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A glimpse of *Escherichia coli* O157:H7 survival in soils from eastern China

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HIGHLIGHTS

- *E. coli* O157:H7 survival times (t_d) varied in soils from eastern China.
- Longer t_d values exist in soils from north-eastern than those from south-eastern China.
- Soil MBC, *Chloroflexi*, TN and amorphous Al_2O_3 are important in affecting the t_d values.
- Results provide information to reduce the potential risks of pathogen contamination.

ARTICLE INFO

ABSTRACT

Escherichia coli O157:H7 (*E. coli* O157:H7) is an important food-borne pathogen, which continues to be a major public health concern worldwide. It is known that *E. coli* O157:H7 survive in soil environment might result in the contamination of fresh produce or water source. To investigate how the soils and their properties affect *E. coli* O157:H7 survival, we studied *E. coli* O157:H7 survival dynamics in 14 soils collected in eastern China from the warm-temperate zone to subtropical zone. Results showed that *E. coli* O157:H7 survival as a function of time can be well described by the Weibull model. The calculated t_d values (survival time to reach the detection limit, 100 colony forming units per gram oven-dried weight of soil) for the test soils were between 1.4 and 25.8 days. A significantly longer survival time (t_d) was observed in neutral or alkaline soils from north-eastern China (the warm-temperate zone) than that in acidic soils from south-eastern China (the subtropical zone). Distinct *E. coli* O157:H7 survival dynamics was related to soil properties. Stepwise multiple regression analysis revealed that the t_d values were significantly enhanced by soil microbial biomass carbon and total nitrogen, but were significantly reduced by amorphous Al_2O_3 and relative abundance of *Chloroflexi*. It should pay more attention to *E. coli* O157:H7 long survival in soils and its potential environmental contamination risk.

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1. Introduction

Escherichia coli O157:H7 (*E. coli* O157:H7) is a food-borne pathogen that can cause watery diarrhea, hemorrhagic colitis, hemorrhagic uremic syndrome, and thrombotic thrombocytopenic purpura (Mead et al., 1999). The minimal infectious dose of *E. coli* O157:H7 for human is as few as 10 cells (Griffin and Tauxe, 1991). The first outbreak of *E. coli* O157:H7 infections caused by contaminated ground beef was reported in the USA in 1982 (Riley et al., 1983). Since then, many outbreaks and numerous sporadic cases of *E. coli* O157:H7 have been reported from all over the world, with several hundred severe outbreaks worldwide at mortality rate as high as 5–10% (Hedden, 2008). In the years from 1986 to 1988, *E. coli* O157:H7 strains in China were isolated from patients with diarrhea in Xuzhou City, Jiangsu Province (Xu et al., 1990). Later, during 1999–2000, several outbreaks in the middle-eastern areas of China, including Jiangsu, and the neighboring provinces of Anhui and Henan were reported (Ma et al., 2009). In recent years, extensive data from the epidemiologic survey disclosed that the presence of *E. coli* O157:H7 in external environments, such as excrements, sewages, foods, and soils for growing vegetables has been widely documented in China and other countries of the world (Banatvala et al., 2001; Islam et al., 2004; Ding et al., 2009; Ma et al., 2009; Brennan et al., 2010; Bradford et al., 2013).

More significantly, the carriage of *E. coli* O157:H7 would most likely result in the contamination of the environment. Studies reported that

the manure-borne zoonotic pathogens (i.e., *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, et al.) can invade into the soil through sewage irrigation, runoff from stored manure, manure application or other processes (Solomon et al., 2002; van Elsas et al., 2011; Bradford et al., 2013). Furthermore, *E. coli* O157:H7 can survive in soil or soil-related (manure) environment for days to more than 1 year (Vidovic et al., 2007; Franz et al., 2008; Patel et al., 2010; Ma et al., 2011; Wang et al., 2013; Yao et al., 2013; Zhang et al., 2013), and it will be potentially transported to surface runoff, leached into groundwater, attached to plants, grew on plants surface, or even became internalized within plant tissue (Solomon et al., 2002; Islam et al., 2004; Brennan et al., 2010; Patel et al., 2010). Hence, a better understanding of the nature of *E. coli* O157:H7 survival in soil or soil-related (manure) environment will help in reducing its potential environmental contamination risk.

Previous studies observed that *E. coli* O157:H7 survival in soils were related to multiple factors, such as soil abiotic (nutrients, pH, moisture, temperature, texture, etc.), biotic parameters (indigenous microbial communities), and management practices (Islam et al., 2004; Vidovic et al., 2007; Franz et al., 2008; Patel et al., 2010; Ma et al., 2011, 2013; Wang et al., 2013; Yao et al., 2013; Zhang et al., 2013). For instances, Vidovic et al. (2007) revealed that the nutrient rich soils, in combination with moisture, might significantly extend *E. coli* O157:H7 survival in soil environment. Our recent findings showed that soil pH, organic carbon, and microbial community structure (represented as the ratio of bacteria phospholipid fatty acids (PLFAs) to fungi PLFAs) are the important factors in controlling *E. coli* O157:H7 survival in soils from vegetable fields (Yao et al., 2013). We also observed that *E. coli* O157:H7 can survive longer in soils under plastic-greenhouse cultivation than that in the corresponding open-field soils (Yao et al., 2013). A longer survival time of *E. coli* O157:H7 in soils implies that there is a high potential infection risk from the pathogen contaminated soils. Likewise, pathogen die-off rates are more critical to develop manure management recommendations. Consequently, the knowledge of *E. coli* O157:H7 survival in soils with different physical, chemical, and biological properties is critical to understand its fate in the environment to minimize the human infection from the pathogen.

The previous study reported that the survival kinetics of *E. coli* O157:H7 EDL933 (ATCC 43895) and its four mutant derivatives in the same soil were similar (Ma et al., 2011). Therefore, this study selected *E. coli* O157:H7 EDL933 (ATCC 43895) as a representative strain to investigate its survival in the 14 test soils from different climate zones from north to south in eastern China. The specific aims and significances of this study were to (1) determine the dynamics of *E. coli* O157:H7 survival in 14 soil samples collected from 12 different provinces in eastern China, (2) investigate the relationships between *E. coli* O157:H7 survival time and soil physicochemical and biological properties, and (3) provide information to understand the potential risks of pathogen contamination from soil and to protect human and environmental health.

2. Materials and methods

2.1. Soils

The 14 soil samples (S1–S14) were collected from north (47.4°N, denoted as north-eastern China) to south (20.0°N, denoted as south-eastern China) in eastern China. Six soils (S1–S6) were from the warm-temperate zone in north-eastern China, while eight soils (S7–S14) were from the subtropical zone in south-eastern China. Specifically, soil S7 was from near the boundary between warm-temperate zone and subtropical zone, and its microbial community structure was found to be similar to the soil samples of warm-temperate zone (Wu et al., 2009). Each sample was collected from the surface horizon (0–15 cm) and was a composite of several individual soil cores taken along a 200-m transect. Soil samples were stored immediately in coolers with ice bags and transported to laboratory. The fresh samples were hand-picked to remove discrete plant residues, sieved to pass

through a 2-mm plastic mesh, homogenized thoroughly, and then stored in a cold room at 4 °C. A sub-sample from each of the 14 soil samples was collected and air-dried for analyzing physical and chemical properties. Briefly, soil pH, organic carbon (OC), available potassium (AK), amorphous and free sesquioxides, texture, and water holding capacity (WHC) were tested with air-dried soil according to the protocols in Agricultural Chemistry Committee of China (1983). Total nitrogen (TN) was determined using Flash 2000 NC Analyzer (Thermo Scientific, MA, USA).

A sub-sample of each test soil was taken out from the cool room (at 4 °C), then incubated for 5 days at the same incubation conditions (21 ± 1 °C, 40% WHC) with the experiments of *E. coli* O157:H7 survival in soils, and subjected to soil microbial biomass carbon (MBC), water soluble organic carbon (WSOC), DNA extraction and 454-pyrosequencing analyses. The total organic carbon (TOC) in the MBC and WSOC extracts were measured by an Apollo 9000 total organic carbon analyzer (Tekmar-Dohrmann, OH, USA) using the high temperature combustion method. Soil DNA was extracted by the Power Soil Extraction Kit (MOBIO Laboratories, CA, USA) and submitted to Research and Testing Laboratory (Lubbock, TX, USA) for bacterial 454-pyrosequencing analysis. The moisture content (MC) of the moist or air-dried soil samples was gravimetrically determined by oven-drying soil at 105 °C for 24 h, and expressed as a percentage of oven-dried weight (Agricultural Chemistry Committee of China, 1983). Soil property analyses were performed on moist or air-dried samples, however, all the results were finally corrected to an oven-dried basis by use of the Moisture Factor (1 + MC / 100). The selected soil properties are shown in Tables 1, 2 and Fig. 1.

2.2. E. coli O157:H7 survival in soils

The *E. coli* O157:H7 EDL933 (ATCC 43895) was obtained from ATCC and then induced to be resistant to 100 μg mL^{-1} of rifampicin (Fisher Scientific, Fair Lawn, NJ, USA) and 25 μg mL^{-1} of nalidixic acid (Sigma-Aldrich, MO, USA). The induced *E. coli* O157:H7 cells were then cultured for inoculation following the method by Wang et al. (2013). The *E. coli* O157:H7 cells were finally re-suspended and adjusted with sterilized deionized water to an optical density (OD) at 600 nm of 0.6 prior to inoculation of the soil samples. The plate counting showed that the concentration in the bacterial suspension was ca. 10^8 CFU mL^{-1} (CFU, colony-forming units).

Although the 14 test soils collected from 12 provinces in eastern China with different mean annual temperature (MAT) and mean annual precipitation (MAP), the laboratory experiments were performed at the fixed temperature (21 ± 1 °C) and moisture conditions (40% WHC) according to the following practical factors. The reports showed that the majority of *E. coli* O157:H7 infections were outbroken in summer seasons (Banatvala et al., 2001; Ma et al., 2009). While the summer mean temperature is above 20 °C in most of areas in eastern China (Sun and Liu, 2008). In addition, the MC in moist soils under field conditions is typically at 40–60% WHC (Ministry of Water Resources, the People's Republic of China, 2008). Therefore, the experimental temperature (21 ± 1 °C) and moisture conditions (40% WHC) are representative for the ecological environment of the sampling locations.

Before the inoculation experiment, soil samples were removed from 4 °C storage and pre-incubated in the dark at 21 ± 1 °C for 5 days to activate the microbial community of the soil samples. The prepared *E. coli* O157:H7 cells were added into the soil samples, thoroughly mixed, and the soil moisture was further adjusted to 40% WHC by adding sterilized deionized water. By considering the MC of the soils, the inoculated concentration of *E. coli* O157:H7 in the soils was approximately 10^6 CFU per gram oven-dried weight of soil (CFU g^{-1}). Forty grams (equivalent to oven-dried weight) of each inoculated soil was placed in a sterilized 50-mL centrifugal tube. While the uninoculated control of each soil sample was treated by adding sterilized deionized water instead of cell suspension. All tubes (triplicates both for

Table 1
Location and properties of soils used in the study.

Soil code	Province	Soil classification (USDA)	Latitude °N	MAT °C	MAP mm	WHC %	pH (H₂O)	OC g·kg⁻¹	TN	WSOC mg·kg⁻¹	MBC	AK	Clay %	Silt	Sand	Fe$_o$ g·kg⁻¹	Al$_o$	Fe$_d$	Al$_d$
S1	Heilongjiang	Argiustoll	47.4	1.2	550	71.4	5.4	31.7	2.6	119.2	366.8	195.8	36.4	55.3	8.3	6.3	2.0	9.2	1.7
S2	Liaoning	Paleboralf	41.8	8.3	500	53.6	6.5	9.4	0.7	82.1	391.9	85.4	24.7	62.2	13.1	4.1	1.2	11.5	1.5
S3	Shandong	Typustalf	35.4	14.3	661	46.1	6.3	9.0	0.7	73.8	427.3	181.7	27.3	37.6	35.1	3.9	1.1	11.7	1.1
S4	Shanxi	Cumuli-Ustic Isohumosols	35.2	9.1	584	44.2	8.4	8.5	0.6	73.4	100.2	239.4	27.2	67.4	5.4	1.7	0.9	11.0	0.5
S5	Henan	Ustifluvent	35.0	13.9	605	45.4	8.6	7.7	0.5	89.9	79.1	91.3	20.4	63.7	15.9	1.6	0.8	8.9	0.8
S6	Henan	Haplustalf	34.8	15.0	402	41.4	8.4	4.2	0.1	42.3	414.9	66.6	19.5	60.7	19.8	1.8	0.9	9.8	0.5
S7	Jiangsu	Typudalf	32.0	15.7	1021	62.0	5.1	15.4	1.3	154.9	572.0	97.6	25.1	68.4	6.5	4.7	1.2	19.3	2.3
S8	Zhejiang	Plinthudult	29.1	16.7	1974	58.4	4.6	3.6	0.3	26.6	177.1	137.9	50.2	41.5	8.3	2.9	1.7	25.0	3.3
S9	Zhejiang	Psammaquent	29.1	16.7	1974	42.5	4.3	1.5	0.1	18.9	156.3	34.7	11.5	14.1	74.4	0.8	0.8	7.2	0.9
S10	Jiangxi	Plinthudult	28.7	17.6	1600	55.9	4.7	6.6	0.4	32.2	309.8	54.6	49.3	46	4.7	3.6	2.0	24.9	3.5
S11	Guizhou	Hapludult	27.0	15.3	1200	62.9	4.1	25.7	1.4	254.7	395.5	60.7	35.1	61	3.9	6.0	2.3	24.6	5.0
S12	Hunan	Plinthudult	26.6	18.2	1426	51.4	4.6	9.1	0.6	60.2	70.3	110.0	46.1	45.9	8.0	3.2	1.2	26.0	4.9
S13	Guangxi	Plinthudult	23.0	21.2	1690	51.4	4.6	10.7	0.5	86.4	281.5	42.8	37.0	28.7	34.3	2.1	1.8	24.9	3.5
S14	Hainan	Tropudult	20.0	23.8	1700	42.5	4.8	24.6	1.5	153.2	434.0	182.6	22.5	13.6	63.9	2.0	4.1	7.0	0.5

MAT, mean annual temperature; MAP, mean annual precipitation; WHC, water holding capacity; OC, organic carbon; TN, total nitrogen; WSOC, water soluble organic carbon; MBC, microbial biomass carbon; AK, available potassium; Fe$_o$, amorphous Fe_2O_3; Al$_o$, amorphous Al_2O_3; Fe$_d$, free Fe_2O_3; Al$_d$, free Al_2O_3.

inoculated soil samples and uninoculated controls) were then incubated at 21 ± 1 °C. Soil moisture content was measured by weighing every 2 days, and the water loss was replenished by adding sterilized deionized water.

Based on the preliminary studies, two sets of time intervals were used to take the soil samples: 0.5-g (equivalent to oven-dried weight) of soil sub-sample was taken from each tube at 0, 0.04, 1, 3, 5, 7, 10, 15, 24, and 36 days after treatment (DAT) for soils with slow decline of *E. coli* O157:H7, and 0, 0.04, 0.125, 0.208, 0.5, 1, and 3 DAT for soils with fast decline of *E. coli* O157:H7. The samples were then subjected to plating analyses to determine the survival of *E. coli* O157:H7 over time as described below.

Each sample was thoroughly mixed with 4.5 mL of 0.1% peptone buffer (Lab M, Lancashire, UK) by inverting the tube and then vortexed for 2 × 30 s. Serial dilutions (1:10) of each sample were prepared with sterilized deionized water, and 0.1-mL portions of the last three of the serial dilutions per sample were surface plated in duplicates on the SMAC (sorbitol MacConkey)–BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) agar (Lab M, Lancashire, UK) with appropriate antibiotics (100 µg mL⁻¹ of rifampicin and 25 µg mL⁻¹ of nalidixic acid) for *E. coli* O157:H7 counting. The inoculated SMAC–BCIG agar plates were incubated at 37 °C for 16 h and then enumerated. In this study, the detection limit of the plating method was 100 CFU g⁻¹, and the cell recovery rate of the method ranged from 92.5 to 104.2%, with a mean ± standard deviation value of 98.7 ± 4.1%. Plating analysis did not continue after plate counts of zero appeared twice in succession during the incubation.

Table 2
Soil bacterial richness and diversity estimates based on operational taxonomic units (OTUs) cluster at 97% sequence similarity according to 454-pyrosequencing.

| Soil code	Sequences	OTUs	Chao1	Shannon	Simpson	Coverage
S1	2899	1398	2969.44	9.859	0.998	0.700
S2	2710	873	1465.55	8.565	0.993	0.827
S3	4543	2254	5712.18	10.176	0.996	0.651
S4	4005	1917	3609.07	10.363	0.999	0.716
S5	4234	1849	3645.93	10.074	0.998	0.737
S6	4142	1734	4037.82	9.832	0.997	0.730
S7	1000	225	467.82	6.018	0.954	0.871
S8	3263	718	1073.84	8.336	0.993	0.906
S9	5637	894	1420.73	8.408	0.993	0.936
S10	6589	1658	2711.03	9.111	0.991	0.876
S11	839	332	557.04	7.480	0.984	0.788
S12	5060	1783	3522.79	9.562	0.994	0.791
S13	6173	1811	2861.00	9.739	0.997	0.858
S14	1004	222	476.20	5.908	0.937	0.876

Soil codes (S1 to S14) are the same as shown in Table 1.

2.3. E. coli O157:H7 enrichment and confirmation

A subsequent enrichment and immunomagnetic separation (IMS) of all inoculated and uninoculated soil samples were carried out at 63 DAT to determine whether the viable cells of *E. coli* O157:H7 were still present in soils. Ten grams of soil sample from each treatment was taken and put into a stomacher bag containing 100 mL 0.1% peptone buffer supplemented with vancomycin (8.0 mg L⁻¹), cefixime (0.05 mg L⁻¹), and cefsulodin (10.0 mg L⁻¹). The soil and buffer were then mixed for 2 min at 200 rpm using a stomacher 400 circulator (Wolf Laboratories Ltd., York, UK). The soil mixture was left on bench top for about 5 min to allow the soil particles to settle. The aqueous phase was then transferred into a 250-mL flask, incubated with rotation (220 rpm) at 37 °C for 16 h, and then performed IMS to recover *E. coli* O157:H7 cells by Dynabeads anti-*E. coli* O157, following the manufacture's protocol (Invitrogen, Carlsbad, CA, USA). After IMS, the bead–bacteria complexes were spread over the SMAC–BCIG agar plate with a sterilized loop. This ensured the separation of the bead–bacteria complexes. The plates were incubated at 37 °C for 24 h. The recovered presumptive *E. coli* O157:H7 colonies were confirmed by the multiplex PCR according to Ma et al. (2011). Since these data are semiquantitative, they were not used in the survival model construction.

2.4. Data analysis

The 454-pyrosequencing data were processed by using Quantitative Insights Into Microbial Ecology (QIIME) platform, version 1.6.0 (Caporaso et al., 2010). After the sequences with chimeras, average quality scores of <25 and <200 bps in length were removed, the
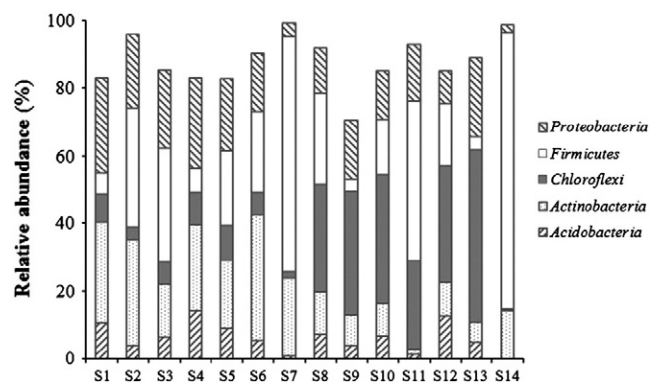
Fig. 1. Relative abundances of dominant bacterial taxa at phylum level in the test soils revealed by 454-pyrosequencing. Soil codes (S1 to S14) are the same as shown in Table 1.

remaining sequences were assigned to operational taxonomic units (OTUs) clustering at 97% similarity level. The taxonomic classification of each phylotype was determined in accordance with Greengenes database (DeSantis et al., 2006) using RDP-classifier (Wang et al., 2007). The OTUs data were further used to calculate the richness and diversity indices of bacterial community (Table 2) and the relative abundances of phylogenetic groups in the soils (Fig. 1).

Bacterial populations were converted to $\log_{10}$ (CFU g^{-1}) before statistical analysis. The survival data were analyzed by the Weibull survival model (Eq. (1)) as followed (Mafart et al., 2002; Geeraerd et al., 2005):

$$\log_{10}(N_t) = \log_{10}(N_0) - \left(\frac{t}{\delta}\right)^p \qquad (1)$$

where N_t represents the number of surviving cells remaining at time t, N_0 is the initial size of the inoculums population; p is a shape parameter, and δ is a scale parameter that represents the time needed for first decimal reduction. Likewise, the time (t_d) when N_t reaches detection limit (100 CFU g^{-1}) can also be calculated from Eq. (1).

In addition, simple correlation analysis, stepwise multiple-linear regression analysis, and path analysis were carried out by using SPSS 18.0 for Windows (SPSS Inc., IL, USA) to better understand how soil properties affected *E. coli* O157:H7 survival time (t_d). Detrended correspondence analysis of the parameters (p and δ) and t_d values was performed by using R software vegan package v2.0-5 (Oksanen et al., 2012; R core team, 2012) to visualize the difference of *E. coli* O157:H7 survival in the test soils. One-way analysis of variance (ANOVA) and Duncan's multiple range test were also carried out to test the differences at 5% significant level of the p, δ and t_d values among soils by SPSS statistic software.

Path coefficients (PC) and determinative coefficients (DC) were also calculated to assess the direct and indirect effects of soil properties on the t_d values following the method by Wright (1934):

$$r_{iy} = PC_{yi} + \sum \left(PC_{yk} \times r_{ik} \right)$$

$$DC_{iy} = \left(PC_{yi} \right)^{2} + \sum \left(PC_{yi} \times PC_{yk} \times r_{ik} \right)$$

where r_{iy} is the simple correlation coefficient between an independent variable (i, a soil property) and a dependent variable (y, t_d), r_{ik} is the simple correlation coefficient between one soil property (i) and another (k), PC_{yi} and PC_{yk} denote the direct effects of one soil property (i) and another (k) on the t_d values as measured by the direct path coefficients, respectively. $\sum (PC_{yk} \times r_{ik})$ is the total indirect effect of the soil property (i) on the t_d values via all other soil properties (k). DC_{iy} and $(PC_{yi})^{\wedge 2}$ represent the total determinative coefficients, and the direct determinative coefficients between the soil property (i) and t_d values, respectively. $\sum (PC_{yi} \times PC_{yk} \times r_{ik})$ is the total indirect determinative coefficient of the soil property (i) on the t_d values via all other soil properties (k). Subscript designations (i, k = 1, 2, 3, 4; i ≠ k) are: 1, pH; 2, TN; 3, MBC; and 4, amorphous Al_2O_3.

3. Results

3.1. Survival dynamics of E. coli O157:H7 in soils

No *E. coli* O157:H7 was detected neither by the plating analysis during the entire incubation period nor by the enrichment technique and multiplex PCR assay at 63 DAT in all inoculated and uninoculated soil samples. As shown in Fig. 2, the inoculated *E. coli* O157:H7 colonies recovered from the soils generally decreased with time over the 36-days incubation period. However, *E. coli* O157:H7 survival dynamics varied among the 14 test soils. *E. coli* O157:H7 declined rapidly in the five soils from south-eastern China (S8–S10, S12, and S13): the colonies
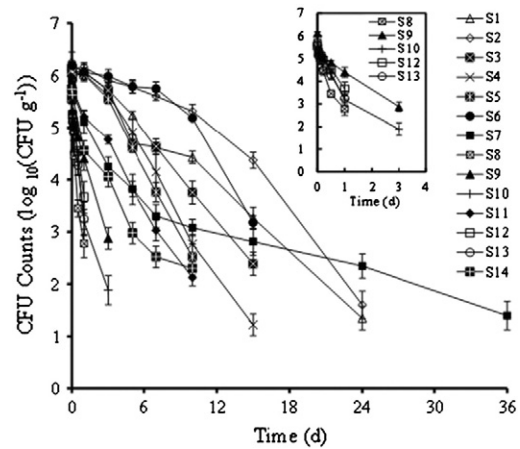

Fig. 2. Survival of *E. coli* O157:H7 in the test soils. Bars are ± the standard deviation of means. CFU g^{-1}, the colony forming unit per gram oven-dried weight of soil. Soil codes (S1 to S14) are the same as shown in Table 1.

survived about 1 to 3 days before reaching the detection limit (100 CFU g^{-1}) in these soils. In comparison, *E. coli* O157:H7 survived longer in other soils (S1–S7, S11, and S14) (Fig. 2). Results of enrichment technique showed that no colony was found on the SMAC–BCIG agar plate with the antibiotics (100 μg mL^{-1} of rifampicin and 25 μg mL^{-1} of nalidixic acid) for most of the inoculated soils at 63 DAT. The presumptive *E. coli* O157:H7 colonies were detected only in the inoculated soils S1, S3 and S7 at 63 DAT. However, multiplex PCR assay finally revealed that the inoculated *E. coli* O157:H7 colonies were still present in soils S1 and S7 at 63 DAT. As shown in Fig. 3, the *E. coli* O157:H7 colonies recovered from soils S1 and S7 (Lanes 3–6) had identical three bands with the *E. coli* O157:H7 wild type strains (Lane 2). The three bands represent the amplicons from *eae*, *stx2*, and *stx1* genes (from top to bottom), with estimated sizes of 384, 255, and 180 bp, respectively.

3.2. Modeling of E. coli O157:H7 survival data

The *E. coli* O157:H7 survival data from all the test soils fitted well to the Weibull model (Eq. (1)) with R^2 ranging from 0.986 to 0.998. The survival time needed to reach the detection limit (t_d) for *E. coli* O157:H7 in the tested soils was also calculated from the Weibull model (Eq. (1)). The average survival time (t_d) for *E. coli* O157:H7 in the test soils ranged from 4.4 to 25.8 days. The p and δ values were higher in the soils S1–S6 from north-eastern China than those in the soils S7–S14 from south-eastern China. Test of homogeneity of variances showed that no significant differences were present in the variances
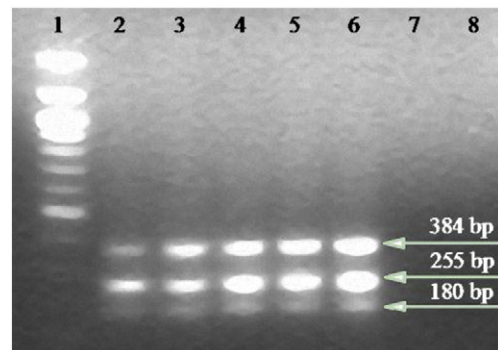

Fig. 3. Multiplex PCR confirmation of *E. coli* O157:H7 in the test soils. Lane 1, 100 bp λDNA ladder; Lane 2, *E. coli* O157:H7 wild type tagged with rifampicin and nalidixic acid resistance; Lanes 3 and 4, *E. coli* O157:H7 colonies recovered from S1; Lanes 5 and 6, *E. coli* O157:H7 colonies recovered from S7; Lanes 7 and 8, no *E. coli* O157:H7 colonies recovered from S3. Soil codes S1, S3, and S7 are the same as shown in Table 1.

of p (P = 0.254), δ (P = 0.134), and t_d (P = 0.231) among all soil samples. ANOVA further indicated that there were differences at 5% significant level in the p, δ and t_d values among the 14 test soils, according to Duncan's multiple range test (Fig. 4). Results of simple correlation analysis revealed that the δ (r = 0.708, P < 0.01) and t_d (r = 0.674, P < 0.01) values were positively correlated with the latitude, which further corroborated the observation that *E. coli* O157:H7 survived significantly longer in the soils from north-eastern China than those from south-eastern China. Moreover, big differences were observed in *E. coli* O157:H7 survival parameters (p and δ) and t_d values between soils from north-eastern China (NE) and from south-eastern China (SE) by detrended correspondence analysis (Fig. 5).

3.3. Relationship between soil properties and E. coli O157:H7 survival

Soil analyses showed that the 14 soil samples have different physico-chemical and biological properties (Tables 1, 2 and Fig. 2). Simple correlation analysis revealed that t_d was correlated with MBC (r = 0.648, P < 0.05), silt fraction (r = 0.544, P < 0.05), the relative abundance of *Acidobacteria* (r = 0.744, P < 0.01) and *Chloroflexi* (r = −0.848, P < 0.001). However, other selected soil properties in this study did not yield strong correlation with t_d. Further analyses of the relationship between t_d and soil properties were conducted using stepwise multiple regression analysis. It showed that soil TN, MBC, amorphous Al_2O_3 and the relative abundance of *Chloroflexi* (*CHL*) were the most important factors impacting *E. coli* O157:H7 survival in the test soils, with TN and MBC displaying positive effects (P < 0.01) but amorphous Al_2O_3 and *CHL* showing negative effects (P < 0.001) on the survival time (t_d) (Table 3).
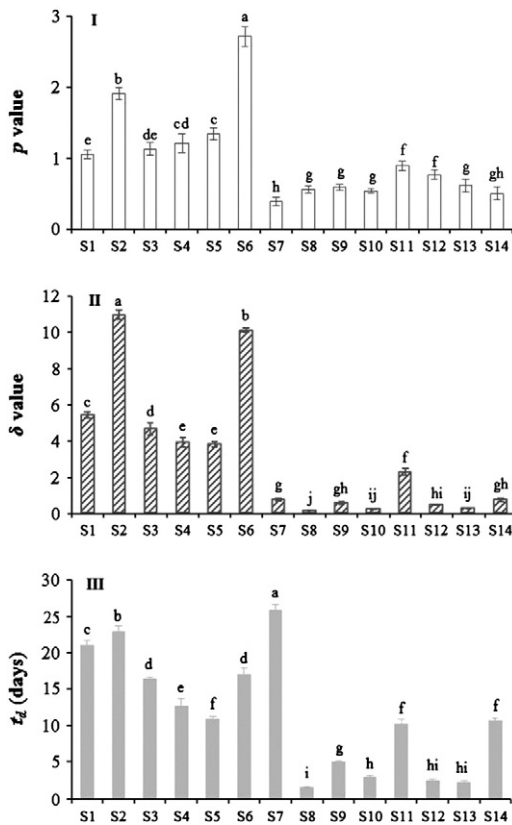

Fig. 4. The Weibull model parameters p (I), δ (II) and survival time (t_d) (III) for *E. coli* O157: H7 survival in the test soils. Bars are ± the standard deviation of means. Without the same lower-case letter marked on the columns indicates significant difference among the test soils at the 0.05 probability level (P < 0.05) by Duncan's multiple range test, and vice versa. Soil codes (S1 to S14) are the same as shown in Table 1.
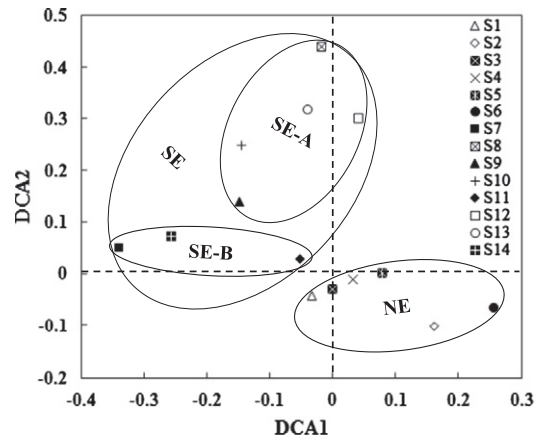

Fig. 5. Detrended correspondence analysis (DCA) of the Weibull model parameters (p and δ) and survival time (t_d) for *E. coli* O157:H7 survival in the test soils. NE, soils collected from north-eastern China; SE, soils collected from south-eastern China; SE-A and SE-B are two sub-groups in SE. Soil codes (S1 to S14) are the same as shown in Table 1.

Path analysis, a straightforward extension of the multiple regression analysis, was used to better understand the relative importance of the direct and indirect effects of each soil property on *E. coli* O157:H7 survival time (t_d). The path coefficients (PC) and determinative coefficients (DC) indicated that the direct effects of soil properties on *E. coli* O157:H7 survival times were higher than those of the indirect effects (Table 4). It was observed that the direct effects follow the order of *CHL* > amorphous Al_2O_3 > MBC > TN. The results also revealed that there are strong interaction indirect effects of soil properties on the overall survival of *E. coli* O157:H7 in the soils. For instances, TN and MBC would mutually promote the positive indirect effects on the t_d values with the DC of 0.067. The negative direct effects of amorphous Al_2O_3 (PC = −0.501) on the t_d values were counteracted by the positive indirect effects by TN (PC = 0.185) and MBC (PC = 0.154). However, the interaction indirect effects of amorphous Al_2O_3 with *CHL* on the t_d values were relatively unimportant (Table 4).

4. Discussion

As a general observation, the survival of *E. coli* O157:H7 showed a progressive decline in the 14 test soils with the incubation time. However, the dynamics of *E. coli* O157:H7 survival differed among the soils under the same temperature and moisture conditions. There existed a lag period at the beginning of inoculation (0–1 DAT) before *E. coli* O157:H7 declined with time in the soils S1–S6 from north-eastern China, while *E. coli* O157:H7 declined rapidly once the cells inoculated into the soils S7–S14 from south-eastern China (Fig. 2). But after 1 DAT, a slow decline of *E. coli* O157:H7 with time was observed in the soils S7, S11, and S14. The presence of a lag phrase in the soils S1–S6 from north-eastern China and a slow decline rate of the pathogen in the soils S7, S11, and S14 were attributed to the more available nutrients (e.g., OC, TN, WSOC) for pathogen survival in these soils, as pointed out by Crane and Moore (1986). Further, detrended correspondence analysis also revealed an obvious difference in *E. coli* O157:H7 survival between soils from north-eastern China (NE) and from south-eastern China (SE) (Fig. 5). The latter could be further divided into two sub-groups: SE-A (S8–S10, S12, and S13) and SE-B (S7, S11, and S14), based on the parameters (p and δ) and t_d values.

The differences in *E. coli* O157:H7 survival among the test soils during the incubation period were associated to their respective soil properties. The survival time (t_d) was longer in soils with a higher level of TN, MBC, and with a lower content of amorphous Al_2O_3 and *CHL*. MBC is commonly used to characterize the readily available C sources in soil system (Vance et al., 1987). Since the availability of nitrogen and carbon substrates can both provide the easily available energy

Table 3
Stepwise multiple-linear regression analysis of soil properties and the survival time (t_d) of E. coli O157:H7 in soils.

Regression equation[a]	R^2	F value	T value and the partial correlation coefficients (r)		
				T value	r
$t_d = 13.914 + 4.146 \times TN + 0.022 \times MBC - 4.674 \times Al_o - 26.065 \times CHL$	0.961	55.870***	TN	3.887**	0.792
			MBC	5.238***	0.868
			Al_o	−6.089***	−0.897
			CHL	−6.608***	−0.911

TN, total nitrogen; MBC, microbial biomass carbon; Al_o, amorphous Al_2O_3; CHL, the relative abundance of Chloroflexi.
[a] t_d, survival time of E. coli O157:H7 to reach the detection limit, 100 colony forming units per gram oven-dried weight of soil.
** Correlation is significant at the 0.01 probability level.
*** Correlation is significant at the 0.001 probability level.

sources for pathogen growth and decrease the competitive pressure between organisms, E. coli O157:H7 can survive longer in nutrient-rich soil environment (Franz et al., 2008; van Elsas et al., 2011; Ma et al., 2013). Path analysis further confirmed the positive direct and indirect effects of MBC and TN on E. coli O157:H7 survival time (t_d). For example, the cumulative positive indirect effects of amorphous Al_2O_3 via TN and MBC (DC = −0.170) were close to the negative direct effects of amorphous Al_2O_3 on the t_d values (Table 4). Namely, the results suggest that the positive effects from MBC and TN would counteract the negative effects of amorphous Al_2O_3 on the t_d values and prolong E. coli O157:H7 survival in nutrient-rich soil.

In addition, many studies pointed out that both the type and population of indigenous microorganisms have distinct impacts on pathogen survival in soils (Takahashi et al., 2008; Kim et al., 2011; van Elsas et al., 2011, 2012; Ma et al., 2013; Yao et al., 2014). Results of this study revealed that the t_d values were positively correlated with relative abundance of Acidobacteria, and negatively correlated with Chloroflexi in the test soils. Ma et al. (2013) also showed that the survival of E. coli O157:H7 was positively correlated with the abundances of Actinobacteria, Acidobacteria, α- and δ-Proteobacteria. However, most of the reports indicated that fungi (Takahashi et al., 2008; van Elsas et al., 2012), actinomycetes (Kim et al., 2011; van Elsas et al., 2012), Firmicutes (Westphal et al., 2011), Bacteroidetes, β- and γ-Proteobacteria (Westphal et al., 2011; Ma et al., 2013) can inhibit E. coli O157:H7 survival. Westphal et al. (2011) proposed that microbial suppression may be harnessed to develop new options for mitigating the risk and dispersal of zoonotic bacterial pathogens in the environment. Thus, further study to assess the impact of indigenous microorganisms on the survival of E. coli O157:H7 in soils would provide support information for reducing the negative environmental risk by the pathogen.

Although soil pH and silt content had no significant effect in the stepwise multiple regression equation, it is interesting to note that E. coli O157:H7 exhibited longer survival time (t_d) in the soils with high silt content and high pH (Table 1, Fig. 2). This is in agreement with previous studies reported that fine particles (silt or clay) can provide more available water, nutrients and protective pore spaces than the coarse particles (sand) can do for E. coli O157:H7 survival in soils (Ma et al., 2011; Wang et al., 2013). Moreover, the t_d values in this study were significantly shorter (P < 0.05) in the south-eastern China soils (S8–S14, low pH) than those in the north-eastern China soils (S1–S7, high pH) (Fig. 4). This might be related to the different soil mineral compositions in the test soils and their corresponding effects on the adsorption or inactivation of E. coli O157:H7.

Generally, the acidic soils from south-eastern China contain higher contents of Fe-/Al- (hydro) oxides and kaolinite, while the neutral soils from north-eastern China contain more montmorillonite (Institute of Science, Chinese Academy of Sciences, 1980). The Fe-/Al- (hydro) oxides and kaolinite are usually carried positive charges at pH of most natural soils, whereas, montmorillonite is negatively charged at the pH from 4.0 to 9.0 (Mills, 2003; Zhao et al., 2012). However, E. coli O157:H7 has an isoelectric point (pI) of about 4.2 (Kim et al., 2009), which is lower than the pH of the test soils, except for soil S11. Therefore, E. coli O157:H7 is negatively charged when the soil pH is higher than pI of E. coli O157:H7, and will be adsorbed by the positively charged soil particles. Moreover, previous studies reported that the adsorption of bacteria to soil particles would increase gradually with decreasing pH (Zhao et al., 2012; Bradford et al., 2013). Cai et al. (2013) found that the adsorption of E. coli O157:H7 on minerals in KCl solution (0.1–100 mM, pH 6) was in the order of goethite > kaolinite > montmorillonite, and the higher E. coli O157:H7 deposition on goethite resulted in a higher detrimental of its viability. Results of this study also showed that E. coli O157:H7 survival in soil was suppressed by amorphous Al_2O_3, which is attributed to the bacteria–mineral adsorption reactions (Yee et al., 2000; Jiang et al., 2009).

Therefore, compared to the soils from the north-eastern China, lower pH but higher contents of Fe-/Al- oxides in the south-eastern China soils (Table 1) might offer the explanation for the observed shorter E. coli O157:H7 survival times in the south-eastern China soils. In contrast, the low adsorption and long survival time of E. coli O157:H7 in the north-eastern China soils may pose a high environmental contamination risk. Until now, very few studies have examined the impact of soil texture and composition on the fate of E. coli O157:H7 in the soil. Further studies are needed to evaluate the complex interactions of soil particles (both texture and composition) and E. coli O157:H7.

The survival time (t_d) in the soils from north-eastern China (the warm-temperate zone) was substantially longer than that in the soils from south-eastern China (the subtropical zone), which agreed with the previous studies that reported the long-term persistence, growth and naturalization of E. coli populations in the soils of temperate and northern temperate regions (Brennan et al., 2010; Ishii et al., 2010). An interesting observation is that the outbreaks of E. coli O157:H7 were mostly occurred in Europe, North America, and higher latitude region in Asia (Money et al., 2010). Nevertheless, results of this study indicated that soil indigenous microorganisms, mineral type varieties, and nutrients had the profound influences on E. coli O157:H7 survival in the test soils under the same temperature and moisture conditions. As the above mentioned, the distribution of soil indigenous microorganisms

Table 4
Path analysis for the effects of soil properties on the survival time (t_d) of E. coli O157:H7 in soils.[a]

	Direct (italic values) and indirect effects			
	TN → t_d	MBC → t_d	Al_o → t_d	CHL → t_d
TN →	*0.343*	0.197	−0.270	0.213
	(0.118)[b]	(0.067)	(−0.093)	(0.073)
MBC →	0.158	*0.426*	−0.182	0.246
	(0.067)	(0.181)	(−0.077)	(0.105)
Al_o →	0.185	0.154	*−0.503*	0.021
	(−0.093)	(−0.077)	(0.253)	(−0.011)
CHL →	−0.138	−0.198	0.020	*−0.529*
	(0.073)	(0.105)	(−0.011)	(0.280)

TN, total nitrogen; MBC, microbial biomass carbon; Al_o, amorphous Al_2O_3; CHL, the relative abundance of Chloroflexi.
[a] t_d, survival time of E. coli O157:H7 to reach the detection limit, 100 colony forming units per gram oven-dried weight of soil.
[b] The data in the parentheses show determinative coefficients of soil properties to E. coli O157:H7 survival time, and the other values in the table represent path coefficients.

and mineral type varieties follows certain geographical patterns (Institute of Soil Science, Chinese Academy of Sciences, 1980; Wu et al., 2009). In this context, it is worth to further investigate whether these specific soil properties determine the geographical patterns for *E. coli* O157:H7 survival in the environment. In this study, the longest survival time of *E. coli* O157:H7 was found in soil S7 collected from Jiangsu province, where several *E. coli* O157:H7 outbreaks have been reported. Increasing evidences show that soil can be a significant source of pre-harvest contamination to fresh produce or water source by this pathogen (Solomon et al., 2002; Islam et al., 2004; Brennan et al., 2010; Patel et al., 2010; Yao et al., 2013). Hereby, special attention should be paid to the fate of *E. coli* O157:H7 in these long survival soils when evaluating the environmental risk associated with *E. coli* O157:H7.

5. Conclusions

Results from this study showed that *E. coli* O157:H7 survival varied in soils from eastern China. The *E. coli* O157:H7 could survive significantly longer in the north-eastern China soils than that in the south-eastern China soils under the experimental temperature (21 ± 1 °C) and moisture conditions (40% WHC). Soil microbial biomass carbon, *Chloroflexi*, TN and amorphous Al_2O_3 were the most important factors impacting *E. coli* O157:H7 survival time (t_d) in the test soils. It is difficult to control the pathogens' dissemination into open environment once they are introduced into the soil. Therefore, the potential environmental risk regarding of *E. coli* O157:H7 by soil should not be overlooked, especially in north-eastern China. Distinct *E. coli* O157:H7 survival dynamics in soils from north to south in eastern China suggest that it needs to develop proper manure management and field application under different geographical regions in order to reduce the potential risks of pathogen contamination. Moreover, the complex interactions among soil particles and indigenous microorganisms with *E. coli* O157:H7 need to be further evaluated under natural conditions, in order to thoroughly investigate if geographical patterns exist for *E. coli* O157:H7 survival in soils with climatic gradient.

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