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Persistence of *Salmonella* Typhimurium in apple-pear (*Pyrus bretschneideri Rehd.*) orchard soils influenced by bacterial communities and soil properties

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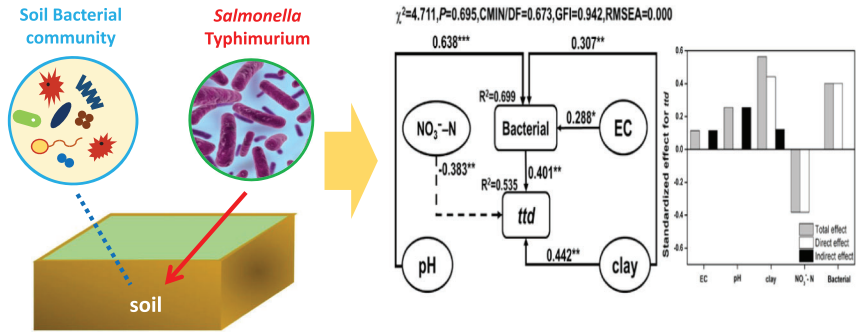
HIGHLIGHTS

- Persistence of *Salmonella* Typhimurium was investigated in apple-pear growing soils.
- Clay and bacterial communities directly related the survival time (*ttd*) of *S.* Typhimurium.
- Salinity and pH indirectly correlated with *ttds* of *S.* Typhimurium via bacterial communities.
- Relative abundances of *Actinobacteria*, *Acidobacteria* and *Deltaproteobacteria* correlated with *ttds*.

GRAPHICAL ABSTRACT



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ABSTRACT

2In this study, we investigated the persistence of *Salmonella* Typhimurium in 26 soil samples from apple-pear orchards in Yanji, Longjing and Helong in northeastern China. The time to reach detection limit (*ttds*) of *Salmonella* Typhimurium in soils varied from 20 to 120 days. Redundancy analysis and variation partition analysis elucidated that bacterial communities, clay content, pH, electrical conductivity (EC) salinity, and NO_3^-–N could explain more than 85% of overall variation of the persistence behaviors. Results of structural equation models and Mantel tests revealed that clay content and EC displayed both direct and indirect effect on *ttds*, while NO_3^-–N and pH exhibited direct and indirect effect on the survival patterns, respectively. Furthermore, *Actinobacteria*, *Acidobacteria* and *Deltaproteobacteria* at class level showed highly close correlations with *ttds*. Our results revealed that certain biotic and abiotic factors could greatly contribute to the overall persistence of *Salmonella* in apple-pear orchard soils.

1. Introduction

Zoonotic pathogens have been shown continuously in the past decades as major agents that impact human health (Riley et al., 1983). *Salmonella*, as a zoonotic foodborne bacterial pathogen, can lead to severe health issues, and the infections caused by *Salmonella* have been reported worldwide. The typical symptoms of the infection including fever,

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vomiting and diarrhea, which could be enhanced among individuals with low immunity, i.e. infants, children and the elderly (Magistrali et al., 2008). In the United States, approximately 1.2 million people got infected by *Salmonella* and there were 450 deaths annually (Scallan et al., 2011). It was reported that *Salmonella* has been isolated from over 600,000 people during 1996–2011 (Boore et al., 2015). In China, *Salmonella* contributed to about 40% of overall bacterial infections per year (Yang et al., 2010). In Sichuan Province, China, an outbreak of *Salmonella* Typhimurium infection caused by the consumption of contaminated cowpea salad, has resulted in 401 hospitalization (Liu et al., 2016).

Salmonella Typhimurium could survive on carrots and radishes for more than 100 days, highlighting its high risk to their potential consumers (Islam et al., 2004). *Salmonella* could transfer to fresh produce via polluted irrigation water or manure applied to the soils, and runoff from polluted areas such as piles of animal wastes and pasture of livestock (Tyler and Triplett, 2008). Furthermore, longer persistence of *Salmonella* in soils might increase the possibility that this pathogen contaminate fruit or vegetables. In such circumstances, it is more likely for people to get infected by consuming contaminated fresh produce. Thus, it is essential to investigate the persistence of *Salmonella* in agricultural soils to better understand the environmental behavior of the pathogen, aiming to decrease the health risks associated with this pathogen.

Soil properties, including biotic and abiotic factors, have a great impact on persistence of the invading pathogens. Biotic factors, such as bacterial, fungal, viral, and protists' communities, are regarded as important elements towards the stability of environmental ecosystems. It is widely known that ecosystems with a high level of biodiversity show high resilience to disturbance (van Elsas et al., 2007). Specifically, persistence of invading microbes may be higher in an ecosystem with low microbial complexity (Tilman, 1997). Recent studies have shown that specific bacterial groups such as *Actinobacteria*, *Acidobacteria*, and *Proteobacteria* had strong correlations with persistence of pathogens (Ma et al., 2013). Therefore, understanding of indigenous bacterial communities is of great value for evaluating the persistence of *Salmonella*. In addition, abiotic factors, including salinity, pH, temperature, nutrients, and trace- and macro- elements are also important environmental parameters in affecting of the persistence of *Salmonella*. It was reported that total organic carbon, pH, NH_4^+–N, and total phosphate showed a positive correlation with persistence of *Salmonella* in water samples (Li et al., 2018). Moreover, persistence of pathogens in soils may be related closely with the total nitrogen and electrical conductivity (Ma et al., 2012). Thus, identifying the crucial abiotic factors could provide more information for persistence of *Salmonella*.

In this study, 26 soil samples were collected from three areas, Yanji, Longjing, and Helong in apple-pear orchards located in the northeast region of China. With the increase demand for organic fruits, fertilizer originated from poultry and livestock started to be amended into the soils, which increases the possibilities for orchard soils to get contaminated by poorly composted animal manure. Under such conditions, pathogens could be transferred from contaminated irrigation water or manure to orchard soils and finally get into the food chain, and finally constitutes a threat to human health. Therefore, it is important to identify and evaluate environmental factors affecting persistence of *Salmonella* in these areas. The objectives of the current study were to 1) investigate the persistence of *Salmonella* Typhimurium in apple-pear orchards soils, and 2) identify the environmental factors that closely linked to the survival parameters of *Salmonella* Typhimurium in apple-pear orchards soils.

2. Materials and methods

2.1. Soil sampling and characterization

Twenty-six soils were collected from three apple-pear orchards located in Northeastern China: eight from Yanji (YJ), nine from Longjing (LJ), nine from Helong (HL). Longitude and latitude coordinates of soil samples are shown in Table S1. The three orchards contribute more than 60% of the apple-pear output of the region. The same apple-pear trees were planted in those orchards that were subjected to the similar agricultural management strategies (major organic fertilizer supplemented with minor chemical fertilizer). In all apple-pear orchards, the distances between plants and rows were 4 m and 5 m, respectively, i.e. 500 plants/acre. Each soil sample (top 0–15 cm) was collected between two neighboring rows in triplicate at 5 m intervals, mixed, and transported to the laboratory on ice. Roots, stones and vegetation were removed and the soil samples were sieved (≤ 2 mm). The sampling sites were about 3 m away from the stem of the plants to avoid the strong effect of the plant roots. The sampling sites were evenly distributed across each orchard according to its shape and scope to ensure their representativeness. A portion of soil samples were air-dried, and about half stored at 4 °C to determine physiochemical properties and the other small portion of soils samples were stored at -80 °C for DNA extraction.

Soil water extracts were made with water to soil ratio of 1:2.5 for EC determination using a conductivity meter (DDS-11A, Shanghai, China) and pH were measured by a glass electrode (PHS-3C, Shanghai, China). Clay, sand and silt contents were measured by a laser particle size analyzer (Bettersize 2000, Dandong, China). Water soluble organic carbon (WSOC) in soil water extracts were qualified by a total organic carbon analyzer (TOC-V, Shimazu, Japan). Total dissolved nitrogen (TDN) was measured by potassium persulfate oxidation spectrophotometry. NO_3^-–N was determined by double wavelength ultraviolet spectroscopy method. NH_4^+–N was measured by Nessler's reagent spectrophotometry method. Total dissolved phosphorus (TDP) was quantified by ammonium phosphomolybdate colorimetry. The results of soil sample characterization are shown in Table S1.

2.2. Bacterial strains

The inoculating strain was *Salmonella enterica* subsp. *enteric* serovar Typhimurium (ATCC 14028) was obtained from American Type Culture Collection (ATCC). Wild type *Salmonella* Typhimurium (*S.* Typhimurium) was tagged with rifampicin (Fisher Scientific, Fair Lawn, NJ, USA) and nalidixic acid (Sigma-Aldrich, Saint Louis, MO, USA) in order to facilitate the numeration on the selective media (Semenov et al., 2009). The growth and survival curves of the tagged *S.* Typhimurium ATCC 14028 strain were identical to those of the non-tagged wild type (Li et al., 2018).

2.3. Survival experiment of S. Typhimurium

Cells of *S.* Typhimurium were streaked on LB (Luria-Bertani) agar (Lab M, Lancashire, UK) and incubated under 37 °C overnight. Single colonies were restreaked on selective LB agar media with 100 mg/L rifampicin and 20 mg/L nalidixic acid and incubated at 37 °C overnight. A single colony was selected and inoculated in 100 mL LB broth under 37 °C for 14–16 h. The cells were washed two times by 0.9% NaCl (Beijing Chemical Factory, China) to remove residues of nutrients from LB medium, and resuspended in sterile deionized water (Li et al., 2018). Stationary phase cells were used and inoculated into soil samples with a final cell density of 10^6 colony forming units (CFU) per gram of dry soil. Triplicate soil samples were individually spiked and stored in sterile plastic bags in dark at room temperature (21 ± 1 °C). The moisture content of soil samples was adjusted to a constant 60% of water holding capacity by adding deionized water during the experiment. Two samples (approximately 1.0 dry weight equivalent) from each triplicate bag were put into sterile test tubes and the cells were extracted with 4 mL 0.1% buffered peptone water (Lab M, Lancashire, UK). The resultant soil suspension was subjected to 10-fold serial dilutions and plated on selective LB agar containing rifampicin (100 mg/L) and nalidixic acid (20 mg/L). The detailed procedures regarding cell extraction and enumeration can be found in our most recent publication (Han et al., 2020; Huang et al., 2020).

2.4. Survival data modeling

Survival of *S.* Typhimurium was analyzed by fitting the experimental data (colony forming unit counts) to the Weibull survival model using GInaFiT version 1.5 (Geeraerd et al., 2005; Mafart et al., 2002). The model was applied based on the hypothesis that survival of *S.* Typhimurium follows a Weibull distribution. The size of the surviving population can be calculated using the following equation:

$$\log(N_t) = \log(N_0) - (t/\delta)^p$$

where N is number of survivors, N_0 is inoculum size, t is time (days) post inoculation, δ is scale parameter representing the time needed for the first decimal reduction, and p is non-unit shape parameter. When $p > 1$, a convex curve is observed, when $p < 1$, a concave curve is observed, and when $p = 1$, a linear curve is observed. The survival parameter *ttd* representing time needed to reach detection limit (days) can be calculated based on the survival data modeling. The detection limit was 100 CFU per gram soil dry weight, gdw^{-1}.

2.5. Soil DNA extraction, sequencing, and sequence data analysis

Community DNA was extracted from 0.5 g soil from each of the 26 soil samples. The quality of the soil DNA was detected by 0.8% agarose gel via electrophoresis, and DNA was quantified using ultraviolet spectrophotometer. The 16S rRNA gene was amplified by using the primers 338F (ACT CCT ACG GGA GGC AGCA) and 806R (GGA CTA CHV GGG TWT CTA AT), and products of PCR was detected by 2% agarose gel via electrophoresis. Sequencing of purified samples was carried out at Shanghai Personal Biotechnology Co. Ltd., China, on an Illumina MiSeq high-throughput sequencing platform. Sequences were identified using QIIME software (Quantitative Insights Into Microbial Ecology, v1.8.0), and chimera sequence was checked and removed with USEARCH (v5.2.236). Operational taxonomic units (OTUs) were clustered and differentiated based on 97% similarity using UCLUST. Taxonomic information was obtained based on the comparison between OTU representative sequence and corresponding model sequence in Greengenes database (Release 13.8). The sequencing data have been deposited with links to BioProject under accession number PRJNA640242.

2.6. Statistical analysis

Bar charts and line charts were plotted using OriginPro 9.0 (OriginLab, Northampton, MA, USA). Principal coordinate analysis (PCoA) and dissimilarity analysis were conducted to analyze the differences of soil bacterial communities in YJ, LJ and HL using R 3.5.2 with vegan package. The dissimilarity tests were conducted by Multi-Response Permutation Procedure (MRPP), Adonis and ANOSIM (both were non-parametric MANOVA) based on four distances metrics, i.e. Bray-Curtis, Horn, Gower and Jaccard. Redundancy analysis (RDA), variation partition analysis (VPA), Mantel and partial Mantel tests were conducted by R 3.5.2 with vegan package in order to obtain the influence of soil physiochemical properties and bacterial communities on survival of *S.* Typhimurium. Structural equation modeling (SEMs) was performed by AMOS 22.0 (Amos Development, Spring House, PA, USA) in order to determine direct and indirect effects of soil physiochemical properties and bacterial communities on survival parameters of *S.* Typhimurium.

Table 1
Dissimilarity analyses of bacterial community structures in soils from three sites. Four distance metrics were used, including Bray, Horn, Gower and Jaccard. Three distance indices were calculated, including mrpp, adonis and anosim. Bolded numbers indicate p values were significant at 0.05 level.

		mrpp		Adonis		anosim	
		δ	p	F	p	R	p
Sites	Bray	0.6595	**0.001**	2.3832	**0.001**	0.5202	**0.001**
	Horn	0.5008	**0.001**	3.0440	**0.012**	0.4339	**0.001**
	Gower	0.1197	**0.001**	1.9178	**0.001**	0.3125	**0.001**
	Jaccard	0.7917	**0.001**	1.8859	**0.001**	0.5202	**0.001**

3. Results

3.1. Compositions and structures of bacterial communities in soils

The relative abundance profiles of bacteria at phylum level in soils YJ, LJ and HL showed that bacterial phyla with the highest relative abundances were *Actinobacteria* (approximately 40%), followed by *Proteobacteria* (about 20%) in soils from all sampling sites. *Chloroflexi*, *Acidobacteria* and *Gemmatimonadetes* also had higher relative abundance in YJ, LJ and HL soils. Therefore, soils from YJ, LJ and HL shared similar dominant bacterial communities according to Fig. 1A. PCoA of YJ, LJ and HL soils was shown in Fig. 1B, where 26 soils were clustered into three groups based on their sampling sites. Soil samples from the same site were clustered together, indicating soils from the same site shared more similarities in comparison with those from the other two sites. In agreement with the outcome of PCoA, results of dissimilarity test (Table 1) displayed that soil samples varied significantly ($p < 0.05$) from each of the sampling sites based on four different distance metrics.

3.2. Survival of S. Typhimurium in soils

Survival profiles of *S.* Typhimurium in 26 soil samples were shown in Fig. 2. Eight *ttd*s were obtained from YJ soils (Fig. 2A), among them YJ2

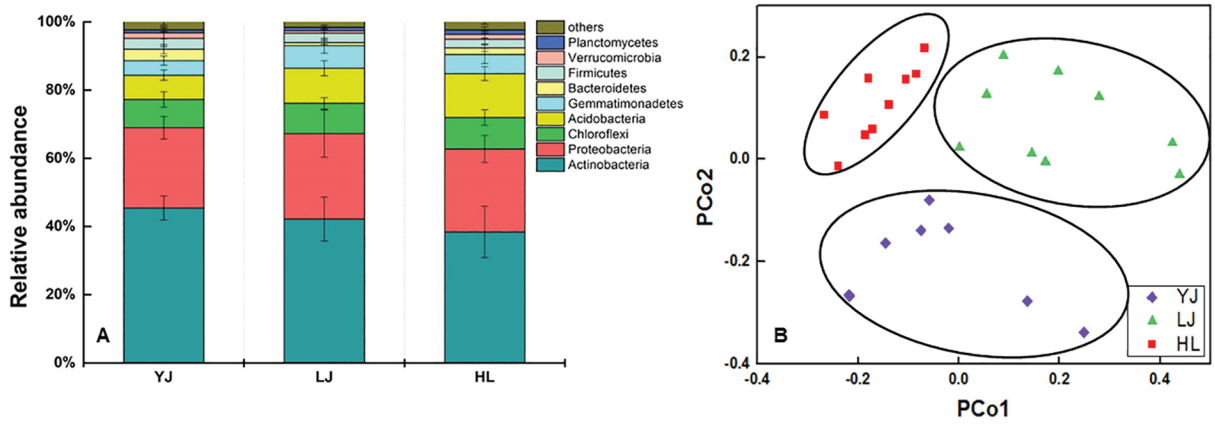

Fig. 1. The relative abundance of dominant bacterial phyla and biplot by principal components 1 and 2 (PCo1 16%, PCo2 10.3%) from Principal coordinate analysis (PCoA) of bacterial community structures in 26 soil samples from Yanji (YJ), Longjing (LJ) and Helong (HL).

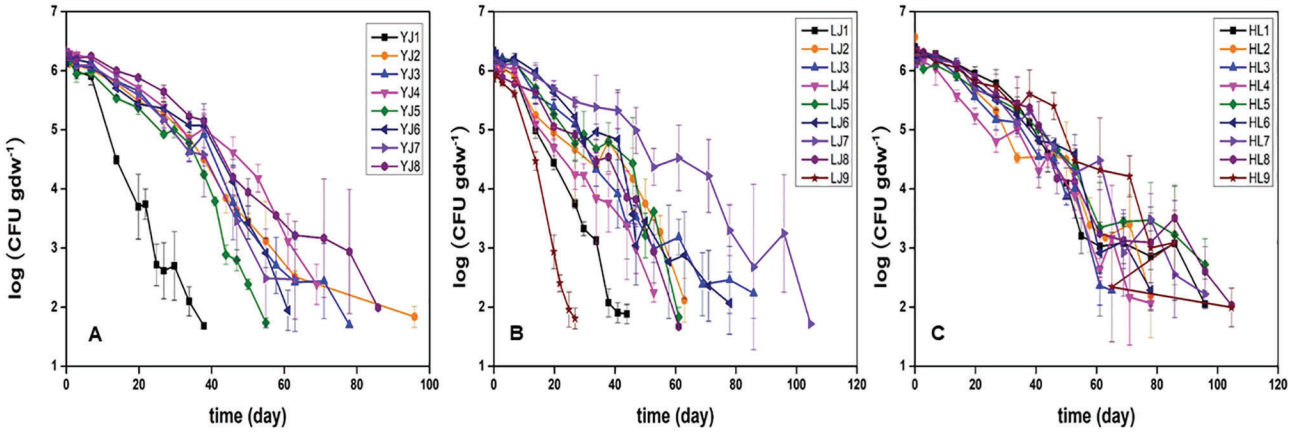

Fig. 2. Survival profiles of *Salmonella* Typhimurium in 8 soils from Yanji (A), 9 soils from Longjing (B) and 9 soils from Helong (C). Standard errors were means of triplicate measurements.

and YJ8 had *ttd*s greater than 80 days, while YJ1 had a *ttd* less than 40 days, and *ttd*s of other soil samples were somewhere in between. YJ1 and YJ2 displayed concave curves, indicating $p < 1$, while others were convex with $p > 1$. For soils from LJ (Fig. 2B), *ttd*s of LJ3 and LJ7 were longer than 80 days and LJ7 showed a *ttd* more than 105 days, while LJ9 had a *ttd* less than 30 days. The rest of the samples had *ttd*s between 30 and 80 days. Only LJ3 showed a concave curve while other samples had convex curves. In addition, *ttd*s of HL soils were quite different from those collected from the other two sites (Fig. 2C). All nine soils had *ttd*s greater than 60 days. In HL5, HL8 and HL9 soils, *S.* Typhimurium persisted for more than 100 days. All samples showed convex curves with $p > 1$. The average *ttd*s were 66.18 days in YJ soils, 64.97 days in LJ soils and 92.15 days in HL soils.

3.3. Mantel and partial mantel analyses

Results of Mantel and Partial Mantel analyses were displayed in Tables 2 and 3, indicating that physical and bacterial factors influenced the survival of *S.* Typhimurium. In Table 2, Mantel analyses showed that *ttd*s had a significant correlation ($p < 0.01$) with physical factors (clay, silt and sand) and bacterial community structure (PCo1, PCo2 and PCo3). The time needed for the first decimal reduction δ were related to physical factors ($p < 0.05$) and bacterial community structure ($p < 0.01$).

In Table 3, Partial Mantel analyses showed that *ttd*s were highly correlated with physical factors ($p < 0.01$) when controlling for bacterial community structures, indicating that physical properties of soils had significantly direct effect on *ttd*s without indirect effect of bacterial factors. Similarly, bacterial community structures showed significant direct effect on *ttd*s ($p < 0.05$) without indirect effect of physiochemical properties. The δ was significantly related with bacterial factors ($p < 0.01$), indicating bacterial community structures had a direct effect on δ.

Overall, Mantel analyses and Partial Mantel analyses showed that soil physical properties and bacterial community structures had direct effect on *ttd*s. In addition, bacterial community structure showed a direct effect on δ, while soil physical properties only indirectly affected δ through influencing bacterial communities.

3.4. Redundancy analysis and variation partition analysis

RDA plots (Fig. 3A) indicated that bacterial community structures (PCo1, PCo2 and PCo3), EC, pH, NO_3^-–N and clay content had effect on the survival parameters of *S.* Typhimurium. According to VPA (Fig. 3B), bacterial community structure explained 21.68% of the overall variation of the survival parameters (*ttd*, *p* and δ), clay content explained 16.90% and other factors (including pH, EC and NO_3^-–N) explained 12.86%, respectively. The interpretation of both bacterial communities and clay content for survival parameters was 12.38%, and the interpretation of bacterial communities and other factors was 16.84%. Bacterial communities, clay content and other factors could explain 4.52% for survival of *S.* Typhimurium. There were 14.82% of the overall variation of survival parameters was left unexplained.

3.5. Structural equation models

Structural equation models were constructed in order to investigate direct and indirect factors influencing the survival of *S.* Typhimurium (Fig. 4). Fig. 4A illustrated the relation among certain soil properties,

Table 2
Mantel analyses and Pearson correlations between survival parameters (*ttd*, *p* and δ) and physical, chemical, bacterial communities. Physical factors included clay, silt and sand contents. Chemical factors included EC, pH, WSOC, DOC, TDN, NH_4^+–N, NO_3^-–N, TDP. Bacterial factors indicated bacterial community structures (PCo1, PCo2 and PCo3). ** and * indicated *p* values were significant at 0.01 and 0.05 level, respectively.

Survival parameters	Environmental factors	Pearson	
		R	*p*
ttd	Physical	**0.4437****	0.001
	Chemical	0.1449	0.158
	Bacterial	**0.2831****	0.002
p	Physical	0.03976	0.298
	Chemical	0.09125	0.234
	Bacterial	−0.03602	0.622
δ	Physical	**0.2226***	0.037
	Chemical	0.1775	0.093
	Bacterial	**0.3624****	0.002

Table 3
Partial Mantel analyses and Pearson correlations between survival parameters (*ttd*, *p* and δ) and physical, chemical, bacterial communities with certain controlling factors. Physical factors included clay, silt and sand contents. Chemical factors included EC, pH, WSOC, DOC, TDN, NH_4^+–N, NO_3^-–N, TDP. Physiochemical factors included chemical and physical factors. Bacterial factors indicated bacterial community structures (PCo1, PCo2 and PCo3). ** and * indicated *p* values were significant at 0.01 and 0.05 level, respectively.

Survival parameters	Environmental factors	Controlling factors	Pearson	
			R	*p*
ttd	Physical	Bacterial	**0.3686****	0.001
	Chemical	Bacterial	0.06984	0.308
	Bacterial	Physicochemical	**0.1572***	0.045
p	Physical	Bacterial	0.06314	0.210
	Chemical	Bacterial	0.1060	0.199
	Bacterial	Physicochemical	−0.08841	0.866
δ	Physical	Bacterial	0.06930	0.217
	Chemical	Bacterial	0.08304	0.249
	Bacterial	Physicochemical	**0.2898****	0.003

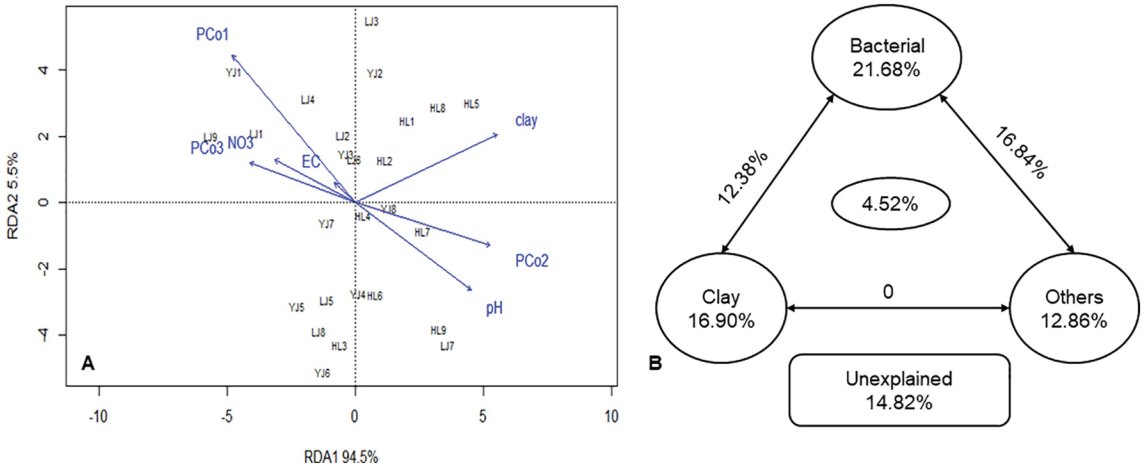

Fig. 3. Redundancy analysis (A) and variation partition analysis (B) of the effects of bacterial community structures (PCoA, including PCo1, PCo2 and PCo3), clay content and others (pH, EC, NO_3^--N) on survival parameters (*ttd*, *p* and δ) of *Salmonella* Typhimurium.

bacterial community structures and *ttd*. It showed that NO_3^-–N was negatively correlated with *ttd* ($p < 0.01$). Clay content had a positive correlation with *ttd* ($p < 0.01$). In addition, clay content influenced bacterial community structures ($p < 0.01$), indicating that clay content influenced *ttd* indirectly by affecting bacterial community structures. Similarly, pH ($p < 0.001$) and EC ($p < 0.05$) had positive correlations with bacterial community structures, which influenced *ttd* ($p < 0.01$). Thus, NO_3^-–N showed a direct negative effect on *ttd*, while EC and pH showed indirect positive effect on *ttd*. Clay content displayed both direct and indirect effect on *ttd*.

Fig. 4B identified the direct and indirect factors influencing shape parameter *p*. NO_3^-–N was positively correlated with *p* ($p < 0.05$). The pH was positively correlated with bacterial community structures ($p < 0.001$), and bacterial communities also had a positive effect on *p* ($p < 0.001$). Moreover, clay content ($p < 0.01$) and EC ($p < 0.05$) affected bacterial community structures positively, indicating an indirect influence on *p*. Both clay content and EC showed negative correlations with *p* ($p < 0.01$). Therefore, NO_3^-–N showed a positive effect directly on *p*, while pH showed a positive effect indirectly on *p*. Clay content and EC displayed both direct and indirect effect on *p*.

Fig. 4C showed the effect of certain environmental factors and bacterial community structures on scale parameter δ. It showed that NO_3^-–N was positively correlated with δ. Clay content ($p < 0.01$) and pH ($p < 0.001$) had significantly positive correlation with bacterial community structures, indicating an indirect influence on δ ($p < 0.001$). EC showed a direct negative relation with δ, and also had a significantly positive correlation with bacterial community structures ($p < 0.05$) indirectly influencing δ. Hence, NO_3^-–N showed direct positive effect on δ, while pH and clay content showed an indirect positive effect on δ. EC displayed both direct and indirect effect on δ.

3.6. Roles of different bacterial classes on the survival of S. Typhimurium

Different bacterial classes showed different relationships with the survival parameters of *S.* Typhimurium. In Fig. 5, *Actinobacteria* and *Acidobacteria* showed negative correlations with *ttds* ($p < 0.05$). *Deltaproteobacteria* was also found to be positively correlated with *ttds* ($p < 0.01$).

4. Discussion

4.1. Biotic characterization of soils

The major bacterial phyla were *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Planctomycetes*, which were consistent with soils collected from northeastern China (Liu et al., 2014). The relative abundances of dominant bacteria were similar to those in soils from the Arctic (Chu et al., 2010). Research on major global bacterial systems found that Proteobacteria and Actinobacteria were the two bacterial phyla with the highest relative abundances (Delgado-Baquerizo et al., 2018), which was consistent with the finding in the current. Alphaproteobacteria and Betaproteobacteria accounted for the highest proportion of Proteobacteria, which was consistent with a previous study on bacterial community composition of apple orchards (Chen et al., 2014). Actinobacteria and Proteobacteria were both dominant phyla and keystone taxa, indicating that they might play an essential role in the ecological function of microbial communities (Essel et al., 2019; Shanmugam et al., 2016). Although there were some differences in bacterial communities, they also shared similarities, e.g. the dominant bacterial groups, this may be due to the same land use types (artificial economic forest) of the soils. On the other hand, the variations in bacterial communities in soils might differently influence the survival of the pathogens invading into the soils.

4.2. The survival patterns of S. Typhimurium

The time needed to reach detection limit (*ttds*) varied from 20 to 120 days, the shape parameter *p* ranged between 0.8 and 2.4, and the first decimal times (δ) were between 7 and 42 days. The survival time was consistent with another research, which showed that *S.* Typhimurium could survive for up to 75 days in waters (Li et al., 2018). The results were also comparable to the survival of *Escherichia coli* O157:H7 in soils (up to 90 days) (Ibekwe et al., 2007). Like other well documented foodborne pathogen, *S.* Typhimurium could be very persistence, thus constitute environmental heal risks.

4.3. Direct and indirect effect of soil properties on the survival of S. Typhimurium

Factors affecting the survival patterns of *S.* Typhimurium were identified based on SEMs. Recent researches have shown that NO_3^-–N, NH_4^+–N and total nitrogen were positively correlated with the survival of invading pathogens in soil or water environment (Franz et al., 2008; Li et al., 2018; Ma et al., 2012). A low level of available nutrients (i.e. nitrogen, dissolved organic carbon, and available phosphorus) could lead to shorter survival of invading pathogens (Ding et al., 2018). The reason may be that a soil system with less nutrients could enhance the competition for food among microorganisms, resulting in the rapid decrease of pathogens in soils (van Bruggena and Termorshuizen, 2003).

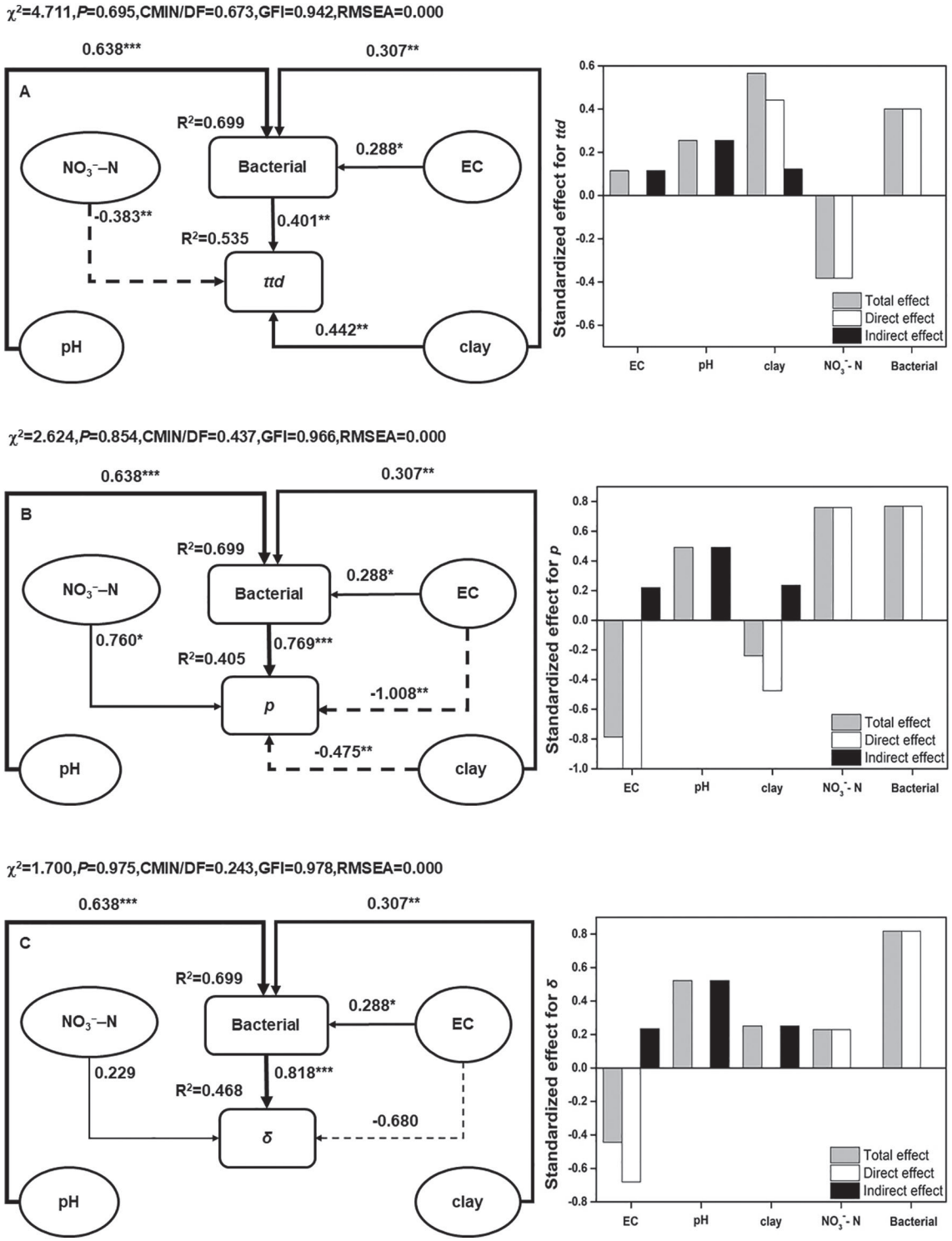

Fig. 4. Structural equation models (SEMs) based on direct and indirect effect of soil properties (pH, EC, clay content and NO_3^-–N) and bacterial community structures (PCo1, PCo2 and PCo3) on the survival parameters *ttd* (A), *p* (B) and *δ* (C) of *Salmonella* Typhimurium. The solid and broken lines indicated the positive and negative path coefficients, respectively. The total effect was the sum of direct and indirect effect. ***, ** and * indicated *P* values were significant at 0.001, 0.01 and 0.05 level, respectively.

Nonetheless, further investigation would be required in terms of the influence of organic form of nitrogen (i.e. amino acids) on the survival of *S.* Typhimurium.

Soil pH exerted an indirect effect on the survival of *S.* Typhimurium by influencing the bacterial community structures ($p < 0.001$). Soil pH might have a close impact on the indigenous bacterial communities by changing soil properties, such as available carbon or nitrogen content and electrical conductivity, and thus influence the survival of inoculated pathogens (Cabral, 2010; Leclerc et al., 2002; Ma et al., 2016). Bacteria more tolerant to pH change might exist longer in soils, while less tolerant ones tend to persist shorter.

In this study, clay content had a close correlation with the persistence of *S.* Typhimurium ($p < 0.01$). The *ttd*s showed a highly positive relation with clay content, which was comparable to the results in recent studies (Ma et al., 2013; Ma et al., 2011). This may be because soil textures have been shown to be important for the survival of

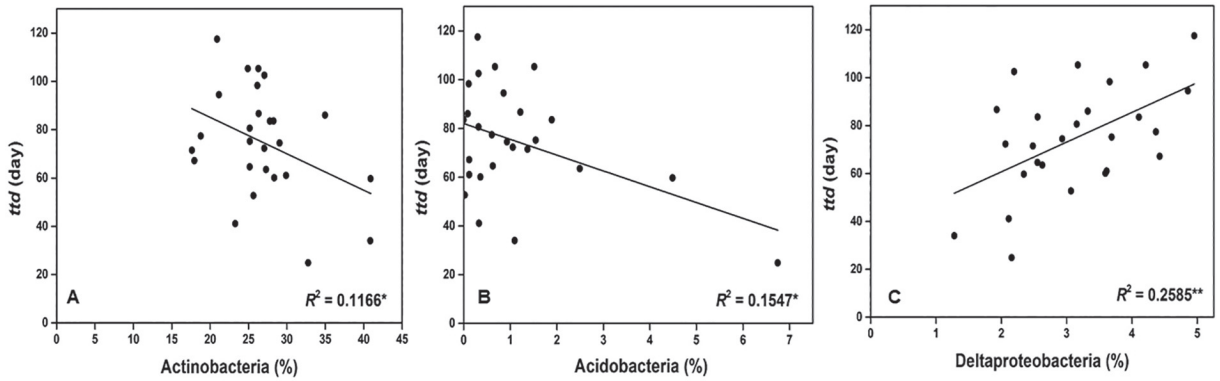

Fig. 5. Correlation of relative abundance of dominant bacterial classes *Actinobacteria* (A), *Acidobacteria* (B) and *Deltaproteobacteria* (C) with the survival of Salmonella Typhimurium. ** and * indicated *P* values were significant at 0.01 and 0.05 level, respectively.

pathogens. Finer textured soils (i.e. clay) tent to provide more pore space for both inoculated pathogens and indigenous bacterial communities, leaving more space for more water interactions (van Veen et al., 1997; Zhou et al., 2002). However, it is interesting to mention that clay content could also exert a negative correlation with the survival of pathogens (Ma et al., 2012). It was not surprising that EC showed a highly negative correlation with the survival (p) of *S.* Typhimurium. The results were similar to the survival of *E. coli* O157:H7 in 32 soils collected from USA (Ma et al., 2012). Higher salinity might result in shorter prolonging of invading pathogens, as soil salinity may interfere with ion transport based on the osmotic effect or certain ion toxicity and inactivate some essential enzymes (Shabala et al., 2009). Salinity was shown to pose great influence on microbial communities as the change of ionic compounds may affect soil organic matter solubility and decomposition (Han et al., 2018; Rath and Rousk, 2015).

4.4. Effect of bacterial communities on the survival of S. Typhimurium

Previous studies have shown that the indigenous microbial communities exerted a significant relation with the survival of inoculated pathogens (Jiang et al., 2002; Wang et al., 2014). This may be due to the complex biological interactions between microbes, such as mutualism, predation and competition (Montoya et al., 2006). Our results revealed that *Actinobacteria* and *Acidobacteria* at class level exhibited significantly negative correlations with the *ttd*s ($p < 0.05$), which could be caused by the microbial suppression (Westphal et al., 2011). Interestingly, positive relations were observed between phylum *Actinobacteria*, *Acidobacteria* and survival of *Escherichia coli* O157:H7, which may be because that both bacterial groups effectively degraded high-molecular-weight organic compounds into low-molecular-weight energy resources for inoculated pathogens (Hugenholtz et al., 1998). *Deltaproteobacteria*, as one of the *Proteobacteria* subclasses, displayed a highly positive relation with *ttd*s ($p < 0.01$), which might be because that *Deltaproteobacteria* and *S.* Typhimurium established a mutualistic behavior. The result was consistent with another study about *E. coli* O157:H7 (Ma et al., 2013). Both *S.* Typhimurium and *E. coli* O157:H7 belongs to *Gammaproteobacteria* class. It is worth mentioning that *Betaproteobacteria* and *Gammaproteobacteria* classes had negative influence on the survival of *E. coli* O157:H7, indicating not all *Proteobacteria* subclasses played the same role in the survival pattern (Westphal et al., 2011). Thus, it is reasonable to presume that the overall influence of phylum *Proteobacteria* on the survival of inoculated pathogens might depend on dominant *Proteobacteria* subclasses. In addition, *Bacteroidetes* and *Firmicutes* also suppressed the survival of *E. coli* O157:H7 in the study above.

Many efforts have been made to elucidate the mechanisms explaining the relationships between *S. Typhimurium* and other bacteria. A recent study revealed that *Lactobacillus plantarum*, a species from phylum *Firmicutes* could negatively affect the survival of *S. Typhimurium*, and such suppression was dependent on cell concentrations (Chen et al., 2020). It was reported that the growth inhibition of *S. Typhimurium* was only observed in co-culture with *E. coli*, which might excrete toxins (Avendano-Perez et al., 2015), and cell-cell contact was believed to be essential for such inhibition (Avendano-Perez and Pin, 2013). In addition, other soil microbes, such as fungi, protists and viruses may also affect the survival of S. Typhimurium. Botrytis cinerea and Rhizopus stolonifer caused a decrease in *S. enterica* population, and moulds also reduced the growth of *S. enterica* (Ortiz-Sola et al., 2020). Both protozoa and bacteriophage could inhibit the persistence of *Salmonella* (Gourabathini et al., 2008; Ye et al., 2019).

5. Conclusion

In summary, we investigated 26 soil samples collected from apple-pear orchards in northeastern China to probe the prevailing biotic and abiotic factors influencing the survival parameters (*ttd*, p and δ) of *S.* Typhimurium. Soils from three sampling sites varied significantly though the dominant bacterial communities remained similar. The *ttd*s were between 20 and 120 days. Further analyses elaborated NO_3^-–N and pH had direct and indirect effect on *ttd*s, respectively. Clay content and EC showed both direct and indirect effect on *S.* Typhimurium survival. In addition, specific bacterial classes (*Actinobacteria*, *Acidobacteria* and *Deltaproteobacteria*) had close relationships with the *ttd*s. Our results demonstrated the understanding of *S.* Typhimurium survival in order to decrease potential health risks, whereas more information and novel approaches should be provided to thoroughly understand the survival patterns of *S.* Typhimurium.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of sequencing data

The sequencing data have been deposited with links to BioProject under accession number PRJNA640242.

CRediT authorship contribution statement

Jiafen Liao: Formal analysis, Writing – original draft. **Jiahang Li:** Methodology, Formal analysis, Writing – original draft. **Ziming Han:** Methodology, Writing – review & editing. **Guangze Lyu:** Writing – review & editing. **A. Mark Ibekwe:** Methodology, Writing – review & editing. **Jincai Ma:** Methodology, Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interest. The funder did not play any role in experimental design, data collection and analysis, decision for publication, and preparation of the manuscript.

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