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Characterisation of *Bt* maize IE09S034 in decomposition and response of soil bacterial communities

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Abstract: Returning straw to the soil is an effective way to improve the soil quality. As genetically modified (GM) crops experience expanded growing scales, returning straw to the soil could also be necessary. However, the impact of GM crop straws on soil safety remains unclear. The environment (including soil types, humidity and temperature) can result in a significant difference in the diversity of soil bacterial communities. Here, we compared the impacts of the straw from *Bt* maize IE09S034 (IE) and near-isogenic non-*Bt* maize Zong31 (CK) on soil bacterial community and microbial metabolic activity in three different environments. Sampling was carried out following 6–10 months of decomposition (May, June, July, and August) in three localities in Chinese cities (Changchun, Jinan, and Beijing). Our results showed that *Bt* maize residues posed no direct impact on soil bacterial communities in contrast to the environment and decomposed time. The microbial functional diversity and metabolic activity showed no significant difference between IE and CK. The results could be a reference for further assessing the effect of *Bt* maize residues on the soil that promotes the commercialisation of *Bt* maize IE09S034.

Keywords: genetically modified maize; litterbags; 16S rRNA; miseq sequencing; biolog eco-plates

Returning straw to the soil was reported to positively improve soil quality by affecting soil aggregation, structure, water-holding capacity, and nutrients availability (Takahashi et al. 2003, Su et al. 2020b). In the agribiotech industry, genetically modified (GM) crops are the fastest adopted commodities, which is a sustainable basis for ensuring the food supply as the global population increases (Kamle et al. 2017). 2.9 million hectares area of biotech crops have been cultivated in China (ISAAA 2018), the need to return the straw of GM crops to the soil after harvest to improve soil quality is increasing. Most of GM insect-resistant plants are environmentally friendly, and they reduce the cost associated with the usage of chemical insecticides. GM maize, introduced with *Bacillus thuringiensis* (*Bt*) insecticidal genes, is one such product that plays an important role already in the agricultural product market (Han et al. 2016). Despite its advantages, the safety issue of *Bt* maize cultivation remains controversial, and the impact of *Bt* maize straw returning to the soil on non-target organisms such as soil bacterial community. In a previous study, about 2–2.5 t/ha (silage maize) to 6 t/ha dry matter was left in the soil after harvest, which provided a way for *Bt* proteins exposing to

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the soil ecosystem through plant root exudates and plant matrix leachates (Zwahlen et al. 2003). These insecticidal proteins may affect the soil ecosystem for extended periods (Losey et al. 1999). Interestingly, a previous study has reported that the decomposition of maize residues with the *Cry3Bb* protein has no significant effects on the soil bacterial community (Xue et al. 2011). However, maize straw expressing the *Cry1Ab* protein was shown to have a short-term influence on microbial communities in the soil (Mulder et al. 2006). Thus, the effects of GM crop residues may need to be reviewed case-by-case.

The *Cry1Ie* anti-insect gene was isolated and cloned by the Chinese Academy of Agricultural Sciences (CAAS) (Song et al. 2003). *Bt* maize IE09S034 was a GM maize line expressing the *Cry1Ie* gene produced by the Institute of Crop Sciences, CAAS. Previous studies showed that no significant difference was observed in rhizosphere bacterial communities between *Bt* maize line IE09S034 and the near-isogenic non-*Bt* line Zong31 (Liang et al. 2018). IE09S034 also has no influence on non-lepidopteran pest abundance, diversity, community composition (Guo et al. 2016), and soil fauna (Fan et al. 2019). However, the effects of IE09S034 straw on soil bacterial communities remain uninvestigated. Here we evaluate if the residues of GM maize line IE09S034 have an influence on soil microbial metabolic activities and bacterial structure and diversities. With litterbag for assessing the effects of degradation of *Bt* maize residues on soil microbial community, we carried out experiments in different areas in three cities to determine if the environment impacts the degradation of straw. We aimed to determine: (1) if *Bt* maize residues impact the soil bacterial community and microbial metabolic activity; (2) if the influence by *Bt* maize residues is different between each city's environment or the decomposition time.

MATERIAL AND METHODS

Plant material and litterbag preparation. The maize lines used in the experiment were GM *Bt* maize IE09S034 (IE) expressing the *Cry1Ie* gene and near-isogenic non-*Bt* line Zong31 (CK). The GM *Bt* maize IE09S034 was provided by the Institute of Crop Science, CAAS. Each cultivar was planted in three plots in Changchun City (43°19'N, 124°29'E) with a randomised block arrangement. The area of each plot was 10 m × 15 m. Maize was maintained by the typical agronomic practice in Northeastern China.

In early November 2015, the maize reaches maturity. Thirty well-grown maize plants were sampled in each plot. The roots, stems and leaves were cleaned up. They were then cut into 5 cm long sections and weighed. Roots (2 g), stems (2 g), and leaves (3 g) were placed into 2 mm mesh decomposition nylon bags (15 cm × 15 cm × 10 cm) and tightly sealed.

Field design and sampling methods. The packed litter decomposition bags were packed with dry ice during transit. These bags were transported and buried in the test fields in November 2015 in different localities in three cities: National transgenic corn and soybean pilot test and industrialisation base, Jilin Academy of Agricultural Sciences, Changchun (43°19'N, 124°29'E) (CC), Beipu experimental base of Institute of Agricultural Sciences, Chinese Academy of Agricultural Sciences, Beijing (39°58'N, 116°19'E) (BJ) and National Huang Huai Hai transgenic maize pilot test and industrialisation base, Shandong Academy of Agricultural Sciences, Jinan (36°46'N, 117°23'E) (JN). The soil type of CC (loam clay) is haplic phaeozems, while BJ (silt loam) and JN (sandy loam) are mollic gleysols. The environment of three localities from May to August was different: monthly average temperature for CC was 21.5 °C, for BJ was 25.6 °C and for JN was 25.2 °C; soil relative humidity for CC was 62.5%, for BJ was 49.9% and for JN was 54.0% (Table 1). 5 L decomposition bags were placed in each plot and three plots for each cultivar. Each plot area was 10 m × 15 m, and the distance between each plot was 2 m. The land was cleaned up without any straws from other crops. When embedding litter decomposition bags, the principle of random embedding was followed. The minimal distance between the two adjacent litter decomposition bags was 50 cm, and the depth was about 10 cm. The location of the buried bags was marked so that litter decomposition bags can be retrieved for sampling. The soil around the litter bag was also sampled in early May (May), June (Jun), July (Jul), and August (Aug) of 2016.

DNA extraction. Each sampling contains 0.5 g soil, and MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA) was used for DNA extraction. The quantity and quality of DNA were measured by NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, USA). DNA was used for PCR amplification when it met requirements: OD260/280 = 1.8–2.0, c ≥ 20 ng/μL.

16S rRNA amplification and sequencing. Gene amplification and sequencing of bacterial 16S rRNA were performed at BGI (Shenzhen, China). V4 re-

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Table 1. Meteorological data of three localities as support information

		Monthly average temperature	Monthly maximum temperature	Monthly minimum temperature	Soil relative humidity (10 cm depth) (%)
		(°C)			
Changchun	May	16.9	22.7	11.6	–
	Jun	21.6	27	16.5	59.34
	Jul	24.3	28.9	20.1	57.69
	Aug	23.1	28	18.5	70.54
	mean	21.475	26.65	16.675	62.52333
Beijing	May	21.5	28.1	14.8	23.7
	Jun	25.9	31.4	20.1	43.34
	Jul	27.4	31.8	23.8	64.36
	Aug	27.5	31.8	23	68.07
	mean	25.575	30.775	20.425	49.8675
Jinan	May	21.1	26.7	15.3	44.49
	Jun	25.6	30.7	20.8	63.11
	Jul	27.7	31.9	23.7	63.88
	Aug	26.4	30.6	22.4	44.44
	mean	25.2	29.975	20.55	53.98

Data of soil relative humidity in 10 cm depth in Changchun in May is missing

gion of 16S rRNA was amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Kozich et al. 2013). Illumina MiSeq was the platform for sequencing. If the two paired-end reads overlapped, they will merge to tag. The tags were clustered into operational taxonomic units (OTUs) at a 97% threshold by UPARSE. OTUs representative sequences were taxonomically classified using Ribosomal Database Project (RDP) Classifier v.2.2 trained on the Greengenes database at 0.6 confidence value.

Microbial community-level physiological profiles. The Biolog Eco-Plates were prepared according to a previous report (Liang et al. 2016). Briefly, 5 g fresh soil was mixed in 45 mL 0.85% sterile saline solution, and the supernatant was isolated and then diluted 100 fold/10^3 dilution was added to each pore of Biolog Eco-Plates and incubated in the dark at 25 °C for 168 h. The color development and absorbance values were recorded every 24 h at 590 nm and 750 nm. The OD value at 72 h was used to calculate indices, including average well color development (AWCD), substrates utilisation of carbon sources, richness index (R), Shannon-wiener diversity index (H) and evenness index (E).

Statistical analysis. One-way ANOVA ($P < 0.01$) in SPSS 17.0 software (SPSS Inc., Chicago, USA) was used to determine the minimum significant difference. The richness estimators (ACE and Chao), diversity indices (Shannon and Simpson), and rarefaction curves were generated using Mothur (v1.31.2) (Schloss et al. 2009). Principal component analysis (PCA) was performed in R version 3.1.1 (R Development Core Team 2011) to compare bacterial community structure across all samples. Each index of Biolog microplate was calculated by Microsoft Excel 2016 (Microsoft, Redmond, USA).

RESULTS AND DISCUSSION

The transgenic technology plays an important role in improving the production and quality of crops. In addition, most of GM crops are usually environmentally friendly due to the reduction of chemical pesticide use (Kumar et al. 2008). However, when GM crops grown in the field, the actual effects on the environment, such as soil, are still equivocal. Some studies reported that the cultivation of GM crops did not affect the soil microbial communities (Sohn 2016, Zhou et al. 2016, Lu et al. 2018a), while some studies found the difference of soil microbial communities between non-GM crops and GM crops (Lu et al. 2018b, Wen et al. 2019). As the soil microbial communities differed with plants, different GM crops

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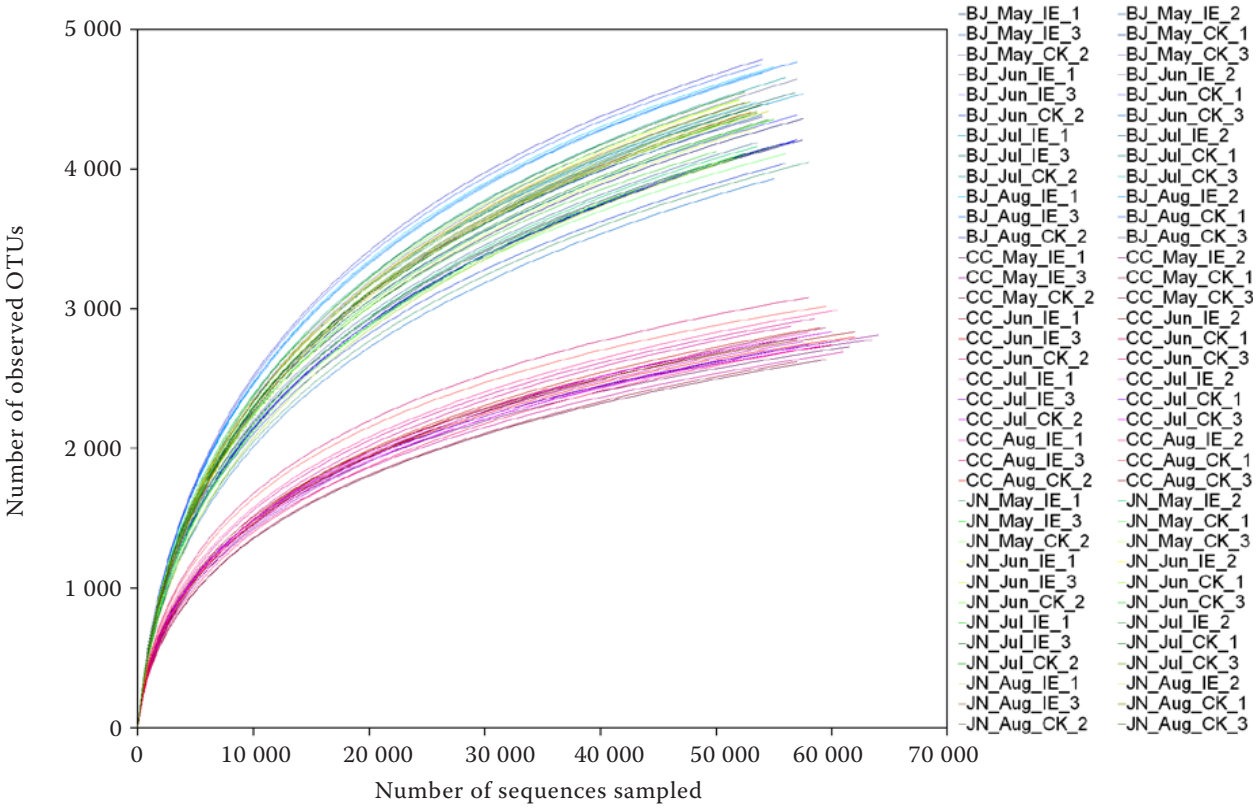

Figure 1. Rarefaction curves of operational taxonomic units (OTUs) clustered at 97% sequence identity across the samples in three localities. The curves were named in the following form: city name, month, and lines replicate. CC – Changchun; BJ – Beijing; JN – Jinan; May – May; Jun – June; Jul – July; Aug – August; IE – *Bt* maize residues; CK – non-*Bt* residues

should be analysed individually so that the effects caused by GM crops can be assessed accurately. Our experiment was a part of the environment safety assessment of *Bt* maize IE09S034, which was aim to ascertain if the residues of *Bt* maize have effects on soil microbial communities. Results indicated that the decomposition of maize residues in different environments and soil was the major factor affecting the soil microbial communities rather than line.

16S rRNA amplicon sequencing. The V4 region of 16S rRNA was amplified and used for sequencing. The sequences were available in NCBI Genebank SRA under BioProject PRJNA613863. The sequencing data showed that 5 759 660 tags were obtained from 72 samples, which were clustered and filtered into 10 936 OTUs at 97% similarity. The OTUs counted from 2 623 to 4 789 per sample, with an average of 3 853 ± 759 OTUs. As shown in Figure 1, the rarefaction curves showed no differences between IE and CK, and the data were sufficient for revealing differences between the lines. In addition, fewer OTUs were identified in CC samples between BJ and JN.

Effects on the diversity of soil bacterial communities. The α-diversity results showed no differences between IE and CK among three localities (Table 2). The ANOVA results indicated that the environment was the parameter affecting α-diversity indices (Table 3). When focused on the community richness (Chao and ACE), it is evident that the species richness of JN and BJ was higher than that of CC. Similarly, the species diversity (Shannon and Simpson) of CC was less than JN and BJ (Figure 2). Sampling in different months showed no significant influence on the α-diversity of soil bacterial communities (Table 3).

β-diversity analysis was used to evaluate differences of samples in species complexity. In the results of PCA, IE and CK had no significant difference in β-diversity (Figure 3A). It was obvious that different sampling localities were the paramount factor to vary species complexity, which divided the scatters into three parts (Figure 3B). Different months of sampling did not significantly influence the β-diversity of the bacterial community (Figure 3C). The PCA results revealed that the significant factors that contributes to

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Table 2. Data summary of α-diversity (mean ± standard deviation) ($P < 0.01$)

			obs	Chao	Ace	Shannon	Simpson
May	CC	IE	2 776.67 ± 41.26^A	3 470.71 ± 98.66^A	3 485.67 ± 79.81^A	6.18 ± 0.05^A	0.01 ± 0.00^A
		CK	2 709.67 ± 136.49^A	3 606.63 ± 197.08^A	3 626.18 ± 208.48^A	5.79 ± 0.07^A	0.02 ± 0.00^A
	BJ	IE	4 169.33 ± 26.27^A	5 500.35 ± 89.46^A	5 435.57 ± 50.60^A	6.95 ± 0.03^A	0.00 ± 0.00^A
		CK	4 207.00 ± 7.21^A	5 545.39 ± 77.16^A	5 545.13 ± 49.75^A	6.93 ± 0.05^A	0.00 ± 0.00^A
	JN	IE	4 110.33 ± 58.14^A	5 297.35 ± 96.60^A	5 363.25 ± 101.56^A	6.91 ± 0.06^A	0.00 ± 0.00^A
		CK	4 091.67 ± 15.95^A	5 292.47 ± 119.25^A	5 339.87 ± 127.34^A	6.98 ± 0.03^A	0.00 ± 0.00^A
Jun	CC	IE	2 829.00 ± 38.51^A	3 586.13 ± 100.62^A	3 614.90 ± 71.66^A	6.02 ± 0.03^A	0.01 ± 0.00^A
		CK	2 965.67 ± 108.97^A	3 796.34 ± 73.82^A	3 780.54 ± 77.75^A	6.23 ± 0.23^A	0.01 ± 0.00^A
	BJ	IE	4 378.00 ± 9.17^A	5 567.61 ± 57.26^A	5 625.79 ± 42.31^A	7.07 ± 0.05^A	0.00 ± 0.00^A
		CK	4 065.33 ± 140.00^A	5 413.22 ± 108.54^A	5 402.75 ± 135.55^A	6.86 ± 0.17^A	0.00 ± 0.00^A
	JN	IE	4 410.33 ± 101.28^A	5 742.57 ± 249.09^A	5 750.12 ± 192.03^A	7.11 ± 0.05^A	0.00 ± 0.00^A
		CK	4 351.67 ± 192.34^A	5 580.34 ± 250.11^A	5 619.29 ± 260.11^A	7.13 ± 0.05^A	0.00 ± 0.00^A
Jul	CC	IE	2 742.67 ± 43.89^A	3 525.82 ± 14.98^A	3 527.99 ± 42.69^A	6.04 ± 0.11^A	0.01 ± 0.00^A
		CK	2 776.67 ± 56.09^A	3 547.61 ± 50.45^A	3 557.04 ± 58.58^A	6.13 ± 0.04^A	0.01 ± 0.00^A
	BJ	IE	4 493.00 ± 87.54^A	5 677.98 ± 60.95^A	5 757.49 ± 71.20^A	7.11 ± 0.04^A	0.00 ± 0.00^A
		CK	4 542.00 ± 100.46^A	5 909.93 ± 151.79^A	5 923.14 ± 119.14^A	7.12 ± 0.04^A	0.00 ± 0.00^A
	JN	IE	4 439.67 ± 102.28^A	5 761.23 ± 81.04^A	5 784.34 ± 111.10^A	7.07 ± 0.04^A	0.00 ± 0.00^A
		CK	4 412.67 ± 71.81^A	5 772.88 ± 66.07^A	5 771.37 ± 91.80^A	7.05 ± 0.03^A	0.00 ± 0.00^A
Aug	CC	IE	2 859.33 ± 123.66^A	3 674.58 ± 101.25^A	3 705.43 ± 96.33^A	6.06 ± 0.24^A	0.01 ± 0.00^A
		CK	2 885.67 ± 119.22^A	3 536.43 ± 125.09^A	3 576.36 ± 113.39^A	6.25 ± 0.20^A	0.01 ± 0.00^A
	BJ	IE	4 734.00 ± 43.03^A	5 889.15 ± 69.52^A	5 959.90 ± 43.11^A	7.22 ± 0.02^A	0.00 ± 0.00^A
		CK	4 729.67 ± 73.82^A	6 000.02 ± 64.03^A	6 005.22 ± 56.39^A	7.20 ± 0.08^A	0.00 ± 0.00^A
	JN	IE	4 337.00 ± 137.70^A	5 566.32 ± 121.89^A	5 649.93 ± 104.78^A	6.88 ± 0.13^A	0.00 ± 0.00^A
		CK	4 457.00 ± 32.36^A	5 720.07 ± 76.83^A	5 773.04 ± 63.87^A	7.05 ± 0.04^A	0.00 ± 0.00^A

CC – Changchun; BJ – Beijing; JN – Jinan; IE – *Bt* maize residues; CK – non-*Bt* residues

the difference in species complexity were the general environment and soil in three localities rather than the degradation of *Bt* maize residues.

The environments and soil of the three test fields affected the soil bacterial community more than line and month. From rarefaction curves or α/β diversity analysis, it was obvious that the soil bacterial community in BJ and JN have more similarities compared with the soil bacterial community in CC. A previous study suggested that geographical distance could not be used as an explanation of the difference in maize rhizosphere microbiota, but some other effectors such as soil pH, moisture content, and geographic patterns were likely to affect the α-diversity of the maize rhizosphere bacterial community (Peiffer et al. 2013). For example, with the decomposition of

Table 3. Alpha diversity indices affecting factors compared by ANOVA

		obs	chao	ACE	Shannon	Simpson
Month	*F*-value	0.564	0.276	0.326	0.354	0.292
	P-value	0.641	0.842	0.807	0.786	0.831
Environment	*F*-value	570.087	849.995	864.974	339.908	113.232
	P-value	0.000*	0.000*	0.000*	0.000*	0.000*
Lines	*F*-value	0.002	0.027	0.008	0.005	0.030
	P-value	0.969	0.871	0.927	0.942	0.864

*$P < 0.01$

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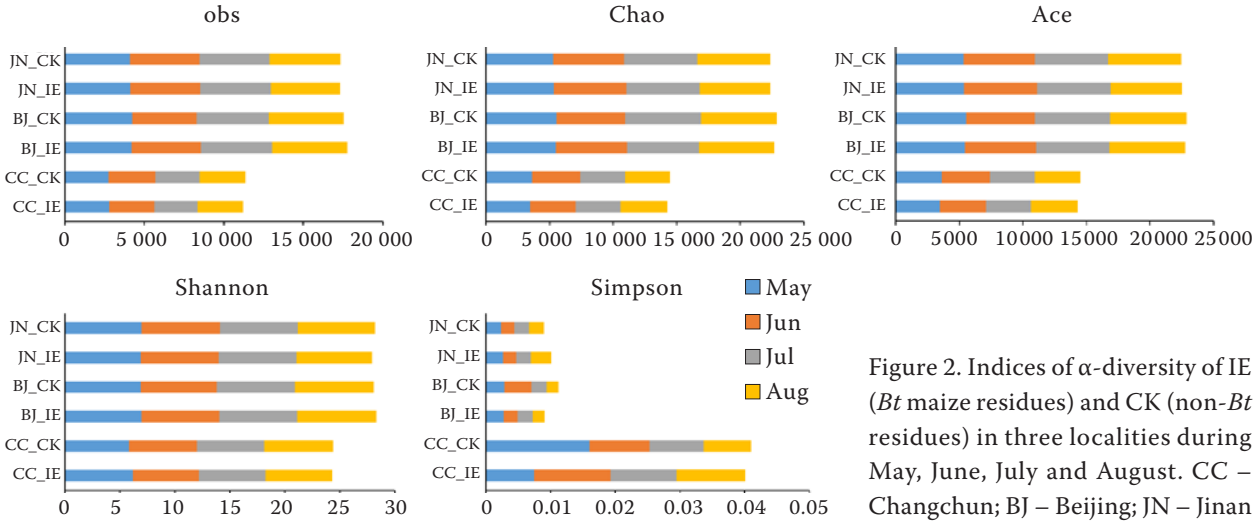

Figure 2. Indices of α-diversity of IE (*Bt* maize residues) and CK (non-*Bt* residues) in three localities during May, June, July and August. CC – Changchun; BJ – Beijing; JN – Jinan

Bt maize residues and the nutrients from straw entering the soil ecosystem, soil nutrient availability might negatively affect the diversity of soil bacteria (Shu et al. 2017). We speculated that the difference between bacterial diversities in our results might also be caused by environmental heterogeneity rather than geographical distance.

Effects on the structure of soil bacterial communities. Compared with fresh straw, long-term (> 10 years) decomposed straw returned to soil was reported to have more positive effects on the soil microbial community due to the nutrient and salinity (Su et al. 2020a). Therefore, the time required for decomposing straw can make a difference in the development of the soil microbial community. According to our experimental results, maize residue decomposing of 6–10 months had significant effects on the main phyla and core genera of the soil bacteria.

Fifty-one different genera of bacteria were obtained from all soil samples. The core genera had no significant difference between IE and CK in CC and JN, but the four dominant bacterial genera in BJ were significantly different between IE and CK (Table 4). These genera of bacteria were distributed in the soil in three areas with distinct differences. In CC, there were 9 core genera with an average of > 1% relative abundance, including *DA101*, *Kaistobacter*, *Rhodanobacter*, *Segetibacter*, *Arthrobacter*, *Burkholderia*, *Candidatus*, *Solibacter*, *Bradyrhizobium*, and *Flavisolibacter*. In JN, only

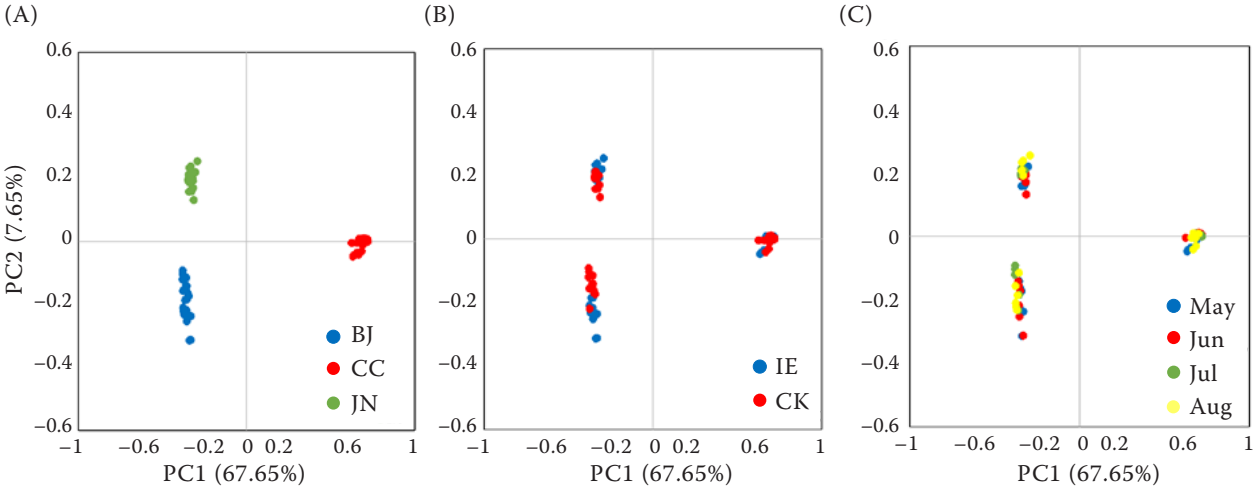

Figure 3. Principal component analysis (PCA) of bacterial community structure. (A) Bacterial community structure between IE (*Bt* maize residues) and CK (non-*Bt* residues); (B) bacterial community structure between different releasing localities; (C) bacterial community structure between various months. CC – Changchun; BJ – Beijing; JN – Jinan

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Table 4. Bacterial relative abundance (%) in Changchun (CC), Jinan (JN) and Beijing (BJ) at the genus level ($P < 0.01$)

City	Core genera	Mean ± SD (%)		Lines		Month	
		IE	CK	*F*	*P*	*F*	*P*
CC	*DA101*	9.33 ± 4.77	11.02 ± 4.70	0.766	0.391	3.001	0.055
	Kaistobacter	5.91 ± 0.99	5.47 ± 2.15	0.408	0.529	6.846	0.002*
	Rhodanobacter	2.12 ± 1.37	1.69 ± 1.42	0.550	0.466	3.276	0.042
	Segetibacter	1.64 ± 0.89	1.55 ± 0.60	0.082	0.777	52.532	0.000*
	Arthrobacter	1.49 ± 0.68	1.50 ± 0.43	0.001	0.974	2.581	0.082
	Burkholderia	1.58 ± 0.79	1.34 ± 0.41	0.948	0.341	9.058	0.001*
	Candidatus_Solibacter	1.32 ± 0.39	1.39 ± 0.26	0.279	0.602	15.588	0.000*
	Bradyrhizobium	1.28 ± 0.19	1.32 ± 0.32	0.126	0.726	5.754	0.005*
	Flavisolibacter	1.04 ± 0.36	1.08 ± 0.35	0.054	0.819	9.644	0.000*
JN	*Steroidobacter*	1.93 ± 0.38	1.81 ± 0.25	0.792	0.383	8.888	0.001*
	Kaistobacter	2.11 ± 1.05	1.56 ± 0.56	2.551	0.124	6.494	0.003*
	Arthrobacter	1.40 ± 0.46	1.54 ± 0.24	0.996	0.366	4.710	0.012
	Skermanella	1.08 ± 0.39	1.20 ± 0.19	0.849	0.367	1.298	0.303
	Bacillus	1.02 ± 0.40	1.03 ± 0.26	0.009	0.925	17.340	0.000*
BJ	*Steroidobacter*	1.80 ± 0.16	2.33 ± 0.51	12.062	0.002*	2.024	0.143
	Skermanella	2.33 ± 0.39	1.76 ± 0.49	9.837	0.005*	1.204	0.334
	Kaistobacter	0.86 ± 0.16	1.48 ± 0.55	13.983	0.001*	1.270	0.312
	Arthrobacter	1.39 ± 0.53	0.78 ± 0.18	14.290	0.001*	3.552	0.033

five dominant genera were observed > 1% relative abundance, including *Steroidobacter*, *Kaistobacter*, *Arthrobacter*, *Skermanella*, and *Bacillus*. Compared with the dominant bacterial genera in JN, *Arthrobacter* was not dominant in BJ (Table 4). The core genera in CC and JN were influenced by sampling months rather

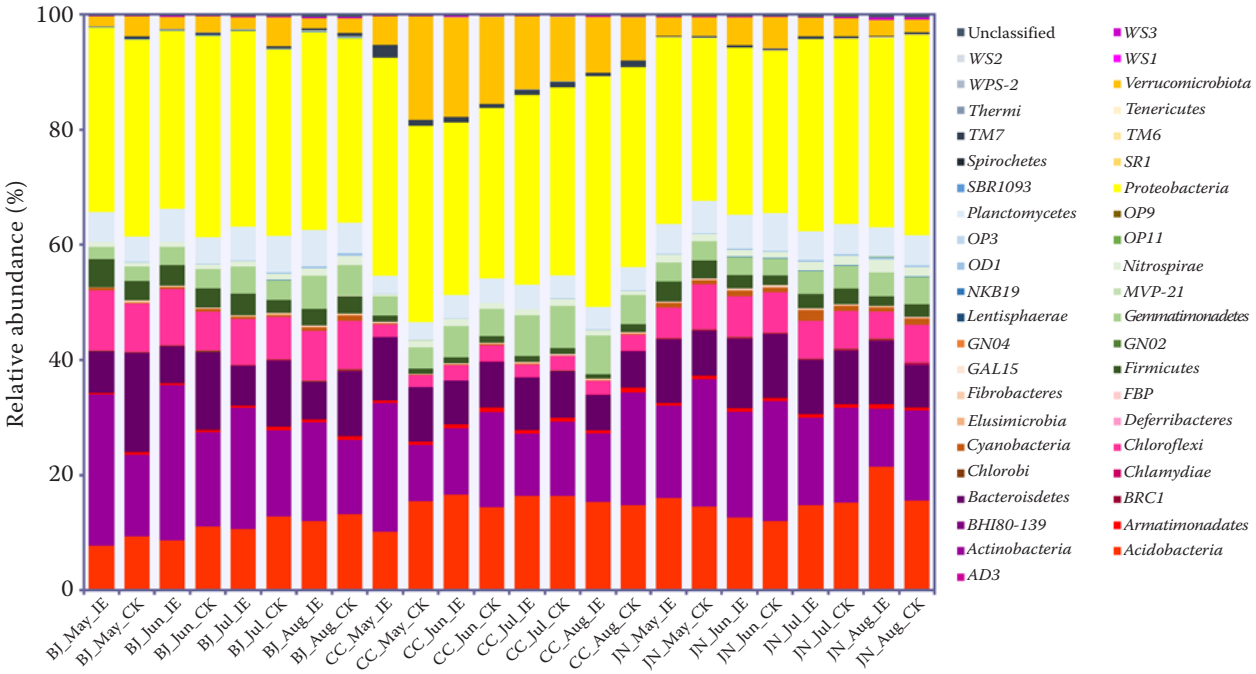

Figure 4. Bacterial composition at the phylum level. The bars were named in the following form: city, month and lines. CC – Changchun; BJ – Beijing; JN – Jinan; May – May; Jun – June; Jul – July; Aug – August; IE – *Bt* maize residues; CK – non-*Bt* residues

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Table 5. One-way ANOVA examining the effects of maize residues on the main soil bacterial phyla in three localities ($P < 0.01$)

	Variable	Acidobacteria	Actinobacteria	Bacteroidetes	Chloroflexi	Firmicutes	Gemmatimonadetes	Nitrospirae	Planctomycetes	Proteobacteria	Verrucomicrobia
Beijing	month	*F* 11.707	1.999	0.810	1.619	2.807	44.199	25.243	5.384	0.033	2.008
		P 0.000*	0.147	0.503	0.217	0.066	0.000*	0.000*	0.007*	0.992	0.145
	lines	*F* 6.122	36.770	58.770	8.356	5.453	0.210	1.046	4.869	0.534	23.580
		P 0.022	0.000*	0.000*	0.008*	0.029	0.651	0.318	0.038	0.473	0.000*
Changchun		Acidobacteria	Actinobacteria	Bacteroidetes	Chloroflexi	Firmicutes	Gemmatimonadetes	Planctomycetes	Proteobacteria	TM7	Verrucomicrobia
	month	*F* 3.065	0.867	9.768	3.602	0.393	26.605	6.277	12.721	4.583	3.601
		P 0.052	0.474	0.000*	0.031	0.760	0.000*	0.004*	0.000*	0.013	0.031
	lines	*F* 0.478	0.070	0.465	1.679	3.582	0.603	0.009	2.890	1.078	0.896
		P 0.497	0.795	0.502	0.208	0.072	0.446	0.926	0.103	0.310	0.354
Jinan		Acidobacteria	Actinobacteria	Bacteroidetes	Chloroflexi	Firmicutes	Gemmatimonadetes	Nitrospirae	Planctomycetes	Proteobacteria	Verrucomicrobia
	month	*F* 5.490	4.541	2.424	1.795	15.364	13.223	11.324	7.498	12.399	37.291
		P 0.006*	0.014	0.096	0.181	0.000*	0.000*	0.000*	0.001*	0.000*	0.000*
	lines	*F* 1.927	5.512	7.879	6.683	0.034	0.228	1.317	1.455	0.872	0.001
		P 0.179	0.028	0.010	0.017	0.855	0.638	0.263	0.241	0.360	0.978

than lines, which was different from BJ (Table 4). *Bradyrhizobium* and *Rhodanobacter* were reported to decrease with increasing heavy metal pollution degrees, while *Arthrobacter* and *Steroidobacter* were abundant in the areas with heavy metal pollution (Hong et al. 2015). This indicated that soil in CC was less affected by heavy metal compared to soil in BJ and JN. The metabolic activity of soil microbes was thought to be varied, with the primary source of being readily available carbon in soil (Cao et al. 2018).

Forty-two different phyla of bacteria were identified in 72 samples (Figure 4). At the phylum level, the relative abundance of main soil bacterial phyla in three localities was not significantly different between IE and CK, except for Actinobacteria, Bacteroidetes, and Chloroflexi in BJ (Table 5, P = 0.000, 0.000 and 0.008, respectively). However, the main phyla of soil bacterial communities in three localities were not exactly the same. As shown in Figure 5, the phyla of relative abundance > 1% were listed. And we found that the ten phyla in BJ and JN were Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes, Firmicutes, Verrucomicrobia, and Nitrospirae. But it was different in CC in which ten main phyla were Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Chloroflexi, TM7, and Firmicutes. The taxonomic composition of soil bacterial communities was different in three localities. Proteobacteria was the most abundant phylum in three places, consistent with a report by Dohrmann et al. (2013). And the relative abundance of Actinobacteria was different between the three localities, possibly caused by soil pH. More specifically, the relative abundance of Actinobacteria was higher in near-neutral pH and lowered at acidic and alkaline pH (Zhang et al. 2017). To test whether pH is also a determining factor, pH was measured, and results showed that the soil in CC was acidic while the soil of the other two areas was alkalescent (Figure 6), which may result in a significant difference in the abundance of Actinobacteria. It was obvious that the relative abundance of main bacterial phyla was affected more by months than lines. There were four phyla in BJ (Acidobacteria, Gemmatimonadetes, Nitrospirae, and Planctomycetes) and CC (Bacteroidetes, Gemmatimonadetes, Planctomycetes, and Proteobacteria), seven phyla in JN (Acidobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, Proteobacteria, and Verrucomicrobia) affected significantly by months

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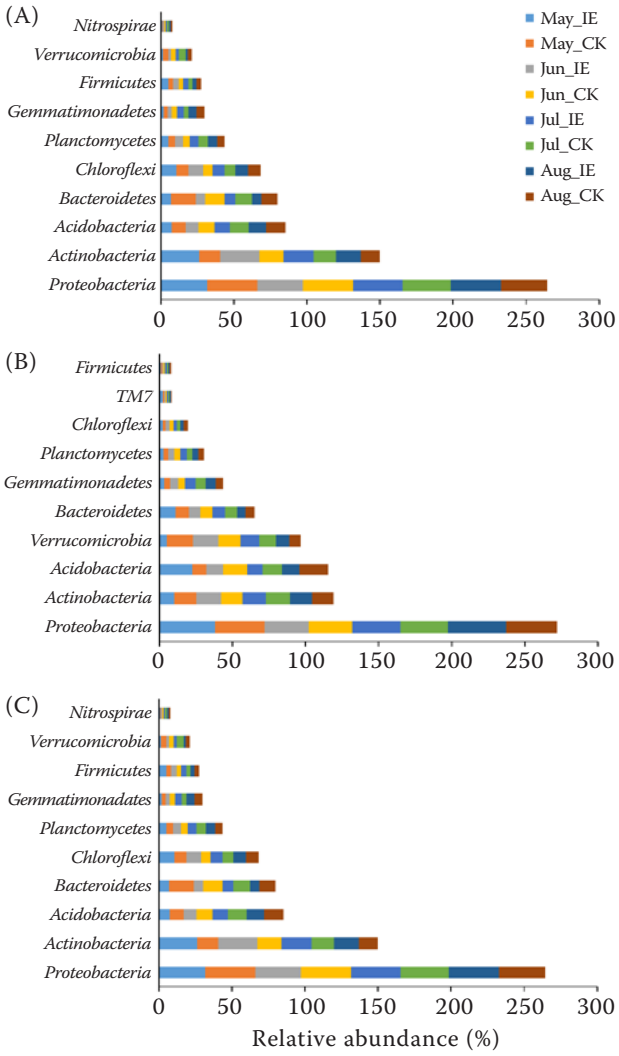

Figure 5. Relative abundance of main phyla in three localities: (A) Beijing; (B) Changchun and (C) Jinan; IE – *Bt* maize residues; CK – non-*Bt* residues) maize

of sampling (Table 5). The relative abundance of Planctomycetes and Gemmatimonadetes was found to be more significantly affected by the environment than months (Figure 7). Gemmatimonadetes prefer to staying in drier soil (DeBruyn et al. 2011), while Planctomycetes prefer to living in the water-saturated habit (Dedysh and Ivanova 2019), indicating that soil moisture can affect the relative abundance of these phyla in the four months. We collected the meteorological data from China Meteorological Administration and found that the relative soil humidity in CC was higher than that in the other two areas (Table 1). It was not consistent with the relative abundance of Planctomycetes and Gemmatimonadetes. But it is reported that the rises in air temperature have positive effects on soil bacterial diversity (Dennis et al. 2019). As the difference of relative abundance of Planctomycetes and Gemmatimonadetes in three areas was more related to the temperature that CC was colder than BJ and JN, we speculated that the soil bacterial communities were affected more seriously by temperature than soil moisture.

The ANOVA comparison indicates a significant difference in the main phyla and core genera of BJ between IE and CK (Tables 4 and 5). As mentioned before, pH could affect this relative abundance as pH between IE and CK was significantly different (Figure 6, $P = 0.004$). The other possible reason might be that the *Bt* residues had a higher lignin content and lignin/N ratio in soil than non-*Bt* residues (Fang et al. 2007). Annual variability was also important when assessing GM crops' environmental effects (Szoboszlay et al. 2019). Further investigation could ascertain if the decomposition of *Bt* maize residues

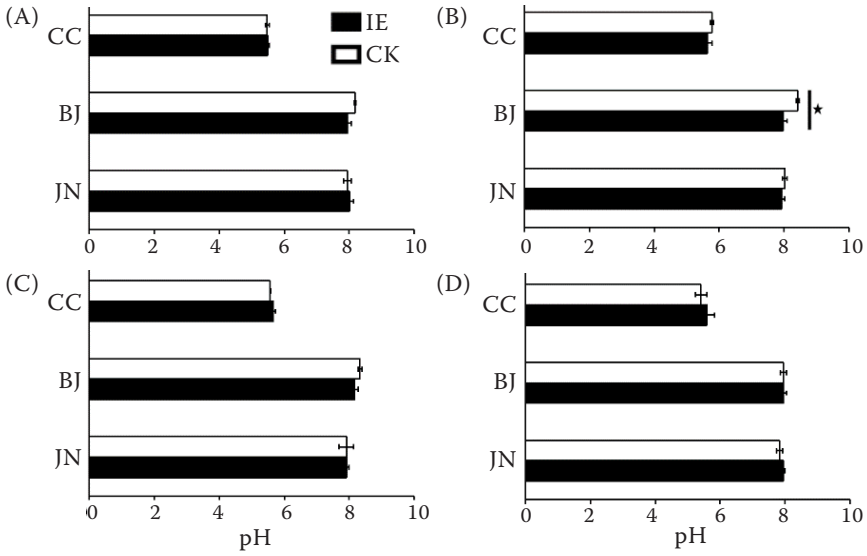

Figure 6. pH of soil under IE (*Bt* maize residues)/CK (non-*Bt* residues) maize degradation in Changchun (CC), Beijing (BJ) and Jinan (JN). pH of the soil in (A) May; (B) June; (C) July, and (D) August. *indicate significant difference according to the ANOVA, $P < 0.01$

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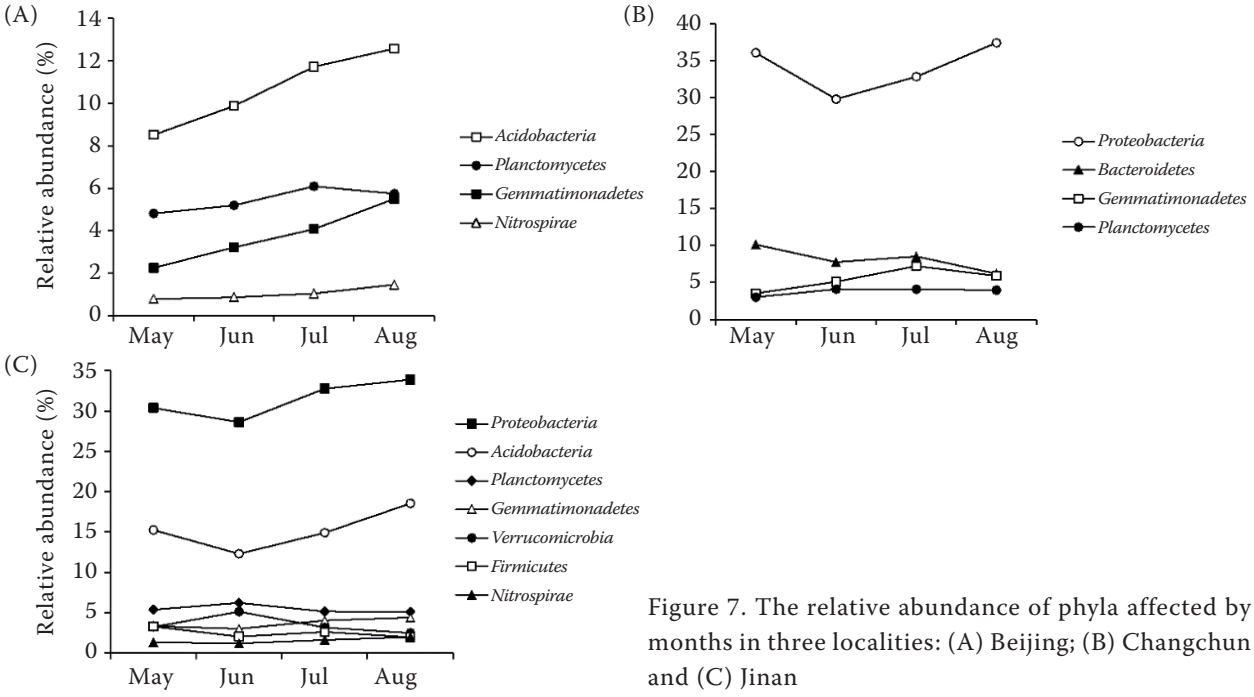

Figure 7. The relative abundance of phyla affected by months in three localities: (A) Beijing; (B) Changchun and (C) Jinan

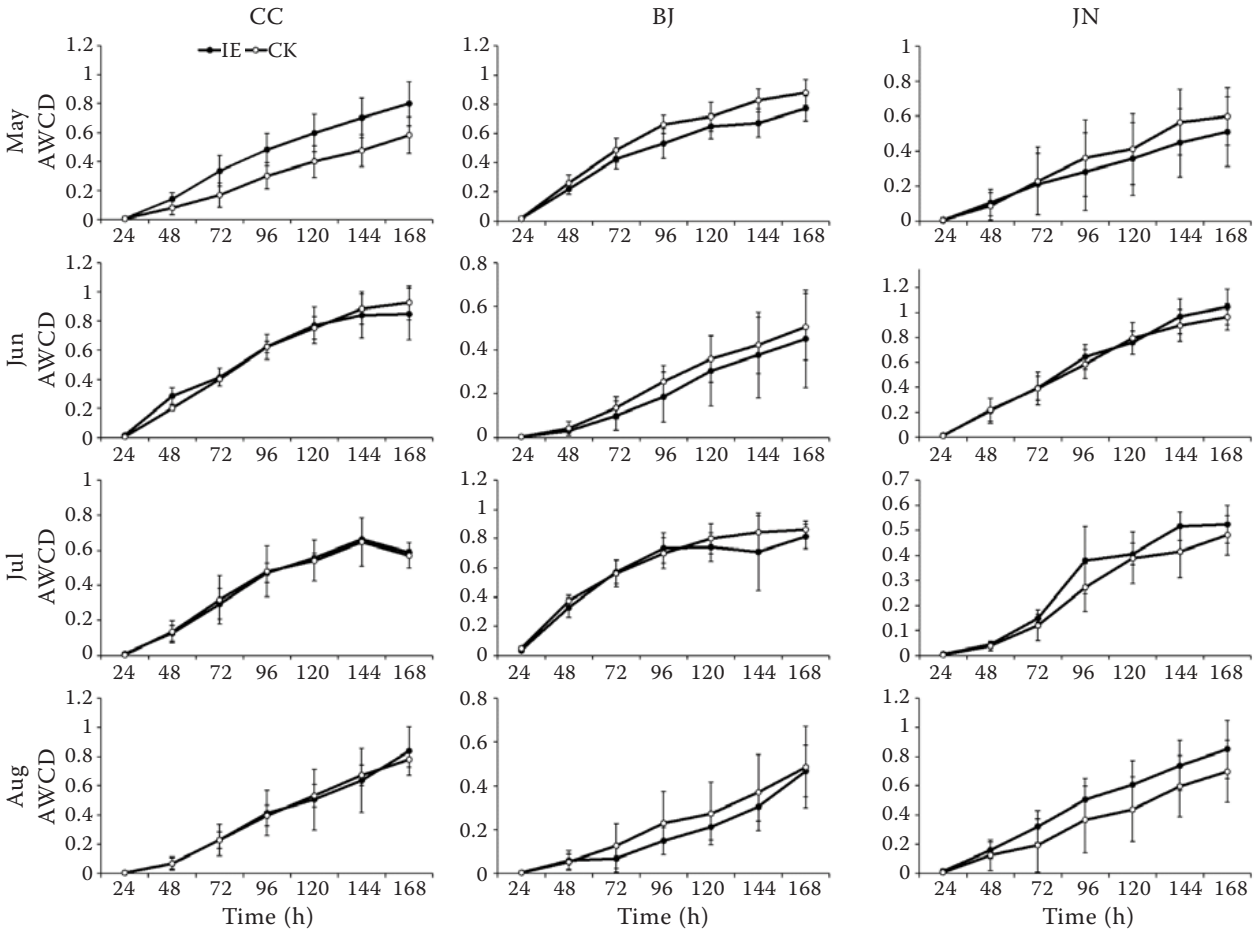

Figure 8. Average well color development (AWCD) of substrates utilisation in Biolog Eco-Plate incubated for 168 h. CC – Changchun; BJ – Beijing; JN – Jinan; IE – *Bt* maize residues; CK – non-*Bt* residues) maize

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Table 6. Data summary of Shannon-wiener diversity index (H), richness index (R), and evenness index (E) ($P < 0.01$)

		May-IE	May-CK	Jun-IE	Jun-CK	Jul-IE	Jul-CK	Aug-IE	Aug-CK
H	CC	2.8 ± 0.1^{A}	2.5 ± 0.3^{A}	2.9 ± 0.2^{A}	3.1 ± 0.1^{A}	2.3 ± 0.2^{A}	2.3 ± 0.4^{A}	2.5 ± 0.4^{A}	2.7 ± 0.1^{A}
	BJ	3.0 ± 0.1^{A}	3.1 ± 0.1^{A}	1.9 ± 0.7^{BC}	2.5 ± 0.3^{AB}	3.1 ± 0.1^{A}	3.0 ± 0.1^{A}	1.5 ± 0.5^{C}	2.0 ± 0.5^{BC}
	JN	2.3 ± 0.5^{A}	2.5 ± 0.5^{A}	2.9 ± 0.1^{A}	2.9 ± 0.1^{A}	2.3 ± 0.2^{A}	2.3 ± 0.5^{A}	2.7 ± 0.2^{A}	2.4 ± 0.6^{A}
R	CC	22.0 ± 1.0^{A}	16.7 ± 5.8^{A}	23.7 ± 3.2^{A}	26.7 ± 0.6^{A}	20.7 ± 1.5^{A}	21.0 ± 6.1^{A}	17.3 ± 6.5^{A}	18.7 ± 1.2^{A}
	BJ	23.3 ± 1.2^{A}	28.0 ± 1.0^{A}	9.0 ± 4.4^{BC}	15.3 ± 4.0^{B}	26.7 ± 2.5^{A}	26.0 ± 2.0^{A}	6.7 ± 3.8^{C}	10.3 ± 4.7^{BC}
	JN	12.7 ± 6.5^{A}	16.3 ± 7.2^{A}	23.7 ± 1.2^{A}	22.7 ± 3.5^{A}	13.7 ± 1.2^{A}	13.7 ± 5.5^{A}	19.3 ± 4.5^{A}	15.3 ± 8.1^{A}
E	CC	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}
	BJ	0.9 ± 0.0^{A}	0.9 ± 0.0^{AB}	0.9 ± 0.1^{AB}	0.9 ± 0.0^{AB}	0.9 ± 0.0^{A}	0.9 ± 0.0^{AB}	0.8 ± 0.0^{B}	0.9 ± 0.0^{AB}
	JN	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}	0.9 ± 0.1^{A}	0.9 ± 0.0^{A}	0.9 ± 0.0^{A}

BJ – Beijing; CC – Changchun; JN – Jinan; IE – *Bt* maize residues; CK – non-*Bt* maize residues

contributes to the difference of dominant phyla and core genera in BJ.

Soil microbial functional diversity. The AWCD value has an increasing trend within 168 h, indicating that the microbial community could keep utilising various carbon sources. And no significant difference in AWCD values was observed between IE and CK (Figure 8). Indices including richness index (R), Shannon-wiener diversity index (H), and evenness index (E) were not statistically different between IE and CK in three localities (Table 6). Our results also indicated that monthly differences in the Shannon-Wiener diversity index, richness index, and evenness index were only seen in BJ (Table 6). The difference was possibly related to the weather, which may influence the properties of rhizosphere soil that indirectly affect the microbial community (Van Wyk et al. 2017). With ANOVA comparison,

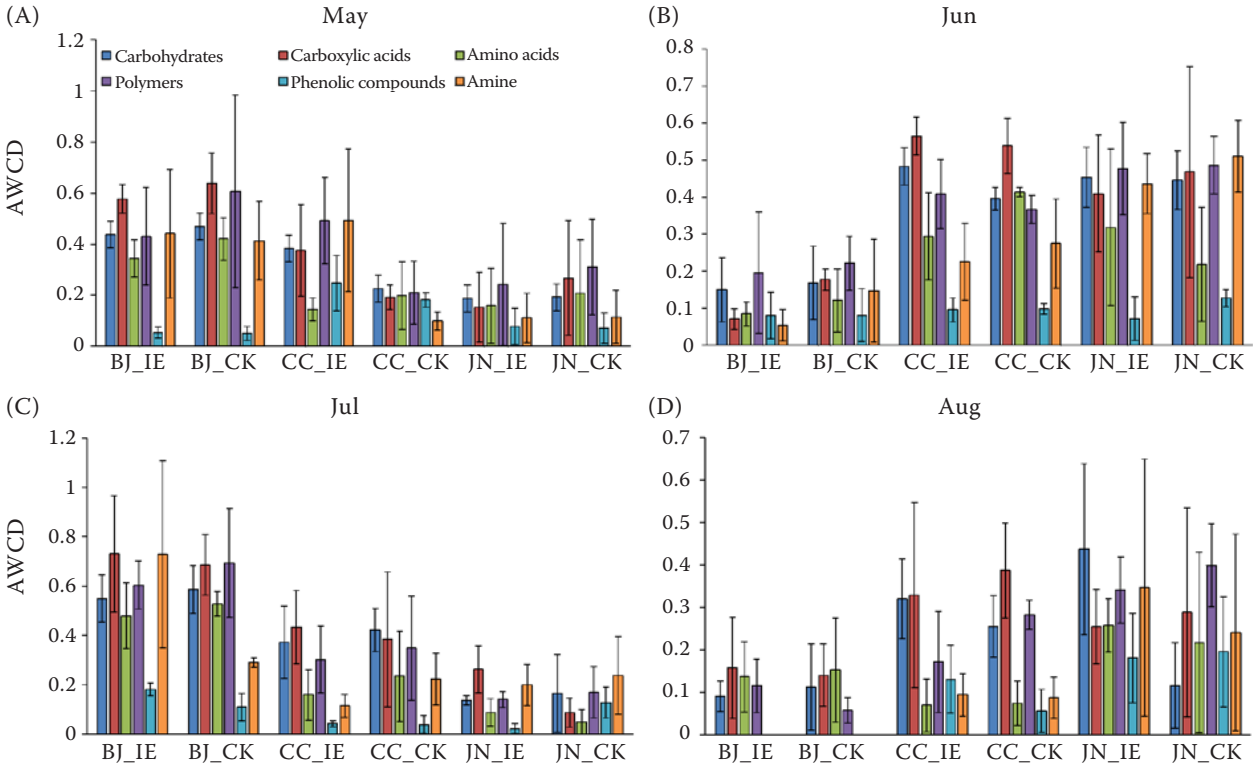

Figure 9. Substrates utilisation of six carbon sources in three localities based on 72-h incubation (n = 3). The bars were named in the following form: city name: BJ – Beijing; CC – Changchun; JN – Jinan; IE – *Bt* maize residues; CK – non-*Bt* maize residues

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Table 7. ANOVA comparison of the diversity indices between cultivar, environment, and month ($P < 0.01$)

Diversity indices	Variable	*F*	*P*
Shannon-wiener diversity index	cultivar	0.407	0.526
	environment	1.791	0.174
	month	2.912	0.041
Richness index	cultivar	0.385	0.537
	environment	1.908	0.156
	month	3.204	0.029
Evenness index	cultivar	0.341	0.561
	environment	0.480	0.621
	month	2.766	0.048

richness index, Shannon-wiener diversity index, and evenness index had no significant differences between lines, environment, and sampling months (Table 7). The 31 carbon sources in Biolog Eco-Plate consisted of 6 carbon sources: carbohydrates, carboxylic acids, amino acids, polymers, phenolic compounds, and amines. The results showed that there was no significant difference existed in six carbon sources between IE and CK, and carbohydrates, carboxylic acids, and polymers were the more popular carbon sources in the soil for microbial community utilisation (Figure 9). The substrates utilisation was different from geographic sites (Figure 9), which was consistent with the study of Luo et al. (2016). Interestingly, the substrates' utilisation of six carbon sources in BJ was higher in May and July than in June and August. Previous works showed that the increase of soil salinity could be the factor resulting in the shift of substrates utilisation (Thottathil et al. 2008). In addition, *Bt* maize straw caused increased microbial consumption of carbohydrates in the short term (less than a month) (Mulder et al. 2006). Based on our results, the difference caused by *Bt* maize residues on soil microbial community was short-termed.

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