



Survival and transfer potential of *Salmonella enterica* serovar Typhimurium colonising polyethylene microplastics in contaminated agricultural soils

Luke Woodford¹ · Rosie Fellows¹ · Hannah L. White¹ · Michael J. Ormsby¹ · Chloe J. Pow¹ · Richard S. Quilliam¹

Received: 8 April 2024 / Accepted: 22 July 2024
© The Author(s) 2024

Abstract

Agricultural environments are becoming increasingly contaminated with plastic pollution. Plastics in the environment can also provide a unique habitat for microbial biofilm, termed the ‘plastisphere’, which can also support the persistence of human pathogens such as *Salmonella*. Human enteric *Salmonella enterica* serovar Typhimurium can enter agricultural environments via flooding or from irrigation with contaminated water. Using soil mesocosms we quantified the ability of *S. Typhimurium* to persist on microplastic beads in two agriculturally relevant soils, under ambient and repeat flood scenarios. *S. Typhimurium* persisted in the plastisphere for 35 days in both podzol and loamy soils; while during multiple flood events was able to survive in the plastisphere for up to 21 days. *S. Typhimurium* could dissociate from the plastisphere during flooding events and migrate through soil in leachate, and importantly could colonise new plastic particles in the soil, suggesting that plastic pollution in agricultural soils can aid *S. Typhimurium* persistence and facilitate further dissemination within the environment. The potential for increased survival of enteric human pathogens in agricultural and food production environments due to plastic contamination poses a significant public health risk, particularly in potato or root vegetable systems where there is the potential for direct contact with crops.

Keywords Flooding · Human pathogens · Leachate · Plastic pollution · Plastisphere · Soil quality

Introduction

Annual global plastic production exceeds 300 million tonnes, and over 32% of all plastic waste ends up in the terrestrial environment (Kumar et al. 2020; Rillig and

Lehmann 2020). The release of plastics into terrestrial environments is four to 23 times higher than in marine environments (Horton et al. 2017), yet the majority of research focuses on the impact of plastics in marine environments (Omeyer et al. 2022). Microplastics (1 µm–5 mm in size) originate from either a primary source, e.g. cosmetic products, or from secondary sources via fragmentation of larger plastics such as plastic mulching sheets used in agriculture (Zhou et al. 2023). Plastic polymers such as polyethylene (PE), polystyrene (PS), and polyvinylchloride (PVC) can enter soil systems from contaminated irrigation or flood-water, agricultural practices (e.g. mulching films and seed dressings), windblown from landfill sites, or contamination of water systems (Kumar et al. 2020; Ren et al. 2021; Steinmetz et al. 2016; Zhang et al. 2020). Livestock frequently ingest microplastics found in feed and feed bags, which subsequently enter agronomic systems via excrement, or via the application of organic fertilisers, manure, or composts (Beriot et al. 2021; Wu et al. 2021; Yang et al. 2021). Livestock faeces can also carry a range of zoonotic pathogens (including *Salmonella* spp. and *Escherichia coli*), which are

Responsible Editor: Diane Purchase

Highlights

- *S. Typhimurium* can persist on plastics in podzol and loamy soils for > 35 days.
- Flooding can lead to dissociation of *S. Typhimurium* from the plastisphere.
- *S. Typhimurium* can migrate through soil and colonise new plastic particles.
- Plastics facilitate pathogen persistence and dissemination in agricultural soils.

✉ Luke Woodford
luke.woodford@stirling.ac.uk

¹ Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK

capable of colonising microplastics (Ormsby et al. 2024a, b, c; 2023).

Once in the environment, plastics rapidly become colonised by microbial biofilms comprising complex microbial communities in a habitat known as the ‘plastisphere’ (Zettler et al. 2013). The plastisphere supports a diverse community of organisms shaped by the specific stressors of the external environment (Basili et al. 2020), and often differs from the surrounding microbial community (Martínez-Campos et al. 2021; McCormick et al. 2014). Soil directly surrounding plastics has been termed the ‘microplastisphere’ and provides a microbial habitat unique from both the rhizosphere and the surrounding bulk soil (Zhou et al. 2021). Differences in microbial community structure and diversity in bulk soil and around the plastisphere are influenced by the surface area, size, and type of plastic polymer, and the level of hydrophobicity (Zhu et al. 2022). Once in soil systems, microplastics can alter the composition of microbial communities (Li and Xiao 2023), leading to altered nutrient availability and carbon and nitrogen cycling. Importantly, the plastisphere commonly contains human pathogens, including viruses, bacteria, and fungi (Gkoutselis et al. 2021; Metcalf et al. 2022; Moresco et al. 2021), although the associated risks with human health are still debated (Beloe et al. 2022). The effects of microplastics in edaphic systems have thus far only focused on the impacts on physicochemical soil properties and changes to soil microbial communities (Ma et al. 2023; Zhang et al. 2020); however, little is known about the capacity of microplastics to act as vectors for human pathogens in soil (Quilliam et al. 2023; Zhu et al. 2022).

Human pathogenic *Salmonella* serovars, such as *Salmonella enterica* serovar Typhimurium, can cause systemic infections and accounts for ca. 594,000 cases and ca. 79,000 deaths a year (‘Global Burden of Disease Study’ 2019). *Salmonella* Typhimurium can enter soil systems via organic fertilisers and animal manure, flooding events, or via contaminated irrigation water; survival and persistence is subsequently influenced by soil type and fertiliser (Jechalke et al. 2019; Pornsukarom and Thakur 2016). It has recently been demonstrated that virulent strains of *S. Typhimurium* can persist on the surface of plastic bags in waste piles (Ormsby et al. 2024a), although how this relates to survival in other environments, particularly soil and agricultural systems, where there are clear pathways for human exposure, is unknown. Therefore, in this study, we aimed to (1) quantify the persistence of *S. Typhimurium* on low-density polyethylene (LDPE) microplastic beads in two contrasting agricultural soil types; (2) quantify the subsequent transfer potential of *S. Typhimurium* from colonised microplastics in simulated floodwater to uncolonised microplastics in soil; and (3) determine the potential for vertical transfer of *S. Typhimurium* from contaminated microplastics through the soil horizon as soil leachate.

Materials and methods

Soil and water

Two typical agricultural soil types (a podzol and a loamy soil) were selected based on their contrasting pH profiles. Both soils were dried at room temperature and sieved to 4 mm before being used. The pH and electrical conductivity (EC) of each soil were measured in distilled water (5:1 v:w) using a portable probe (Hanna Instruments Ltd., UK). Moisture content was calculated by oven drying at 105 °C and total organic matter was estimated from loss on ignition (LOI) using a Carbolite muffle furnace. Total nitrogen (N) and total carbon (C) were determined using a Flash smart NC-org -elemental analyser (Thermo Fisher, UK) (Table S1).

River water was collected from the Allan Water (Bridge of Allan, Scotland) in plastic carboys. Prior to use, river water and soils were screened for the presence of chloramphenicol resistant bacteria on Luria–Bertani (LB) agar (Invitrogen, UK) with chloramphenicol (25 mg/mL), with no growth detected after 24 h at 37 °C (data not shown).

Biofilm generation and inoculation of plastic and glass beads

Low-density polyethylene (LDPE) plastic beads (Goodfellow, UK) and glass beads (Hecht Karl, Germany), both with a diameter of 4 mm, were placed in spherical stainless steel metal cages (45-mm high, 38-mm diameter, 1-mm pore size; Golf, China) and added to replicate glass tanks containing 7 L river water with additional trace metals (added as a 1 mL/L solution of CuCl₂·2H₂O, 15 mg/L; NiCl₂·H₂O, 25 145 mg/L; Na₂MoO₄·2H₂O, 25 mg/L; ZnCl₂, 70 mg/L; MnCl₂·4H₂O, 100 mg/L; CoCl₂·6H₂O, 120 146 mg/L; FeCl, 4 g/L; EDTA, 2 g/L; HCl [25%], 6.5 mL/L) (Eguchi et al. 1996) and aerated continuously using a pond air pump (Swell, UK). The tanks were covered with black plastic to prevent evaporation and left at ambient room temperature (ca. 18–21 °C) for 72 h to allow a natural biofilm to develop.

The pathogen used in all experiments in this study was an isolate of chloramphenicol-resistant *Salmonella enterica* serovar Typhimurium D23580 (hereby referred to as *S. Typhimurium*), which prior to use was grown in LB broth at 37 °C with shaking at 120 rpm, unless otherwise stated. An overnight culture of *S. Typhimurium* was centrifuged at 4000 rpm for 5 min, resuspended in 20 mL PBS, and added into each replicate glass tank (to give a final concentration of ca. 2.8×10^5 CFU/mL). After 96 h, all the LDPE and glass beads were removed from the tanks and

lightly rinsed in Milli-Q water (Millipore Milli-Q Synthesis System, USA) to remove lightly attached microbes not part of the plastisphere, and then weighed prior to being used in mesocosms.

Design of soil mesocosms to quantify *S. Typhimurium* persistence

Replicate sterile glass mesocosm jars (total volume 150 mL) contained 120 g of either podzol or loam soil and 3 g (2.5% w/w) of either plastic or glass beads inoculated with *S. Typhimurium*. Subsequently, 25 mL of river water was added to each mesocosm and the contaminated beads thoroughly mixed into the soil. An *S. Typhimurium*-only ‘culture control’ was made from an overnight culture diluted 1:100 in fresh, pre-warmed LB broth (to 37 °C), and grown to an approximate OD_{600nm} of 0.2. Twenty millilitres of this diluted culture was added to 2 L of river water to give a final concentration of ca. 10^6 CFU/mL. From this, 25 mL was added to each ‘culture-control’ mesocosm containing 120 g soil, thoroughly mixed, and subsequently used as *S. Typhimurium*-only controls (Fig. 1A). All mesocosms were stored in the dark at room temperature (18–21 °C), with their lids loosely attached.

At each sampling time point (1, 2, 3, 4, 7, 10, 14, 21, 28, and 35 days), sterile forceps were used to remove all of the plastic and glass beads from the soil (four replicate mesocosms at each time point), and placed into individual sterile 30-mL glass universal tubes (one for each replicate mesocosm), mixed with 10 mL of Milli-Q water, inverted five times (to remove loosely adhering bacteria), and the liquid discarded; a fresh 5 mL of Milli-Q water was then added to each tube. For the ‘culture control’, 3 g of the soil

was removed from each mesocosm and mixed with 5 mL Milli-Q water. Universal tubes containing LDPE, glass, or culture control samples were vortexed for 5 min at 1500 rpm to disrupt the biofilm. The Milli-Q water was then serially diluted in PBS and plated out on LB chloramphenicol media, incubated overnight at 37 °C and *S. Typhimurium* colony forming units (CFU) enumerated.

Soil flooding and the subsequent transfer of *S. Typhimurium* from colonised plastic and glass beads

To understand the potential for plastic and glass particles to facilitate the survival and transfer of *S. Typhimurium* through flooded soils, two different mesocosm experiments were designed. Firstly, the effect of floodwater moving down through a soil column containing *S. Typhimurium*-contaminated plastic or glass particles was quantified in replicate mesocosms (plastic pipes, 10 cm × 6.5 cm, with 3 × 3 mm holes in the base, sterilised with 70% EtOH before use; Fig. 1B) containing 200 g of soil and 5 g (2.5% w/w) of LDPE or glass beads (colonised with a natural biofilm and inoculated with *S. Typhimurium* as described above) and mixed with 25 mL of river water. Replicate ‘culture-only control’ (no plastic or glass beads) mesocosms consisted of an overnight culture of *S. Typhimurium* added to 25 mL river water and mixed with the soil. Stainless steel wire mesh (0.125-mm mesh size; Amazon, UK) was placed around the base of each mesocosm to retain the soil but allow the leachate to flow through (Fig. 1B). At each designated time point (1, 2, 3, 4, 7, 10, 14, and 21 days), 50 mL of river water was added to the top of each pipe and the leachate collected in a Petri dish. Mesocosms were typically left for approx. 4 h to allow sufficient time for leachate to run through. Viable

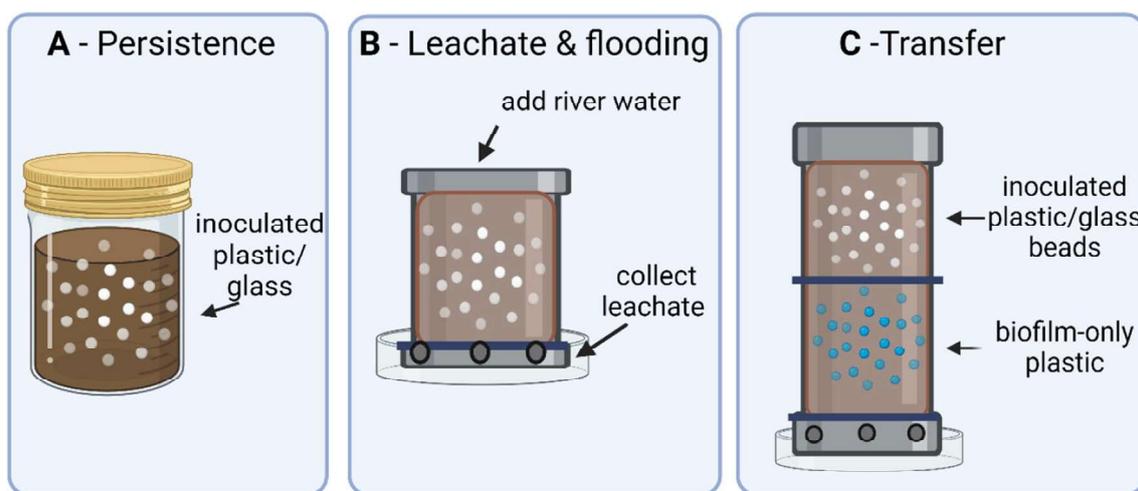


Fig. 1 Schematic of the different experimental designs. **A** Persistence of *S. Typhimurium* on plastic and glass beads in different soil types; **B** flooding and soil leachate collection; **C** transfer of *S. Typhimurium*

from inoculated plastic or glass beads to biofilm-only plastics through a soil column flooded with river water

cells of *S. Typhimurium* in the leachate were subsequently enumerated on LB chloramphenicol media as described above.

Secondly, we quantified whether *S. Typhimurium* could be transferred from *S. Typhimurium*-colonised plastic and glass beads through soil and colonise new plastic beads. To achieve this, mesocosms were designed whereby the top compartment contained plastic or glass beads (10 g equal to 5% w/w) colonised by a natural biofilm and contaminated by *S. Typhimurium* (10^4 – 10^5 CFU/g) or a ‘culture-only control’ (culture mixed with 200 g of soil). The bottom compartment of each mesocosm contained 200 g of soil mixed with 5 g (equal to 2.5% w/w) of LDPE plastic beads colonised by a natural biofilm (generated as described above). The two halves of each column were partitioned using a stainless-steel mesh (0.125-mm pores) to retain the soil but allow the transfer of liquid; all mesocosms were positioned in Petri dishes to collect leachate (Fig. 1C). At each time point (1, 2, 3, 4, 7, 10, and 14 days), 50 mL of river water was poured through four replicate columns for each treatment in each soil type (24 total) and the leachate was discarded. At 7 and 14 days, the columns were disassembled and the LDPE and glass beads in the top compartment, and the plastic beads in the bottom compartments were carefully removed with sterile forceps. For the ‘culture-only control’, 5 g of soil was removed from the top of each replicate mesocosm. The concentration of *S. Typhimurium* was determined as described above. The same weight of soil (5 g) was removed from the bottom compartment and analysed by the same method (data not shown).

PCR confirmation of *S. Typhimurium*

PCR of the *ttr* gene (encoding tetrathionate reductase; *ttr*_for: 5'-CTCACCAGGAGATTACAACATGG-3'; *ttr*_rev: 5'-AGCTCAGACCAAAAGTGACCATC-3' (Hopkins et al. 2009)) was conducted on a selection of colonies throughout the study, with all samples testing positive for *Salmonella*. Briefly, reactions consisted of a 2 × master mix (Qiagen, Germany), 0.4 μM forward and reverse primer, and a single colony, made to a final volume of 25 μL using nuclease-free water. Cyclic conditions of 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min were run for 30 cycles, with an initial 5 min at 95 °C and final extension of 72 °C for 5 min. All samples were analysed by 1% gel electrophoresis.

Statistical analyses

Differences in *S. Typhimurium* concentrations between time points in the simulated soil flooding mesocosm experiment were assessed by one-way analysis of variance (ANOVA) and a Tukey post-hoc multiple comparison test (GraphPad Prism Software v. 9.5.1). To calculate *S. Typhimurium*

die-off rates, CFU concentrations were normalised by transforming to \log_{10} CFU/mL or \log_{10} CFU/g. Linear regression analysis, carried out in Minitab version 18 (Minitab Inc.; State College, PA, USA), was used to describe the dynamics of *S. Typhimurium* die-off as previously described (Ormsby et al. 2024c, 2024a). Briefly, a log linear regression model was fitted to the \log_{10} transformed data, which is described by the equation

$$\text{Log}_{10}(C) = \text{Log}_{10}(C_0) - kt$$

where C_0 is the cell concentration at $t=0$ and k is a die-off rate constant (day⁻¹).

The percentage decrease in *S. Typhimurium* concentration per unit time was determined as constant using the log linear model. Following a log-linear die-off profile, decimal reduction times (D -values; the number of days to reduce viable bacteria by 90%) were calculated based on the decline rates for each population. Analysis of variance (ANOVA) was used to assess the effect of different material types on K values, and Tukey post-hoc tests were used for comparing means. Pearson correlation coefficients were used to investigate the linear correlation between the K value and D value from the linear decline rate analysis for each material. From this, 95% confidence ellipses were calculated and generated using R (Kassambara 2020; R Core Team 2021).

Results

Persistence of *S. Typhimurium* in the plastsphere of two different soil types

S. Typhimurium was able to persist on the surface of both plastic and glass beads in podzol and loam soil. After 35 days, the concentration of *S. Typhimurium* persisting on the plastic beads was higher in both soil types compared to the glass, with 5×10^4 CFU/g (58% of the original inoculum) and 1×10^3 CFU/g (0.9% of the original inoculum) on plastic beads in the podzol and loam, and 2×10^3 CFU/g (1.1% of the original inoculum) and 1×10^2 CFU/g (0.06% of the original inoculum) on glass beads in the podzol and loam (Fig. 2). In the mesocosms containing ‘culture-only control’, no colonies were recovered in the podzol soil from 7 days onward, although *S. Typhimurium* was still detectable at 4×10^1 CFU/g (0.0001% of the original inoculum) after 35 days in the loam soil.

Log linear regression models were used to determine the linear decline rate constants (K) and decimal reduction times (D -values) of *S. Typhimurium* colonising plastic and glass beads, or in the culture-only control. Both the soil type and the type of colonised material influenced the rate of decline, with *S. Typhimurium* D -values of 49.5 and 22.3 for podzol

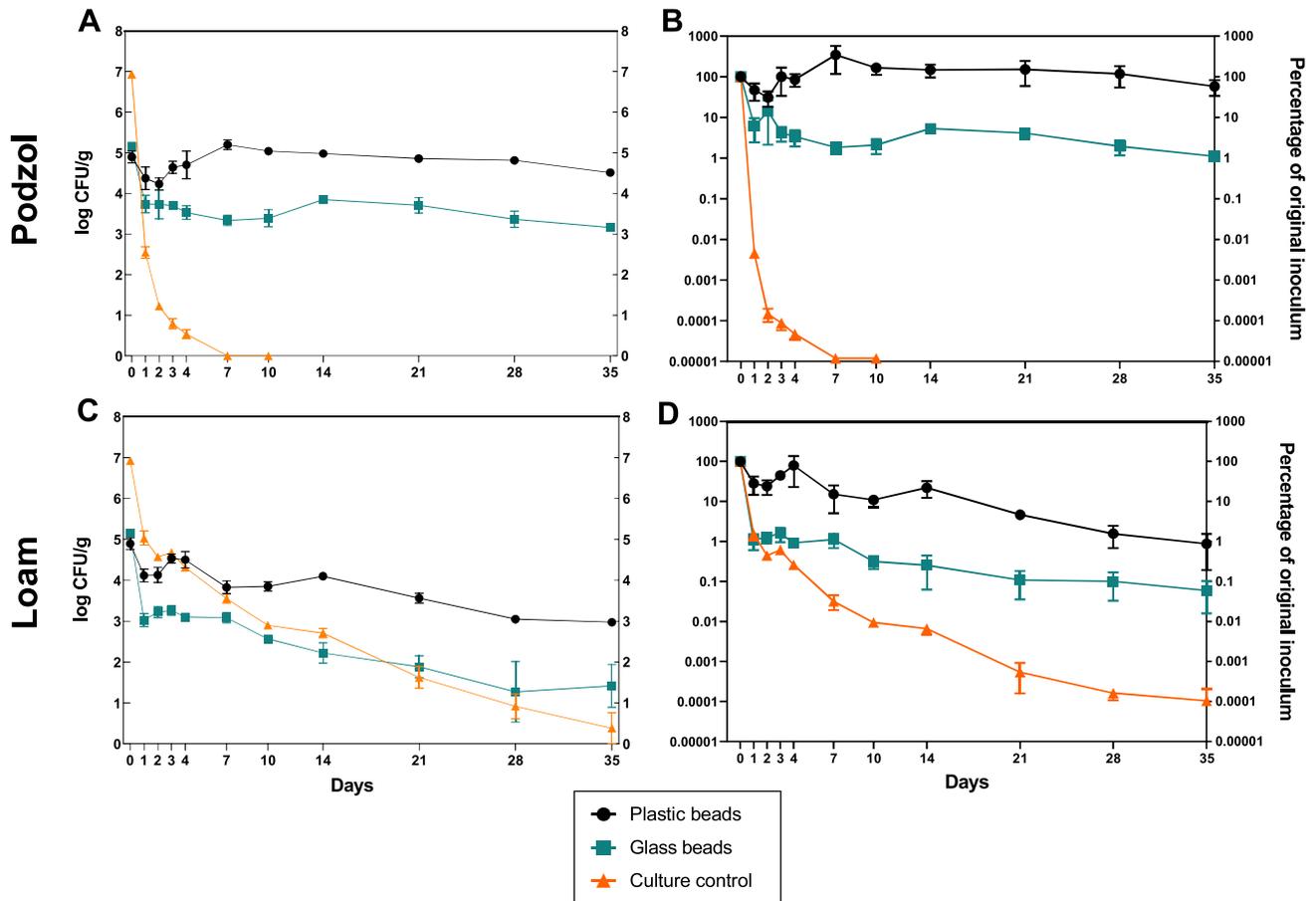


Fig. 2 Persistence of *S. Typhimurium* on plastic and glass beads in different soil types. *S. Typhimurium* was enumerated from the surfaces of plastic beads and glass beads, and from a ‘culture-only control’ recovered from podzol (A, B) and loam (C, D) soils. Persistence

is presented as the log CFU/g (A, C) and the percentage survival compared to the starting inoculum (B, D). Data points represent the mean ($n=4$) \pm SEM

and loam, respectively, on the plastic beads, compared to *D*-values of 0.7 and 6.1 for the ‘culture-only control’ in podzol and loam, respectively (Table S2). The *K* value was significantly higher ($P < 0.0001$) for *S. Typhimurium* in both soil types for the ‘culture-only control’ compared to the *S. Typhimurium* concentration on plastic or glass, indicating a more rapid rate of decline when not associated with a biofilm on plastic or glass beads (Fig. S1).

Persistence of *S. Typhimurium* on plastic and glass beads after flooding

Persistence of *S. Typhimurium* bound to plastic and glass beads or in ‘culture controls’ mixed into the soils was quantified after repeatedly flooding each mesocosm with river water. There was no difference in the concentration of *S.*

Typhimurium recovered from the surface of the plastic beads in the podzol between days 7 and 21; although there was a significant decrease in *S. Typhimurium* on the plastic beads between days 14 and 21 in the loam soil ($P < 0.01$) (Fig. 3A and D). In the ‘culture only’ control, the concentration of *S. Typhimurium* decreased significantly at each time point in the podzol ($P < 0.001$) and decreased between 14 and 21 days in the loam soil ($P < 0.001$) (Fig. 3C and F). On the plastic beads there was a lower linear decline *K* value and higher *D* value for *S. Typhimurium* in both soil types compared to the glass beads or the ‘culture control’ (Table S3). There was a significant difference in *K* values ($P < 0.0001$) for *S. Typhimurium* concentration in the ‘culture control’ and the concentration of *S. Typhimurium* on inoculated glass and LDPE beads, with a strong negative Pearson correlation coefficient between *K* and *D* values for all samples (Fig. S2).

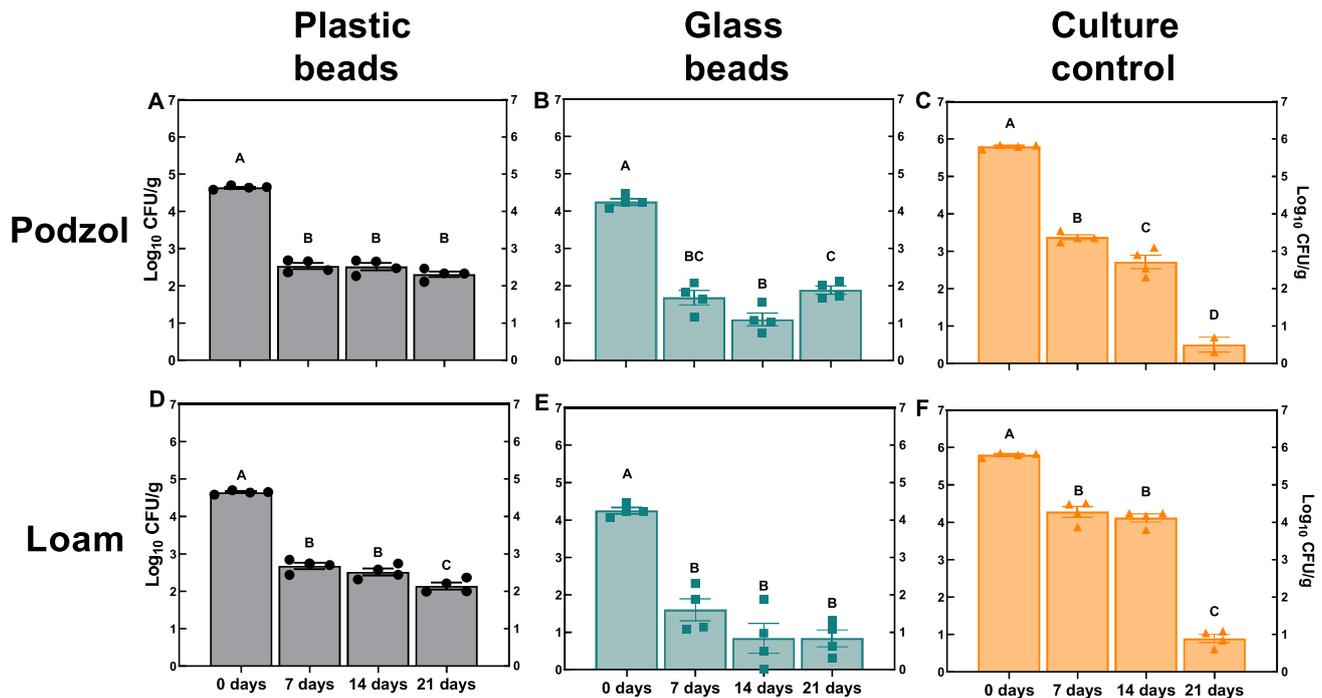


Fig. 3 The influence of plastic and glass beads on the persistence of *S. Typhimurium* exposed to repeated flooding events. *S. Typhimurium* was enumerated after multiple flood events in podzol (A–C)

and a loamy soil (D–F). Each bar represents the mean of four replicates \pm SEM. Bars with different letters are significantly different to each other ($P < 0.01$)

Transfer of *S. Typhimurium* from plastic beads in floodwater to uncontaminated plastic beads in soil

S. Typhimurium colonising plastic or glass beads in both soil types were able to detach from the biofilm and be transported through the soil following a simulated flooding event and subsequently colonise new plastic beads in the bottom compartment of the column after 7 days in the flooded soil (Fig. 4A, B, D, and E). However, after 14 days in the flooded soil, *S. Typhimurium* was only detected on plastics in the bottom compartment of the mesocosms containing loam soil (Fig. 4D). In the mesocosms containing the ‘culture control’, *S. Typhimurium* was also transferred through both soil types during simulated flooding and readily colonised the new plastics in the bottom compartment in the podzol and loam soils (Fig. 4C and F).

Concentration of *S. Typhimurium* in soil leachate following simulated flooding events

Within 24 h, *S. Typhimurium* was detectable in leachate from both soil types in all mesocosms (Fig. 5) and remained detectable throughout the 21 days of the experiment, albeit at low concentrations (e.g., by day 21, less than 1 Log₁₀ CFU/mL recovered from any of the replicates). In the

‘culture-only’ control in the loam soil, *S. Typhimurium* also continued to be detected in the leachate for up to 21 days; however, by day 10 in the podzol leachate *S. Typhimurium* was no longer detected. Despite similar concentrations of *S. Typhimurium* being recovered in the leachate from mesocosms containing colonised plastic or glass beads, the final concentration of *S. Typhimurium* was higher on the plastic beads compared to the glass beads in both soil types (Fig. 5). However, the rate of decline (*D*-value) for the *S. Typhimurium* in the leachate was slowest for those cells that dissociated from the glass and plastic beads in the podzol (15.8 and 11.2 days, respectively), while in the loam soil the *D*-value for *S. Typhimurium* that dissociated from the glass beads was the same as the ‘culture control’ (4.2 days), while for the plastic beads it was higher (7.9 days) (Table S4; Fig. S3).

Discussion

The plastisphere can provide a protective environment for *S. Typhimurium* in soil

This study has demonstrated that the plastisphere provides significant protection for *S. Typhimurium* in the soil environment, allowing it to persist in two contrasting soil types at significantly higher concentrations than when added

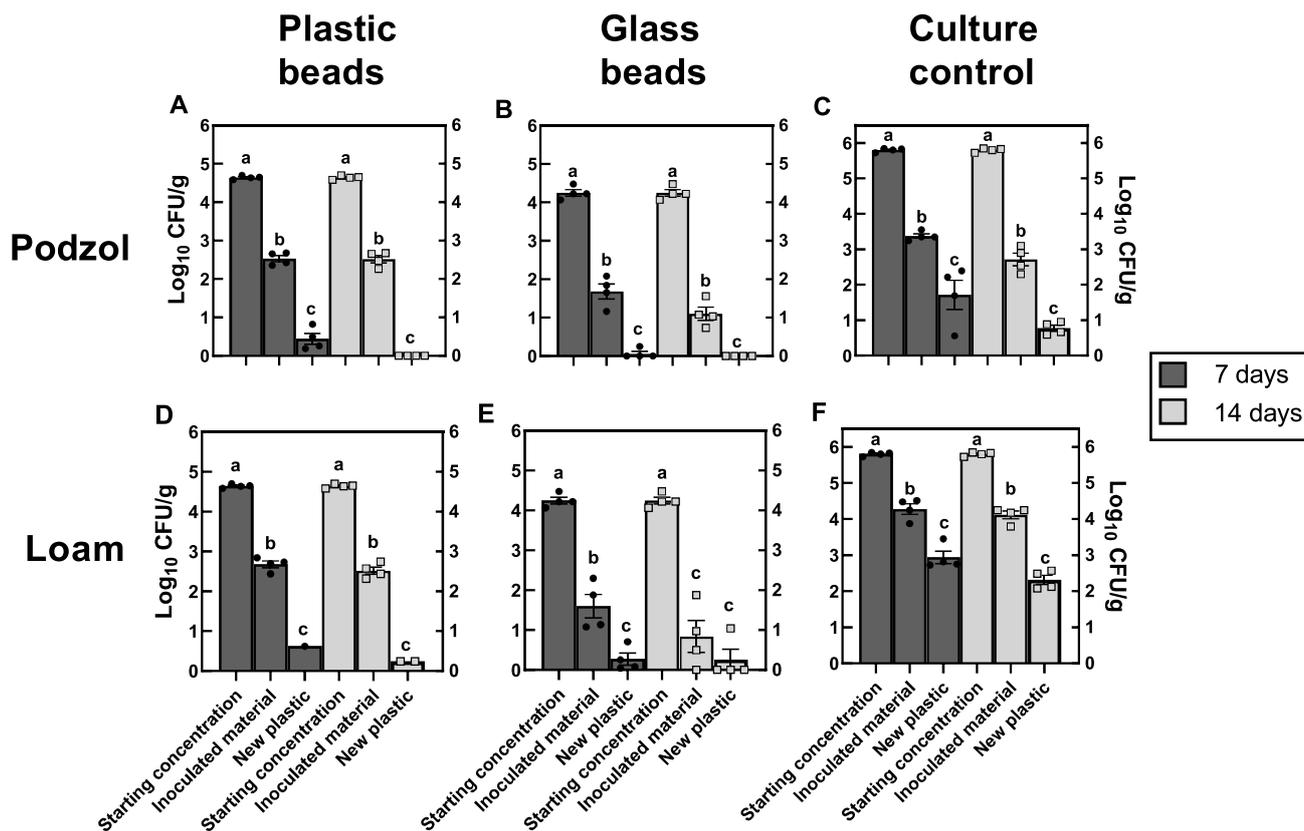


Fig. 4 Transfer of *S. Typhimurium* to new plastic beads following a simulated flooding event. The ‘starting concentration’ represents colonisation of *S. Typhimurium* on plastic, glass, and in the ‘culture control’ inoculated soil prior to the experiment beginning. The ‘inoculated material’ is the concentration of *S. Typhimurium* on the plastic and glass beads or in the soil for the ‘culture only’ control after the

specified period. ‘New plastic’ refers to the *S. Typhimurium* concentration on the new plastic beads placed in the bottom half of the mesocosm. Each bar represents the mean of four replicates \pm SEM. Different letters above the bars indicate significant differences ($P < 0.01$) from ANOVA and Tukey post-hoc test

directly to soil. Furthermore, *S. Typhimurium* cells were subsequently able to dissociate from the plastisphere and transfer to surrounding soil, which demonstrates the potential co-pollutant risk of plastics and human pathogens in soil systems. *S. Typhimurium* was detectable in loamy soil for at least 35 days, although when added directly to the podzol, *S. Typhimurium* rapidly became undetectable, which was likely a response to the acidic pH of this soil type. The ability of *Salmonella* spp. to form biofilms and survive in the soil environment is influenced by soil moisture, pH, and mineral content (Alegbeleye et al. 2018), but being in the plastisphere can increase the persistence of *S. Typhimurium* and allow it to survive at significantly higher concentrations in both soil types.

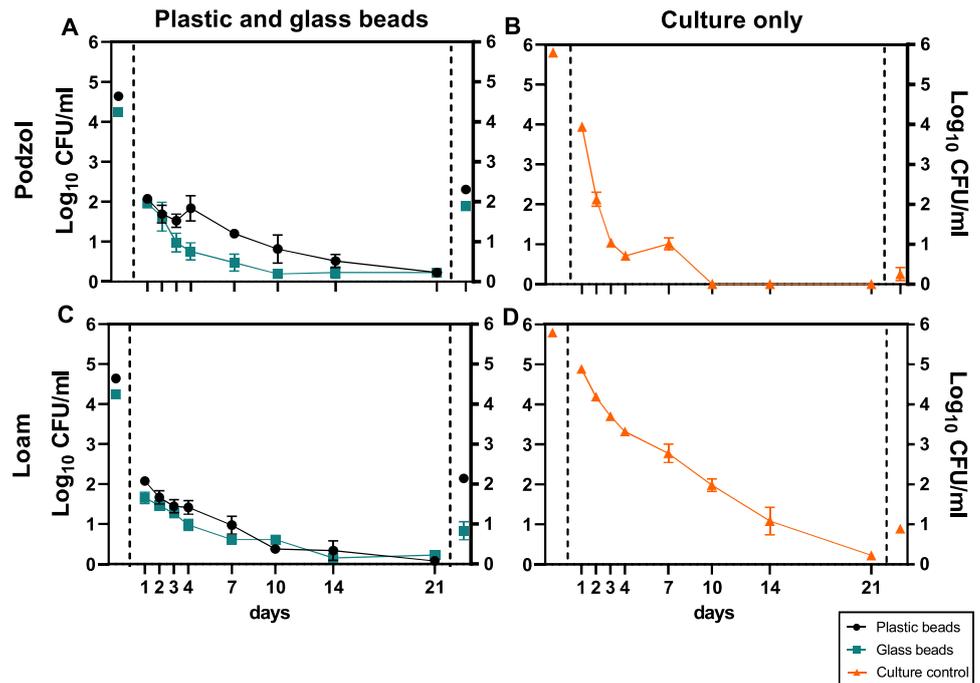
The dynamic and cyclical (re)contamination of plastics in agricultural soils from either contaminated floodwater or irrigation water has important implications for environmental and human health risks. The pathogenic potential of soils used for crops or livestock could be significantly increased if they are contaminated with plastic pollution (Quilliam

et al. 2023), particularly if this is acting as a protective reservoir for *S. Typhimurium*. As crops are being increasingly grown in microplastic contaminated soils (Chen et al. 2020; Li et al. 2022), potential human health risks could be further compounded by the facilitated increased survival of human enteric pathogens in agricultural environments with potential consequences for crops, livestock, and food safety.

Persistence of *S. Typhimurium* in the plastisphere during flooding and further dissemination into the environment

The plastisphere aided the persistence of *S. Typhimurium* when the soil was repeatedly flooded, compared to *S. Typhimurium* inoculated freely in the soil, likely due to the protection biofilms provide bacterial communities from environmental stressors (Yin et al. 2019; Zhu et al. 2022). Flooding of soils can affect the availability and composition of nutrients, which together with changes in soil physical properties can have a significant impact on *Salmonella*

Fig. 5 Recovery of *S. Typhimurium* in soil leachate. The concentration of recovered *S. Typhimurium* from plastic and glass in podzol (A) and loam (C) and the culture control in podzol (B) and loam (D). The data points on the far left of the dashed line are the concentrations of *S. Typhimurium* colonising the plastic and glass beads at day zero (A, C), and the data points on the far right of the dashed line are the concentrations of *S. Typhimurium* remaining on the plastic and glass beads after 21 days (A, C); or the concentration of the inoculum added to the soil at day 0 and the concentration remaining in the soil at the end (B, D). Each data point represents the mean of four replicates \pm SEM



spp. survival (Jechalke et al. 2019; Peng et al. 2022). In the podzol, the freely inoculated *S. Typhimurium* reduced in concentration quickly compared to when bound to the plastic sphere, due to being washed from the soil by the repeated addition of flood water. Soils with high sand content typically have lower cation exchange capacity (CEC) compared to clay-based soils, as clay soil particles are negatively charged and hold on to cations in the soil. Podzols are less likely to retain floodwater compared to loamy soils because of the difference in CEC, which may reflect the differences in *S. Typhimurium* persistence when freely inoculated into these two soils.

The two different soil types will also contain contrasting concentrations of microbial biomass that may respond differently to waterlogging (Khan et al. 2022). During severe flooding, soil enzyme activity often increases, probably due to the addition of organic matter and detritus in floodwater (Macé et al. 2016). The lower CEC of podzols, e.g., due to a higher proportion of sand, make them less likely to retain water and more likely to have a reduced microbial biomass, resulting in potentially harsher conditions for the survival of human pathogens. Microbial biomass, the availability of nutrients, and extracellular enzyme activity can also be affected by microplastics in the soil (Zhang et al. 2024); therefore, a combination of flooding and plastic contamination could lead to a hostile environment for the survival of enteric pathogens not associated with the plastic sphere.

Soil leachate collected from the contaminated plastics in both soil types still contained viable *S. Typhimurium*,

which indicates that *S. Typhimurium* can dissociate from the plastic sphere and be washed through the soil profile. This demonstrates that microplastics can not only simultaneously increase the duration of *S. Typhimurium* persistence in soil but also facilitate their further dissemination within the environment. Soil type can influence the movement of human pathogens, with loamy soils less likely to facilitate the rapid movement of floodwater compared to a podzol, which will have a higher volume of small pores and a larger total pore area (Lipiec et al. 2018). As well as affecting the movement of water, soil porosity and texture will also influence the ability of the microbial community to access nutrients through the pore network (Patel et al. 2021). Following the dissociation of *S. Typhimurium* from the plastic sphere during flooding and subsequent waterlogging of soil, it could be rapidly transported through the soil and into waterbodies, further increasing its contamination potential (Callahan et al. 2017).

In this study, LDPE plastic beads were added to soil at 5% w/w, which is reflective of the quantity of plastic found in some agricultural settings (Palansooriya et al. 2022). As they are typically lightweight, microplastics can decrease soil bulk density, influence soil texture, and increase soil aeration (De Souza MacHado et al. 2018), all of which will contribute to the rapid movement of water through soil during flooding. This can result in mineral depletion of soil, with lower concentrations of total organic C and N in flooded soils (Saint-Laurent et al. 2014); however, whether repeated flooding and soil saturation reduced the survival of *S. Typhimurium* inoculated directly into the soil remains

unclear. Compositional differences between the two soil types can also influence the movement of *S. Typhimurium* during flooding. When applied to different soil types as part of a liquid manure, *S. Typhimurium* was more likely to be found in the top 20 cm of soil compared to lower depths in soils (Bech et al. 2010). The concentration of human pathogens in the top of the soil profile increases the risk of contact with the aerial parts of crops, e.g., due to splash from rain or irrigation water, but also the (re)colonisation of plastic waste at the soil surface.

***S. Typhimurium* can be transferred from the plastisphere to uncontaminated plastics in a shared soil environment**

When placed in soil directly contaminated with *S. Typhimurium* or with plastic beads contaminated with *S. Typhimurium*, uncontaminated plastics became colonised with the pathogen. As floodwater passed through the soil, it transported cells of *S. Typhimurium* through the soil profile and delivered them to the new plastic beads where they subsequently became incorporated into the plastisphere. This suggests that microplastics in agricultural soil, e.g., from degrading plastic mulches, can act as sinks for colonisation by human enteric pathogens that have become resuspended and disseminated by either flood or irrigation water. Such transfer to uncolonised microplastics in the field may lead to the enhanced persistence of human pathogens and an increased risk of contact with livestock or humans (Ormsby et al. 2024b).

Once disassociated from the plastisphere and transferred into the soil, *S. Typhimurium* survival will be influenced by autochthonous microbial communities, which will be different in the two soil types used in this study (Liao et al. 2021). Interactions occurring at the soil-plastic interface may affect the subsequent persistence of *S. Typhimurium* in the plastisphere. The micro-plastisphere can produce enriched microbial hotspots in soil due to the presence of bioavailable carbon compounds in plastics (particularly bioplastics) (Zhou et al. 2021) that could be used by *S. Typhimurium*, which can alter its carbon metabolism in response to locally available sources of carbon in the soil (Han et al. 2023). The colonisation of new plastics by *S. Typhimurium* following flooding may be influenced by the available C or specific microbial hotspots associated with these plastics, particularly if farmers are increasingly using bioplastics to cover and protect crops. The soil plastisphere can also be a hotspot for horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) (Rillig et al. 2019; Zhu et al. 2022). HGT within the plastisphere is particularly likely to occur when manure is added to the soil, or at elevated temperature or moisture (Zhu et al. 2022).

Conclusions

The ability of *S. Typhimurium* to persist on plastics in the soil has significant implications for food production. Nano- and microplastics can bind to leaves and fruits of crops and be taken up by roots (Azeem et al. 2021), and the subsequent persistence of human pathogens in the plastisphere will determine the public health risk from both soils and crops (Quilliam et al. 2023), including the ability of pathogens to transfer from plastics onto the surface of edible salad leaves (Woodford et al. 2024). Microplastics represent a novel and rapidly increasing environmental concern in soil systems, particularly for agriculture. Plastics can alter soil physicochemical properties and autochthonous microbial communities and have the potential to deliver harmful human pathogens into the food chain. Given the lightweight and durable nature of plastics, pathogens found in the plastisphere could spread further and last longer than other potentially contaminated materials, posing a greater threat to human health.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-024-34491-4>.

Acknowledgements We would like to acknowledge Ian Washbourne and Emily Bonsall for advice and assistance with soil analysis.

Author contribution LW, RSQ: conceptualisation; LW, RF, HW, MO, CP: data curation; LW: formal analysis; LW: writing—original draft preparation; RSQ: supervision; LW, RF, HW, MO, CP, RSQ: writing, reviewing, and editing; RSQ: funding acquisition.

Funding This work was supported by the UKRI Natural Environment Research Council (NERC) as part of the GCRF SPACES project [grant number NE/V005847/1].

Data availability All data is either presented in the manuscript and supplementary files, or will be made available upon request.

Declarations

Ethical approval No ethical approval was required for this study.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will

need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alegbeleye OO, Singleton I, Sant'Ana AS (2018) Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. *Food Microbiol* 73. <https://doi.org/10.1016/j.fm.2018.01.003>
- Azeem I, Adeel M, Ahmad MA, Shakoor N, Jiangcuo GD, Azeem K, Ishfaq M, Shakoor A, Ayaz M, Xu M, Rui Y (2021) Uptake and accumulation of nano/microplastics in plants: a critical review. *Nanomaterials*. <https://doi.org/10.3390/nano1112935>
- Basili M, Quero GM, Giovannelli D, Manini E, Vignaroli C, Avio CG, De Marco R, Luna GM (2020) Major role of surrounding environment in shaping biofilm community composition on marine plastic debris. *Front Mar Sci* 7:262. <https://doi.org/10.3389/FMARS.2020.00262/BIBTEX>
- Bech TB, Johnsen K, Dalsgaard A, Laegdsmand M, Jacobsen OH, Jacobsen CS (2010) Transport and distribution of salmonella enterica serovar typhimurium in loamy and sandy soil monoliths with applied liquid manure. *Appl Environ Microbiol* 76. <https://doi.org/10.1128/AEM.00615-09>
- Beloe CJ, Browne MA, Johnston EL (2022) Plastic debris as a vector for bacterial disease: an interdisciplinary systematic review. *Environ Sci Technol* 56:2950–2958. <https://doi.org/10.1021/acs.est.1c05405>
- Beriot N, Peek J, Zornoza R, Geissen V, Huerta Lwanga E (2021) Low density-microplastics detected in sheep faeces and soil: a case study from the intensive vegetable farming in Southeast Spain. *Sci Total Environ* 755. <https://doi.org/10.1016/J.SCITOTENV.2020.142653>
- Callahan MT, Micallef SA, Buchanan RL (2017) Soil type, soil moisture, and field slope influence the horizontal movement of salmonella enterica and citrobacter freundii from floodwater through soil. *J Food Prot* 80. <https://doi.org/10.4315/0362-028X.JFP-16-263>
- Chen Y, Leng Y, Liu X, Wang J (2020) Microplastic pollution in vegetable farmlands of suburb Wuhan, central China. *Environ Pollut* 257. <https://doi.org/10.1016/j.envpol.2019.113449>
- De Souza MacHado AA, Lau CW, Till J, Kloas W, Lehmann A, Becker R, Rillig MC (2018) Impacts of microplastics on the soil biophysical environment. *Environ Sci Technol* 52. <https://doi.org/10.1021/acs.est.8b02212>
- Eguchi M, Nishikawa T, Macdonald K, Cavicchioli R, Gottschal JC, Kjelleberg S (1996) Responses to stress and nutrient availability by the marine ultramicrobacterium *Sphingomonas* sp. strain RB2256. *Appl Environ Microbiol* 62. <https://doi.org/10.1128/aem.62.4.1287-1294.1996>
- Gkoutselis G, Rohrbach S, Harjes J, Obst M, Brachmann A, Horn MA, Rambold G (2021) Microplastics accumulate fungal pathogens in terrestrial ecosystems. *Sci Rep* 11. <https://doi.org/10.1038/s41598-021-92405-7>
- Global Burden of Disease Study [WWW Document] (2019) Global burden of disease study results. URL <https://vizhub.healthdata.org/gbd-results/> (accessed 8.2.23).
- Han M, Schierstaedt J, Duan Y, Nietschke M, Jechalke S, Wolf J, Hensel M, Neumann-Schaal M, Schikora A (2023) Salmonella enterica relies on carbon metabolism to adapt to agricultural environments. *Front Microbiol* 14. <https://doi.org/10.3389/fmicb.2023.1213016>
- Hopkins KL, Peters TM, Lawson AJ, Owen RJ (2009) Rapid identification of *Salmonella enterica* subsp. *arizonae* and *S. enterica* subsp. *diarizonae* by real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 64. <https://doi.org/10.1016/j.diagmicrobio.2009.03.022>
- Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci Total Environ* 586:127–141. <https://doi.org/10.1016/J.SCITOTENV.2017.01.190>
- Jechalke S, Schierstaedt J, Becker M, Flemer B, Grosch R, Smalla K, Schikora A (2019) Salmonella establishment in agricultural soil and colonization of crop plants depend on soil type and plant species. *Front Microbiol* 10. <https://doi.org/10.3389/fmicb.2019.00967>
- Kassambara A (2020) ‘ggpubr’: “ggplot2” based publication ready plots. R package version 0.2.5. <https://doi.org/10.32614/CRAN.package.ggpubr>
- Khan SU, Hooda PS, Blackwell MSA, Busquets R (2022) Effects of drying and simulated flooding on soil phosphorus dynamics from two contrasting UK grassland soils. *Eur J Soil Sci* 73. <https://doi.org/10.1111/ejss.13196>
- Kumar M, Xiong X, He M, Tsang DCW, Gupta J, Khan E, Harrad S, Hou D, Ok YS, Bolan NS (2020) Microplastics as pollutants in agricultural soils. *Environ Pollut* 265:114980. <https://doi.org/10.1016/J.ENVPOL.2020.114980>
- Li W, Xiao Y (2023) Microplastics increase soil microbial network complexity and trigger diversity-driven community assembly. *Environ Pollut* 333. <https://doi.org/10.1016/j.envpol.2023.122095>
- Li S, Ding F, Flury M, Wang Z, Xu L, Li S, Jones DL, Wang J (2022) Macro- and microplastic accumulation in soil after 32 years of plastic film mulching. *Environ Pollut* 300. <https://doi.org/10.1016/j.envpol.2022.118945>
- Liao J, Li J, Han Z, Lyu G, Ibekwe AM, Ma J (2021) Persistence of *Salmonella Typhimurium* in apple-pear (*Pyrus bretschneideri* Rehd.) orchard soils influenced by bacterial communities and soil properties. *Sci Total Environ* 768. <https://doi.org/10.1016/j.scitotenv.2020.144458>
- Lipiec J, Świeboda R, Chodorowski J, Turski M, Hajnos M (2018) Pore size distribution and stability of ortstein and overlying horizons in podzolic soils under forest. *Geoderma* 310. <https://doi.org/10.1016/j.geoderma.2017.09.021>
- Ma J, Xu M, Wu J, Yang G, Zhang X, Song C, Long L, Chen C, Xu C, Wang Y (2023) Effects of variable-sized polyethylene microplastics on soil chemical properties and functions and microbial communities in purple soil. *Sci Total Environ* 868. <https://doi.org/10.1016/j.scitotenv.2023.161642>
- Macé OG, Steinauer K, Jousset A, Eisenhauer N, Scheu S (2016) Flood-induced changes in soil microbial functions as modified by plant diversity. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0166349>
- Martínez-Campos S, González-Pleiter M, Fernández-Piñas F, Rosal R, Leganés F (2021) Early and differential bacterial colonization on microplastics deployed into the effluents of wastewater treatment plants. *Sci Total Environ* 757. <https://doi.org/10.1016/j.scitotenv.2020.143832>
- McCormick A, Hoellein TJ, Mason SA, Schluep J, Kelly JJ (2014) Microplastic is an abundant and distinct microbial habitat in an urban river. *Environ Sci Technol* 48:11863–11871. <https://doi.org/10.1021/es503610r>
- Metcalfe R, Oliver DM, Moresco V, Quilliam RS (2022) Quantifying the importance of plastic pollution for the dissemination of human pathogens: the challenges of choosing an appropriate ‘control’ material. *Sci Total Environ* 810. <https://doi.org/10.1016/j.scitotenv.2021.152292>
- Moresco V, Oliver DM, Weidmann M, Matallana-Surget S, Quilliam RS (2021) Survival of human enteric and respiratory viruses on

- plastics in soil, freshwater, and marine environments. *Environ Res* 199. <https://doi.org/10.1016/j.envres.2021.111367>
- Omeyer LCM, Duncan EM, Aiemsomboon K, Beaumont N, Bureekul S, Cao B, Carrasco LR, Chavanich S, Clark JR, Cordova MR, Couceiro F, Cragg SM, Dickson N, Failler P, Ferraro G, Fletcher S, Fong J, Ford AT, Gutierrez T, Shahul Hamid F, Hiddink JG, Hoa PT, Holland SI, Jones L, Jones NH, Koldewey H, Lauro FM, Lee C, Lewis M, Marks D, Matallana-Surget S, Mayorga-Adame CG, McGeehan J, Messer LF, Michie L, Miller MA, Mohamad ZF, Nor NHM, Müller M, Neill SP, Nelms SE, Onda DFL, Ong JLL, Pariatamy A, Phang SC, Quilliam R, Robins PE, Salta M, Sartimbul A, Shakuto S, Skov MW, Taboada EB, Todd PA, Toh TC, Valiyaveetil S, Viyakarn V, Wonnapijit P, Wood LE, Yong CLX, Godley BJ (2022) Priorities to inform research on marine plastic pollution in Southeast Asia. *Sci Total Environ* 841. <https://doi.org/10.1016/j.scitotenv.2022.156704>
- Ormsby MJ, White HL, Metcalf R, Oliver DM, Quilliam RS (2023) Clinically important *E. coli* strains can persist, and retain their pathogenicity, on environmental plastic and fabric waste. *Environmental Pollution* 326:121466. <https://doi.org/10.1016/j.envpol.2023.121466>
- Ormsby MJ, White HL, Metcalf R, Oliver DM, Feasey NA, Quilliam RS (2024a) Enduring pathogenicity of African strains of *Salmonella* on plastics and glass in simulated peri-urban environmental waste piles. *J Hazard Mater* 461:132439. <https://doi.org/10.1016/j.jhazmat.2023.132439>
- Ormsby MJ, Woodford L, Quilliam RS (2024b) Can plastic pollution drive the emergence and dissemination of novel zoonotic diseases? *Environ Res* 246:118172. <https://doi.org/10.1016/j.envres.2024.118172>
- Ormsby MJ, Woodford L, White HL, Fellows R, Oliver DM, Quilliam RS (2024c) Toxigenic *Vibrio cholerae* can cycle between environmental plastic waste and floodwater: implications for environmental management of cholera. *J Hazard Mater* 461:132492. <https://doi.org/10.1016/j.jhazmat.2023.132492>
- Palansooriya KN, Shi L, Sarkar B, Parikh SJ, Sang MK, Lee SR, Ok YS (2022) Effect of LDPE microplastics on chemical properties and microbial communities in soil. *Soil Use Manag* 38. <https://doi.org/10.1111/sum.12808>
- Patel KF, Fansler SJ, Campbell TP, Bond-Lamberty B, Smith AP, Roychowdhury T, McCue LA, Varga T, Bailey VL (2021) Soil texture and environmental conditions influence the biogeochemical responses of soils to drought and flooding. *Commun Earth Environ* 2. <https://doi.org/10.1038/s43247-021-00198-4>
- Peng S, Song D, Zhou B, Hua Q, Lin X, Wang Y (2022) Persistence of *Salmonella* Typhimurium and antibiotic resistance genes in different types of soil influenced by flooding and soil properties. *Ecotoxicol Environ Saf* 248. <https://doi.org/10.1016/j.ecoenv.2022.114330>
- Pornsukarom S, Thakur S (2016) Assessing the impact of manure application in commercial swine farms on the transmission of antimicrobial resistant salmonella in the environment. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0164621>
- Quilliam RS, Pow CJ, Shilla DJ, Mwesiga JJ, Shilla DA, Woodford L (2023) Microplastics in agriculture – a potential novel mechanism for the delivery of human pathogens onto crops. *Front Plant Sci* 14. <https://doi.org/10.3389/fpls.2023.1152419>
- R Core Team, 2021. R core team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>. Accessed Nov 2023
- Ren Z, Gui X, Xu X, Zhao L, Qiu H, Cao X (2021) Microplastics in the soil-groundwater environment: Aging, migration, and co-transport of contaminants – a critical review. *J Hazard Mater* 419. <https://doi.org/10.1016/j.jhazmat.2021.126455>
- Rillig MC, De Souza Machado AA, Lehmann A, Klümper U (2019) Evolutionary implications of microplastics for soil biota. *Environ Chem* 16. <https://doi.org/10.1071/EN18118>
- Rillig MC, Lehmann A (2020) Microplastic in terrestrial ecosystems research shifts from ecotoxicology to ecosystem effects and Earth system feedbacks. *Science* 1979(368):1430–1431. <https://doi.org/10.1126/science.abb5979>
- Saint-Laurent D, Gervais-Beaulac V, Berthelot JS (2014) Variability of soil properties in different flood-risk zones and link with hydroclimatic changes (Southern Québec, Canada). *Geoderma* 214–215. <https://doi.org/10.1016/j.geoderma.2013.09.025>
- Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Tröger J, Muñoz K, Frör O, Schaumann GE (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Sci Total Environ* 550:690–705. <https://doi.org/10.1016/J.SCITOTENV.2016.01.153>
- Woodford L, Fellows R, White HL, Ormsby MJ, Quilliam RS (2024) *Salmonella* Typhimurium and *Vibrio cholerae* can be transferred from plastic mulch to basil and spinach salad leaves. *Heliyon* 10:e31343. <https://doi.org/10.1016/j.heliyon.2024.e31343>
- Wu RT, Cai YF, Chen YX, Yang YW, Xing SC, Di LX (2021) Occurrence of microplastic in livestock and poultry manure in South China. *Environ Pollut* 277. <https://doi.org/10.1016/J.ENVPOL.2021.116790>
- Yang J, Li R, Zhou Q, Li L, Li Y, Tu C, Zhao X, Xiong K, Christie P, Luo Y (2021) Abundance and morphology of microplastics in an agricultural soil following long-term repeated application of pig manure. *Environ Pollut* 272:116028. <https://doi.org/10.1016/J.ENVPOL.2020.116028>
- Yin W, Wang Y, Liu L, He J (2019). Biofilms: the microbial “protective clothing” in extreme environments. *Int J Mol Sci* 20. <https://doi.org/10.3390/ijms20143423>
- Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the “plastisphere”: microbial communities on plastic marine debris. *Environ Sci Technol* 47:7137–7146. <https://doi.org/10.1021/es401288x>
- Zhang L, Xie Y, Liu J, Zhong S, Qian Y, Gao P (2020) An overlooked entry pathway of microplastics into agricultural soils from application of sludge-based fertilizers. *Environ Sci Technol* 54:4248–4255. <https://doi.org/10.1021/acs.est.9b07905>
- Zhang H, Huang Y, An S, Wang P, Xie C, Jia P, Huang Q, Wang B (2024) Mulch-derived microplastic aging promotes phthalate esters and alters organic carbon fraction content in grassland and farmland soils. *J Hazard Mater* 461. <https://doi.org/10.1016/j.jhazmat.2023.132619>
- Zhou J, Jia R, Brown RW, Yang Y, Zeng Z, Jones DL, Zang H (2023) The long-term uncertainty of biodegradable mulch film residues and associated microplastics pollution on plant-soil health. *J Hazard Mater*. <https://doi.org/10.1016/j.jhazmat.2022.130055>
- Zhou J, Gui H, Banfield CC, Wen Y, Zang H, Dippold MA, Charlton A, Jones DL (2021) The microplastisphere: biodegradable microplastics addition alters soil microbial community structure and function. *Soil Biol Biochem* 156. <https://doi.org/10.1016/j.soilbio.2021.108211>
- Zhu D, Ma J, Li G, Rillig MC, Zhu YG (2022) Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens. *ISME J* 16:521–532. <https://doi.org/10.1038/s41396-021-01103-9>