA) on the relative abundance of bacterial genera using Canoco 4.5 (Figure 4). Data are presented as a 2D plot to better illustrate the relationship. The PC1 and PC2 accounted for 40 % and 25 % of the total variation, respectively. There were significant differences between the five treatments. The bacterial community of straw treatments has more similarity than the communities of the CK and M treatments.

Differences were found in the relative abundance of top 35 class, family and genera between different treatments (Figure 5A-C). *Sva0725*, *Nitrospira*, *MB-A2-*

Table 3 – Comparison of the estimated operational taxonomic unit (OTU) richness and diversity indexes of the 16S rRNA gene libraries for clustering at 97 % identity as obtained from the Illumina MiSeq (250) analysis.

Treatments	Observed OTUs	Shannon	Chao1
CK	2,513	9.11	2,825.6
WC	2,964	9.73	3,217.6
WN	3,137	9.80	3,413.8
M	2,948	9.85	3,241.2
WCN	3,264	9.98	3,766.5

Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

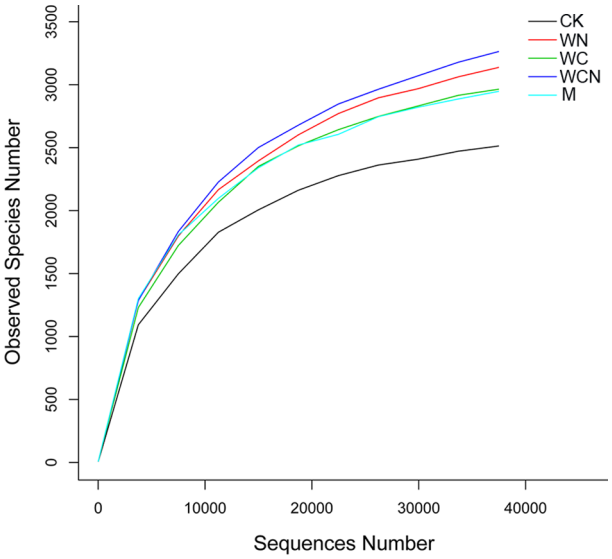

Figure 1 – Rarefaction on species-abundance data; Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

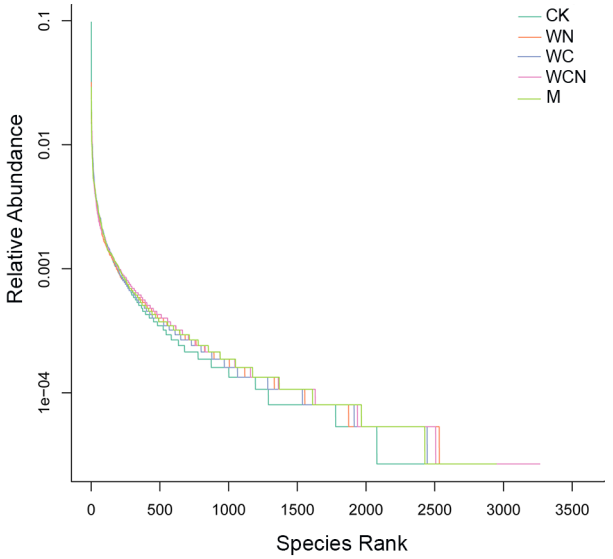

Figure 2 – Rank Abundance curve of different treatments; Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

108, *RB25*, *Bacilli*, *JL-ETNP-Z39* and *Thaumarchaeota* were the dominant class in the CK treatment. *Chloracidobacteria* and *Acidimicrobiia* were the dominant class in the WN treatment. *Acidobacteria-6* was the dominant class in the WCN treatment. *OM190* and *S1a-1H* were the dominant class in the WC treatment. *Alphaproteobacteria* and *Sphingobacteriia* were the dominant class in the M treatment (Figure 5A). *Syntrophobacteraceae*, *Nitrospiraceae*, *Nitrososphaeraceae* and *Lactobacillaceae* were the dominant family in CK treatment. *Chitinophagacea* and *Sinobacteraceae* were the dominant family in the M treatment. *Gaiellaceae* and *RB40* were the dominant family in the WCN treatment. There was no obvious dominant family in the WN treatment. *Coxiellaceae* and *Moraxellaceae* were the dominant family in the WC treatment (Figure 5B). *Cuoriavidus*, *Nitrospira*, *Lactobacillus*, *Pediococcus*, and *Candidatus_Nitrososphaera* were the dominant genera in the CK treatment. *Stenotrophomonas*, *Pseudo-*

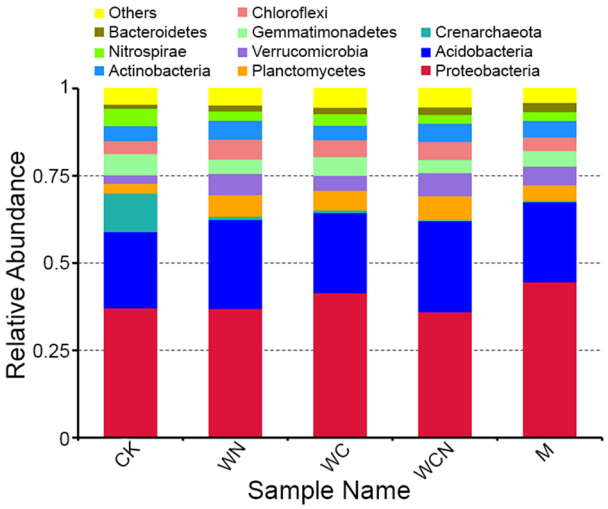

Figure 3 – Comparison of the bacterial communities at the phylum level; Relative read abundance of different bacterial phyla within the different communities. Sequences that could not be classified into any known group were labeled "Others". Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

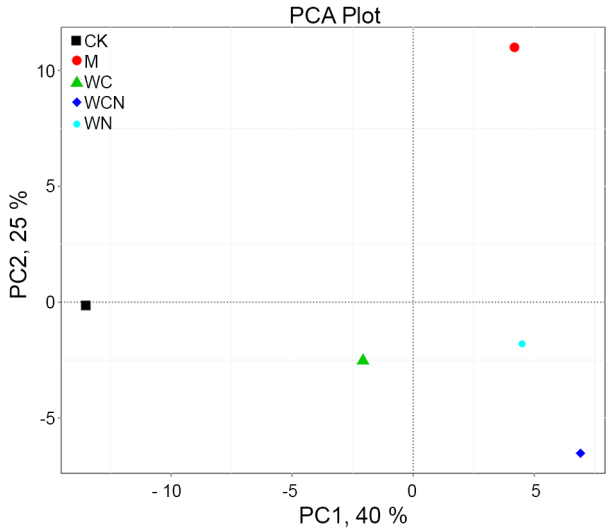

Figure 4 – Principal component analysis (PCA) on the relative abundance of bacterial genera; Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

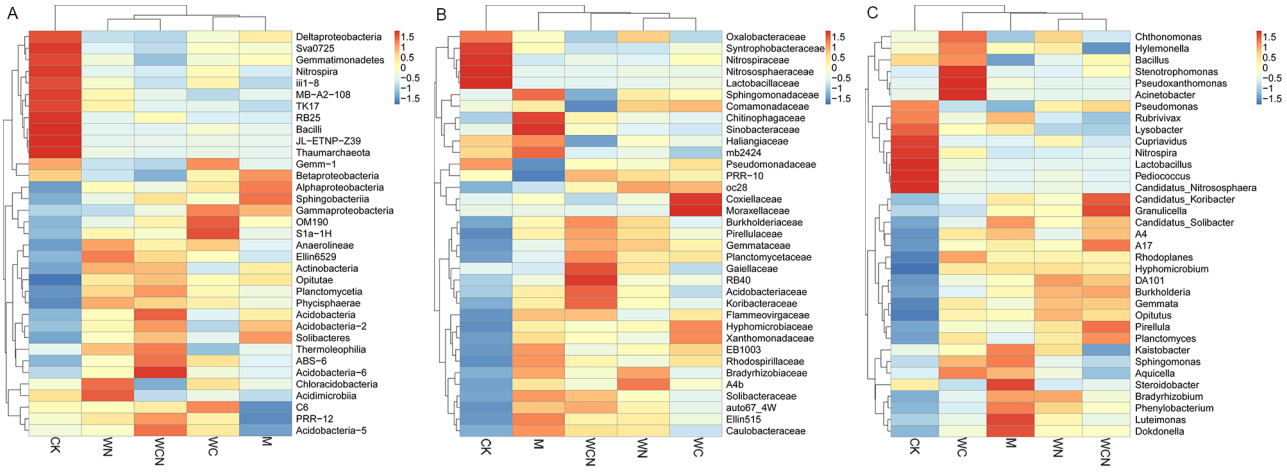

Figure 5 – Relative abundance of top 35 class (A), family (B) and genera (C) in different treatments; Treatments: CK = Soil without fertilizer; WC = Wheat and corn straw returning; WN = Wheat straw returning with 276 kg N ha^{-1}; M = Manure, 60,000 kg ha^{-1}, pig manure compost; WCN = Wheat and corn straw returning with 276 kg N ha^{-1}.

xanthomonas, and *Acinetobacter* were the dominant genera in the WC treatment. *Steroidobacter*, *Luteimonas*, and *Dokdonella* were the dominant genera in the M treatment. There were no obvious dominant genera in the WN treatment. *Candidatus*, *Koribacter* and *Granulicella* were the dominant genera in the WCN treatment (Figure 5C). Relative abundance of other class, family and genera were all changed under the straw returning and manure treatments, indicating that straw returning and manure treatments significantly changed the bacterial community composition structure.

Discussion

Organic fertilizers can promote the accumulation of organic matter and the major soil macronutrients of N, P, and K in the long-term experiments (Bastida et al., 2007; Elfstrand et al., 2007). Consistent with previous studies, soil organic carbon, and total N were significantly increased after six years application of straw and manure (Table 1). However, compared with long-term organic fertilization (M) treatment, straw returning with inorganic amendments did not increase total N, but significantly enhanced the concentrations of available P and K (Table 1). These results illustrate that organic manure plays a more important role in improving soil fertility than do mineral fertilizers (Mäder et al., 2002).

Soil enzymes are crucial indicators of the soil biochemistry, involved with biological cycling and the development of fertility (Aon and Colaneri, 2001). Nannipieri et al. (2012) further indicated that soil enzyme activities controlled the rate of soil organic matter decomposition and nutrient cycling processes. Urease catalyzes the hydrolysis of urea to produce ammonia and carbamate, and it is thus recognized as an important indicator of soil health (Sun et al., 2014b). In this study, long-term straw returning and manure significantly increased urease activity (Table 2), indicating that after the application of straw and manure, the soil might support a new and different functional microbial community that was responsible for this apparent increase in mineralization and result in a better supply of available nutrients. This result was supported by the data on maize yield (Table 1), which, when compared with the CK treatment, maize yield significantly increased 8 %, 116 %, 161 %, and 154 % in the WC, WN, M and WCN, respectively. Soil invertase is an important factor affecting hydrolysis of sucrose into glucose and fructose (Ross, 1983) and soil cellulase is involved in breaking down cellulose (Sinsabaugh and Linkins, 1988). They were both higher in the long-term straw returning with N fertilizer and manure, than when applied alone under straw returning, and the CK treatment (Table 2). This could be related to the abundant decomposed organic matter in the M treatment and inorganic N fertilizer reduced soil C/N and promote straw decomposition in the straw returning with inorganic N fertilizer treatments (WN and WCN) (Kieft, 1994). Marschner et al. (2003) and Zhong et al. (2010) reported that long-term fertilization strongly affects the soil microbial community structure. In the present study, our analysis is just descriptive, because we did not have enough samples to perform confidential statistical tests compared with the CK treatment, straw returning and manure treatments which improved diversity within the soil bacterial community (Table 3). This may be caused by not only the input of available nutrients (Fierer et al., 2007), but also by bacteria added with the straw and organic manure. However, this type of top 10 phylum variety in the bacterial community was not affected by straw returning and manure treatments (Figure 3), indicating that soil bacterial environment is a continuous process, and different fertilization measurements will have a similar effect (Mazzola and Strauss, 2013). The distribution of each phylum varied (Figure 3), which was consistent with Teng et al. (2009) who suggested that adding organic manure to soil can stimulate certain bacteria species and render them the dominant species. Planctomycetes is a kind of anaerobic ammonium oxidizing bacteria, which can be used to participate in the soil carbon and nitrogen cycle (Koji and Kamagata, 2014). Application of straw and manure can provide a large amount of carbon and N sources, resulting in the increasing relative abundance of Planctomycetes (WC: 3 %, WN: 3 %, M: 2 %, WCN: 4 %) compared to the CK treatment (3 %) (Figure 3). *Verrucomicrobia* were abundant within the environment, and important to soil cultures, but they are still poorly characterized at present (Hou et al., 2008). In apple orchard soil, Sun et al. (2014b) found that an optimal manure ratio resulted in abundance increasing from 1 % to 2 % of Verrucomicrobia compared with soil with no manure applied. This may explain the increasing relative abundance from 2 % to 4 % of Verrucomicrobia with the use of straw and manure compared to the CK treatment (Figure 3).

Principal component analysis on the genus level showed that N fertilizer had an impact on the soil microbial community, and the changes from WC treatment to WCN treatment differed (Figure 4). *Stenotrophomonas*, *Pseudoxanthomonas*, and *Acinetobacter* were the dominant genera in the WC treatment, while *Candidatus*, *Koribacter* and *Granulicella* were the dominant genera in the WCN treatment (Figure 5C). *Stenotrophomonas*, *Pseudoxanthomonas*, and *Acinetobacter* all belonging to the genus of Gram-negative bacteria (Palleroni and Bradbury, 1993), are sensitive to copiotrophic conditions (Esperschütz et al., 2009), are often stimulated by added organic matter, and have straw-induced long-term effects (Buyer et al., 2010; Larkin et al., 2006) and replicated aerobic wheat straw enrichment cultures (Jiménez et al., 2014), which were much more abundant in the WC treatment than in other treatments. *Candidatus*, *Koribacter* and *Granulicella* all belonging to an unclassifed Acidobacteria, may be important contributors to ecosystems, since they are particularly abundant within fertilized soils (Eichorst et al., 2007), such as in the WCN treatment in this study. However, little was known about their function in soil until now.

Conclusion

Long-term applications of straw and manure enhanced soil fertility and enzyme activity, especially for straw returning with N fertilizer and improved diversity within the soil bacterial community, clearly changed the distribution of each phylum and genera, but did not affect the type of dominated phylum variety in the soil bacterial community. Application of N fertilizer at the straw returning soil had a considerable impact on the soil microbial community. The soil bacterial communities of the WC treatment and the WCN treatment responded differently, resulting in a more distinct community structure. To summarize, straw returning over two seasons (wheat and maize) with N fertilizer would be the optimum proposal for improving soil quality and yield in non-calcareous fluro-acquic-wheat and maize cultivated soils in the North China Plain in China in the future.

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