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The shift of bacterial community composition magnifies over time in response to different sources of soybean residues amended to a Mollisol

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ABSTRACT

The transformation of plant residues by soil microorganisms contributes significantly to soil carbon (C) sequestration. Although the contribution of different soybean residue sources (leaf, stalk and root) to soil organic C have been quantified in the highly productive Mollisols, the temporal dynamics of microbial community composition during the transformation has not. This study examined the effect of the soybean residues and incubation time on the bacterial community in a Mollisol during 150 days of incubation. Compared to the non-residue control, the presence of soybean residues increased abundances of Bacteroidetes, Actinobacteria and Firmicutes, but decreased those of Proteobacteria and Acidobacteria 15 days after residue amendment. The bacterial community composition differed between the residue sources after 60 days of incubation with the abundance of *Niastella* increasing in the leaf (from 4.5% to 19.1%) and stalk (from 6.5% to 25.7%) but not root treatments. The abundance of *Bacillus* significantly decreased in the stalk (from 12.2% to 2.2%) and root (from 4.0% to 1.6%) but not leaf-residue treatments. The abundance of bacterial genera was significantly associated with soil chemical variables including soil C, N, and pH. Overall, chemical variables drove the temporal response of the bacterial community succession, which provides insight into bacterial contribution to C turnover in soils.

Key words: Illumina-sequencing; Soil chemical property; Carbon; Nitrogen; Residue return

1. Introduction

Soil carbon (C) sequestration is significantly influenced by the decomposition of crop residues (Lal, 2004; Lenhart et al., 2016), and contributes to improvement of physiochemical properties of soil and soil fertility in agricultural systems (Lu et al., 2009; Rui et al., 2009; Le Guillou et al., 2012). Soil microorganisms act to decompose residues by enzymatic degradation of recalcitrant plant-derived compounds, such as lignin, and utilization of carbon substrates (Romani et al., 2006; Masai et al., 2007; Paul, 2007). Understanding the microbial community succession during C cycling of fresh residue incorporation in soils needs intensive investigation.

Residue quality, such as C/N ratio and/or lignin content, greatly influences the decomposition processes. Recalcitrant compounds often require specific microbial community members for degradation, delaying turnover (Palm et al., 2001). Compared to other crop residues, soybean is considered to be easily degradable due to a low C/N ratio (Cadisch et al., 1998). A number of studies indicate that the leaf, stalk and root residues of soybean decompose at different rates, and that various residue types have different retention rates in soil C pools (Gale *et al.*, 2000; Lu et al., 2003; Loya et al., 2004; Lian et al., 2016). The dynamics of microbial community response to different residues of soybean is poorly understood. *Arthrobacter*, *Streptomyces*, *Bacillus* and *Saccharopolyspora* have been found as dominant genera during degradation of soybean leaf residue in a Vertisol (España et al., 2011).

Mollisols, the main soil type in northeast China, have undergone a considerable decrease in soil organic C (SOC) due to intensive farming practices (Liu et al., 2006). Soybean is a major crop of northeast China where the crop residue are a major resource of C input into soils (Fan et al., 2011). Clarifying the succession of microbial community during the decomposition of soybean residues is useful to develop management strategies that sustain soil productivity of Mollisols. Although a number of studies have examined the bacterial diversity in the Mollisols in northeast China by using denaturing gradient gel electrophoresis (DGGE) (Xu et al., 2010; Mi et al., 2012) and high throughput sequencing technologies (Liu et al., 2016), no information is available on microbial community response to soybean residue incorporation.

This study aimed to understand the effect of soybean residue incorporation on the dynamics of microbial community in a Mollisol. We hypothesized that 1) the amendment of soybean residue would shift soil bacterial community composition, and 2) the shift would depend on residue sources (leaf, stalk or root).

2. Materials and methods

An incubation experiment consisted of four treatments in three replicates. The four treatments were 1) no residue-amended control; 2) leaf, 3) stalk and 4) root residues. Cores of 20 g of sieved soil (< 2 mm) mixed with 0.8 g residues (estimated to be equivalent to 22 t ha^{-1}) each were placed in 0.25-L Mason jars and incubated at 80% field water capacity under constant 25°C. Soil samples were collected 15, 30, 60, 90 and 150 days after the incubation started.

Total soil DNA was extracted, the V4 hypervariable region of 16S rRNA gene was amplified

with barcoded primers. Amplicons were sequenced on MiSeq sequencer and then the quality of all sequence reads was evaluated using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (version 1.17; http:// qiime.org/). The MOTHUR program (http://www.mothur.org) was used to estimate Shannon diversity index and evenness (Chelius and Triplett, 2001). Principal coordinate analysis (PCoA) (Lozupone et al., 2005; Li et al., 2014) was deployed to indicate patterns of similarity (Bray-Curtis similarity) in bacterial community composition between the treatments over time. A correlation analysis was performed to associate the dominant genera with soil characteristics such as SOC, dissolved organic C (DOC), total N, NH_4^+, NO_3^- and soil pH across five sampling times. Detailed experimental information and methodology were presented in the supplementary section.

3. Results

3.1. Bacterial community in response to residue amendment

Residue amendment decreased the richness and diversity of bacterial community, as indicated by the significant decreases in the number of OTUs, and Shannon diversity index, compared to the non-residue control. Furthermore, residue amendment significantly decreased the Shannon evenness, indicating that the decrease in Shannon diversity was due to the decrease in the community richness and evenness (Table 1). Decreases were less pronounced in the root residue treatment than the stalk or leaf residue treatments. Decreases were also greater at day 150 than days 90, leading to the significant interaction between residue and incubation time ($p < 0.05$).

The PCoA analysis indicated significant differences in the bacterial communities treated with leaf, stalk and root residues compared to the control (Fig. 1) ($p < 0.05$; Table S1). The soil bacterial communities of the three residue treatments were similar during the initial 60 days of incubation but differed significantly by 150 days. Over the incubation period, the bacterial community in the control did not change significantly (data not shown).

Compared to the no-residue control, residue amendment substantially increased relative abundances of Bacteroidetes, Actinobacteria, Firmicutes and Acidobacteria, but decreased abundances of Gemmatimonadetes and Chloroflexi (Fig. 2). Five phyla, i.e. Bacteroidetes, Proteobacteria, Firmicutes, Acidobacteria and Actinobactia exhibited significantly different abundances between the residue-amended treatments, and the abundances altered over time. In particular, the abundance of genera *Niastella* affiliated to Bacteroidetes increased over time in the leaf (from 4.5% to 19.1%) and stalk (from 6.5% to 25.7%) residue treatments but not in the root treatment (9.5% on average) (Tables S1, S2). Residue amendment significantly decreased the abundance of Proteobacteria (29.5% to 17.1%). Especially, the abundance of genera *Steroidobacter*, *Thermomonas*, *Agrobacterium* and *Balneimonas*. *Bacillus* affiliated to Firmicutes were significantly decreased in the stalk (from 12.2% to 2.2%) and root (from 4.0% to 1.6%) residue amendment but not in the leaf treatment with an average of 8.0%. Over the 150 days of incubation, residue amendment significantly decreased the abundance of Actinobactia (Table S2) to which *Streptomyces* and *Nonomuraea* were the major genera affiliated. Over time, the abundance of *Streptomyces* decreased in all residue treatments. The

abundance of *Nonomuraea* appeared to decrease over time in the root-residue treatment but did not change in the leaf and stalk treatments. Similarly, Acidobacteria showed a significant decrease over time. A correspondent response to residue treatments also occurred at the OTU level (Table S3).

3.2. Relationship between bacterial community composition and soil properties

The abundances of *Bacillus*, *Actinomadura*, *Nocardia* correlated positively with DOC, while only the abundance of one genus, *Actinomadura,* correlated positively with soil C content ($p < 0.05$) (Table 2). A number of genera such as *Steroidobacter*, *Luteibacter*, *Phenylobacterium*, *Nonomuraea* and *Actinomadura* were positively associated with soil total N content ($p < 0.05$).

Soil pH correlated positively with the abundance of genera *Chitinophaga*, *Steroidobacter*, *Phenylobacterium* and *Actinomadura* but negatively with that of *Candidatus Koribacter* and *Nocardia* ($p < 0.05$). The concentration of NO_3^- in soil correlated positively with the abundances *Segetibacter*, *Steroidobacter*, and *Streptomyces*, while it correlated negatively with the relative abundance of *Niastella*, *Thermomonas Nannocystis*, *Bacillus*, *Nocardia*. Furthermore, genera *Agrobacterium* and *Nonomuraea* had positive relations with the concentration of NH_4^+. However, no genus had any significant association with C/N in soil (Table 2).

4. Discussion

Residue sources markedly impacted the soil bacterial community over time, forming a significant grouping compared to unamended soils. In general, the input of plant residue into soil shifts the relative abundance of the different phyla and favors copiotrophic bacteria such as Actinobacteria and Firmicutes (Ramirez-Villanueva et al., 2015). In our study, *Streptomyces*, *Nonomuraea* and *Bacillus* were enriched during the initial stage after residue amendment compared to the no-residue control (Table S2). This is likely attributed to an increase of labile-C concentration in soil during residue decomposition (Lian et al., 2016), which favors the enrichment of copiotrophic groups in soil. It was evident of higher abundances of these groups in soils with high C availability either as an intrinsic property of the soil or as a result of sucrose amendments (Fierer et al., 2007). Moreover, the order Bacillales was identified as a major component in composting processes and enriched in a ^{13}C cellulose-amended soil (Eichorst and Kuske, 2012). However, the abundances of some genera in this order decreased over time except for the leaf-residue treatment. This is probably attributed to the decrease of DOC with time, which is related to the residue quality varying among residue types (Lian et al., 2016), because of readily consumable energy sources depleted by microbes with time. Although Proteobacteria are well known to respond readily to labile-C sources and *Steriodobacter* belonging to this phylum was enriched in the residue-amended soil, the abundance of *Steriodobacter* was below 2%, indicating that Proteobacteria was not the major bacterial group in response to the amendment of soybean residues in the Mollisol.

In contrast to Actinobacteria and Firmicutes, *Niastella* affiliated to Bacteroidetes was enriched over time, especially in the leaf- and stalk-residue treatments. The abundance of *Niastella* became the largest in the community at the end of incubation, indicating that the genus *Niastella* was the major component in response to residue-induced characteristics in the Mollisol. Compared to the root-amended soil, the greater abundance in the leaf- and stalk-amended soil implies that *Niastella* may exert different functions regarding the fate of residue-C since the more root-derived C was sequestrated in organic fractions of Mollisols over time (Lian et al., 2016). Weon et al. (2006) observed that several species within this genus exhibited the ability to hydrolyze carboxymethylcellulose. However, this genus did not show any significant association with DOC or C content in soil (Table 3). Due to the variation in residue quality between residue types (Kögel-Knabner et al., 1993; Dignac et al., 2005; Rasse et al., 2006), it is unknown whether the organic C components such as cellulose and starch in residues contribute to the marked increase in the *Niastella* abundance. Further investigation is required on the association of specific compounds with the response of bacterial species.

Compared to organic C in soil, the N limitation was more likely to contribute to the shift of bacteria in response to residue amendment. In this present study, the concentrations of NH_4^+ and NO_3^- were lower in the residue-amended soils than the control (data not shown), while the effect of residue amendment on DOC was opposite (Lian et al., 2016). This relatively low N availability may greatly favour the enrichment of major genera such as *Niastella* and *Bacillus* in soil because significant genus-N relationships were observed in this study (Table 2). Long et al. (2012) found that the ratio of DOC to DON (dissolved organic N) in an Ultic Alfisol soil negatively affected the abundance of bacteria, supporting this point view on the N limitation to the bacterial community. However, the function of these genera in C/N cycling needs further investigation.

Residue-induced change of soil pH was likely to be one of main factors to shift the bacterial community. As the soil pH increased under three residue treatments compared to the control (Lian et al., 2016), a number of the genera, especially *Chitinophaga* and *Steroidobacter*, exhibited a similar trend in relative abundance (Table 2). This indicates that these genera and their relevant OTU/species may link with pH change in the Mollisol. In addition, as a major community component in Acidobateria in response to residue amendment, *Candidatus Koribacter* had a negative association with soil pH, which was supported by Liu et al. (2016) who experimentally showed that pH greatly affected acidobacterial community composition, and had a negative association with Acidobacteria subgroup 1 which *Candidatus Koribacter* in this study belonged to.

In summary, the residue amendment significantly impacted the bacterial community in Molllisols, resulting in Bacteroidetes, Actinobacteria, Firmicutes and Acidobacteria enriched at the initial stage after amendment compared to the non-residue control. There was no considerable difference between the residues initially, however, the shift of bacterial community over time depended on residue types. Major genera such as *Niastella*,

Steroidobacter, *Thermomonas*, *Agrobacterium*, *Balneimonas*, *Bacillus Streptomyces* and *Nonomuraea* contributed to various responses of bacterial community to residue amendment. Not only the increase of soil organic C due to residue amendment, but also soil N availability and pH in the amended soil drove the shift of bacterial community composition. Fungal community in response to residue amendment over time was not examined in this study but is worth further investigation due to its role in the turnover of the recalcitrant residue-C.

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Table 1. The rarefaction coverage, number of OTUs, and Shannon diversity index and Shannon evenness in the leaf-, stalk- and root-residue-amended and the control soils after 150 days of incubation at 25ºC. Values are means of three replicates.

Incubation time (d)	Residue treatments	Coverage	Number of OTUs	Shannon diversity index	Shannon evenness
15	Control	0.81	1225	8.66	0.85
	Leaf	0.90	673	6.47	0.70
	Stalk	0.87	827	6.78	0.71
	Root	0.86	916	7.06	0.74
30	Control	0.78	1373	8.90	0.86
	Leaf	0.90	649	6.23	0.67
	Stalk	0.86	891	6.98	0.74
	Root	0.87	839	7.07	0.74
60	Control	0.83	1105	8.20	0.83
	Leaf	0.89	734	6.37	0.69
	Stalk	0.88	733	6.14	0.66
	Root	0.87	821	7.09	0.74
90	Control	0.84	1087	8.33	0.84
	Leaf	0.88	729	6.43	0.69
	Stalk	0.87	830	6.87	0.72
	Root	0.87	846	7.42	0.77
150	Control	0.76	1503	9.18	0.87
	Leaf	0.88	784	6.52	0.70
	Stalk	0.87	844	6.54	0.69
	Root	0.81	1213	8.32	0.82
	LSD(p=0.05)	0.03	152	0.70	0.056
Significance level	Time	0.003	0.002	0.073	<0.001
	Residues	<0.001	<0.001	<0.001	<0.001
	Time×Residues	0.006	0.010	0.048	0.067

Table 2. Pearson correlation (*r*) between soil properties and relative abundances of dominant genera across all time points (n=20). Those in bold are significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Phyla	Genus	SOC	DOC	TotalN	C/N	NH_4^+	NO_3^-	pH
Bacteroidetes	*Chitinophaga*	-0.07	-0.03	0.04	-0.08	0.07	-0.06	**0.34***

	Niastella	0.13	0.05	0.06	0.11	0.10	**-0.34***	0.25
	Segetibacter	0.11	0.08	0.25	0.05	0.19	**0.33***	0.27
Proteobacteria	*Steroidobacter*	-0.18	-0.03	**0.37***	-0.27	0.09	**0.47****	**0.41****
	Thermomonas	0.07	0.09	-0.15	0.11	0.19	**-0.35***	0.04
	Agrobacterium	0.16	0.09	0.27	0.08	**0.36***	-0.25	0.24
	Luteibacter	0.30	0.12	**0.31***	0.21	0.11	-0.22	-0.02
	Phenylobacterium	-0.08	0.20	**0.32***	-0.16	0.14	-0.05	**0.42****
	Lysobacter	-0.08	-0.13	**-0.50****	0.05	-0.21	-0.09	-0.22
	Nannocystis	0.13	0.15	**-0.52****	0.27	0.02	**-0.34***	-0.26
Acidobacteria	*CandidatusKoribacter*	-0.03	0.22	**-0.41****	0.08	-0.08	-0.10	**-0.47****
	Edaphobacter	0.28	-0.08	0.01	0.27	0.24	**-0.31***	-0.08
Firmicutes	*Bacillus*	0.13	**0.33***	-0.16	0.17	0.14	**-0.41****	-0.29
Actinobacteria	*Streptomyces*	-0.21	-0.11	-0.16	-0.17	0.01	**0.42****	0.06
	Nonomuraea	0.20	0.07	**0.40****	0.09	**0.31***	0.20	0.22
	Actinomadura	**0.37***	**0.39****	**0.53****	0.23	0.21	-0.17	**0.32***
	Nocardia	0.10	**0.32***	**-0.38***	0.21	0.07	**-0.49****	**-0.39****

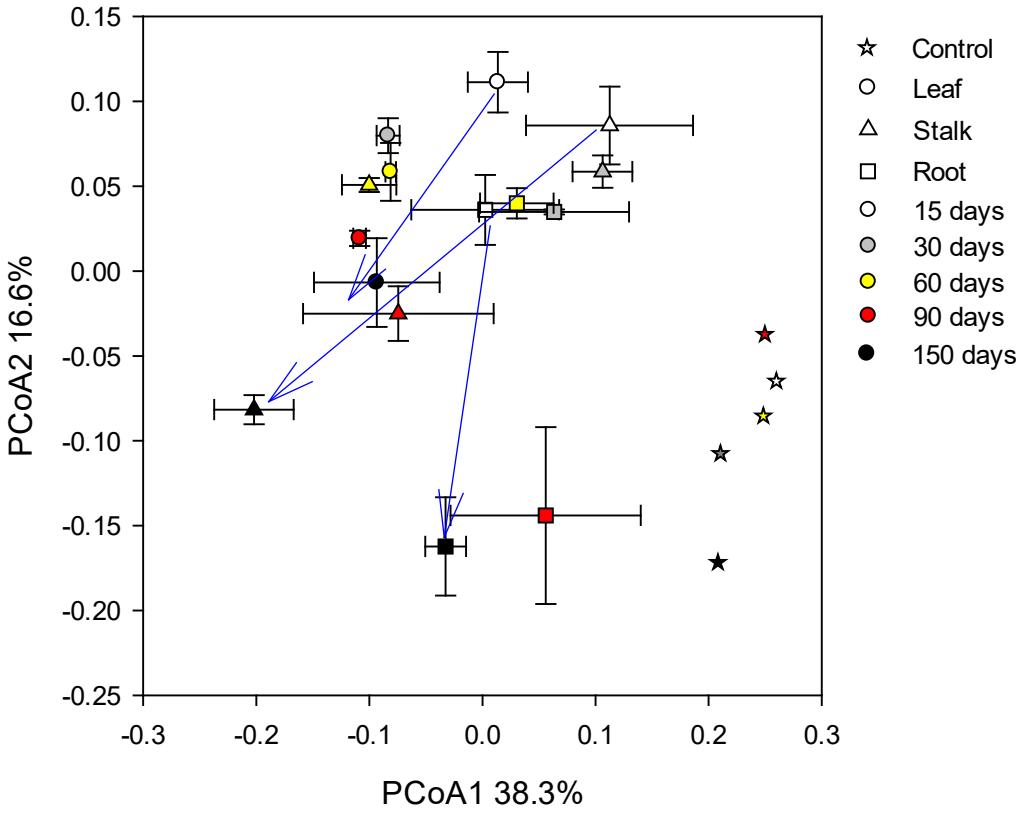

Fig. 1. The principal coordinates analysis (PCoA) of bacterial communities in the leaf-, stalk-, root-residue-amended and the control soils after 150 days of incubation at 25ºC. Error bars on data points represent the standard error of the mean (n=3). The arrows indicate the progression of the microorganisms over the incubation time. PCoA score plot based on weighted UniFrac metrics.

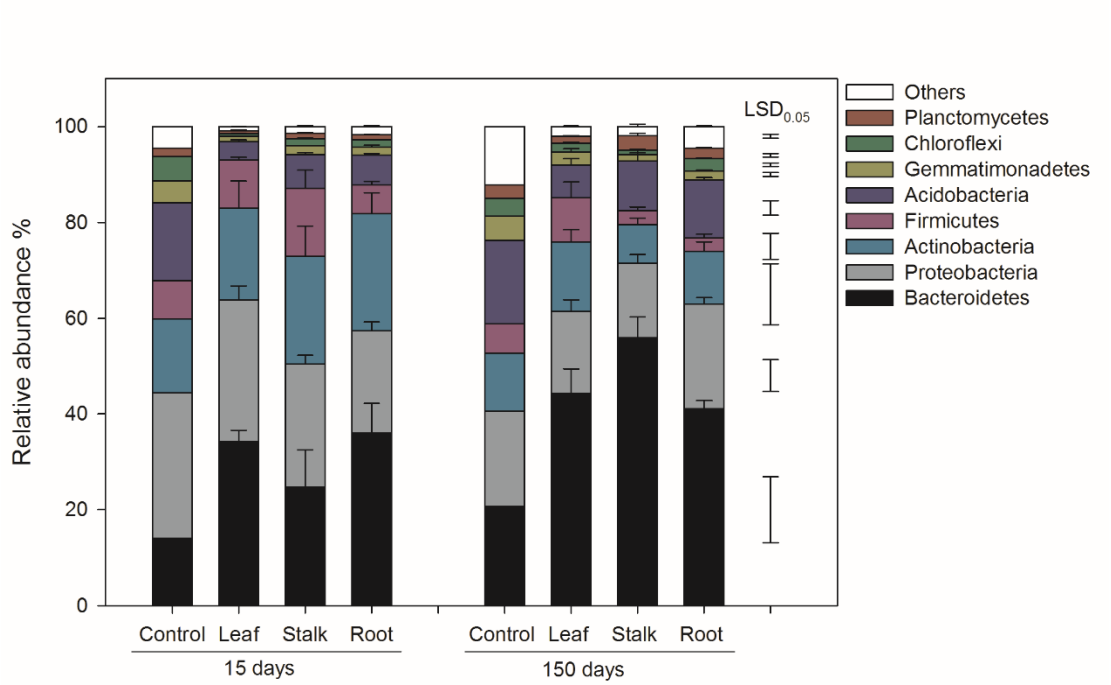

Fig. 2. Relative abundances (% total reads) of bacterial phyla in the leaf-, stalk-, root-residue-amended soils after 15 and 150 days of incubation at 25ºC. The no-residue control was the average over time. Error bars on data points represent the standard error of the mean (n=3). LSD (p=0.05) bars for individual phyla are also presented.

Supplementary materials

Materials and Methods

Experimental design and soil sampling

This incubation experiment consisted of four treatments and three replicates in a randomized complete block design. The four treatments were (1) no residue-amended control, (2) soybean leaf, (3) stalk and (4) root residues in three replicates. Soil samples were destructively taken on day 15, 30, 60, 90 and 150.

The original soil was collected from the top 10 cm of farmland (47°26′ N, 126°38′ E), Hailun, Heilongjiang Province, where soybean was the major crop. The soil is classified as Mollisol or Phaeozem (FAO-UNESCO, 1974), and the soil chemical properties are as follows: pH (in H_2O), 5.16; total C, 30.0 g kg^{-1}; total N, 2.1 g kg^{-1}; total P, 0.9 g kg^{-1}; available N, 234 mg kg^{-1}; Olsen-P, 25.8 mg kg^{-1} and available K, 191 mg kg^{-1}. Nitrogen concentrations in soybean leaf, stalk and root residues were 34, 13 and 16 mg g^{-1}, C/N 13, 33 and 26, and lignin concentration 89, 153 and 236 mg g^{-1}, respectively (Lian et al., 2016).

Zero point eight of ground dry residues were added into 20 g of sieved soil and fully mixed. The residue-soil mixture was loaded into PVC cores (4.5-cm height, 2-cm diameter) with nylon mesh bottoms, and the cores were then placed in 0.25-L wide-mouth mason jars. In each jar, there was also a vial filled with 10 ml of water to maintain the humidity. Eighty percent of field capacity of the soil was maintained by watering to the target weight. The incubation temperature was maintained at 25°C with mason jars kept in a dark incubator.

At each sampling time, soil in each PVC core was sampled and separated into three parts: around 16 g of soil was kept at 4 °C for measurements of nitrate (NO_3^--N), ammonium (NH_4^+-N) and dissolved organic C (DOC); 3 g of soil was air-dried and used for measurements of total C and N concentration, and remaining 1 g was placed in liquid nitrogen for 20 min, and stored at -80 ºC for the DNA extraction. Total soil C and N were determined using An Elementar III analyser (Hanau, Germany). The DOC in the soil was extracted in 0.5 M K_2SO_4 (1:5 = w:v) and determined using a TOC analyzer (Shimadzu, TOC-VCPH, Japan) (Domanski et al., 2001). Soil NH_4^+ and NO_3^- were extracted by shaking 20 g of fresh soil in 20 mL of 2 M KCl for 30 min, and their concentrations in the extracts were then determined using a continuous flow analytical system (SKALAR SAN++, the Netherlands). The pH was measured using a Wettler Toledo 320 pH meter after shaking with water (1:5 = w:v) for 30 min.

DAN extraction and sequencing of amplicons

Using a Fast DNA SPIN Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA), soil DNA was extracted and dissolved in double-deionised H_2O. The quantity of the extracted DNA was determined with a NanoDrop spectrophotometer (Bio-Rad Laboratories Inc.).

The amplification of bacterial 16S rRNA genes were performed with the primers 515F (5′-GTGYCAGCMGCCGCGGTA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′), targeting bacterial V4–V5 region in the 16S rRNA gene. A 10-mer barcode was added at the 5′ end of primer 515F (Osburn et al., 2011). Twenty two point five microliter of Platinum PCR SuperMix (TaKaRa, Dalian, China), 0.5 μL of each primer (10 μM) and 2μL of DNA (10 ng) were mixed to make a 25 μL PCR reaction system. The PCR amplification program started with initial denaturation at 95 °C for 10 min, followed by 28 cycles of 95 °C for 15 s, 60 °C for 10 s and 72 °C for 20 s, and an extension at 72 °C for 10 min (Muyzer et al., 1993). The amplification of each DNA sample was performed twice, and then the two PCR products were combined. The Agarose Gel DNA purification kit was used to clean up PCR products (TaKaRa, Dalian, China). According to the standard protocol of the MiSeq platform, the PCR amplicons of each sample were sequenced at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Using Quantitative Insights Into Microbial Ecology (QIIME) pipeline (version 1.17; http://qiime.org/), the raw sequences were processed to sort treatments according to barcodes, trim for sequence quality and denoise after DNA sequencing (Caporaso et al., 2011). Sequences with more than 97% of similarity were treated as one operational taxonomic unit (OTU). Less than 1% of OTUs were identified as archaea and they were removed before further processing the bacterial community data. The Shannon diversity index (Shannon and Weaver, 1963) was obtained using the Ribosomal Database Project pipeline (RDP) (http://pyro.cme.msu.edu/) and Shannon evenness was calculated as Shannon diversity index divided by the total number of phylotypes (Shannon and Weaver, 1963). In total, there were 257,875 of high quality and chimera-free reads obtained from MiSeq sequencing of 16S rRNA gene. Among the samples, the sequence number varied from 4,195 to 5,699. A subset of 4,195 reads was randomly selected for each sample before further analysis.

Statistical analyses

The index of community distances between each pair of samples was generated using UniFrac statistical analysis online at http://bmf.colorado.edu/unifrac/ (Lozupone and Knight, 2005; Hamady and Knight 2009). Based on this, principal coordinates analysis (PCoA) (Lozupone et al., 2011; Li et al., 2014) was deployed to display patterns of similarity (Bray-Curtis similarity) in the bacterial community composition between the soils amended without and with leaf, stalk and root residues. Mantel test was performed to examine the relationship between the whole bacterial community dissimilarities and soil characteristics. In addition, the relationship between the 17 most abundant genera at five sampling times and correspondent soil properties at these time points were also examined by performing a Pearson correlation (SPSS Statistics 17.0). The PCoA and mantel test were done with the program R for Windows (version 3.1.2, R Development Core Team 2010).

With the analysis of variance (ANOVA) using Genstat 13 (VSN International, Hemel Hemspstead, UK), the effects of soybean residue (including no residue control), incubation time and their interaction on the relative abundance of bacterial groups at the phylum, genus

and OUT levels were assessed (Steel and Torrie, 1980). The significance of variables among treatments was estimated according to the least significant difference (LSD) at the significant level of $p \leq 0.05$.

The relative abundances of genera and the number of OTUs in each genus were shown in this study. All sequences were uploaded to the short-read archive SRP071095 in GenBank.

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Table S1. The effects of incubation time, residue type and their interaction on the abundance of various bacterial phyla. The *p* values less than 0.05 were indicated in bold.

	Time	Residues	Time × Residues
Bacteroidetes	**0.002**	0.171	0.094
Proteobacteria	**0.010**	0.499	0.191
Actinobacteria	**0.028**	0.077	0.737
Firmicutes	**<0.001**	**<0.001**	**0.028**
Acidobacteria	**<0.001**	**<0.001**	0.487
Gemmatimonadetes	0.091	0.076	**0.024**
Chloroflexi	**<0.001**	**<0.001**	**0.001**
Planctomycetes	**<0.001**	**0.001**	0.176
other	**<0.001**	**<0.001**	**<0.001**

Table S2. The effects of incubation time, residue type and their interaction on the abundance of bacterial genera after 15, 30, 60, 90 and 150 days of incubation. The data are means of three replicates. The *p* values less than 0.05 were indicated in bold.

	Incubation day (d)	15				30				60				90				150				*ANOVA* (*p*-values)			
Phylum	Genus	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	LSD (p = 0.05)	Time (T)	Residue (R)	T×R
Bacteroidetes	*Niastella*	2.31	4.45	6.52	9.24	4.24	8.76	7.96	6.86	1.93	10.89	15.4	7.77	1.71	18.33	15.62	3.39	3.82	19.09	25.70	9.80	6.90	**<0.001**	**<0.001**	**0.003**
	Chitinophaga	0.87	8.17	1.49	2.35	1.20	7.99	1.75	2.75	0.75	3.73	3.67	2.41	0.79	6.46	5.90	1.82	0.75	2.25	7.00	2.20	5.26	0.910	**0.021**	0.086
	Flavisolibacter	2.47	0.95	1.5	2.04	3.31	0.87	1.61	1.97	3.91	0.95	0.88	1.83	1.89	0.52	0.78	1.51	3.26	1.48	1.39	2.50	0.50	**<0.001**	**<0.001**	0.270
	Flavobacterium	0.07	0.87	0.03	0.21	0.02	0.65	0.01	0.07	0.06	0.28	0.02	0.18	0.10	0.31	0.04	0.13	0.02	0.33	0.04	0.10	0.53	0.551	**0.001**	0.703
	Segetibacter	1.59	0.37	0.48	0.74	2.45	0.40	0.44	0.65	2.41	0.45	0.24	0.87	1.95	0.18	0.32	0.67	2.92	0.38	0.42	0.74	0.31	0.487	**<0.001**	0.620
Proteobacteria	*Steroidobacter*	0.50	2.03	0.51	2.07	0.51	3.23	0.65	3.99	0.45	2.22	0.93	4.94	0.59	2.11	1.02	1.81	0.37	0.79	0.44	1.02	1.10	**<0.001**	**<0.001**	**<0.001**
	Thermomonas	2.27	1.77	2.61	1.38	2.04	1.27	2.26	1.05	4.38	1.14	1.31	1.51	3.07	0.30	0.71	0.60	1.51	0.30	0.61	0.62	0.68	**<0.001**	**0.002**	0.053
	Agrobacterium	0.28	1.70	0.40	0.63	0.21	2.01	0.49	0.78	0.20	0.85	0.70	0.61	0.35	0.37	0.93	0.44	0.12	0.11	0.61	0.24	0.76	**0.012**	**0.019**	**0.005**
	Balneimonas	0.37	1.45	0.81	0.61	0.19	1.23	0.71	0.55	0.34	0.83	0.44	0.37	0.30	0.39	0.29	0.39	0.22	0.29	0.25	0.39	0.35	**<0.001**	**<0.001**	**0.014**
	Luteibacter	1.13	1.25	1.77	1.30	0.67	0.96	1.38	0.97	1.13	1.11	0.93	1.34	0.55	0.25	0.40	0.64	0.17	0.36	0.23	0.55	0.77	**<0.001**	0.536	0.681
	Phenylobacterium	0.22	0.74	0.36	0.35	0.28	0.51	0.42	0.34	0.26	0.47	0.53	0.33	0.10	0.26	0.61	0.19	0.29	0.23	0.42	0.34	0.26	0.197	**0.020**	**0.016**
	Corallococcus	0.13	0.55	0.30	0.12	0.32	0.24	0.17	0.06	0.22	0.40	0.16	0.30	0.14	1.07	0.29	0.15	0.22	0.37	0.07	0.29	0.32	**0.010**	**<0.001**	**0.013**
	Nannocystis	0.04	0.36	0.05	0.10	0.05	0.74	0.05	0.07	0.02	0.31	0.05	0.14	0.04	0.17	0.02	0.05	0.02	0.10	0.01	0.04	0.20	**0.003**	**<0.001**	**0.003**
	Bradyrhizobium	0.48	0.35	0.42	0.35	0.32	0.49	0.41	0.38	0.41	0.39	0.48	0.28	0.61	0.87	1.13	0.49	0.51	0.39	0.76	0.32	0.25	**<0.001**	**<0.001**	**0.028**
Firmicutes	*Bacillus*	3.80	7.94	12.16	3.97	4.00	8.96	13.88	6.19	3.36	9.26	16.77	8.02	4.43	6.04	5.16	2.33	3.24	7.82	2.21	1.59	4.59	**<0.001**	**<0.001**	**0.016**
	Paenibacillus	0.72	0.83	0.43	0.46	0.42	0.34	0.36	0.43	0.32	0.21	0.23	0.39	0.55	0.27	0.17	0.52	0.32	0.14	0.11	0.17	0.32	**<0.001**	0.174	0.211

Acidobacteria	*Candidatus Koribacter*	1.53	0.34	0.83	0.45	1.18	0.40	1.20	0.61	1.07	0.55	1.17	0.77	1.54	0.50	1.78	0.68	1.10	0.87	1.83	0.90	0.58	**0.005**	**<0.001**	0.561
	Edaphobacter	0.07	0.33	0.33	0.13	0.09	0.20	0.39	0.11	0.18	0.21	0.24	0.14	0.08	0.08	0.18	0.06	0.19	0.07	0.10	0.05	0.16	**<0.001**	**<0.001**	0.352
Actinobacteria	*Streptomyces*	1.94	7.25	7.35	9.03	1.97	4.38	5.19	10.43	1.44	3.37	2.37	6.32	2.66	3.40	3.72	5.82	0.73	2.04	1.62	1.99	4.95	**0.002**	**0.029**	0.832
	Nonomuraea	0.61	2.95	1.46	4.43	0.65	2.35	2.09	5.32	0.73	3.09	0.51	4.03	0.75	3.89	0.56	2.38	0.34	3.54	0.35	0.99	2.10	0.089	**<0.001**	**0.029**
	Actinomadura	0.39	1.31	0.94	1.19	0.37	1.52	1.18	1.07	0.32	1.74	0.36	1.11	0.26	1.57	0.37	0.57	0.15	1.95	0.24	0.32	1.01	0.499	**<0.001**	0.38
	Nocardia	0.13	0.82	0.18	0.21	0.09	0.85	0.15	0.17	0.04	0.38	0.06	0.09	0.06	1.08	0.10	0.12	0.00	0.73	0.12	0.11	0.31	**0.039**	**<0.001**	0.141
	Dactylosporangium	0.11	0.22	0.08	0.22	0.09	0.35	0.15	0.17	0.10	0.50	0.08	0.38	0.10	0.43	0.14	0.42	0.05	0.55	0.20	0.51	0.22	**0.006**	**<0.001**	0.486

Table S3. The effects of incubation time, residue type and their interactions on the abundance of bacterial community at the OTU level after 15, 30, 60, 90 and 150 days of incubation. The data are means of three replicates. The *p* values less than 0.05 were indicated in bold.

	Incubation day (d)		15				30				60				90				150				*ANOVA* (*p*-values)			
Phylum	Genus	OTU ID	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	Control (%)	Leaf (%)	Stalk (%)	Root (%)	LSD (p = 0.05)	Time (T)	Residue (R)	T×R
Bacteroidetes	*Chitinophaga*	7268	0.13	0.58	0.35	0.34	0.09	0.49	0.27	0.16	0.06	0.22	1.01	0.32	0.06	0.05	0.39	0.06	0.07	0.05	0.47	0.15	0.40	0.030	0.007	0.031
		21210	0.39	3.84	0.31	0.64	0.36	4.20	0.28	1.14	0.32	2.11	0.25	0.69	0.40	5.21	0.48	0.49	0.29	1.75	0.21	0.56	1.98	0.155	<0.001	0.219
		24162	0.04	0.78	0.14	0.40	0.34	0.57	0.31	0.43	0.08	0.20	0.38	0.23	0.06	0.18	0.92	0.27	0.17	0.09	0.43	0.57	0.53	0.707	0.815	0.031
	Niastella	40325	1.76	3.08	4.51	6.92	3.12	6.85	6.26	5.24	1.46	8.61	12.46	6.12	1.17	16.39	12.6	2.68	2.55	15.70	20.7	7.06	5.76	<0.001	<0.001	0.002
	UnclassifiedChitinophagaceae	5550	0.00	0.19	0.14	0.10	0.05	0.12	0.04	0.10	0.02	0.17	0.32	0.17	0.00	0.11	0.13	0.05	0.02	0.11	0.09	0.15	0.14	0.012	0.530	0.270
		11780	0.06	0.62	1.39	1.25	0.30	1.14	1.53	0.67	0.16	1.24	1.29	0.56	0.26	0.67	1.24	0.22	0.36	0.70	1.81	0.81	1.04	0.616	0.006	0.657
		16327	0.04	0.75	0.17	0.20	0.07	0.69	0.16	0.20	0.06	0.55	0.27	0.13	0.06	0.52	0.37	0.08	0.02	0.10	0.09	0.11	0.29	0.020	<0.001	0.075
		19220	0.02	0.51	0.07	0.11	0.00	0.56	0.05	0.22	0.02	0.64	0.02	0.13	0.04	0.58	0.07	0.04	0.00	0.32	0.02	0.09	0.27	0.432	<0.001	0.673
		36736	1.81	12.6	8.82	12.53	2.05	16.24	6.01	10.38	1.38	16.86	13.49	10.53	2.01	8.91	9.27	2.08	1.74	10.34	8.21	4.51	5.84	0.001	0.001	0.085
Proteobacteria	*Agrobacterium*	16368	0.13	1.15	0.13	0.41	0.14	1.56	0.09	0.46	0.14	0.60	0.11	0.40	0.22	0.26	0.10	0.26	0.07	0.07	0.04	0.07	0.45	<0.001	<0.001	0.002
	Balneimonas	7691	0.15	0.22	0.20	0.10	0.00	0.24	0.17	0.10	0.12	0.20	0.12	0.04	0.06	0.11	0.10	0.06	0.05	0.07	0.07	0.02	0.10	<0.001	<0.001	0.740
		22023	0.09	0.80	0.35	0.32	0.09	0.62	0.30	0.32	0.18	0.40	0.17	0.21	0.16	0.19	0.10	0.26	0.07	0.14	0.05	0.17	0.18	<0.001	<0.001	0.006
	Bradyrhizobium	14037	0.47	0.35	0.41	0.35	0.32	0.49	0.40	0.37	0.40	0.38	0.48	0.28	0.60	0.86	1.12	0.48	0.50	0.39	0.74	0.31	0.25	<0.001	<0.001	0.025
	Luteibacter	17323	1.07	1.21	1.70	1.25	0.59	0.93	1.32	0.93	1.08	1.05	0.88	1.26	0.50	0.25	0.39	0.61	0.14	0.35	0.22	0.51	0.74	<0.001	0.584	0.707
	Lysobacter	42251	0.00	0.35	0.04	0.07	0.02	0.20	0.03	0.04	0.02	0.16	0.00	0.04	0.00	0.03	0.02	0.05	0.00	0.03	0.00	0.05	0.07	<0.001	<0.001	<0.001
	Steroidobacter	35682	0.19	0.93	0.18	0.52	0.11	1.26	0.23	0.96	0.14	0.91	0.46	1.31	0.10	0.65	0.48	0.30	0.07	0.16	0.15	0.18	0.33	<0.001	<0.001	<0.001
		29659	0.15	0.53	0.10	0.36	0.16	1.10	0.17	0.82	0.12	0.72	0.13	1.11	0.18	0.92	0.12	0.68	0.12	0.38	0.10	0.29	0.39	<0.001	<0.001	0.054
	Thermomonas	25283	2.21	1.76	2.57	1.36	2.00	1.23	2.21	1.04	4.31	1.12	1.27	1.48	3.04	0.30	0.70	0.59	1.45	0.29	0.59	0.62	0.66	<0.001	0.002	0.050

	UnclassifiedComamonadaceae	45955	4.71	6.75	5.19	2.88	3.87	3.88	3.34	2.57	5.85	3.52	2.21	2.51	2.57	2.84	1.86	1.23	1.00	1.62	0.91	1.28	1.48	<0.001	<0.001	0.080
	UnclassifiedEnterobacteriaceae	9942	0.00	0.38	0.02	0.01	0.00	0.13	0.02	0.03	0.02	0.08	0.01	0.01	0.04	0.01	0.01	0.00	0.00	0.01	0.01	0.02	0.07	<0.001	<0.001	<0.001
	UnclassifiedOxalobacteraceae	9672	0.71	0.66	0.60	0.47	0.39	0.26	0.46	0.12	0.72	0.14	0.22	0.14	0.08	0.04	0.08	0.16	0.07	0.05	0.13	0.12	0.30	<0.001	0.343	0.631
Actinobacteria	*Actinomadura*	28530	0.34	1.23	0.91	1.09	0.34	1.31	1.12	1.01	0.32	1.47	0.33	1.02	0.24	1.47	0.37	0.55	0.12	1.83	0.21	0.29	0.95	0.562	<0.001	0.336
	Nocardia	6543	0.04	0.33	0.07	0.07	0.02	0.38	0.06	0.08	0.02	0.16	0.04	0.03	0.02	0.32	0.03	0.04	0.00	0.16	0.05	0.04	0.16	0.139	<0.001	0.503
		40962	0.09	0.48	0.11	0.14	0.07	0.47	0.09	0.08	0.02	0.21	0.02	0.05	0.04	0.76	0.06	0.07	0.00	0.55	0.06	0.07	0.17	0.004	<0.001	0.007
	Nonomuraea	9732	0.60	2.54	1.25	3.90	0.41	1.91	1.56	4.44	0.56	2.59	0.40	3.32	0.66	3.17	0.47	2.03	0.26	2.89	0.27	0.80	1.81	0.090	<0.001	0.041
	Streptomyces	2002	0.39	0.55	1.56	0.84	0.30	0.46	0.59	0.76	0.22	0.25	0.24	0.48	0.91	0.34	0.61	0.91	0.12	0.39	0.24	0.29	0.76	0.023	0.226	0.409
		8080	1.16	5.90	4.65	5.52	1.34	2.90	3.70	5.87	0.94	2.12	1.70	3.89	1.33	2.43	2.64	4.29	0.43	0.93	1.03	1.32	3.52	0.003	0.136	0.943
		49871	0.00	0.16	0.04	0.07	0.00	0.08	0.01	0.08	0.00	0.14	0.02	0.09	0.00	0.03	0.01	0.02	0.00	0.04	0.01	0.01	0.08	0.009	0.003	0.420
	UnclassifiedMicromonosporaceae	36120	0.39	0.42	0.39	0.44	0.57	0.40	0.29	0.30	0.32	0.22	0.07	0.28	0.28	0.24	0.04	0.17	0.38	0.21	0.11	0.16	0.21	<0.001	0.043	0.867
	UnclassifiedNocardiopsaceae	26164	0.02	0.28	0.02	0.00	0.05	0.39	0.01	0.01	0.02	0.13	0.01	0.02	0.04	0.01	0.03	0.03	0.00	0.01	0.00	0.03	0.09	<0.001	<0.001	<0.001
	UnclassifiedStreptomycetaceae	31882	0.30	0.76	5.13	1.01	0.23	0.56	3.62	0.78	0.28	0.27	1.08	0.50	0.64	0.09	2.43	0.49	0.41	0.15	1.53	0.41	1.88	0.016	<0.001	0.179
	UnclassifiedStreptosporangiaceae	36802	0.37	1.04	0.59	1.20	0.23	1.22	0.69	1.04	0.14	1.28	0.35	1.45	0.18	0.82	0.26	0.24	0.07	0.90	0.11	0.26	0.49	<0.001	<0.001	0.052
Firmicutes	*Bacillus*	2174	3.03	7.38	9.85	3.38	3.23	8.61	12.56	5.38	2.86	8.80	15.77	7.10	3.54	5.68	4.73	1.79	2.50	7.37	1.94	1.40	3.80	<0.001	<0.001	0.006
	Shimazuella	36513	0.04	0.36	0.11	0.07	0.02	0.20	0.10	0.15	0.02	0.22	0.14	0.20	0.06	0.47	0.06	0.03	0.00	0.01	0.01	0.04	0.24	0.094	0.007	0.146
Acidobacteria	*Edaphobacter*	45006	0.06	0.32	0.32	0.12	0.09	0.19	0.38	0.10	0.18	0.19	0.22	0.14	0.06	0.07	0.16	0.04	0.19	0.06	0.08	0.04	0.15	<0.001	<0.001	0.253
	UnclassifiedSolibacterales	3525	1.14	0.56	0.50	0.61	1.07	0.86	0.53	0.96	1.48	1.33	0.58	1.25	1.19	0.71	0.53	0.95	1.19	1.41	0.44	1.23	0.39	<0.001	<0.001	0.052