Persistence of *E. Coli* O157:H7 in Urban Recreational Waters From Spring and Autumn: A Comparison Analysis

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Abstract

Persistence of *E. coli* O157: H7 (EcO157) in 48 water samples (24 Spring samples and 24 Autumn samples) from 3 urban recreational waters in Changchun City was investigated, and multivariate statistical analysis was performed to correlate survival data with water physicochemical properties and bacterial communities. Our data showed that EcO157 survived longer in Spring samples than in Autumn samples regardless of the lakes. Results revealed that recreational water physicochemical properties and bacterial community in Spring samples were different from those in Autumn samples. Mantel and Partial Mantel tests, as well as co-occurrence network analysis illustrated that EC salinity, TOC and bacterial community were correlated with survival time (*ttd*) ($p < 0.05$). Variation partition analysis (VPA) indicated that bacterial community, EC, TOC and TN explained about 64.81% of overall *ttd* variation in Spring samples, and bacterial community, EC, pH and TP accounted for about 56.59% of overall *ttd* variation in Autumn samples. Structural equation model (SEM) illustrated that EC indirectly positively affected *ttd* through bacterial community. The correlation between bacterial community and *ttd* was negative in Spring samples and positive in Autumn samples. TN appeared a direct positive effect on *ttd* in Spring samples. TP displayed a direct negative effect on *ttd* in Autumn samples. Our results concluded that there was seasonal variation in environmental factors that directly or indirectly affected the survival of EcO157 in urban recreational waters.

Introduction

E. coli O157:H7 (EcO157) is a pathogenic, rod-shaped, gram-negative, and Shiga toxins producing bacterium belonging to phylum *Proteobacteria*. EcO157 was firstly identified during the investigation of a major gastrointestinal illness outbreaks caused by undercooked hamburger patties in the United States (Riley et al. 1983). The infectious dose of EcO157 was very low, and as low as 10 cells was believed to cause infection in human beings (Bach et al., 2002). When get infected by this pathogen, people could display typical clinical symptoms including bloody diarrhea, vomiting, which could ultimately develop into hemolytic uremic syndrome (HUS) (Jones 1999), a leading cause of life-threatening kidney failure in children and the elder (Mead and Griffin 1998). Therefore, the United States (Siegler et al. 1994), Canada (Rowe et al., 1991), and Europe (Bitzan et al. 1993) have regarded EcO157 as one of the major food pathogens. Reducing the hazard of EcO157 to human health was considered as an urgent public health problem that needs to be solved (Griffin and Tauxe 1991). It has been reported that in the United States alone, over 73,000 cases of human infection with EcO157 occur every year (Nataro and Kaper 1998). In 1999, an outbreak of EcO157 infection occurred in Jiangsu, China, over 20,000 people were infected, and among them 177 died (Cookson et al. 2006). Foodborne infection was one of the EcO157 outbreaks (Ferens and Hovde 2011), mainly caused by undercooked ground beef or dairy products (Caprioli et al. 2005). The consumption of contaminated fermented sausages proved to be related to the large-scale outbreak of EcO157 infections in southern Sweden during Autumn 2002 (Sartz et al. 2008). In addition, waterborne outbreaks of EcO157 are extremely threatening. A large-scale infection caused by unchlorinated drinking water source once occurred in Cabool, Missouri, resulting in 243 infections and

four deaths (Swerdlow et al. 1992). A fecal-contaminated water park near Atlanta, Georgia triggered an EcO157 outbreak, affecting residents in up to eight states (Bach et al. 2002). It can be seen that the investigation of EcO157 infection is necessary to protect human health.

The major origin of EcO157 strains were cattle, and the count of this pathogen in the feces of super shedders could be as high as 10^8 CFU (colony forming units)/g (Fukushima and Seki, 2004). Other warm-blooded animals including pets, birds, sheep, as well as cold-blooded animals such as snakes, frogs and fish (Ferens and Hovde 2011) were also potential EcO157 carriers. Animal manure with EcO157, if abused or improperly removed, could spread into other water bodies (e.g. reservoirs, lakes, and ponds) through wind and surface runoff from rain (Johnson et al. 1999), thereby causing EcO157 water contamination. Plenty of evidences have indicated that EcO157 has a strong ability to survive in water. It has been demonstrated that EcO157 can survive for weeks and up to several months in water (Rice et al. 1992), such as rivers, lakes, even in nutrient-poor bottled mineral water (Kerr et al., 1999). Moreover, protozoan predation (Artz and Killham 2002) and sediments in streams (Pachepsky et al. 2006) also made significant effects on the persistence of EcO157 in waters. Obviously, waterborne transmission will increase the risk of human infection. Thus, it is of great significance to study the persistence of EcO157 in water environment to reduce the risk of human infection.

Urban recreational waters are used for leisure and entertainment and the main places for citizens to take water activities. Surrounding animal manure, pathogens carried by the wind, wild animals and pet feces with EcO157 might invade the water and became sources of contamination (Wyness et al. 2018). Recreational waters were considered to be prone to pathogen infections and outbreaks of EcO157 due to human hydrophilic activities (Hunter et al. 2011), such as swimming, fishing, boating, surfing and water playgrounds (Swinscoe et al. 2018). Outbreaks of EcO157 related to recreational water were reported for the first time in 1991 (Rangel et al. 2005), 14 cases in lakes or ponds, and 7 cases in swimming pools. The reports of recreational water outbreaks among pools (Hildebrand et al. 1996) and lakes (Ackman et al. 1997) have never been decreased. Infections might be caused by swimming in and swallowing water (Paunio et al. 1999). As a potential biohazard, EcO157 could enter into the recreational waters in populous metropolitan area via different pathways as discussed above, therefore it is worthwhile to investigate its environmental behavior in the environment.

The outbreak of EcO157 might have seasonal characteristics (Ailes et al. 2008). Previous studies reported the prevalence of this pathogen in countries with temperate climates in late Spring and early fall (Hermos et al. 2011), suggesting seasonal variation in EcO157 infection. It was reported that the survival rate of EcO157 in the edible parts of lettuce was higher in Autumn than that in Spring and environmental factors played crucial roles (Oliveira et al. 2012). However, the seasonal variation of EcO157 in recreational water has been rarely studied. In northeast Changchun city, China, Spring and Autumn are the peak seasons for water recreational activities. In winter, there are few visitors because the temperature is too low and lakes are frozen. Consequently, in order to protect public health, it is useful to investigate the survival profiles of EcO157 in different seasons in urban surface recreational waters.

In our study, 48 samples were collected from Nanhu Lake, Jingyue Lake, Beihu Lake, respectively. Those lakes are primary urban recreational waters for Changchuners. The collection time was October 2018 (early Autumn) and May 2019 (late Spring). The purposes of this study were to, 1) compare physicochemical properties, bacterial community and EcO157 persistence in urban recreational waters between Spring and Autumn, and 2) reveal the possible mechanisms causing the seasonal variation in EcO157 persistence in urban recreational waters in NE China.

Materials And Methods

Water sampling and characterization

A total of 48 water samples were collected in Spring and Autumn from three main urban recreational waters in Changchun City. 8 samples from Nanhu Lake (NH), 8 samples from Jingyue Lake (JY), 8 samples from Beihu Lake (BH) were obtained in the Autumn of 2018 and Spring of 2019, respectively. The sampling sites in Spring and Autumn were the same. The specific longitude and latitude coordinates were shown in (Table S1). Water samples were collected at 0.2 m below the surface of the lakeshore with a fixed-depth water sampler. A total of 5 L water sample was collected from each site. The samples were transported to the laboratory on ice within 8 h. 1 L sample was saved under 4 °C temporally for survival study. 2 L water sample was filtered through 0.22 μm membrane, and the samples were saved in -80 °C fridge for community DNA extraction. Water physicochemical properties were determined with well-developed methods (see below). TN (total soluble nitrogen) was qualified by potassium persulfate oxidation-double wavelength spectrophotometry method. NH_4^+-N (ammonia nitrogen) was qualified by Nessler reagent spectrophotometry. TP (total phosphorus) was measured by molybdenum-antimony-ascorbic acid spectrophotometric. TOC (total organic carbon) was measured by a total organic carbon analyzer (TOC-VCPH, Shimazu, Japan). The EC (electrical conductivity) was determined by s conductivity meter (DDS-11A, Rex, China). The pH was determined by a pH meter (FE20, Mettler, China).

Bacterial Strains

The strain used in this experiment was *E. coli* O157:H7 EDL931 (ATCC 35150), conferring toxic genes including stx_1, stx_2, and *eae*. The EDL931 was originally obtained from human feces (Beery et al., 1984). The EDL931 wild type was tagged with rifampicin resistance for ease of counting (Ma et al. 2011).

Survival experiment of *E. coli* O157:H7

The EcO157 cells were initially inoculated in LB liquid medium and incubated at 37 °C with shaking (220 $r{\cdot}min^{-1}$) for 15 h. The cells were harvested by centrifugation at 4 ℃, 18,000 g for 10 min. Then the cell pellet was washed three times by 0.9% sodium chloride buffer, and finally resuspended in sterile deionized water. The cells were then starved for 2 h in dark under 4 °C. The starved cells were then inoculated into lake water samples, and the concentration of EcO157 solution was about $1.0{\times}10^8$ CFU/mL. The inoculated water samples were sampled periodically, and subjected to 10-fold

serial diluted. Fifty microliters of the two highest dilutions were plated in duplicate onto the SMAC (sorbitol MacConkey) agar (Lab M, Lancashire, UK) containing 100 mg/L of rifampicin (Ma et al. 2011). The plates were incubated at 37 °C for EcO157 counting. The detailed process of count could be found in our previous publications (Han et al., 2021; Ma et al., 2014).

Survival data modeling

The modeling EcO157 survival was achieved by fitting the experimental data to the Weibull model (Mafart et al., 2002) using GInaFiT Excel add-in (Geeraerd et al. 2005). The model was established based on the assumption that the survival of EcO157 follows the Weibull distribution. The number of the survivors can be quantified by using the following equation:

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

where N_t, N_0, andt represent survivor counts, inoculum size, and inoculation time, respectively. The δ is scale parameter representing the time needed for the first decimal reduction, p is the shape parameter. Survival curves display a convex, concave, and linear shape when $p > 1$, < 1, and $= 1$, respectively. The survival time (*ttd*) represents time (days) needed to reach the detection limit could also be obtained by fitting the Weibull model.

Water DNA extraction, sequencing, and sequencing data processing

Water community DNA was extracted from prepared fiber membranes from each of the 48 water samples. The V3 and V4 regions of 16S rDNA were amplified using forward primers containing the sequence “5’-CCTACGGRRBGCASCAGKVRVGAAT” and reverse primers containing the sequence “5’-GGACTACNVGGGTWTCTAATCC”. PCR products were detected by 1.5% agarose gel electrophoresis. DNA libraries concentration was validated by Qubit 3.0 Fluorometer. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to the instructions (Illumina, San Diego, CA, USA) (Caporaso et al., 2012). PE250/300 paired-end was used to sequence. Image analysis and base calling were conducted by the MiSeq Control Software (MCS) embedded in the MiSeq instrument. The purified chimeric sequences were used for OTU clustering by VSEARCH clustering (1.9.6) (sequence similarity is set to 97%). The used 16s rRNA reference database was Silva, 132. Bayesian algorithm was used to analyze representative sequences and classify community composition under different species classification level. The sequencing data have been deposited with links to NCBI under accession number PRJNA725369.

Statistical analysis

The bar charts of survival parameters, physicochemical properties and relative abundances of major bacterial phyla were plotted by OriginPro 9.1 (OriginLab, USA). Principal component analysis (PCA) was constructed to visualize the differences of physicochemical properties in different samples with the

vegan package of R 4.0.4. Principal coordinate analysis (PCoA) was performed to appear the differences of bacterial community in Bray-Curtis conversion by vegan package. Multivariate analysis of variance (MANOVA) was conducted with SPSS 26 (IBM, USA) in order to test the effects of lake, season and interaction (Lake×Season) on survival parameters of EcO157 and water physicochemical properties. Dissimilarity analysis was applied to indigenous bacterial community by Multi-Response Permutation Procedure (mrpp), Permutational MANOVA (adonis) and Analysis of Similarities (anosim) based on four distance conversion measures of Bray-Curtis, Horn, Gower and Jaccard (vegan package of R 4.0.4).

Mantel and partial Mantel tests (non-parametric statistical methods) were calculated with the vegan package of R 4.0.4. The Mantel test is a correlation test between two matrices including measured variables (Guillot and Rousset, 2013). Partial mantel test also measures correlation, but controls for the effect of a third matrix including other variables (Guillot and Rousset, 2013). The influence of bacterial community and physicochemical properties on the survival of EcO157 was tested by Mantel and partial Mantel. In addition, Pearson correlation between *ttd* and relative abundances of phylum bacterial taxa in Spring and Autumn samples was calculated respectively.

To clearly display the correlations between survival parameters, physicochemical properties and bacterial community, co-occurrence network analysis based on Pearson correlation ($p < 0.05$) was conducted. In order to simplify the network, the correlations between bacteria were deliberately removed. Co-occurrence network analysis and plotting were jointly completed through the Hmisc package of R 4.0.4 and Gephi 0.9.2(Bastian et al. 2009).

In order to further reveal the influence of environmental factors, variation partition analysis (VPA) was used to quantify the explanatory contribution of partitioning factor matrices to the overall *ttd* variation of EcO157. VPA was performed after obtaining the corrected R^2 (total explanatory proportion) of Redundancy analysis (RDA) with R 4.0.4 (Dixon, 2003) using vegan package. VPA can be output in the style of Venn diagram. The overlap in the Venn diagram indicated combined contributions of multiple matrices (Peres-Neto et al., 2006).

The structural equation model (SEM) can more accurately identify the direct and indirect effects of environmental factors on the persistence of EcO157. SEM was performed by AMOS 22.0 (Amos Development, Spring House, PA, USA). Direct effect, indirect effect and total effect were calculated based on standard path coefficients (λ) which reflect the strength of the relationship between variables (Igolkina and Samsonova, 2018). Model fitness is the degree of consistency between the hypothetical theoretical model and the actual data (Wyeld and Nakayama, 2019). The model fitness parameters and criteria are as follows: $\chi^2 > 0.05$, chi-square; $p > 0.05$, p value; CMIN/DF < 3, ratio of χ^2 and degrees of freedom; GFI > 0.9, goodness of fit index; RMSEA < 0.08, root mean square error of approximation.

Results

Water properties, dominant bacterial phyla and *E. coli* O157:H7 survival parameters

The water physicochemical properties were listed in Table S2. Fig. 1 displayed the variation of physicochemical properties. The result showed that EC levels were higher in Spring samples than in Autumn samples ($p < 0.05$) (Fig. 1b). Regardless of seasons, EC levels were the highest in BH (Fig. 1b). TN levels were higher in Autumn samples than in Spring samples ($p < 0.05$) (Fig. 1c). TOC was higher in Spring samples than in Autumn samples ($p < 0.05$). In Spring samples, TOC was the highest in BH ($p < 0.05$). However, TOC were not significantly different in Autumn samples (Fig. 1d). For TP and NH_4^+-N, the highest concentrations were showed in NH ($p < 0.05$) (Fig. 1e and 1f).

Fig. 2 showed the variation of relative abundances of major bacterial phyla. Relative abundance of *Proteobacteria* was the highest in BH for Spring samples ($p < 0.05$) and NH for Autumn samples ($p < 0.05$) (Fig. 2a). For Spring samples, BH had the lowest relative abundance of *Bacteroidetes* ($p < 0.05$), while in Autumn samples JY had the lowest one ($p < 0.05$) (Fig. 2b). Relative abundance of *Actinobacteria* was highest in BH in Autumn samples ($p < 0.05$) (Fig. 2c).

Survival parameters (*ttd*, δ, *p*) were calculated according to survival profiles of EcO157 (Fig. S1). Variation of EcO157 survival parameters was shown in Fig. 3. On average, the survival time (*ttd*) in urban recreational waters was 64.6 days when both seasonal samples were taken into account. For Spring samples, *ttd*s in NH, JY and BH were 70 days, 58.9 days and 80 days, respectively, with average being 69.7 days. For Autumn samples, the *ttd*s in NH, JY and BH were 52.8 days, 51.6 days and 75 days, respectively, and the average was 59.8days. Overall, the survival time was longer in Spring samples compared with Autumn ones (Fig. 3a). For individual recreational water, EcO157 survived for the longest in BH, regardless of seasons (Fig. 3a). The δ of EcO157 was longer in Autumn samples than in Spring samples (Fig. 3b). Survival profiles of EcO157 mostly displayed a concave curve, and *p* was less than 1 in most cases (Fig. 3c).

Principal component analysis visualized the variation in physicochemical properties of different samples. The closer the distance between sample points, the more similarity shared by those samples, and vice versa. PCA showed that the most Spring samples could be distinguished from the Autumn samples (Fig. 4a). However, the variation between lakes were not observed since the sampling points were not well separated from each other. Similarly, seasonal variation was observed among bacterial communities in all samples (Fig. 4b).

MANOVA was performed on survival parameters and physicochemical properties of all 48 samples. MANOVA results revealed that NH_4^+-N, pH, EC, TOC, TN, *ttd* and δ were largely affected by seasons ($p < 0.05$) and the interaction (Lake× Season) could be observed for NH_4^+-N and pH (Table 1). The *p* was neither affected by lakes nor seasons. Dissimilarity test revealed that bacterial community was significantly different ($p < 0.05$) between Spring and Autumn samples (Table 2). Overall, seasonal variation was noticed in recreational water physicochemical properties, bacterial community and EcO157 survival time.

Multivariate statistics on environmental factors and *E. coli* O157:H7 survival parameters

The result of Mantel and partial Mantel tests for all 48 samples revealed that *ttd* was correlated with the indigenous bacterial community, EC and TOC ($p < 0.05$) (Table 3). When the effects of other factors were excluded, coefficients between *ttd* and bacterial community, EC and TOC became smaller, but remained statistically significant. Bacterial community and physicochemical properties showed no significant effect on p and δ (data not shown).

Pearson correlation showed that the bacterial phyla correlated with *ttd* varied greatly in Spring and Autumn samples (Table 4). In Spring samples, relative abundances of *Proteobacteria* and *Bacteroidetes* were positively and negatively correlated with *ttd*, respectively ($p < 0.05$) (Table 4). For Spring samples, most bacterial taxa excluding *Proteobacteria* were negatively correlated with *ttd* ($p < 0.05$). In Autumn samples, *Actinobacteria* was positively correlated with *ttd* ($p < 0.05$) (Table 4). Almost all major bacterial taxa in Autumn samples were positively correlated with *ttd* ($p < 0.05$).

Co-occurrence network analysis displayed the interactions among EcO157 survival parameters, water physicochemical properties and indigenous bacterial community. When all 48 samples were considered, *ttd* was positively linked to TOC and EC (Fig. 5a). Networks of Spring (24 samples) and Autumn (24 samples) were less complex with fewer links, lower average degree, and lower density (Fig. 5d). Connections profiles between *ttd* and other nodes representing water properties and bacterial phyla were seasonally differentiated between Spring and Autumn samples (Fig. 5b and 5c). On the other hand, δ and p had fewer links in the networks (Fig. 5b and 5c).

Variation partition analysis (VPA) was used to quantify the contribution of environmental factors to the *ttd* variation of EcO157. When all 48 samples were considered, VPA showed that the measured environmental factors explained approximately 47.49% of the overall *ttd* variation, leaving 52% unexplained. The combined contribution by bacterial community and water properties explained about 23.74% of *ttd* variation (Fig. 6a). Fig. 6b and 6c highlighted the seasonal differences in VPA between Spring and Autumn samples. In Spring samples, TOC and TN accounted for 36% of overall *ttd* variation. The individual contribution of bacterial community was only 3.88%, but the combined contribution reached 22.09%. About 64.81% of overall *ttd* variation was explained by measured environmental factors (Fig. 6b). In Autumn samples, pH and TP explained 10.42% of overall *ttd* variation (Fig. 6c). The bacterial community alone explained 24.11%. About 56.59% of overall *ttd* variation was explained by measured environmental factors (Fig. 6c).

The effects of bacterial community and water properties on the *ttd* of EcO157 could be either direct or indirect effects through SEM (Fig. 7). TOC had a direct positive effect on *ttd* ($p < 0.05$) and EC had an indirect effect on *ttd* through bacterial community (Fig. 7a). Fig. 7b and 7c emphasized the seasonal differences in SEM between Spring and Autumn samples. It was showed that TN had a positive effect on *ttd* ($p < 0.05$) in Spring samples, while TP had a negative effect on *ttd* ($p < 0.05$) in Autumn samples (Fig. 7b and 7c). The effect of EC on bacterial community was negative in Spring samples and positive in Autumn samples, respectively. Similarly, EC had an indirect positive effect on *ttd* through bacterial community, regardless of seasons (Fig. 7e and 7f). Interestingly, although bacterial community had

effects on *ttd*, the effect was negative in Spring samples and positive in Autumn samples, respectively (Fig. 7b and 7c).

Discussion

Seasonal variation in water properties and bacterial community

Our data revealed that EC and TOC levels were higher in Spring samples and TN levels were higher in Autumn samples. The possible reasons might lie in the changes of climate conditions associated with different seasons. As a rainy season of Changchun City, Autumn had higher precipitation and lake water volume, which might directly change some water physicochemical properties (e.g. pH, EC) (Stocker et al., 2019). Chemical components that reflected lake water EC salinity may be diluted due to increased water volume. The nutrients (e.g. nitrogen, phosphorus) from lake sediment and surrounding environment might be released into the lake with direct rainfall and surface runoff (Shen et al., 2021). Low temperature in Autumn was considered to be beneficial to maintain TN concentration (Mu et al., 2021). In Spring, there was plenty of sunlight and the temperature is higher. Although temperature had little direct effect on water total organic carbon, a recent study showed that the temperature was related to carbon efficiency of bacteria (Smith et al., 2021). Therefore, high temperature in Spring might indirectly affect the concentration and composition of total organic carbon in the water.

We also found that different bacterial phyla varied seasonally, e.g. *Bacteroidetes*. As the dominant bacterial phylum, the relative abundance of *Bacteroides* was higher in Spring samples than in Autumn samples. Previous research showed that *Bacteroidetes* might be more suitable for growth at the intermediate temperature (Wang et al. 2021a) in Spring rather than the low temperature in Autumn. Temperature variation would cause bacteria to adjust the transport and metabolism of amino acid and carbohydrate, which ultimately led to changes in bacterial community structure and behavior (Wang et al., 2021c). Available nutrients variation would significantly affect bacterial diversity and composition, especially on the dominant bacterial groups (e.g. *Proteobacteria*, *Actinobacteria*) (Wang et al. 2021b). In addition, seasonal variation in human activities might also have unpredictable influence on recreational waters.

Seasonal differences in environmental factors correlated with E. coli O157:H7 survival

Our data showed that EcO157 survival time (*ttd*) was longer in Spring samples (69.7 days) than in Autumn samples (59.8 days), both of which were longer than 30.1 days in well water from another research (Ding et al. 2018). Therefore, the risk of EcO157 transmission through recreational water was worth noting. Many studies have suggested that season variation was related to the infection of *E. coli* (Sarr et al. 2020). Research based on epidemiology revealed that *E. coli* outbreaks linked to green leafy vegetables had seasonal characteristics, with more cases in Spring and Autumn than other seasons (Marshall et al. 2020). Compared with dry season, the prevalence of *E. coli* was higher in the rainy season,

which might be due to climate conditions such as humidity and temperature (Desiree et al. 2021). Our results demonstrated that the reasons might be that different seasons had the differences in multiple environmental factors that affected the survival rate of EcO157 as discussed below.

Our results showed that most bacterial phyla (e.g. *Bacteroidetes*) were negatively correlated with *ttd* in Spring samples ($p < 0.05$). In spring samples, bacteria, especially dominant bacteria grew vigorously and demanded more resources. As an invading pathogen, EcO157 might have antagonism, predation and resource competition relationships with parts of dominant bacterial phyla (e.g. *Bacteroidetes*) (Semenov et al., 2007; van Elsas et al. 2007). These relationships weakened the ability of EcO157 in nutrients competition (Williams et al., 2012), leaving fewer niches for EcO157 to occupy, thus greatly inhibited its survival (Xing et al., 2019). Previous research found that EcO157 had many similarities with *GammaProteobacteria* in physiology and ecology and inhibited the persistence of EcO157 (Ma et al., 2013). It was reported that *GammaProteobacteria* showed negatively correlated with *ttd* (Han et al. 2021). However, in our study, *GammaProteobacteria* exhibited positively correlated with *ttd* in Spring samples, which was contrary to previous studies. The reason might be that the combination of environmental factors including the relative abundance of *GammaProteobacteria* might vary simultaneously, and such variation would have opposite effects on the EcO157 persistence (Stocker et al. 2019).

In Autumn samples, most bacterial phyla (e.g. *Actinobacteria*) were positively correlated with *ttd* ($p < 0.05$). Compared with Spring samples, the activity of the bacterial communities in Autumn samples dropped and the nutrient requirement decreased. EcO157 might benefit from nutrients released by certain bacteria during cell lysis and death (Fremaux et al., 2008; Maule, 2000). In addition, research showed that *Actinobacteria* had the ability to decompose high-molecular organic matter, which could provide the required low-molecular nutrients for EcO157 persistence (Ma et al., 2013). Therefore, the persistence of EcO157 was promoted due to increased resource availability and competition release (Xing et al., 2019), which explained why *ttd* was positively correlated with bacterial community in Autumn samples.

We found that the similarity between the Spring and Autumn samples was that EC exhibited an indirect positive effect on *ttd* through bacterial community. EC salinity was considered as a powerful factor affecting bacterial composition, diversity, richness and function (Rath and Rousk 2015). Previous studies showed that higher EC level directly inhibited the *ttd* of EcO157 (Ma et al. 2012). Because osmotic effect or certain ion toxicity of EC would interfere with EcO157 cell metabolism by suppressing necessary enzymes activity (Shabala et al. 2009). But EC level in water was much lower than that in soil, it had little direct inhibition on the EcO157 persistence. Dissimilarly, EC had a negative effect on the indigenous bacterial community in Spring samples, but a positive effect in Autumn samples. It was possible that not only the EC levels but also the chemical composition that made up EC had changed greatly between Spring and Autumn samples. In different habitats, the correlation between bacterial community and EC might show an obvious opposite trend due to the difference in the composition of EC (Stocker et al. 2019).

In Spring samples, TN displayed a significant positive effect on the persistence of EcO157, which was consistent with previous studies (Ma et al. 2012). A recent study demonstrated that NH_4^+-N had the ability to influencing the persistence of pathogens in well water (Li et al. 2018). But in our study, the correlation between NH_4^+-N and *ttd* was not observed in urban recreational waters. Previous studies have pointed out that dissolved organic nitrogen (DON) and NO_3^--N could contribute to EcO157 persistence (Franz et al. 2008). The composition of TN must be further investigated to ascertain which forms of nitrogen affect *ttd* of EcO157 in recreational waters.

In Autumn samples, TP exhibited an adverse effect on *ttd*. Surprisingly. TN showed no effect on *ttd*, although TN concentration was higher in Autumn samples than in Spring samples. Due to high TN concentration, TP might act as a limiting factor for microbial growth in recreational waters in Autumn samples. In addition, TP was negatively correlated with *Actinobacteria*. The partial negative impact of TP on *ttd* might be manifested in the inhibition of certain beneficial bacterial phyla that had positive effects on *ttd.*

When all 48 samples were considered, TOC was positively correlated with *ttd*. TOC was identified as an important factor in determining microbial growth, including the assimilable organic carbon (AOC) (Li et al. 2020) and dissolved organic carbon (DOC) (Vila-Costa et al. 2020), which are easy to be absorbed and utilized by microorganisms. It was established that AOC and DOC were both positively correlated with the persistence of EcO157 in sterile freshwater (Vital et al. 2008). It has been reported that higher levels of available carbon sources may relax biological competition for energy, thereby promoting the persistence of EcO157 (Franz et al. 2008). As one of the most important nodes of co-occurrence network, TOC was also crucial to indigenous bacterial community. When the available carbon sources are not enough for both EcO157 and indigenous bacterial community, the impact of TOC on *ttd* may be weakened due to competition.

It should be noted that some abiotic and biotic factors were not characterized, and their contribution to the overall seasonal variation on persistence of EcO157 could not be ignored. The biotic factors include, but not limited to fungal, algal, viral, protest communities, and even zooplankton, could exert significant influence on the persistence of EcO157 in urban recreational waters. The effect of the fungal community on the persistence of EcO157 has also been confirmed (Huang et al. 2020). The effect of algae on EcO157 persistence is also worth studying (Lin and Ju 2017). For abiotic factors, e.g. dissolved oxygen, should be considered when conducting EcO157 survival studies.

Conclusion

In summary, seasonal variation of EcO157 persistence in urban recreational waters was observed. EcO157 survived longer in Spring samples than in Autumn samples. Through multivariate statistics, it was found that EcO157 survival was jointly affected by physicochemical properties and bacterial community. In Spring samples, survival time of EcO157 was determined by EC, TN, *Proteobacteria* and

Bacteroidetes. In Autumn samples, survival time of EcO157 was affected by EC, pH, TP and *Actinobacteria*.

Declarations

Authors' contributions: Yuang Xie: methodology, formal analysis, and writing—original draft preparation; Liyue Zhu: formal analysis and writing—reviewing and editing; Guangze Lyu: methodology and writing—reviewing and editing; Lu Lu: methodology and writing—reviewing and editing; Jinhua Ma: writing—reviewing and editing; Jincai Ma: project administration, funding acquisition, resources, and writing—reviewing and editing. All authors read and approved the final manuscript.

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Compliance with ethical standards

Competing interests: The authors declare that they have no competing interests.

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Tables

Table 1 MANOVA of water properties and survival parameters with different lakes and seasons

Variables	Lake	Season	Lake× Season
NH_4^+-N	**0.001**	**0.001**	**0.025**
pH	0.464	**0.001**	**0.001**
EC	**0.001**	**0.001**	0.222
TOC	**0.006**	**0.001**	0.076
TN	0.050	**0.001**	0.809
TP	**0.001**	0.948	0.380
ttd	**0.001**	**0.019**	0.416
δ	0.362	**0.005**	0.864
p	0.465	0.506	0.309

NH_4^+-N, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; *ttd,* time needed to reach the detection limit; δ, scale parameter; p, shape parameter. Statistically significant numbers ($p < 0.05$) were bolded.

Table 2 Dissimilarity analysis of indigenous bacterial community between Spring and Autumn samples.

		mrpp		adonis		anosim	
		δ	p	F	p	R	p
Spring and Autumn samples	Bray-Curtis	0.5239	**0.001**	10.55	**0.001**	0.4651	**0.001**
	Horn	0.4115	**0.001**	9.4247	**0.001**	0.2739	**0.001**
	Gower	0.1830	**0.001**	8.8664	**0.001**	0.3858	**0.001**
	Jaccard	0.6759	**0.001**	8.2248	**0.001**	0.4651	**0.001**

Four distance measures of Bray-Curtis, Horn, Gower and Jaccard were used. Three distance tests of mrpp, adonis and ansim were calculated. Statistically significant numbers ($p < 0.05$) were bolded.

Table 3 Mantel and partial Mantel tests between *ttd* and environment factors for total 48 samples

	Bacterial community	NH_4^+-N (mg/L)	pH	EC (μS/cm)	TOC (mg/L)	TN (mg/L)	TP (mg/L)
Mantel	0.1265*	-0.0470	-0.0419	0.1486**	0.0890*	-0.0513	-0.0518
Partial Mantel	0.1046*	-0.0724	-0.0567	0.1230*	0.0833*	-0.0785	-0.0482

The first and second coordinates of principal coordinate analysis (PCoA) were used to represent bacterial community. NH_4^+-N, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; *ttd*, time needed to reach the detection limit. Asterisk meant the following: * $p < 0.05$, ** $p < 0.01$.

Table 4 Pearson correlation between *ttd* and relative abundances of bacterial taxa (Pearson's $p < 0.05$)

Taxa at phylum level	Relative abundance (%)	*ttd*	
		r	*p*
Spring season			
Proteobacteria	33.357	0.439	0.032
GammaProteobacteria	26.400	0.486	0.016
Bacteroidetes	26.242	-0.413	0.045
Chloroflexi	0.393	-0.414	0.047
Planctomycetes	0.018	-0.500	0.013
Latescibacteria	0.002	-0.497	0.047
Autumn season			
Actinobacteria	30.048	0.454	0.026
Gemmatimonadetes	0.289	0.519	0.011
Spirochaetes	0.021	0.529	0.008

Figures

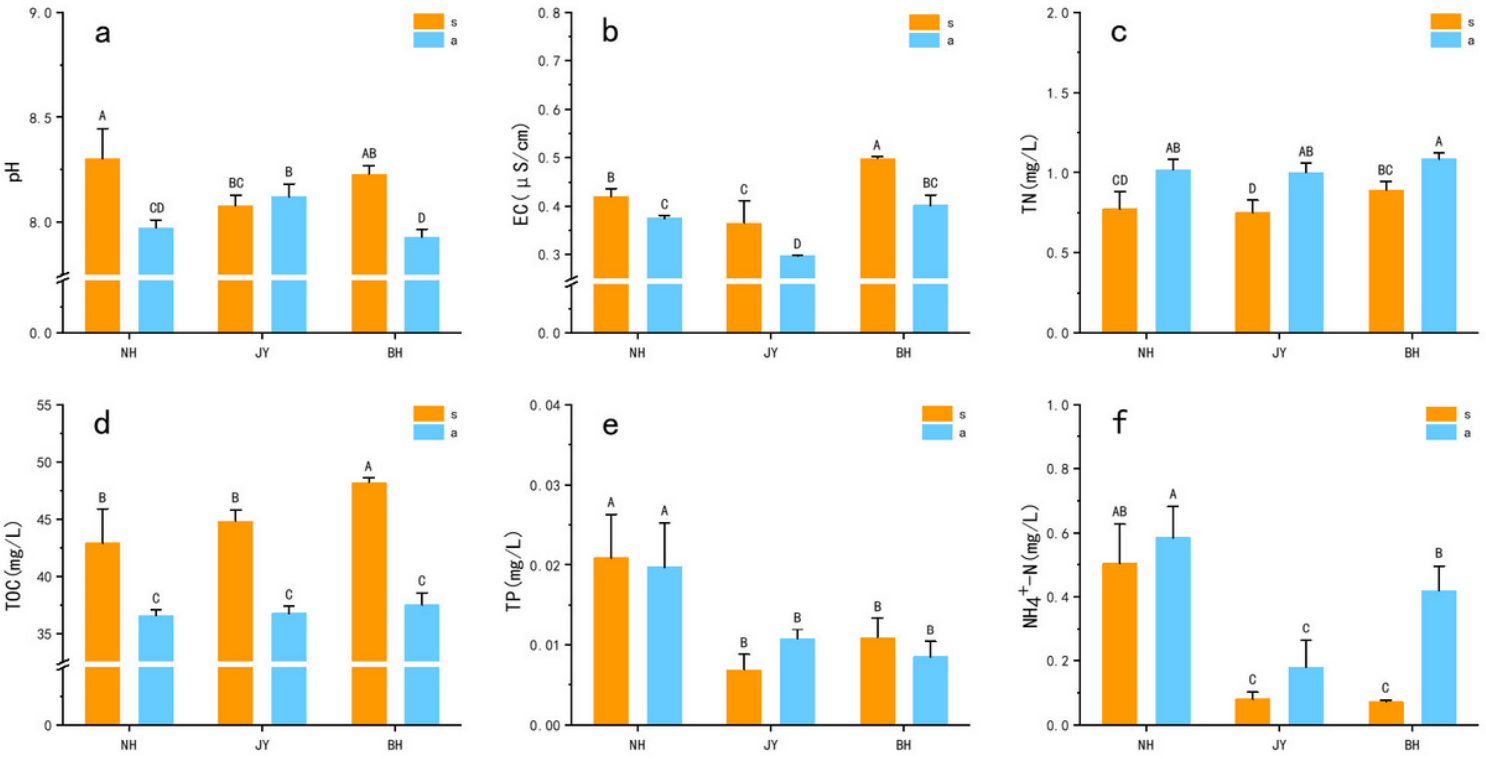

Figure 1

The variation of physicochemical properties in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY and BH represented Nanhu Lake, Jingyue Lake and Beihu Lake, respectively. NH4+-N, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus. Samples with different capital letters indicated significant difference at 0.05 level.

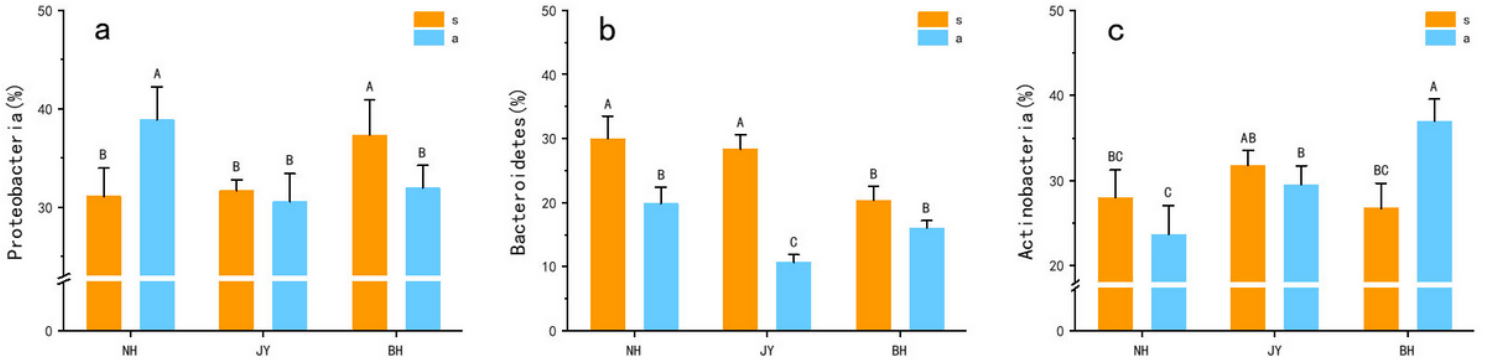

Figure 2

The variation of relative abundances of major bacteria at phylum level in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY and BH represented Nanhu Lake, Jingyue Lake and Beihu Lake, respectively. Samples with different capital letters indicated significant difference at 0.05 level.

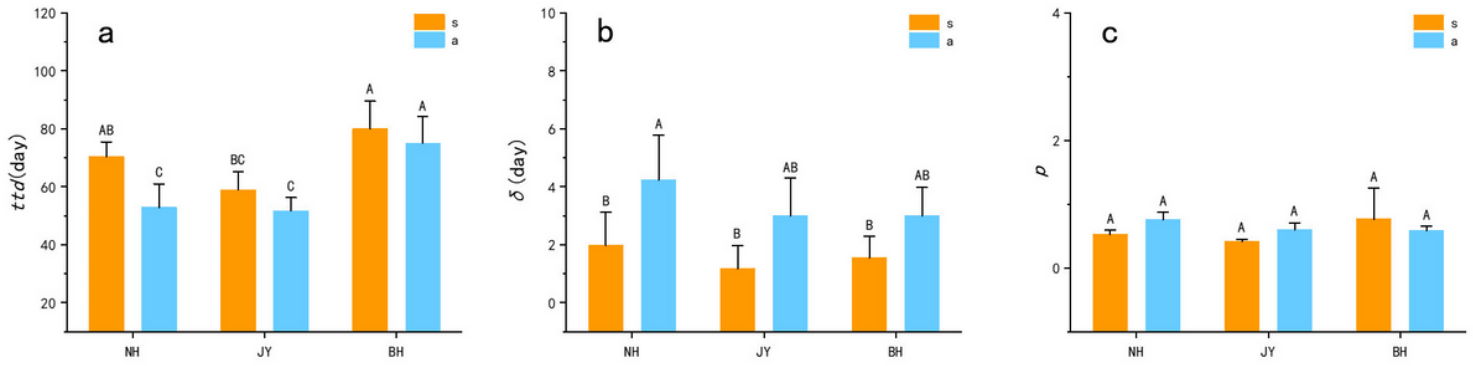

Figure 3

The variation of survival parameters (ttd, δ and p) of E. coli O157:H7 in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY and BH represented Nanhu Lake, Jingyue Lake and Beihu Lake, respectively. ttd, time needed to detection limit; δ, scale parameter; p, shape parameter. Samples with different capital letters indicated significant difference at 0.05 level.

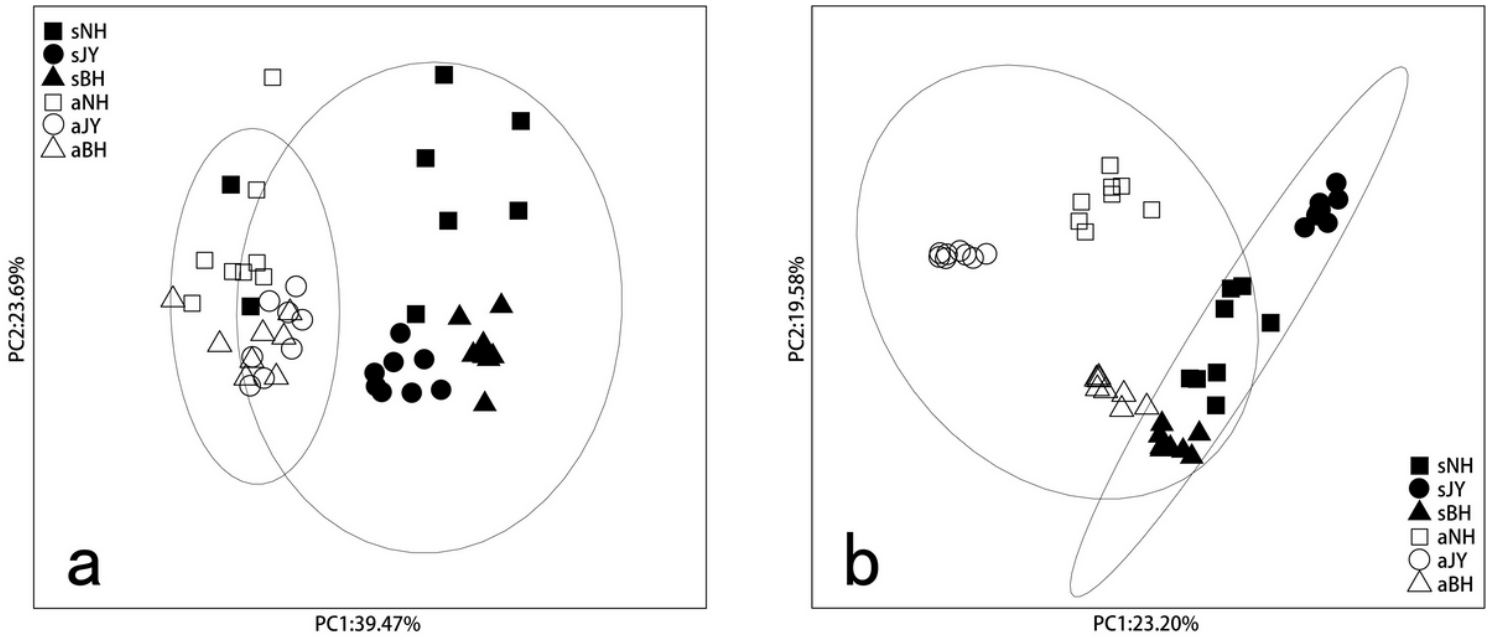

Figure 4

Principal component analysis (PCA) of physicochemical properties (a). The contributions of PC1 and PC2 were 39.47% and 23.69% (a). Principal coordinate analysis (PCoA) of bacterial community (b). The contributions of PC1 and PC2 were 23.20% and 19.58% (b). sNH, sJY and sBH represented Spring water samples from Nanhu Lake (NH), Jingyue Lake (JY), Beihu Lake, respectively. aNH, aJY and aBH represented Autumn water samples from Nanhu Lake (NH), Jingyue Lake (JY), Beihu Lake, respectively.

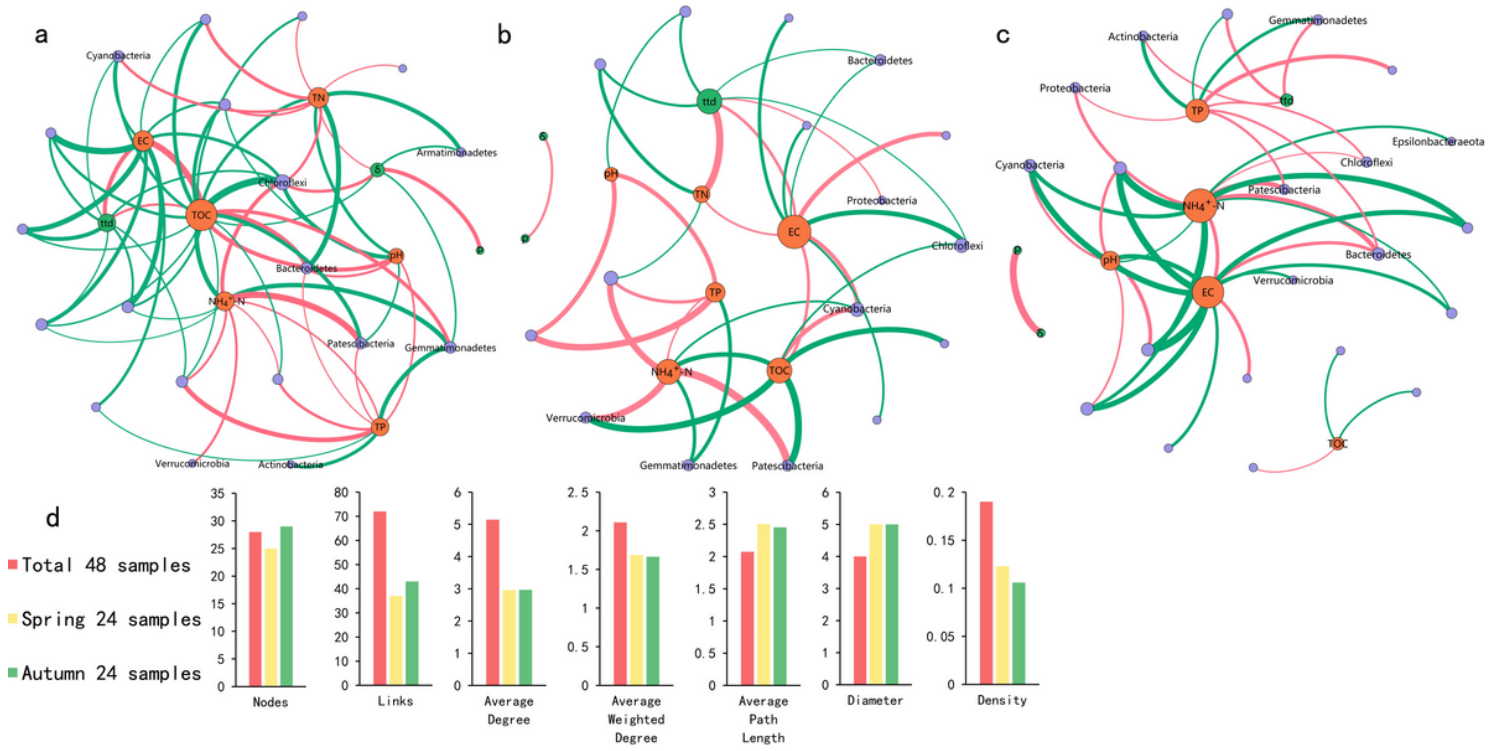

Figure 5

Co-occurrence network based on Pearson correlation ($p < 0.05$) was constructed. The network depicted the interactions of bacterial community (at phylum level), survival parameters, and water physicochemical properties. Correlations between bacteria were not considered. Bacteria phyla (relative abundance > 0.1%), physicochemical properties and survival parameters were labeled. The nodes of bacteria, physicochemical properties, and survival parameters were filled with purple, orange, and green, respectively. The size of the node was proportional to the degree of the node (sum of the weights of correlated links). The links were undirected and weighted. The thickness of link represented the absolute value of Pearson's correlation coefficient. Red link represented a positive correlation. Green link represented a negative correlation. ttd, time needed to reach detection limit; δ, time needed for first decimal reduction; p, shape parameter; EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; NH4+-N, ammonium nitrogen; TOC, total organic carbon. (a) total 48 samples, (b) Spring 24 samples, (c) Autumn 24 samples, (d) Nodes, links and topology parameters (average degree, average weighted degree, average path length, diameter and density).

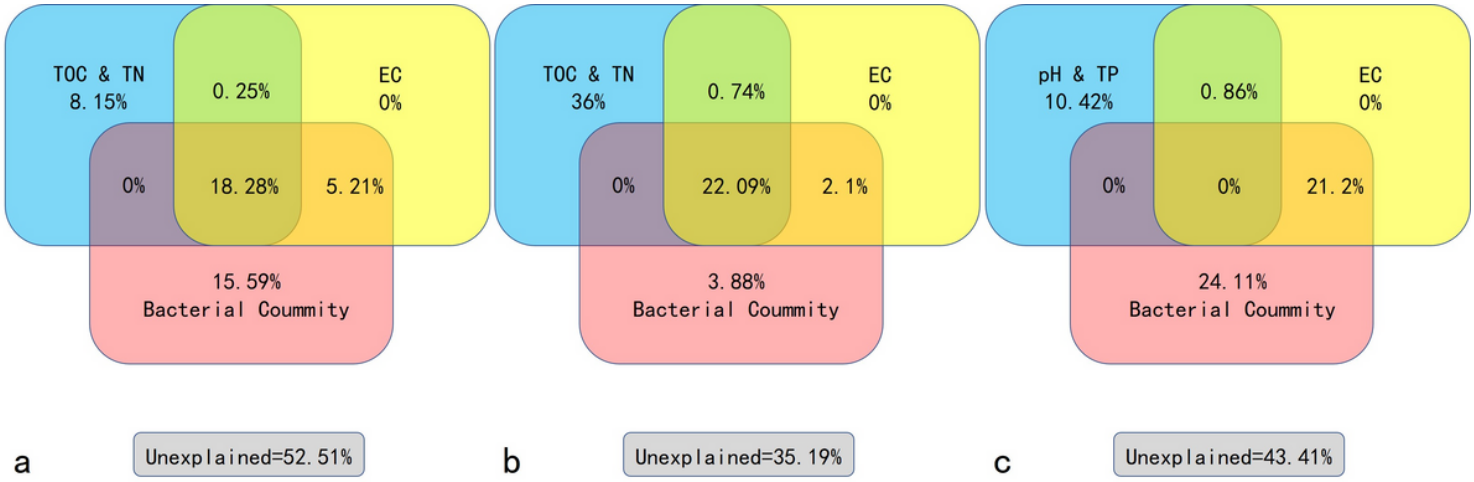

Figure 6

Variation partition analysis (VPA) was used to quantify the contribution of environmental factors to ttd variation of E. coli O157:H7. The first and second coordinates of principal coordinate analysis (PCoA) were used to represent bacterial community. The size of each area had no correlation with the contribution proportion. EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; TOC, total organic carbon. (a) total 48 samples, (b) Spring 24 samples, (c) Autumn 24 samples.

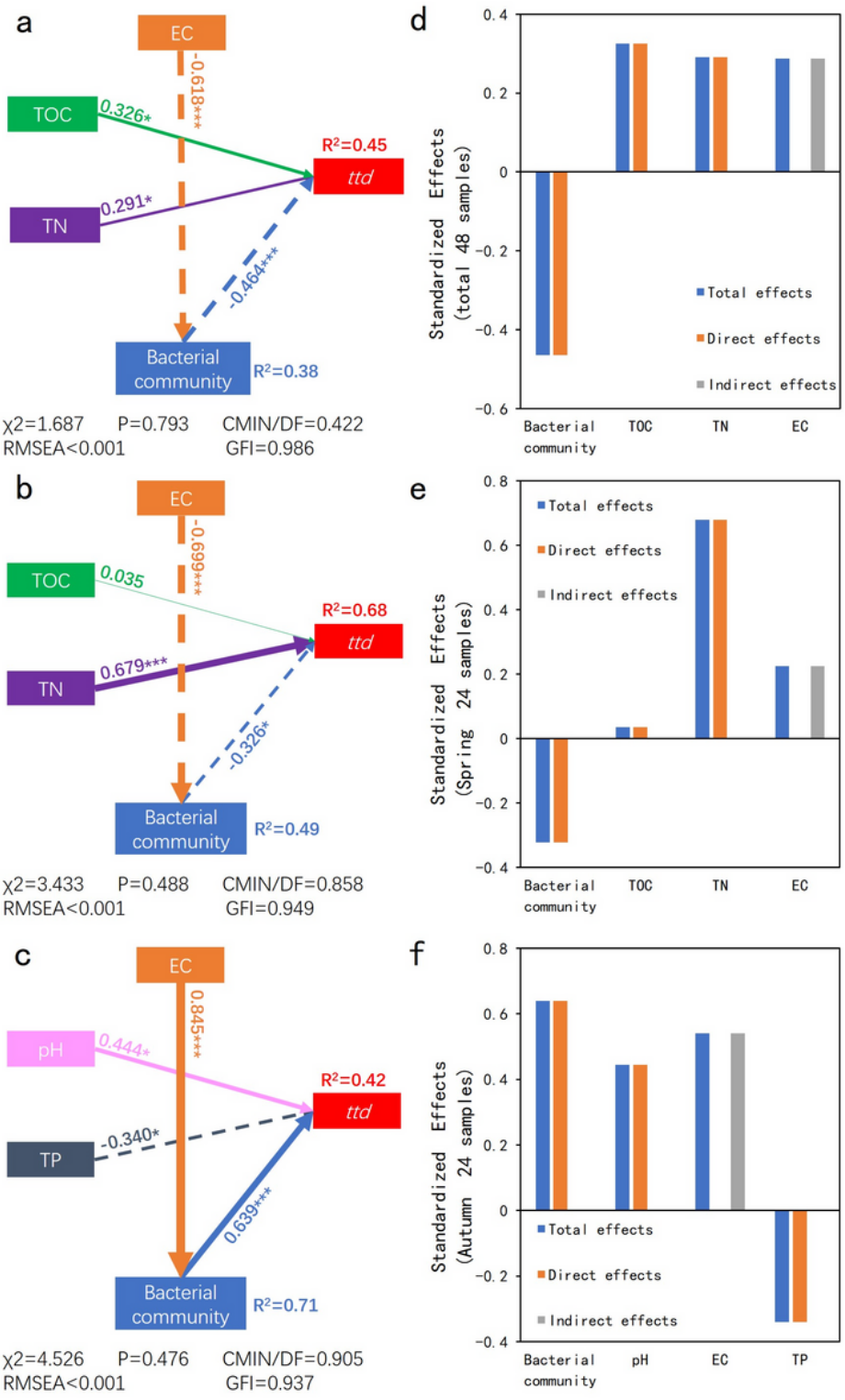

Figure 7

Structural equation model (SEM) was used to identify the direct and indirect effect of environmental factors on the ttd of E. coli O157:H7. The coordinates of principal co-ordinate analysis (PCoA) were used to represent bacterial community. The thickness of arrows represented the absolute value of the standard path coefficient (λ). The solid and dashed lines indicated the positive and negative path coefficients, respectively. R2 value represented the variance explanation of each environmental factor. The total effect

was the sum of direct and indirect effect. The standard path coefficient was labeled on the arrow and marked with significance level as followed: *($p < 0.05$) **($p < 0.01$) ***($p < 0.001$). $\chi 2$, chi-square; P, a p value for testing the hypothesis that the model fits perfectly in the population; CMIN/DF, ratio of $\chi 2$ and degrees of freedom; GFI, goodness of fit index; RMSEA, root mean square error of approximation. EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; TOC, total organic carbon. (a) total 48 samples, (b) Spring 24 samples, (c) Autumn 24 samples, (d) standardized effects of total 48 samples, (e) standardized effects of Spring 24 samples, (f) standardized effects of Autumn 24 samples.

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