University of Groningen

Soil pH and microbial diversity constrain the survival of E-coli in soil

Xing, Jiajia; Wang, Haizhen; Brookes, Philip C.; Salles, Joana Falcao; Xu, Jianming

Published in:
Soil biology & biochemistry

DOI:
[10.1016/j.soilbio.2018.10.013](https://doi.org/10.1016/j.soilbio.2018.10.013)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Xing, J., Wang, H., Brookes, P. C., Salles, J. F., & Xu, J. (2019). Soil pH and microbial diversity constrain the survival of E-coli in soil. *Soil biology & biochemistry*, 128, 139-149.
<https://doi.org/10.1016/j.soilbio.2018.10.013>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Soil pH and microbial diversity constrain the survival of *E. coli* in soil

Jiajia Xing^{a,b}, Haizhen Wang^{a,b}, Philip C. Brookes^{a,b}, Joana Falcão Salles^{c,**}, Jianming Xu^{a,b,*}

^a Institute of Soil and Water Resources and Environmental Science, College of Environment and Resource Sciences, Zhejiang University, Hangzhou 310058, China

^b Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Hangzhou 310058, China

^c Department of Microbial Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, 9747 AG Groningen, the Netherlands

ARTICLE INFO

Keywords:

E. coli O157:H7
Biotic drivers
Abiotic drivers
Community niche
Survival
Invasion

ABSTRACT

The presence of *Escherichia coli* (*E. coli*) leads to potential outbreaks of disease, demonstrating the importance of understanding how such organisms survive in secondary environments such as soil. Biotic and abiotic soil characteristics play a role in *E. coli* survival, but it remains unclear how these two aspects interact with survival and whether it is linked to toxin genes. Here we evaluated the survival of three *E. coli* O157:H7 strains: Shiga toxin-producing *E. coli*, its mutant without *stx* genes and strain without virulence genes in 4 distinct Chinese soils. To further disentangle the effects of microbial diversity, soils were manipulated to generate a gradient of microbial diversity (10^{-1} , 10^{-6} , and a sterile soil [γ -irradiated control]). Overall, our results showed the *E. coli* O157:H7 survival time decreased in all treatments, ranging from 8.23 ± 5.42 to 62.33 ± 35.80 days. The fastest decline was with the Shiga toxin-producing strain at 10^{-1} dilution, whereas the strain without virulence genes, persisted the longest 178 days in the γ sterilized control. These results confirmed the importance of biodiversity upon *E. coli* invasion and revealed virulence genes negatively influenced survival. The negative correlation between community niche or niche breadth of soil communities and survival, indicated that resource competition also was the major driver of *E. coli* O157:H7 survival. Moreover, path analyses revealed that soil pH exerted a critical role on the persistence of *E. coli* O157:H7, higher pH values produced longer survival time in each strain. These conclusions are of relevance for agricultural situations, where anthropogenic influences lead to decreased soil diversity, increased soil pH and resource input through manure application, which can potentially increase the survival time of *E. coli* O157:H7, the expanding the window of opportunity for food contamination.

1. Introduction

There is a general awareness that the fecal bacteria *Escherichia coli* O157:H7 can be transferred into vegetables, animals and cropland (Centers, 1999), leading to human outbreaks with many casualties (Bielaszewska et al., 2011). *E. coli* can persist in several environments e.g. sewage, plants, manure, fruit and vegetables, undercooked meats and unpasteurized milk, increases the risk of infection (Ferens and Hovde, 2011; Gagliardi and Karns, 2002; Ibekwe et al., 2006; van Hoek et al., 2013; Wang et al., 2014a). Soils represent a common denominator among these environmental sources of contamination, serving as a secondary habitat for *E. coli* (Fremaux et al., 2007; Mallon et al., 2015a).

Soils are thus constantly subjected to invasion by *E. coli*, whose

survival is driven by biotic and abiotic characteristics such as microbial community, temperature, oxygen, pH and soil texture. But it still remains unclear how these parameters interact with each other and whether *E. coli* survival is linked to the presence of toxin genes.

From the abiotic perspective, it is known that organic matter additions as manure, and low oxygen concentrations, improve persistence whereas low soil pH often has a deleterious effect on *E. coli* survival (Foppen and Schijven, 2006). Franz et al. (2005) showed that soil pH and the fiber content of manure accounted for the rates of decline of *E. coli* O157:H7. Jiang et al. (2002) also found *E. coli* O157:H7 persisted longer in soil amended with manure even under dry conditions, and higher pH changing under different cultivation temperatures favored the strain survival. From the biotic perspective, the indigenous soil microbial community plays a crucial role in controlling invasion—an

* Corresponding author. Institute of Soil and Water Resources and Environmental Science, College of Environment and Resource Sciences, Zhejiang University, Hangzhou 310058, China.

** Corresponding author. Department of Microbial Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, 9747 AG Groningen, the Netherlands.

E-mail addresses: j.falcao.salles@rug.nl (J.F. Salles), jmxu@zju.edu.cn (J. Xu).

<https://doi.org/10.1016/j.soilbio.2018.10.013>

Received 27 July 2018; Received in revised form 15 October 2018; Accepted 20 October 2018

Available online 24 October 2018

0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

effect that is greater when biodiversity is higher (Mallon et al., 2015b; van Elsas et al., 2012). The mechanisms behind this negative diversity-survival relationship are associated with resource competition (Mallon et al., 2015a) and antagonistic interactions with the indigenous microbial community (Moynihan et al., 2015).

The survival of *E. coli* in soils might also be regulated by strain-specific traits. For instance, strains with higher genotypic and phenotypic plasticity might cope better or adapt faster to suboptimal environmental conditions (Litchman, 2010; Mallon et al., 2015b). However, genes associated with biotic interactions might also play a role. For instance, the Shiga toxin is considered to be the major virulence factor in *E. coli*, leading, for example, to haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Brooks et al., 2005; Hauser et al., 2016; Scallan et al., 2011; Tarr et al., 2005; Zheng and Sadler, 2008). The extent to which the Shiga toxin can influence the persistence of *E. coli* in the environment remains unknown.

In this study, we aimed to separate how biotic and abiotic factors influence *E. coli* invasions in soil and to what extent the presence of Shiga toxin genes influence these patterns. We thus monitored the survival of 3 invading strains of *E. coli* O157:H7, carrying two or null Shiga toxin genes or null toxic genes, in four agricultural soils – each containing a gradient of microbial diversity induced by dilution by the extinction approach and varying in both biotic and abiotic parameters. We hypothesized that the diversity of the indigenous soil communities will be the major driver of survival, and that the response will vary according to soil type. We expected that the presence of Shiga toxin genes would increase soil survival, due to potential negative effects of this toxin on native soil microbial communities.

2. Material and methods

2.1. Soil collection and microcosms

The 4 soils were collected from 4 natural forestry sites across eastern China: Hainan laterite (HN), Wenzhou yellow soil (WZ), Nanjing yellow cinnamon soil (NJ) and Penglai cinnamon soil (PL). At each location, five soil cores were taken from 0 to 20 cm depth, sieved field moist > 2 mm and thoroughly mixed. Part of the soil was air-dried and analyzed for physical-chemical properties: soil pH, total nitrogen, soil texture, field capacity (soil water content at –33 kPa), dissolved organic carbon, fluvic acid and humic acid, soil organic carbon, Free Fe and Free Al (Agricultural Chemistry Committee of China, 1983). Soil water content at –33 kPa was measured with a pressure membrane apparatus (Soil Moisture Equipment Corp, Santa Barbara, CA, USA) as described by Richards (1949). The remaining soil was incubated at 4 °C and a portion sterilized by gamma irradiation (50 kGy) and stored in closed plastic bags, also at 4 °C. Fifty g portions of sterilized soil (oven-dry weight) were put into 100 ml sterile plastic bottles and the water contents adjusted to 100% soil water holding capacity (WHC) at –33 kPa with sterile deionized water.

2.2. Dilution to extinction experiment and inoculation

Each soil microcosm was inoculated with 1 ml soil solution (non-sterilized soil), prepared from 1:10 serially diluted soil in sterile water diluted to 10^{-6} . Thus, for each soil we generated 3 treatments, in triplicates: sterile soil (not inoculated), and 10^{-1} and 10^{-6} dilutions, creating a gradient of diversity. These were stored at 25 ± 1 °C for 30 days to permit the inoculums to colonize and establish stable microbial communities. At 30 days, 3 *E. coli* O157:H7 strains were introduced into the soils at a density of 10^7 CFU/g (oven-dry weight). The *E. coli* O157:H7 strains were: shiga toxin-producing *E. coli* strain EDL933 (ATCC43895) harboring all virulent genes (Shiga toxin-producing strain), shiga toxin mutant of strain *E. coli* O157:H7 EDL933 (Δ Stx₁₋₂ mutant, (Ma et al., 2011)) and non-virulent *E. coli* O157:H7 derivative strain T (Ritchie et al., 2003; Mallon et al., 2015a). Sterile water was

added to the control flasks, instead of cell suspensions. We adjusted the soil moisture to 100% of WHC at –33 kPa and then compensated for moisture loss every 5 days with sterilized deionized water.

We monitored the survival of *E. coli* O157:H7 at 0, 0.25, 1, 4, 8, 11, 15, 30, 50, 75, 90, 120 days after inoculation, by taking approximately 0.5 g soil from each flask, which was diluted in 0.1% peptone buffer (Lab M, Lancashire, UK). Serial dilutions were plated in SMAC-BCIG (sorbitol MacConkey-5-bromo-4-chloro-3-indoxyl- β -D-glucuronide) agar plates containing antibiotics (see Table 3 for strain-specific conditions) and incubated for 18 h at 37 °C. Survival data were fitted to the Weibull model (Ma et al., 2011; Zhang et al., 2013):

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

where p is the shape parameter, δ is the scale parameter, t is the time that *E. coli* O157:H7 survives in soil and N_t represents the cell number of strain remaining at time t , N_0 represents the initial inoculum concentration of the strain.

2.3. Metabolic potential of the soil communities

At 4 days after inoculation 5 g of soil samples (oven-dry weight) were diluted in 45 ml sterile physiological saline (0.85%, W/V), shaken for 1 h at 200 rpm at 25 ± 1 °C on an orbital shaker, followed by starvation for 30 min without shaking. We then transferred 1 ml of the soil suspension to 9 ml sterile saline water and serially diluted to 10^{-3} , from which 150 μ l was used to inoculate BiologTMEcoplate (BIOLOG Inc., Hayward CA., USA) with an 8-channel pipette (Fang et al., 2015). Carbon utilization metabolism of the soil samples was determined by using BiologTMEcoplate (BIOLOG Inc., Hayward CA., USA), consisting of 96 wells with 31 different carbon sources and one blank control in triplicate. The plates were inoculated in aerobic conditions at 25 ± 1 °C and color development measured in each well at 4, 24, 48, 72, 96, 120, 144, 168 and 192 h using a BIOLOG reader (BIO-TEK Instruments, Winooski, VT, USA) at 590 nm. The community niches were calculated from the sum of observed carbon sources utilized by strains in respective communities to calculate the community niches as described in Mallon et al., 2015a).

2.4. DNA extraction and sequencing of soil bacterial communities

Total soil DNA was extracted using a Fast DNA SPIN Kit for soil (Qbio-gene, Carlsbad, CA, USA), following the manufacturer's instructions, using 0.5 g of soil collected at days 0, 4, 40, 120. After the DNA was extracted, it was quantified by Nanodrop (Thermo Fisher Scientific, Wilmington, Delaware, USA). The DNA samples were then sent to Novogene (Beijing, China), where the V4-5 region of the 16S rRNA genes was amplified using the primers 515F: GTGCCAGCMGCGCGG TAA and 907R: CCGTCAATTCCTTTGAGTTT. The PCR products were sequenced using an Illumina HiSeq platform (250 bp paired-end reads).

2.5. PCR amplification and quantification of 16S rRNA genes

PCR was performed in 20 μ l reactions containing 10 μ l Premix Taq DNA Polymerase, 0.1 μ l forward primer (100 μ M), 0.1 μ l reverse primer (100 μ M), 8.8 μ l double distilled water (ddH₂O), and 1 μ l DNA template under the conditions of 95 °C for 5 min, 40 cycles of 94 °C for 30 s, at 54 °C for 30 s, and 72 °C for 40 s, and then completed by a final extension at 72 °C for 7 min. Quantitative PCR (qPCR) was performed with a real-time PCR detection system (Light Cycle 480; Roche). The bacterial quantified based on 16S rRNA gene using the primers 515F: GTGCCAGCMGCGCGGTAA and 907R: CCGTCAATTCCTTTGAGTTT. Each sample was prepared in three replicates and put in a 20 μ l reactions, containing 10 μ l SYBR Premix Mix (TaKaRa, Dalian, China), 0.2 μ l (50 μ M) forward and reverse primer, 1 μ l DNA template, and 8.6 μ l ddH₂O. Thermal conditions for 16S rRNA gene were set as follows:

5 min at 95 °C, 45 cycles of 10 s at 95 °C, 45 s at 53 °C, 45 s at 72 °C. Standard curves for qPCR were created using an up to 10-fold dilution series of PCR product containing a fragment with known 16S rRNA gene copy numbers.

2.6. Sequencing analyses

Sequences were analyzed using UPARSE software and filtering of noising sequencing. Also, the first sequences selected depended on their barcodes and were removed from further analysis if their length were shorter than 200 bp. Chimera checking was performed using UCHIME. All raw sequences were first analyzed and the Operational Taxonomic Unit (OTU) table was created by the UPARSE pipeline. OTUs were assigned from the effective sequences at 97% similarity. The phylogenetic tree was built using FastTree.

2.7. Statistical analyses

The survival of the three strains of *E. coli* O157:H7 in soils were measured using the Weibull model. One-way ANOVA test with diversity treatment and gradient was used to examine the different survival times of the three *E. coli* O157:H7 strains in the various soils. The survival time (td) was calculated from the correlation coefficient (r) between soil abiotic and biotic factors using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA). Path analysis between the strain survival and soil characteristics were also processed using SPSS 19.0, which uses linear stepwise regression after a normality test to calculate the path coefficient, including the direct and indirect path coefficients. 16S rRNA gene sequencing data were analyzed using UPARSE software to create the OTU table. Sequences with $\geq 97\%$ similarity were assigned to the same OTUs. A *de novo* chimera removal was performed using UCHIME (Edgar et al., 2011). Barcodes were removed from further analysis with lengths of less than 200 bp. Then, relevant relative abundance was calculated using R studio where phyloseq, Deseq2, ggplot2 was used. In addition, canonical analysis of principle coordinates (CAP) was obtained using the CAP method of the Phyloseq package. All of the correlation analyses in this study were also processed by R studio drawn by ggplot2.

3. Results

3.1. Total bacterial abundance

Data from qPCR revealed that the number of 16S rRNA gene copies ranged from 1.88×10^9 to 1.45×10^{10} copies/g soil in HN soil, 3.79×10^9 to 1.07×10^{10} copies/g soil in WZ soil, 1.03×10^{10} to 2.44×10^{10} copies/g soil in NJ soil, and 2.06×10^9 to 2.75×10^{10} copies/g soil in PL soil. All copy number did not significantly differ between treatments in all four soils (ANOVA, $P > 0.05$), indicating that soil bacterial communities in 10^{-1} and 10^{-6} dilution treatments had reached similar abundances at the onset of the inoculation of invader strains. These results indicated that differences between 10^{-1} and 10^{-6} dilution treatments are due to species diversity and composition rather than abundance.

3.2. Survival of the *E. coli* O157:H7 strains over time

The soils used for the microcosms differ greatly in their biological, physical and chemical properties (Fig. S1, Table 1), which led to different survival rates for the three *E. coli* O157:H7 strains (Fig. 1). Diversity significantly influenced all samples, with survival being highest in the sterile soil and lowest in soils inoculated with the 10^{-1} dilution (highest microbial diversity; Tukey's test, $P < 0.05$). The greatest effect (most rapid decline) was observed in HN and WZ soils regardless of the *E. coli* strains inoculated (survival time: HN 8.23 ± 5.42 days, WZ 9.39 ± 4.08 days; average $\pm$ standard error), probably due to their low soil pHs. The best soil for *E. coli* O157:H7 was PL (survival

Table 1
Soil physico-chemical characteristics of the four soils used in this study.

Sampling locations	Latitude (°N)	Longitude (°E)	pH	Dissolved organic C (g kg ⁻¹)	Clay (%)	Silt (%)	Sand (%)	Humic (mg kg ⁻¹)	Fulvic (mg kg ⁻¹)	Humic acid (mg kg ⁻¹)	Free Iron (mg kg ⁻¹)	Free Aluminum (mg kg ⁻¹)	Total N (g kg ⁻¹)	Soil organic content (g kg ⁻¹)
Hainan	19.74	109.71	4.30 $\pm$ 0.05	21.87 $\pm$ 1.95	42.96 $\pm$ 1.01	13.0 $\pm$ 0.50	41.6 $\pm$ 1.04	1.09 $\pm$ 0.09	4.76 $\pm$ 0.21	1.13 $\pm$ 0.09	102.98 $\pm$ 5.88	10.27 $\pm$ 0.26	1.43 $\pm$ 0.08	1.78 $\pm$ 0.20
Wenzhou	27.64	120.29	4.51 $\pm$ 0.05	37.50 $\pm$ 0.66	34.10 $\pm$ 1.00	34.6 $\pm$ 0.76	29.9 $\pm$ 0.50	0.27 $\pm$ 0.08	5.12 $\pm$ 0.33	0.23 $\pm$ 0.42	35.26 $\pm$ 1.63	8.05 $\pm$ 0.20	1.33 $\pm$ 0.09	1.33 $\pm$ 0.11
Nanjing	32.06	118.85	6.53 $\pm$ 0.06	27.23 $\pm$ 1.46	20.05 $\pm$ 0.50	46.8 $\pm$ 1.01	30.6 $\pm$ 1.00	1.88 $\pm$ 0.10	0.73 $\pm$ 0.12	1.99 $\pm$ 0.11	9.19 $\pm$ 0.72	1.16 $\pm$ 0.19	1.18 $\pm$ 0.08	1.35 $\pm$ 0.07
Pengtai	37.78	120.77	7.23 $\pm$ 0.12	104.05 $\pm$ 1.71	17.66 $\pm$ 1.00	23.9 $\pm$ 0.58	57.3 $\pm$ 1.15	1.19 $\pm$ 0.05	2.61 $\pm$ 0.25	1.26 $\pm$ 0.06	22.23 $\pm$ 1.03	2.75 $\pm$ 0.16	1.47 $\pm$ 0.06	1.32 $\pm$ 0.09

Table 2

The survival time of *E. coli* O157:H7 in four soils needed to reach the detection limit (td, days) described by statistical measures of Weibull model.

Soil type	Treatment		
	10^{-1}	10^{-6}	γ sterile
STEC HN soil	4.59 ± 0.13 ^{Aa}	5.51 ± 0.06 ^{Ab}	9.21 ± 0.48 ^{Ac}
STEC WZ soil	7.84 ± 1.00 ^{Aa}	12.33 ± 1.00 ^{Ab}	14.78 ± 1.54 ^{Ac}
STEC NJ soil	21.95 ± 0.63 ^{Aa}	34.65 ± 0.27 ^{Ab}	47.85 ± 0.68 ^{Ac}
STEC PL soil	25.06 ± 0.30 ^{Aa}	53.62 ± 1.11 ^{Ab}	65.37 ± 0.46 ^{Ac}
non virulent strain HN soil	12.59 ± 0.90 ^{Ba}	17.29 ± 1.16 ^{Bb}	15.54 ± 0.08 ^{Bc}
non virulent strain WZ soil	11.08 ± 0.34 ^{Ba}	15.01 ± 0.87 ^{Bb}	26.49 ± 0.79 ^{Bc}
non virulent strain NJ soil	85.35 ± 2.36 ^{Ba}	96.62 ± 0.84 ^{Bb}	120.47 ± 0.78 ^{Bc}
non virulent strain PL soil	88.82 ± 6.14 ^{Ba}	118.34 ± 2.38 ^{Bb}	178.28 ± 3.61 ^{Bc}
ΔStx STEC HN soil	4.22 ± 0.04 ^{Aa}	5.19 ± 0.43 ^{Ab}	4.49 ± 0.34 ^{Ca}
ΔStx STEC WZ soil	4.83 ± 1.19 ^{Ca}	5.25 ± 0.38 ^{Ca}	10.96 ± 1.01 ^{Cb}
ΔStx STEC NJ soil	28.97 ± 0.56 ^{Ca}	40.91 ± 0.71 ^{Cb}	73.77 ± 4.77 ^{Cc}
ΔStx STEC PL soil	29.30 ± 0.12 ^{Aa}	58.84 ± 0.27 ^{Cb}	83.75 ± 4.12 ^{Cc}

^Avalues with different letters within a column were significantly different ($p < 0.05$) between same soil of three strains; ^a Values with different letters within a row were significantly different ($p < 0.05$). 10^{-1} , 10^{-1} dilution treatment; 10^{-6} , 10^{-6} dilution treatment; γ sterile, γ sterile control treatment. STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; ΔStx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) ΔStx1–2 mutant.

time = 62.33 ± 35.80 days). Shiga toxin-producing strain was the poorest invader, lasting about 4–25 days at 10^{-1} dilution, 5–53 days at the 10^{-6} dilution and 9–65 days in the control (Fig. 1). The non virulent strain was the best survivor of the three tested, lasting 12–88 days at the 10^{-1} dilution, 17–118 days at the 10^{-6} dilution and 15–178 days in the γ sterilized control (Fig. 1). The survival dynamics of the three strains of *E. coli* O157:H7 in all soils, as described by the Weibull model, is given in Table 2.

3.3. Survival of the *E. coli* O157:H7 strains as a function of abiological soil properties

The soils inoculated with *E. coli* O157:H7 were subjected to correlation analyzes. Higher soil pH values produced longer survival time in each strain (Fig. 2). The same soil pH condition produced higher survival times in the low diversity communities. The γ sterilized control survived the longest and the other strains died quickest in high diversity community of 10^{-1} dilution. Overall, pH exerted a critical role on the persistence of *E. coli* O157:H7 in soils.

As outlined in Fig. S1, pH played an important role in the distribution of samples. In addition, the native soil communities varied from each other due to various soil types and properties. The HN and WZ soil distributed together on the left while the NJ and PL soil occurred on the right region of the CAP figure, in accord with the low pH

Table 3

Bacterial strains and antibiotics.

Strain	Antibiotics			
	Rifampin ($\mu\text{g mL}^{-1}$)	Nalidixic acid ($\mu\text{g mL}^{-1}$)	Kanamycin ($\mu\text{g mL}^{-1}$)	Chloramphenicol ($\mu\text{g mL}^{-1}$)
STEC	100	25	–	–
Non virulent strain	10	–	50	–
ΔStx STEC	–	–	50	25

STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain Tn5 lux CDAEB; ΔStx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) ΔStx1–2 mutant.

in the HN and WZ soil and observed shorter *E. coli* O157:H7 persistence time. Total nitrogen, soil organic C content, sand content and sampling time also influenced the distribution in varied degrees as showed in Fig. S1. Multiple regression analysis is presented in scatterplots Fig. S5 by selecting pH, total nitrogen (TN), soil organic content (soc) and sand content as four relevant factors for the correlation. The black border indicates that pH exerted a critical influence on the survival of *E. coli* O157:H7 strains, independently of dilution treatments or soil types.

3.4. Survival of the *E. coli* O157:H7 strains as a function of biological soil properties

To verify the importance of soil diversity on *E. coli* survival, we correlated the survival time of each strain and the bacterial diversity at day 0, for each soil, as determined by 16S rRNA gene sequencing. As expected, *E. coli* strain persistence in the soil was strongly and negatively correlated with bacterial richness (OTU numbers), indicating that the species richness gradient established in this study strongly and consistently affected strain persistence (negative diversity-invasion relationship; Fig. 3). The strongest negative effects were observed in soils PJ and NJ with higher pH, with all strains, indicating an interaction between soil pH and diversity. There was no significant difference between the 10^{-1} and 10^{-6} dilution treatments, although they varied in community composition (Fig. S1). Similar results were observed for the Shannon diversity index and phylogenetic diversity (Figs. S2 and S3).

It was previously shown that the negative effect of soil diversity on invaders survival can be explained by the amount of resources consumed by the native communities. In order to verify this hypothesis the niche breadth (number of carbon sources) used by native soil communities was measured using BIOLOG plates, to calculate the community niche of each treatment. Correlation analyzes of both measurements and survival time indicated a significant negative relationship with *E. coli* O157:H7 survival, pointing out the importance of resource competition in microbial invasions (Fig. 4). Thus, the higher the number of carbon sources used by the indigenous communities, the lower the number of resources that will be available for utilization by invasive *E. coli* strains. In addition, the slope of the regression analysis varied according to soil, the steepest slope being found with the PL soil. The amount of variance explained (R^2 values) by niche breadth on *E. coli* survival was often higher in the NJ and PL soils, regardless of the strain. The three strains responded similarly to the variation in niche breadth in each soil. The results from the community niche calculated based on the Biolog data were similar to those observed for niche breadth (Fig. S4), indicating that the total amounts of carbon sources utilized by the community also influence survival.

3.5. Effect of biotic and abiotic soil properties on *E. coli* survival

Given the interactions between biotic and abiotic properties, we used path analysis to separate their direct and indirect effects on the survival of the *E. coli* O157:H7 strains. We concentrated on the effect of soil properties on the three strains separately (Fig. 5A–C). The survival of Shiga toxin-producing strain was related to soil pH and number of carbon sources used by the indigenous microbial communities, Free Fe and the abundance of Proteobacteria (Fig. 5A). Among them, soil pH ($r = 0.855$) had positive effects whereas the number of carbon sources ($r = -0.260$), Free Fe concentrations ($r = -0.805$) and Proteobacteria abundance ($r = -0.072$) were negatively correlated with survival. With the non-virulent strain, soil pH ($r = 0.816$) showed direct positive effects on survival whereas the community niche ($r = -0.365$), Free Fe concentration ($r = -0.468$) and Acidobacteria abundance ($r = -0.510$) negatively influenced its survival (Fig. 5B). Regarding ΔStx strain, pH ($r = 0.827$) and community niche ($r = -0.299$) were the main factors, each positively or negatively influencing survival time (Fig. 5C). Overall, although path analyses were different for each of the three *E. coli* O157:H7 strains, soil pH was the main factor that was

Fig. 1. Progressive decline in the survival of three *E. coli* O157:H7 strains (CFU per gram of dry soil) across a gradient of species richness, in 4 different soils: HN (A), WZ (B), NJ (C) and PL (D). Gradient in richness was created through the dilution to extinction approach, by inoculating gamma irradiated (sterilized) soils with dilutions 10⁻¹ or 10⁻⁶. STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; Δ Stx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) Δ Stx1–2 mutant. Time d, days; the error bars are $\pm$ the standard errors; detection limit is 200 CFU/g soil. Triangles, sterilized soil; circles, dilution 10⁻⁶; square, dilution 10⁻¹.

positively related with survival, whereas the number of carbon sources or community niche was the other largest contributors, although in opposite direction to pH.

4. Discussion

The probability of *E. coli* outbreaks due to food contamination is directly linked to its survival in secondary environments such as soil, which is, in turn, dependent on soil biotic and abiotic characteristics (Gagliardi and Karns, 2002; Johannessen et al., 2005; Zhang et al., 2013). However, most studies have focused upon only one of the components (Franz et al., 2008; Mubiru et al., 2000), making it difficult to predict which factor plays the most important role in *E. coli* survival, and to what extent these results are dependent on *E. coli* O157:H7 strain. Here we provide extensive and concomitant analyses of the survival of 3 strains of *E. coli* O157:H7 in 4 different soils, each of which was manipulated by the dilution to extinction method to disentangle separate the effects of microbial diversity from soil abiotic factors.

4.1. Soil pH is the major abiotic driver of *E. coli* survival

Soil type is an important factor, directly or indirectly influencing the structure of microbial communities on the basis of various physico-chemical properties (Garbeva et al., 2004). For example, the relative abundance of the different type of soil particles (sand, different types of clay, and silt) might influence the attachment of microbial cells, which can ultimately influence the metabolism of these organisms as well as the different survival time of *E. coli* O157:H7 in soil (Liu et al., 2017). Different soils types also vary in chemical composition, such as the amount of dissolved organic carbon or pH, which are likely to influence the survival of *E. coli* O157:H7. To account for the soil effects, we performed the experiment in 4 different soils that were sampled from

various locations in China. In addition to differences in soil properties, these soils also contained different vegetation types, as cover vegetation varies with latitude and rainfall (Chokngamwong and Chiu, 2008; Suepa et al., 2016). Other studies indicated that vegetables or other crops can prolong the survival time of *E. coli* O157:H7 (Gagliardi and Karns, 2002; Islam et al., 2004).

Our results highlighted the overriding effect of pH over the other soil abiotic parameters in affecting *E. coli* survival. The 3 strains persisted for a shorter time in the HN laterite and WZ yellow soil than in the NJ yellow cinnamon soil and PL cinnamon soil. Other studies showed that *E. coli* O157:H7 declined faster in south-eastern than north-eastern China, signifying significantly longer persistence in natural and alkaline soils than in acidic ones (Wang et al., 2014a). Jiang et al. (2002) found that relatively higher soil pH might promote the survival of *E. coli* O157:H7 while Ma et al. (2012) found that pH may negatively affect the persistence of the strains in soils of pH value 6.7 and 8.0. Soil pH also influenced the persistence of *E. coli* O157:H7 in acidic, neutral or alkaline soils. Survival time decreased with decreasing pH in acidic soils but did not significantly change with pH in neutral or alkaline soils (Wang et al., 2014b). Possibly the various properties of the laterite soil we used differed. The pH of the HN laterite (about 4.36) in our work was much lower than their lateritic soils (about 5.97). This is in accordance with decreased survival time in the acidic soils after their pH was reduced artificially (Wang et al., 2014b). The HN laterite and WZ yellow soil pH values ranged from 4.30 to 4.51 while those in the NJ yellow cinnamon soil and PL cinnamon soil ranged from 6.53 to 7.23. Although our data was consistent across all different strains, the non virulent strain was more resistant to low pH indicating that the genetic load influences susceptibility to pH. Balamurugan et al. (2015) found that the survival of *E. coli* O157:H7 and six non-O157 STECs responded similarly to pH changes while water activity, temperature changes would not necessarily be valid for all serotypes.

Fig. 2. Persistence of *E. coli* O157:H7 STEC (A), non virulent strain (B) and Δ Stx STEC (C) in soils as a function of soil pH. Colors represent diversity treatments 10^{-1} , 10^{-6} and sterilized control. td, survival time (days). STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; Δ Stx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) Δ Stx1–2 mutant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.2. Soil bacterial diversity drives *E. coli* survival through resource competition

Previous studies reported a negative relationship between the persistence of *E. coli* O157:H7 in soil and microbial diversity (Mallon et al., 2015a; van Elsas et al., 2012), a result that was consistent with ours,

irrespective of strain or soil. Overall, we observed the highest survival in our γ sterilized control soils occurred in the 10^{-1} and 10^{-6} dilution treatments. When used as an explanatory variable in regression analyzes, bacterial richness (number of OTUs) was negatively correlated with the survival of the 3 strains in all soils, indicating that survival is strongly affected by bacterial richness. However, the slope of the

Fig. 3. Persistence of *E. coli* O157:H7 STEC (A), non virulent strain (B) and Δ Stx STEC (C) in soils: HN, WZ, NJ and PL as a function of soil OTU numbers. Colors represent soil samples HN, WZ, NJ and PL. td, survival time (days). STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; Δ Stx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) Δ Stx1–2 mutant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

regression line was steeper in PL and NJ at soil at pH > 6, indicating an interaction between the effects of pH and diversity (see next section). It has been proposed that the mechanism behind this negative diversity-invasion relationship is associated with resource availability. Recent studies on *Pseudomonas fluorescens* invasions suggest that the factors and mechanisms governing invasion of macro- and micro-organisms are similar, as increased disturbance led to increased invasion

success, possibly due to increased resource availability and competition release (Liu et al., 2012). In the case of *E. coli*, Mallon et al. (2015b) pointed that higher microbial diversity lead to an increase in resource competition, which in turn, suppressed colonization by invasive species. Also, the addition of carbon sources released the suppression, allowing the invasive species to increase in abundance. In order to verify whether the same mechanism was driving our diversity-invasion

Fig. 4. The survival of *E. coli* O157:H7 STEC (A), non virulent strain (B) and Δ Stx STEC (C) in soils is correlated with the niche breadth (number of carbon sources) of the native soil community. Colors represent soil samples HN, WZ, NJ and PL. td, survival time (days). Survival is measured as CFU/g soil. Niche breadth was calculated using Biolog plates, measured at the end of cultivation 120 days. STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; Δ Stx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) Δ Stx1–2 mutant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

relationship we applied two metrics, niche breadth and community niche, which provided an overview of the number of resources that a community can use and the overall potential of resource exploitation, respectively. Both metrics were negatively and significantly correlated with *E. coli* survival, in all soils and strains, leading to the conclusion that diverse communities exploit resources more efficiently, leaving fewer niches available for invading strains, causing their lower survival rates. Previous studies revealed bacterial strains with different functions, due to high diversity in the community, permitting more efficient exploitation of the environment, leaving decreased availability of

resources for invasive species (Eisenhauer et al., 2013). A recent study revealed the invasion of *E. coli* O157:H7 does relate to soil niche breadth (Mallon et al., 2018).

4.3. The presence of toxin genes is negatively correlated with *E. coli* survival in soils

Enterohemorrhagic *E. coli* (EHEC) strains are characterized by their unique toxic factors including the *eae* gene, which encodes Intimin, and *ehxA* genes which encodes for Enterogemolysin, as well as *stx* toxic

Fig. 5. Path analysis of *E. coli* O157:H7 STEC (A), non virulent strain (B) and Δ Stx STEC (C) indicating the effects of soil abiotic and biotic characteristics on the survival time (td) of three strains of *E. coli* O157:H7 in soils. STEC: *E. coli* O157:H7 EDL933 (ATCC43895); non virulent strain: *E. coli* O157:H7 derivative strain T; Δ Stx STEC: *E. coli* O157:H7 EDL933 (ATCC43895) Δ Stx1–2 mutant.

genes, especially those coding for the phage-borne proteins shiga toxins Stx1 and Stx2. The stx2 genes are traits of EHEC which are most often implicated in human diseases (Feng et al., 2001). The three strains of *E. coli* O157:H7 we used were native ones (Shiga toxin-producing strain), *E. coli* O157:H7 derivative strain T (non virulent strain) and *E. coli* O157:H7 Δ Stx1–2 mutant (Δ Stx strain), which represent a gradient of toxin genes – whereas Shiga toxin-producing strain has all the toxic

genes (*eae*, *stx*, *hlyA* and *rfbEO157*) and the non virulent strain have none, Δ Stx strain does not harbor *stx1* & *stx2* genes. The survival time (td) in all soils cultivated with non virulent strain were significantly different from Shiga toxin-producing strain and Δ Stx strain. Meanwhile, survival time between Shiga toxin-producing strain and Δ Stx strain exerted an obvious difference in WZ and NJ soil but not HN and PL soils. Although each of these strains persisted for different times in the

test soils, the survival time was much longer in the strain with no toxin genes, whereas the difference between Shiga toxin-producing strain and Δ Stx strain were smaller compared to the non virulent strain, which suggests that toxin genes, except the Shiga toxin gene, may be related to survivability. Similar survival times have been observed for Shiga toxin positive and negative *E. coli* O157 strains (Bolton et al., 1999), which is in accordance with Ma et al. (2011), who showed that the possession of Shiga toxins and Intimin in *E. coli* O157:H7 might not play an important role in its persistence in soils. Our results not only corroborate the idea that Shiga toxin genes do not provide a competitive advantage in certain soils like HN and PL, but also indicated the presence of other toxin genes can influence the survival of *E. coli* O157:H7. Thus, they could represent a genetic burden that might reduce bacterial fitness, given that the non virulent strain, which does not possess toxic genes, lived significant longer than the other two strains. Shiga toxin genes played an important role in the persistence of *E. coli* O157:H7 in WZ and NJ soils for example. Shiga toxin genes can affect the survival time of *E. coli* O157:H7 depending on the soil type. In addition to the presence of toxin genes, the specific characteristics of other strains influenced survival, as indicated by the different correlations with soil biotic and abiotic properties (see next section).

4.4. The relationship between biotic and abiotic soil drivers and their effect on *E. coli* O157:H7 survival

The survival time of *E. coli* O157:H7 cultivated under anaerobic conditions is much longer compared to aerobic conditions – whereas the changes in temperature have a bigger impact on the survival time than changes in oxygen concentrations (Semenov et al., 2010). There are many other factors which also affect the fate of *E. coli* O157:H7 such as physical and chemical characteristics of soil, weather or atmospheric conditions, biological interactions and agricultural and livestock management practices. The fate of such enteric pathogens is also affected by biotic, abiotic, edaphic and climatic factors in different geographical regions (Ongeng et al., 2015). Variations in bacterial characteristics of the strains cause variations of persistence when invaded into soil. For example, animal and clinical human *E. coli* O157:H7 isolates revealed that phenotypic diversity generates various colonization abilities, acid resistance and toxic production (Franz et al., 2011). Our study also found that *E. coli* O157:H7 strains carrying different toxic genes survived differently, which demonstrated that these toxic genes provide considerable pathogen survival in soil. In the sections above we discussed the major abiotic and biotic drivers of *E. coli* survival in soil. However these parameters interact and also influence *E. coli* persistence in soils. In order to disentangle these interactions we used path analysis, which was performed for each strain separately, taking into account all 4 soils. In each case, soil pH and carbon availability, either through the niche breadth or community niche, were the major positive and negative drivers, respectively. Interestingly, the strains with lower survival time were less affected by pH than non virulent strains, which survived the longest. The effects of these factors on Δ Stx strain were less than the other two and were only related to pH and community niche. This supports our hypothesis that if *E. coli* O157:H7 lost their toxic genes, its survival time would increase in soil sand, or, the interaction with indigenous microbes would change. Bacteria in natural communities compete for space and resources with their neighbors and can suppress and kill introduced species and the interactions between or within bacterial species affect the results of invasion and competition (Hibbing et al., 2010).

In conclusion, this study showed that both soil pH and the amount of available resources in soils are important drivers of *E. coli* O157:H7 survival, regardless of the number of toxin genes. Nevertheless, non-virulent *E. coli* O157:H7 strains survived longer than those carrying virulent genes. These results are of relevance for areas under agricultural production where the addition of manure can promote soil invasion by pathogenic *E. coli*, potentially leading to disease outbreaks.

In this context, in soils with low pH, the use of crops that are adapted to these conditions might reduce the risk of contamination. Also, anthropogenic disturbances that lead to an increase in nutrient output in soils or resource pulses might promote invasions by changing resource competition between native microbial communities and invasive species (Li and Stevens, 2012).

Acknowledgments

We thank Tianyue Qin, Xiu Jia, Shanshan Sun and Kankan Zhao for their help in laboratory and with data analyses. This work was supported by the National Key Research and Development Program of China (2016YFD0800207), the National Natural Science Foundation of China (41721001) and KNAW-CSC grant Joana Falcao Salles and Jianming Xu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2018.10.013>.

Supplementary materials related to this article includes details about CAP analyses during Shiga toxin-producing *E. coli* invasion; the relationships between *E. coli* survival and soil shannon index, phylogenetic diversity as well as community niche; pairwise relationships among *E. coli* survival time and some abiotic properties. All of these showed in Figs. S1–S5.

References

- Balamurugan, S., Ahmed, R., Gao, A., 2015. Survival of Shiga toxin-producing *Escherichia coli* in broth as influenced by pH, water activity and temperature. *Letters in Applied Microbiology* 60, 341–346.
- Bielaszewska, M., Mellmann, A., Zhang, W., Koeck, R., Fruth, A., Bauwens, A., Peters, G., Karch, H., 2011. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolyticuraemic syndrome in Germany, 2011: a microbiological study. *The Lancet Infectious Diseases* 11, 671–676.
- Bolton, D.J., Byrne, C.M., Sheridan, J.J., McDowell, D.A., Blair, I.S., 1999. The survival characteristics of a non-toxicogenic strain of *Escherichia coli* O157: H7. *Journal of Applied Microbiology* 86, 407–411.
- Brooks, J.T., Sowers, E.G., Wells, J.G., Greene, K.D., Griffin, P.M., Hoekstra, R.M., Strockbine, N.A., 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *Journal of Infectious Diseases* 192, 1422–1429.
- Centers, F.D.C.U., 1999. Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington county fair - New York, 1999. *Morbidity and Mortality Weekly Report* 48, 803–804.
- Chokngamwong, R., Chiu, L.S., 2008. Thailand daily rainfall and comparison with TRMM products. *Journal of Hydrometeorology* 9, 256–266.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200.
- Eisenhauer, N., Schulz, W., Scheu, S., Jousset, A., 2013. Niche dimensionality links biodiversity and invasibility of microbial communities. *Functional Ecology* 27, 282–288.
- Fang, H., Lian, J., Wang, H., Cai, L., Yu, Y., 2015. Exploring bacterial community structure and function associated with atrazine biodegradation in repeatedly treated soils. *Journal of Hazardous Materials* 286, 457–465.
- Feng, P., Dey, M., Abe, A., Takeda, T., 2001. Isogenic strain of *Escherichia coli* O157: H7 that has lost both Shiga toxin 1 and 2 genes. *Clinical and Diagnostic Laboratory Immunology* 8, 711–717.
- Ferens, W.A., Hovde, C.J., 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathogens and Disease* 8, 465–487.
- Foppen, J., Schijven, J.F., 2006. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research* 40, 401–426.
- Franz, E., Semenov, A.V., Termorshuizen, A.J., de Vos, O.J., Bokhorst, J.G., van Bruggen, A.H.C., 2008. Manure-amended soil characteristics affecting the survival of *E. coli* O157: H7 in 36 Dutch soils. *Environmental Microbiology* 10, 313–327.
- Franz, E., van Diepeningen, A.D., de Vos, O.J., van Bruggen, A., 2005. Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar typhimurium in manure, manure-amended soil, and lettuce. *Applied and Environmental Microbiology* 71, 6165–6174.
- Franz, E., van Hoek, A.H.A.M., Bouw, E., Aarts, H.J.M., 2011. Variability of *Escherichia coli* O157 strain survival in manure-amended soil in relation to strain origin, virulence profile, and carbon nutrition profile. *Applied and Environmental Microbiology* 77, 8088–8096.
- Fremaux, B., Delignette-Muller, M.L., Prigent-Combaret, C., Gleizal, A., Vernozy-Rozand, C., 2007. Growth and survival of non-O157: H7 Shiga-toxin-producing *Escherichia coli* in cow manure. *Journal of Applied Microbiology* 102, 89–99.
- Gagliardi, J.V., Karns, J.S., 2002. Persistence of *Escherichia coli* O157: H7 in soil and on

- plant roots. *Environmental Microbiology* 4, 89–96.
- Garbeva, P., van Veen, J.A., van Elsas, J.D., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* 42, 243–270.
- Hauser, E., Bruederle, M., Reich, C., Bruckbauer, A., Funk, J., Schmidt, H., 2016. Subtilase contributes to the cytotoxicity of a Shiga toxin-producing *Escherichia coli* strain encoding three different toxins. *International Journal of Food Microbiology* 217, 156–161.
- Hibbing, M.E., Fuqua, C., Parsek, M.R., Peterson, S.B., 2010. Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews Microbiology* 8, 15–25.
- Ibekwe, A.M., Shouse, P.J., Grieve, C.M., 2006. Quantification of survival of *Escherichia coli* O157: H7 on plants affected by contaminated irrigation water. *Engineering in Life Science* 6, 566–572.
- Islam, M., Morgan, J., Doyle, M.P., Jiang, X.P., 2004. Fate of *Escherichia coli* O157: H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. *Journal of Food Protection* 67, 574–578.
- Jiang, X.P., Morgan, J., Doyle, M.P., 2002. Fate of *Escherichia coli* O157: H7 in manure-amended soil. *Applied and Environmental Microbiology* 68, 2605–2609.
- Johannessen, G.S., Bengtsson, G.B., Heier, B.T., Bredholt, S., Wasteson, Y., Rorvik, L.M., 2005. Potential uptake of *Escherichia coli* O157: H7 from organic manure into crisphead lettuce. *Applied and Environmental Microbiology* 71, 2221–2225.
- Li, W., Stevens, M.H.H., 2012. Fluctuating resource availability increases invasibility in microbial microcosms. *Oikos* 121, 435–441.
- Litchman, E., 2010. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecology Letters* 13, 1560–1572.
- Liu, M., Bjornlund, L., Ronn, R., Christensen, S., Ekelund, F., 2012. Disturbance promotes non-indigenous bacterial invasion in soil microcosms: analysis of the roles of resource availability and community structure. *PloS One* 7.
- Liu, X., Gao, C., Ji, D., Walker, S.L., Huang, Q., Cai, P., 2017. Survival of *Escherichia coli* O157:H7 in various soil particles: importance of the attached bacterial phenotype. *Biology and Fertility of Soils* 53, 209–219.
- Ma, J., Ibekwe, A.M., Crowley, D.E., Yang, C., 2012. Persistence of *Escherichia coli* O157:H7 in major leafy green producing soils. *Environmental Science & Technology* 46, 12154–12161.
- Ma, J., Ibekwe, A.M., Yi, X., Wang, H., Yamazaki, A., Crowley, D.E., Yang, C., 2011. Persistence of *Escherichia coli* O157:H7 and its mutants in soils. *PloS One* 6.
- Mallon, C.A., Poly, F., Le Roux, X., Marring, I., van Elsas, J.D., Salles, J.F., 2015a. Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. *Ecology* 96, 915–926.
- Mallon, C.A., Roux, X., Doorn, G.S., Dini-Andreote, F., Poly, F., Salles, J.F., 2018. The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche. *The ISME Journal* 12, 728.
- Mallon, C.A., van Elsas, J.D., Salles, J.F., 2015b. Microbial invasions: the process, patterns, and mechanisms. *Trends in Microbiology* 23, 719–729.
- Moynihan, E.L., Richards, K.G., Brennan, F.P., Tyrrel, S.F., Ritz, K., 2015. Enteropathogen survival in soil from different land-uses is predominantly regulated by microbial community composition. *Applied Soil Ecology* 89, 76–84.
- Mubiru, D.N., Coyne, M.S., Grove, J.H., 2000. Mortality of *Escherichia coli* O157: H7 in two soils with different physical and chemical properties. *Journal of Environmental Quality* 29, 1821–1825.
- Ongeng, D., Geeraerd, A.H., Springael, D., Ryckeboer, J., Muyanja, C., Mauriello, G., 2015. Fate of *Escherichia coli* O157:H7 and *Salmonella enterica* in the manure-amended soil-plant ecosystem of fresh vegetable crops: a review. *Critical Reviews in Microbiology* 41, 273–294.
- Richards, L.A., 1949. Methods of measuring soil moisture tension. *Soil Science* 68, 95–112.
- Ritchie, J.M., Campbell, G.R., Shepherd, J., Beaton, Y., Jones, D., Killham, K., et al., 2003. A stable bioluminescent construct of *Escherichia coli* O157:H7 for hazard assessments of long-term survival in the environment. *Applied and Environmental Microbiology* 69, 3359–3367.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases* 17, 7–15.
- Semenov, A.V., Franz, E., van Bruggen, A.H.C., 2010. COLIWAVE a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil. *Ecological Modelling* 221, 599–609.
- Suepa, T., Qi, J., Lawawirojwong, S., Messina, J.P., 2016. Understanding spatio-temporal variation of vegetation phenology and rainfall seasonality in the monsoon Southeast Asia. *Environmental Research* 147, 621–629.
- Tarr, P.I., Gordon, C.A., Chandler, W.L., 2005. Shiga-toxin-producing *Escherichia coli* and haemolyticuraemic syndrome. *Lancet* 365, 1073–1086.
- van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the United States of America* 109, 1159–1164.
- van Hoek, A.H.A.M., Aarts, H.J.M., El, B., van Overbeek, W.M., Franz, E., 2013. The role of *rpoS* in *Escherichia coli* O157 manure-amended soil survival and distribution of allelic variations among bovine, food and clinical isolates. *FEMS Microbiology Letters* 338, 18–23.
- Wang, H., Zhang, T., Wei, G., Wu, L., Wu, J., Xu, J., 2014a. Survival of *Escherichia coli* O157:H7 in soils under different land use types. *Environmental Science and Pollution Research* 21, 518–524.
- Wang, H.Z., Wei, G., Yao, Z.Y., Lou, J., Xiao, K.C., Wu, L.S., Wu, J.J., Xu, J.M., 2014b. Response of *Escherichia coli* O157:H7 survival to pH of cultivated soils. *Journal of Soils and Sediments* 14, 1841–1849.
- Zhang, T., Wang, H., Wu, L., Lou, J., Wu, J., Brookes, P.C., Xu, J., 2013. Survival of *Escherichia coli* O157:H7 in soils from Jiangsu Province, China. *PloS One* 8.
- Zheng, X.L., Sadler, J.E., 2008. Pathogenesis of thrombotic microangiopathies. *Annual Review of Pathology: Mechanisms of Disease* 249–277.