# Survival of *Escherichia coli* O157:H7 in Soils from Jiangsu Province, China

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# Abstract

*Escherichia coli* O157:H7 (*E. coli* O157:H7) is recognized as a hazardous microorganism in the environment and for public health. The *E. coli* O157:H7 survival dynamics were investigated in 12 representative soils from Jiangsu Province, where the largest *E. coli* O157:H7 infection in China occurred. It was observed that *E. coli* O157:H7 declined rapidly in acidic soils (pH, 4.57 - 5.14) but slowly in neutral soils (pH, 6.51 - 7.39). The survival dynamics were well described by the Weibull model, with the calculated  $t_d$  value (survival time of the culturable *E. coli* O157:H7 needed to reach the detection limit of 100 CFU g<sup>-1</sup>) from 4.57 days in an acidic soil (pH, 4.57) to 34.34 days in a neutral soil (pH, 6.77). Stepwise multiple regression analysis indicated that soil pH and soil organic carbon favored *E. coli* O157:H7 survival, while a high initial ratio of Gram-negative bacteria phospholipid fatty acids (PLFAs) to Gram-positive bacteria PLFAs, and high content of exchangeable potassium inhibited *E. coli* O157:H7 survival profiles in soils with high pH were different from those with low pH.

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# Introduction

Applications of animal manure as fertilizers or soil amendments to agricultural soils are routine, world-wide. In the UK, the annual amount of animal manure applied to land was recently estimated at  $4.3 \times 10^5$  tons dry weight [1]. Though animal manure can provide nutrients, a variety of pathogenic bacteria may survive in the manure, which in turn may serve as a primary hazardous material for environmental contamination and as a public health threat [2]. For example, Escherichia coli O157:H7 (E. coli O157:H7), which can cause severe hemorrhagic colitis and haemolytic uraemia in humans, can persist in soil for days to more than 1 year following manure application to land [3]. It was reported that 20 people were infected with E. coli O157:H7 through manurecontaminated soil after camping on a field in Scotland that was previously grazed by sheep [4]. Increasing evidence shows that soil and animal manures are the main transport agents of E. coli O157:H7 to contaminate fresh vegetables, fruits and drinking water [1], [5], [6]. Therefore, it is important to understand the nature of E. coli O157:H7 survival and its infective risk in soil or soil-related (manure) environments.

In America, about 63,000 human cases of *E. coli* O157:H7 infections have been reported every year [7]. Many studies have focused on the survival of *E. coli* O157:H7 in soil, manure, water, and vegetables [1], [2], [4], [5], [8], [9]. However, very little attention has been paid to *E. coli* O157:H7 survival and its potential environmental contamination risk in the soils in the areas where outbreaks of *E. coli* O157:H7 infection have occurred. Previous studies found that the survival time of *E. coli* O157:H7 in soils depends on soil type, physicochemical properties, indigenous

microorganisms, etc. [2], [9-15]. Franz et al. (2008) pointed out that higher amounts of dissolved organic carbon and dissolved organic nitrogen were the best predictors for long E. coli O157:H7 survival time in organically managed soils [2]. In several experiments, E. coli O157:H7 persisted longer in silty clay soils than in sandy soils [2], [10], [11]. E. coli O157:H7 survived for up to 77, 226, and 231 days at 5, 15, and 25°C in manure-amended autoclaved soil, respectively [13]. E. coli O157:H7 survived significantly longer under anaerobic than under aerobic conditions in manure and slurry [14]. Yao et al. (2012) and van Elsas et al. (2012) proved E. coli O157:H7 survival was affected by indigenous microorganisms in soil [9], [15]. Different E. coli O157:H7 survival rates indicate the different potential risks of the pathogen contamination under various soil environments. Consequently, a better understanding of E. coli O157:H7 survival in soils will help in reducing the potential risk of pathogen contamination and avoiding human infection from the pathogen.

In the present study, experiments were carried out to investigate *E. coli* O157:H7 survival in 12 soils taken from Jiangsu Province, China. In 1987, researchers firstly detected *E. coli* O157:H7 from the fecal samples of patients in Jiangsu Province, where the largest *E. coli* O157:H7 outbreak in China occurred [16], [17]. Later, Xu et al.(1990) found that the biochemical reactions of five strains of *E. coli* O157:H7, which were isolated from 486 stool specimens of patients with diarrhea in Xuzhou City, Jiangsu Province, were almost identical with those of the well-known *E. coli* O157:H7 (strain EDL933) [18]. Recently, numerous researchers reported that *E. coli* O157:H7 (strain EDL933) had been detected in excrement, sewage, foods, and soils from many provinces of

China, including Jiangsu Province [16], [19–21]. Thus, *E. coli* O157:H7 (strain EDL933) was selected as a representative strain in this study.

Most *E. coli* O157:H7 outbreaks occur in summer [17-19]. The summer mean temperature in Jiangsu Province is about 26°C [22]. Furthermore, the water content under -33 kPa, indicating the water holding capacity of the soil and representing the highest available water contents in soil, was generally used to simulate field conditions [23]. Hence, our simulation experiments also used incubation conditions of  $25\pm1^{\circ}$ C and water content under -33 kPa. The aims of this study were to (1) investigate *E. coli* O157:H7 survival dynamics in soils, (2) identify the relationships between *E. coli* O157:H7 survival time and soil physicochemical properties and microbial community structure, and (3) understand the possible risks of pathogen contamination to prevent further disease outbreaks from *E. coli* O157:H7.

#### **Materials and Methods**

#### **Ethics Statement**

The samples were not collected from national parks, protected areas or private land. Hence, no specific permission was required to obtain these samples. The sampling did not cause any disturbance to the environment or to protected species at the sampling sites.

#### Soils

The 12 soils (S1–S12) used in this study were taken from Jiangsu Province, China (32.05°N - 34.70°N). Three replicates were sampled at each soil site from the surface horizon (0-20 cm) and each sample was a composite of several individual soil cores taken at 5-m interval. After sampling, the 36 individually bulked fresh soil samples were immediately taken to the laboratory in coolers containing ice packs. The samples were then hand-picked to remove discrete plant residues, sieved <2 mm, homogenized thoroughly, and then stored at 4 °C. According to the protocols of the Agricultural Chemistry Committee of China [24], a subsample of the sieved soil from each sample was air-dried for physical and chemical analyses, including pH, total organic carbon (OC), exchangeable potassium (K), humic acid, fulvic acid, sand, clay and silt. Total nitrogen (TN) was determined following digestion using a Kieldahl apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland). Total dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured by a Multi N/C TOC analyzer (Analytic Jena AG, Jena, Germany). Exchangeable potassium (K) was extracted with 1 M ammonium acetate and measured by Flame Atomic Absorption Spectrophotometry (Analytik Jena AG, Jena, Germany). The soil water content at -33 kPa was determined using a pressure membrane apparatus (Soil Moisture Equipment Corp, CA,USA) as described by Richards [23].

Another portion of each sieved fresh sample was frozen at  $-80^{\circ}$ C and then freeze-dried (FreeZone Freeze Dry Systems LABCONCO Corp, MO, USA) for phospholipid fatty acids (PLFAs) analysis [25]. The PLFAs are the specific components of cell membranes that are only found in intact (viable) cells [26]. Many studies have widely used PLFAs to express the biomass and composition of microbial communities in soils [9], [27], [28]. Thus, PLFAs were used to determine the effects of soil indigenous microorganisms on *E. coli* O157:H7 survival in this study. According to Ding et al. (2009) [29] and Ying et al. (2013) [30], the PLFA biomarkers i15:0, a15:0, 15:0, i16:0, 16:1 $\omega$ 7c, i17:0, a17:0, cy17:0, 17:0, 18:1 $\omega$ 7c, cy19:0 $\omega$ 8c were used to represent bacterial biomass; i15:0, a15:0, i16:0, i17:0, and a17:0 were used

to indicate Gram-positive (G<sup>+</sup>) bacteria biomass; 16:1 $\omega$ 7c, 18:1 $\omega$ 5c, cy17:0, and cy19:0 indicated Gram-negative (G<sup>-</sup>) bacterial biomass. Polyenoic, unsaturated PLFA 18:2 $\omega$ 6,9c was used to represent fungal biomass. The fatty acids 10Me 16:0, 10Me 17:0, 10Me 18:0 were used to indicate actinomycetic biomass. Based on the studies of the Institute of Soil Science, Academia Sinica [31], the 12 soils used were classified into acidic soils (pH < 6.5) and neutral soils (pH > 6.5). Selected soil properties are shown in Table 1.

#### Incubation experiments

The preparation of *E*. coli O157:H7 (strain EDL933) and inoculums were described previously by Wang et al. (2013) [32]. *E. coli* O157:H7 cells in sterile deionized water were inoculated into soils to achieve a cell density of about  $10^7$  colony-forming units per gram soil oven-dry weight (CFU g<sup>-1</sup>). The incubation experiments were conducted on each of the three soil replicates taken from each of the twelve sampling sites (a total of 36 soil samples). All the inoculated and uninoculated control soil samples were incubated in the dark at  $25\pm1^{\circ}$ C and kept at soil moisture of -33 kPa.

The inoculated soils were sampled at 0, 0.25, 1, 2, 3, 5, 7, 10, 15, 20, 25, 30 and 35 days after inoculation (DAI), and *E. coli* O157:H7 was extracted by 0.1% peptone buffer (Lab M, Lancashire, UK). The resulting soil suspension was then subjected to 10-fold serial dilutions and *E. coli* O157:H7 enumerated at the last three of the serial dilutions. The detection limit of the plating technique is about 100 CFU g<sup>-1</sup>. The sampling was stopped after plate counts of zero appeared twice in succession during the incubation.

#### Statistical analysis

The survival data was converted to  $\log_{10}$  (CFU g<sup>-1</sup>) and then analyzed by the Weibull survival model (Equation 1), as described by Wang et al. (2013) [32] and Ma et al. (2013) [33].

$$\log 10(Nt) = \log 10(N0) - \left(\frac{t}{\delta}\right)^p \tag{1}$$

 $N_t$  represents the number of surviving cells remaining at time t,  $N_0$  is the initial cells of the inoculum population; p is the shape parameter;  $\delta$  is the scale parameter that represents the time needed for the first decimal reduction. The time when  $N_t$  reaches the detection limit (100 CFU g<sup>-1</sup>) of the culturable *E. coli* O157:H7,  $t_{d}$  can also be calculated from Equation (1).

In addition, principal component analysis (PCA) of the parameters (p and  $\delta$ ) and  $t_d$  values were performed using the R software vegan package v 2.0–5 [34] to visualize survival patterns of *E. coli* O157:H7 in the test soils. Stepwise multiple-linear regression analysis was carried out by using SPSS 13.0 for Windows (SPSS Inc., IL, USA) to better understand how soil properties affected the survival of *E. coli* O157: H7 in soils. Analysis of variance (ANOVA) was also carried out to test the differences at the 5% significant level in the survival parameters (p and  $\delta$ ) and  $t_d$  values among soils by SPSS statistic software.

#### Results

#### Survival of E. coli O157:H7 in soils

The *E. coli* O157:H7 population declined by 1.14  $\log_{10}$  (CFU g<sup>-1</sup>) within the first day after inoculation in all the test soils. After 1 day, two different survival dynamics of *E. coli* O157:H7 were observed (Fig. 1). *E. coli* O157:H7 declined rapidly in the acidic

Soil Code	Location	Ħ	#00	Ĭ	N/D	¥	DOC	TDN	FA	НА	HA/FA	PLFA-T	PLFA-F	PLFA-A	PLFA-B	PLFA-G <sup>-</sup>	PLFA-G⁺	9/_	Clay	Silt	Sand
			g kg <sup>-1</sup>			$mg.kg^{-1}$			%			n mol g <sup>1</sup>							%		
S1	Lianyungang	4.57	20.3	1.51	13.44	89.10	51.97	7.46	6.40	43.25	6.75	35.83	0.58	4.24	16.63	7.29	7.67	0.95	17.13	16.72	66.15
S2	Xuzhou	7.27	25.4	2.44	10.41	194.86	28.55	14.97	6.26	22.28	3.56	67.19	1.68	8.52	22.95	10.47	10.77	0.97	31.73	35.19	33.08
S3	Xuyi	6.77	34.4	3.14	10.96	207.22	88.06	6.70	4.68	17.41	3.72	125.46	1.61	14.61	42.25	13.12	27.47	0.47	40.52	32.74	26.74
S4	Yangzhou	4.90	11.6	1.24	9.35	53.72	29.41	2.94	7.67	51.72	6.74	38.45	1.09	4.31	15.71	7.70	7.75	0.99	23.51	48.91	27.58
S5	Nanjing	6.61	8.1	1.01	8.02	123.16	19.83	5.74	23.09	16.91	0.73	21.39	0.21	2.75	9.63	2.50	5.69	0.43	41.12	39.69	19.19
S6	Nanjing	7.39	15.2	1.63	9.32	106.51	30.64	10.05	21.05	3.36	0.16	85.11	1.85	10.31	37.54	19.96	16.71	1.19	35.69	34.66	29.65
S7	Yangzhou	5.14	16.1	1.71	9.42	144.06	61.28	5.66	8.88	38.57	4.34	43.93	1.17	5.43	19.68	8.83	10.62	0.83	27.48	44.04	28.48
S8	Jurong	5.03	15.4	1.42	10.85	98.37	42.58	3.41	11.88	37.14	3.12	46.21	1.31	5.37	18.75	9.50	8.94	1.06	33.77	40.24	25.99
S9	Nanjing	4.66	51.5	3.40	15.15	165.56	134.27	29.42	18.46	16.42	0.89	106.54	1.64	10.91	44.16	22.62	20.81	1.09	18.01	39.13	42.86
S10	Nanjing	6.51	13.7	1.30	10.54	67.67	26.85	3.70	5.62	14.38	2.56	27.91	0.42	3.45	11.87	4.71	6.14	0.76	20.57	47.80	31.63
S11	Jiangyin	4.68	19.4	1.43	13.56	108.82	33.67	5.49	7.16	43.40	6.05	26.6	0.91	3.47	11.67	5.50	6.01	0.92	33.06	39.91	27.03
S12	Jurong	6.78	61.4	5.00	12.28	325.53	115.06	14.27	4.30	20.99	4.89	121.5	3.40	23.21	44.41	16.48	25.9	0.63	24.61	40.51	34.88
# Total : ratio of h bacteria doi:10.13	soil organic carbu numic acid to org phospholipid fat 71/journal.pone.	on (OC); Janic car tty acids .0081178	total soil bon (HA); (PLFA-G 3.t001	l nitrogen ; total phc ]; Gram-p	ו (TN); the ospholipic oositive b	ratio of OC I fatty acids acteria pho	to TN (C, (PLFA-T); spholipid	/N); exch fungus f fatty aci	angeable shospholij ds (PLFA⊣	potassiur pid fatty a G ]; the	m (K); disso acids (PLFA ratio of PLI	lved organi -F); actinom -A-G <sup>-</sup> to PI	c carbon (E ıyces phos	00C); total d pholipid fatt _/G <sup>†</sup> );	lissolved nit y acids (PLF	rogen (TDN) A-A); bacteri	; the ratio of ia phospholi	<sup>:</sup> fulvic aci oid fatty a	d to orga cids (PLF/	nic carboı A-B); Gram	ו (FA); the -negative

Table 1. Physical, chemical, and biological properties of the soils used in this study.



Figure 1. Survival of *E. coli* O157:H7 in the tested soils. Solid line: neutral soils; dashed line: acidic soils. doi:10.1371/journal.pone.0081178.g001

soils (S1, 4, 7, 8, 9, 11) and was not detectable after 7 days. In the neutral soils (S2, 3, 5, 6, 10, 12), *E. coli* O157:H7 declined much more slowly during 1 - 3 days, and then entered a relatively rapid decline period, reaching the detection limit after 30 days.

#### Modeling of survival data

The statistical measures and parameter values of the fitted Weibull model describing the survival of *E. coli* O157:H7 in the test soils are presented in Table 2. The *E. coli* O157:H7 survival dynamics in all soils were accurately described by the model, with  $R^2$  ranging from 0.84 to 0.99 (P < 0.001), while the first decimal reduction time ( $\delta$ ) and the shape parameter (p) varied in the test soils (Table 2). Survival times ( $t_d$ ) of *E. coli* O157:H7 in the 12 soils were calculated from the Weibull model (Equation 1). The survival times ( $t_d$ ) varied considerably among the soils, with  $t_d$  of  $6.50\pm1.34$  days in acidic soils with pH of 4.57 to 5.14 and significantly longer (32.79±1.16 days, P < 0.05) in neutral soils (pH, 6.57–7.39). A long survival time of 32.07 days was detected in soil from Xuzhou (S2), where the biggest infection ever reported in China.

# The relationship between soil properties and the survival of *E. coli* O157:H7

For all soils, stepwise regression analysis showed that soil pH, the ratio of G<sup>-</sup> bacteria PLFAs to G<sup>+</sup> bacteria PLFAs (G<sup>-</sup>/G<sup>+</sup>); exchangeable K and OC were the key factors affecting the survival of *E. coli* O157:H7. Soil pH (P < 0.001) and OC (P < 0.05) had positive effects on *E. coli* O157:H7 survival time ( $t_d$ ). In contrast, a high G<sup>-</sup>/G<sup>+</sup> ratio, and high exchangeable potassium concentration decreased the survival time. The results also suggested that soil pH and G<sup>-</sup>/G<sup>+</sup> were the most important factors determining the survival of *E. coli* O157:H7 in the test soils (Table 3).

## Discussion

The results indicated that *E. coli* O157:H7 survival times  $(t_d)$  mainly depended on the initial soil pH. Principal component analysis (PCA) clearly showed a substantial difference in *E. coli* O157:H7 survival times between acidic and neutral soils (Fig. 2).

**Table 2.** Statistical measures and fitted parameter values of the Weibull model describing the survival of *E. coli* O157:H7 in soils.

Soil Code.	<i>t<sub>d</sub></i> #	б	p	R <sup>2</sup>
S1	4.57±0.23e*	1.01±0.04ef	0.97±0.06ab	0.89±0.01
S2	32.07±0.43c	$3.25\!\pm\!0.58cd$	0.72±0.05def	0.97±0.01
S3	34.34±0.86a	5.76±0.66b	0.90±0.06ebc	0.97±0.01
S4	8.09±0.35d	0.97±0.09ef	0.80±0.04cd	0.99±0.01
S5	$34.03 \pm 1.60 ab$	6.78±1.00a	1.01±0.11ab	0.97±0.01
S6	31.55±0.89c	3.50±0.38c	0.77±0.04cde	0.99±0.01
S7	7.01±0.23d	1.36±0.37ef	1.00±0.17ab	$0.91 \pm 0.01$
S8	7.15±0.22d	1.57±0.14e	1.08±0.06a	0.96±0.01
S9	7.00±0.22d	$0.55 \pm 0.05 f$	0.64±0.01f	$0.91 \pm 0.01$
S10	31.96±1.02c	2.56±0.08d	0.67±0.02ef	0.96±0.02
S11	5.16±0.12e	1.03±0.07ef	0.88±0.04bc	0.84±0.01
S12	32.77±1.16bc	5.22±0.43b	0.90±0.04bc	0.98±0.01

# Survival time to reach detection limit ( $t_d$ ); time needed for first decimal reduction in *E. coli* O157:H7 population ( $\delta$ ); shape parameter (p). \*Significant differences (p < 0.05) indicated by different letters. doi:10.1371/journal.pone.0081178.t002

In the acidic soils, *E. coli* O157:H7 could not be detected by 7 days after inoculation (Fig.1). However, in the neutral soils, *E. coli* O157:H7 declined much slowly than in the acidic soils and survived for up to 38 days. There have been previous reports which linked *E. coli* O157:H7 survival times ( $t_d$ ) with soil pH [9], [12], [32], [35], However, our data showed an exceptionally highly statistically significant correlation between these two parameters. It therefore appears that the different physical, chemical and biological properties of acidic and neutral soils resulted in the remarkable differences in *E. coli* O157:H7 survival. Stepwise multiple regression analysis indicated that *E. coli* O157:H7 survival time was significantly and positively correlated with soil pH and OC (Table 3). In addition, the initial G<sup>-</sup>/G<sup>+</sup> and exchangeable K were negatively correlated with *E. coli* O157:H7 survival time ( $t_d$ ).

There may be several reasons why soil pH had the most significant positive correlation with E. coli O157:H7 survival time  $(t_d)$ . Firstly, most soil microbial species are generally adapted to neutral or slightly alkaline environments and the quantity of soil bacteria increases from pH 4 to 7, and they can readily adapt to changing pH within this range [36], [37]. Secondly, pH can affect the adsorption of bacteria to soil minerals. The adsorption decreased gradually with increasing pH [38], [39]. For example, Zhao et al. (2013) found that more E. coli was absorbed by soil colloids when solution pH decreased from 9 to 4 [38]. Also, Cai et al. (2013) showed that large decreases in the viability of E. coli O157:H7 can be caused by the sorption of E. coli O157:H7 to soil minerals [40]. Therefore, a large amount of E. coli O157:H7 adsorbed to soil minerals would result in a high loss of viability of E. coli O157:H7 in low pH soils. Also, the low biological availability of phosphorus and organic nitrogen and the high toxicity of Al and Mn in the soil with low pH [1], [41-43], might indirectly affect E. coli O157:H7 survival and activity in the acidic soils

Because of competition for nutrients and niche space, as well as predation, the survival of introduced pathogens can be affected by the diverse coexisting populations of the indigenous microorganisms [9], [15]. The stepwise multiple regression analysis showed **Table 3.** Stepwise multiple-linear regression analysis of soil properties and the survival time  $(t_d)$  of *E. coli* O157:H7 in the test soils.

			T value of the pa	rtial regression coefficient
Regression equations #	R <sup>2</sup>	F value		T value
			Constant	-13.208
t <sub>d</sub> = −34.188+12.360pH −17.856(G <sup>-</sup> /G <sup>+</sup> ) −0.076K+0.301OC	0.998	451.782***	рН	32.277***
			G-/G+	-10.970**
			К	-6.450****
			OC	6.494****

# Survival time to reach detection limit ( $t_d$ ), the ratio of Gram-negative bacteria phospholipid fatty acids (PLFAs) to Gram-positive bacteria PLFAs (G<sup>-</sup>/G<sup>+</sup>); exchangeable potassium (K); total soil organic carbon (OC); correlation is significant at the 0.001 probability level(\*\*\*).

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that the initial  $G^{-}/G^{+}$  ratio was negatively correlated with *E. coli* O157:H7 survival time  $(t_d)$  (Table 3). van Elsas et al. (2012) and Ma et al. (2013) also indicated that survival of the invading E. coli O157:H7 was negatively correlated with soil microbial diversity, due to the competition between native bacterial communities and the introduced species for nutrients and niche space [15], [44]. The G<sup>-</sup> bacteria are known to out-compete G<sup>+</sup> bacteria for nutrients in soil [45]. Furthermore, the production of bacteriocins to kill closely related species drives the negative interspecies interactions in bacterial systems, which plays an important role in determining the fate of invading bacteria [46]. E. coli O157:H7 belongs to G<sup>-</sup> bacteria, therefore we surmised that G<sup>-</sup> bacterial species in soil would have a stronger antagonism to E. coli O157:H7 than G<sup>+</sup> bacteria. Another study confirmed that Bacteroidetes, Gammaproteobacteria and Firmicutes, mainly G<sup>-</sup> bacteria, could inhibit E. coli O157:H7 survival due to antagonism [47]. Therefore, the greater direct or indirect antagonism of G bacteria than G<sup>+</sup> bacteria to E. coli O157:H7 might account for the statistically significant negative correlation between E. coli O157:H7 survival time  $(t_d)$  and the initial  $G^-/G^+$  ratio (Table 3).



Figure 2. Principal component analysis (PCA) of the survival parameters ( $\delta_r p$  and  $t_d$ ).  $\delta_r p$  and  $t_d$  are the same as shown in Table 2. A-S: acidic soils; N-S: neutral and slight alkaline soils. doi:10.1371/journal.pone.0081178.g002

E. coli O157:H7 survival times in the test soils were decreased by increasing exchangeable K. A recent study revealed that  $t_d$  values decreased significantly (P < 0.05) with increasing electrical conductivity and concentrations of individual soil cations, e.g.  $K^{+},\,Na^{+},\,\acute{Ca}^{2+},\,and\,Mg^{2+}.$  This could interfere with ion transport, enzyme activity, and crucial protein synthesis in E. coli O157:H7, and finally result in reduced E. coli O157:H7 survival in soils [33]. In addition, our results showed that soil OC was significantly correlated with E. coli O157:H7 survival time  $(t_d)$ . Soil organic matter is a major energy source for microorganisms, and can provide carbon sources for the growth and survival of E. coli in soil and water [48]. Furthermore, abundant organic carbon can decrease the competitive pressure between organisms through providing easily available energy sources in soil, and thus possibly enhance the persistence of E. coli O157:H7 [15]. Soil organic carbon also helps to improve soil structure by forming multi-pored aggregates which serves as microbial habitats [49]. Therefore, the abundance of organic carbon in soil can provide more nutrients, water, air and biological niches for E. coli O157:H7 and decrease the competition with indigenous microorganisms, slowing the decline of E. coli O157:H7 [3], [15].

#### Conclusions

This research has enhanced our understanding of the survival of E. coli O157:H7 in soils. E. coli O157:H7 could survive for  $32.79\pm1.16$  days in neutral soils, and only  $6.5\pm1.34$  days in acidic soils. Special attention should be paid to the different survival times of E. coli O157:H7 in acidic and neutral soils when evaluating the environmental risk associated with it. The survival of E. coli O157:H7 in soils might relate to the interactions between numerous physical, chemical and biological factors. Our findings suggested that high soil pH and organic carbon could prolong E. coli O157:H7 survival times ( $t_d$ ), while the initial G<sup>-</sup>/G<sup>+</sup> ratio and exchangeable K were negatively correlated with survival times. Soil pH governs the fate of E. coli O157:H7 directly and indirectly through the adsorption/desorption of soil minerals, nutrition availability, and metal toxicity. However, the experiment was carried out under laboratory conditions. In order to better understand the survival dynamics of E. coli O157:H7 and provide precise information to assess the possible risk of contamination by this pathogen, further research should be done under natural conditions.

## **Author Contributions**

Conceived and designed the experiments: JX HW. Performed the experiments: TZ. Analyzed the data: TZ HW JL. Contributed reagents/  $\,$ 

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