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ENTEROPATHOGEN SURVIVAL IN SOIL FROM DIFFERENT LAND-USES IS PREDOMINANTLY REGULATED BY MICROBIAL COMMUNITY COMPOSITION

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ABSTRACT (200 WORDS)

Microbial enteropathogens can enter the environment via landspreading of animal slurries and manures. Biotic interactions with the soil microbial community can contribute to their subsequent decay. This study aimed to determine the impact of microbial community structure associated with soils derived from 12 contrasting land-uses on model enteropathogen survival. The phenotypic profiles of these soil microbial communities were

49 determined by phospholipid fatty acid (PLFA) profiling, and soils were also characterized for
50 a range of physicochemical properties. The persistence of *Salmonella* Dublin, *Listeria*
51 *monocytogenes,* and *Escherichia coli* was measured over 110 days within soil microcosms.
52 Physicochemical and biotic data were used in stepwise regression analysis to determine the
53 predominant factor related to pathogen-specific death rates. Phenotypic structure, associated
54 with a diverse range of constituent PLFAs, was identified as the most significant factor in
55 pathogen decay for *S.* Dublin, *L. monocytogenes,* non-toxigenic *E. coli* O157 but not for
56 environmentally-persistent *E. coli*. This demonstrates the importance of entire community
57 interactions in pathogen suppression, which are context-specific.
58
59
60
61 **1. INTRODUCTION**
62 Microbial enteropathogens are released in faecal waste of both animals and humans, and enter
63 the soil environment either directly via faecal shedding, or indirectly via the application of
64 slurry, manure and sewage sludge. In addition, wild animals and birds contribute to
65 enteropathogen load in the environment (Jones, 2001; Jiang et al., 2007; Benskin et al., 2009),
66 and there is evidence to suggest that potentially pathogenic enteric bacteria can exist as
67 naturalised populations within the soil matrix (Texier et al., 2008; Ishii et al., 2006; Brennan
68 et al., 2010). Enteropathogens can pose a serious public health risk, contingent on
69 survivability within the soil environment. Viable pathogens may be transmitted to humans by
70 direct contact with contaminated surfaces and accidental ingestion of faeces or contaminated
71 soil particles (Davis et al., 2005). Pathogens can also be transported via overland or
72 subsurface flow to surface and groundwaters, and infection may arise via ingestion of
73 contaminated water, e.g. Walkerton Outbreak, Ontario in 2000 (Hrudey et al., 2003). It is also

74 possible that pathogens could be present on the crop surface following manure application. In
75 this case, a person may become infected if they consume the contaminated produce, as
76 demonstrated by the 2011 *E. coli* O104 outbreak in Germany, associated with consumption of
77 contaminated beansprouts (Böhmer et al., 2011).
78
79 To date, enteropathogen survival in soil has been mostly investigated in relation to
80 prevailing physicochemical conditions. Factors known to affect pathogen survival include
81 moisture, temperature, texture, pH, cation exchange capacity (CEC), UV irradiation, organic
82 matter (OM) and soil nutrient status (summarised by van Elsas et al., 2011). For example,
83 persistence is favoured by cool moist conditions (Cools et al., 2001), where exposure to UV is
84 limited (Hutchison et al., 2004b). Typically, the survival of enteric bacteria is reduced at low
85 pH, and tends to increase when approaching a neutral to alkaline state (Sjogren, 1994). Fine
86 textured soils with well-developed microstructure and high clay content offer habitat, water
87 and nutrients, which can sustain pathogens introduced via manure application (England et al.,
88 1993).
89 Soil biology also plays an important function in regulating pathogen survival; however
90 research on interactions with the soil community has been comparatively limited. Pertinent
91 biotic interactions include predation (Sørensen et al., 1999), antagonism from indigenous
92 microorganisms (Garbeva et al., 2004) and competition for resources (Irikiin et al., 2006). It
93 has been found that bacteria introduced into soil decline more rapidly when other microbes
94 are present. This decline is apparently accelerated when the indigenous microbial community
95 is increasingly diverse. A range of experimental approaches have been used to manipulate
96 microbial diversity with a view to investigating the relationship between diversity and *E. coli*
97 survivability (Vivant et al, 2013; Yao et al., 2013; Ma et al., 2013; Korajkic et al., 2013;
98 Erickson et al., 2013). All of these studies showed an inverse relationship between community

99 complexity and pathogen survival, attributed to progressively increasing competition for
100 resources and antagonistic interactions associated with greater diversity.
101 The soil microbial community is typically sensitive to changing environmental
102 conditions (Waldrop and Firestone, 2006), and consequent shifts in community structure
103 could influence the survival behaviour of introduced enteric pathogens. Land-use and
104 management has been implicated in shaping the microbial community by modulating the
105 physicochemical environment (Lauber et al., 2008). It has been shown that intensity of land-
106 use (Jangid et al., 2008), length of time under a particular management (Buckley and Schmidt,
107 2001), substrate addition (Degens et al., 2000) and the presence of a plant rhizosphere
108 (Garbeva et al., 2004) can contribute to defining microbial community structure. Some work
109 has been carried out to demonstrate the effects of land-use and management on pathogen
110 suppression (van Elsas et al., 2002; Williams et al., 2007; Franz et al., 2008; Yao et al., 2013).
111 However, the pathogen survival response is often variable and difficult to predict within a
112 framework of complex interactions between site-specific factors, including current and
113 historical land-use, the physicochemical environment, predominant management strategies
114 and resultant impact on community composition. In addition, these studies focused solely on a
115 single pathogen, namely *E. coli* O157, despite the fact that survival and behavioural profiles
116 within soil are species, and even strain-specific (Topp et al., 2003). This is because
117 enteropathogens have different physiological properties and life cycles which will influence
118 survivability within the soil matrix (Winfield and Groisman, 2003).
119 It is therefore unclear whether physicochemical or biotic factors play a dominant role
120 in governing pathogen survival, particularly as few studies have considered both in a coherent
121 manner. Therefore, the aim of this study was to conduct a microcosm study to investigate
122 pathogen survival in relation to naturally-contrasting community phenotypes derived from
123 different land-uses. We hypothesised that soil biology, specifically the phenotypic microbial

community structure, would be more significant in regulating pathogen decay than soil physicochemical composition. We prescribed the phenotype as the operationally important entity in this context, as it represents the literal manifestation of the microbial community which the introduced bacteria would have encountered.

2. MATERIALS AND METHODS

2.1 Soil collection and initial screening

Thirty-nine sites across Ireland were initially prescribed based on contrasting land-use, soil type and management regime. Sites consisted of a single uniform field, free of livestock, which was divided into 3 sections. Approximately 20 cores were taken from the top 15 cm of soil (A horizon) across the W transect from each section, and were combined to yield a composite sample. Soils from these sites were then homogenised and sieved to 4 mm. Sub-samples of approximately 5-10 g freeze-dried soil were weighed out and analysed for community composition by PLFA, as described by Frostegård et al. (1997). Soils were also tested for pH using an automated Aqualyser pH meter, % OM (Davies, 1973), and were assessed by hand texturing (DEFRA, 2010). These data were used to select a suite of 12 contrasting soil types for use in a microcosm experiment investigating pathogen death rates. These 12 soils were comprehensively characterised for a range of physicochemical parameters including total exchange capacity (Ross, 1995), pH (McLean, 1982), % OM (Schulte and Hopkins, 1986), Olsen P (Olsen and Sommers, 1982); extractable ions (Mehlich, 1984); inorganic nitrogen (Dahnke, 1990); total carbon and nitrogen (Nelson and Sommers, 1996) and soil texture (ASTM D422, 2000) using sieved, air-dried soil. Fresh soil was also tested for microbial biomass carbon, according to the method described by Vance et al., 1987.

Average PLFA profiles for the initial 39 soils were used in principal components (PC) analysis. First and second PC scores were ordinated to visualise the distribution of soils

149 according to community composition, and were labelled according to soil ID, land-use,
150 texture, pH and % OM (Supp. Fig. 1a-e, respectively). By comparing these graphs, it was
151 possible to visualise community differences with respect to physicochemical properties and
152 thus prescribe a broad range of naturally-derived contexts to subsequently characterise
153 pathogen survival.
154
155 **2.2 Microcosm establishment**
156 The water holding capacity (WHC) for each prescribed soil was determined by the method
157 described in Franz et al. (2011). Moisture content was then adjusted so that soils exhibited
158 similar cohesiveness to achieve standard friability between different soil types, by wetting-up
159 or restricted slow drying on the bench as appropriate. Following adjustment, soil moisture
160 was measured by oven-drying at 105^{o}C for 24 hours, and expressed as a percentage of WHC.
161 Microcosms designed to quantify pathogen survival were established by weighing out
162 aliquots of 5 g soil into sterile 40 ml polypropylene tubes. The tubes were covered with
163 Parafilm to prevent moisture loss during incubation. Caps were then loosely replaced to allow
164 for gas exchange, whilst minimising the risk of contamination. All tubes were stored at 10^{o}C
165 until inoculated with the pathogen suspension. This temperature was selected as it reflects the
166 average annual topsoil soil (0-10 cm) temperature in Ireland. Pathogen inoculation was
167 staggered over a 4-week period, with exactly one week between each inoculation. Therefore
168 the precise community configuration to which each pathogen was exposed was determined by
169 undertaking PLFA analysis at the outset of each inoculation, in order to capture any microbial
170 changes associated with storage and physical alteration, including sieving and moisture
171 adjustment.
172
173 **2.3 Pathogen inoculation and enumeration**

174 Four model pathogens were selected to investigate community interactions, namely an
175 environmentally-persistent *E. coli* (Brennan et al., 2013), *Salmonella* Dublin (NCTC 9676),
176 *Listeria monocytogenes* (Strain no. 1778) and non-toxigenic lux-marked *E. coli* O157 (Strain
177 no. 3704), which has been shown to be a representative proxy for the toxigenic O157 strain of
178 clinical importance (Bolton et al., 1999). These model organisms were considered relevant in
179 terms of public health significance, and they also represented contrasting cellular structures
180 and growth strategies (Winfield and Groisman, 2003).

181 Pathogen inoculum cultures were prepared overnight in Luria-Bertani broth at 37^{o}C,
182 and washed 3 times in ¼ strength Ringer's solution. Microcosms were individually inoculated
183 with approximately 10^{8} cells of each pathogen, which constituted 10^{7} cells g^{-1} soil (dry
184 weight). Final soil moisture following inoculation, at which soils were incubated, was then
185 determined as a percentage of WHC. Pathogen inoculation was staggered into pathogen-
186 specific batches involving all twelve soils simultaneously. These batches were inoculated
187 weekly over a 4-week period, for reasons of practicality. For each pathogen batch, a pool of
188 96 microcosms per soil type were inoculated at three instances selected at random from the
189 whole (remaining) pool after 2 hours (denoted T_0) and 2, 4, 8, 16, 32, 64 and 110 days
190 (denoted T_2, T_4, T_8, T_{16}, T_{32}, T_{64}, T_{110}). Soils continued to be incubated at 10^{o}C throughout
191 these experimental periods.

192 Enumeration was carried out by suspending the soil in 10 ml of ¼ strength Ringer's
193 solution, vortexing briefly and shaking on an end-over-end shaker for 15 minutes. These
194 suspensions were then used to create serial dilutions, which were then spread-plated onto
195 Sorbitol MacConkey, XLD or Oxford agars (Oxoid) for *E. coli* spp., *S.* Dublin and *L.*
196 *monocytogenes*, respectively. All plates were incubated at 37^{o}C for 24 hours, with the
197 exception of *L. monocytogenes* – these were incubated at 37^{o}C for 48 hours. All soils were
198 screened for bacterial targets prior to the experiment, to ensure background levels were

199 negligible. *L. monocytogenes* could not be quantified at T_{110}, due to excessive growth of
200 background microflora on Oxford agar plates. Therefore, survival data for this organism are
201 only presented to T_{64}.
202
203 **2.5 Data analysis**
204 Pathogen survival data were collected by counting characteristic colonies. Triplicate counts
205 for each soil treatment were averaged and were plotted as CFU g^{-1} (dry weight). These data
206 were used to fit exponential decay curves and calculate the average death rate for each
207 pathogen within the context of each soil treatment, according to the following equation:
208 $y=a+b*e^{-kt}$, where y represents the population (CFU g^{-1} soil dry weight) at a given time t, a+b
209 denotes the apparent starting concentration of cells (i.e. intercept with the y-axis), a denotes
210 the asymptote of the final population concentration, and k denotes the death rate (d^{-1}). This
211 function has been used previously to estimate pathogen death rates (Mubiru et al., 2000;
212 Oliver et al., 2006). PLFA profiles were analysed by principal components (PC) analysis, and
213 relative PLFA abundances were ordinated for each soil independently for each batch. PC
214 analysis was also applied to the entire dataset across the four batches, and the relative PLFA
215 abundances were ordinated accordingly. Physicochemical, community and k-values were
216 averaged per soil, and entered into a forward stepwise regression model (Statistica v. 11) to
217 investigate the predominant influential factor regulating pathogen death rates across the 12
218 soil types.
219
220 **3. RESULTS**
221
222 **3.1 Soil community profiling**

223 The prescribed 12 soils were labelled alphabetically (Table 1) and PLFAs were labelled
224 numerically (Table 2) to aid visualisation during PC analysis. When PLFA profiles were
225 analysed collectively across all four batches, there was a highly significant effect of batch
226 ($P<0.001$) and soil ($P<0.001$), but no significant interaction between these terms ($P=0.2$-0.5)
227 for any of PC1-4, which accounted for 66% of the variability between soils. Ordination of
228 mean scores for each batch showed significant separation of all four circumstances, with
229 Batch 3 being notably separated by PC1, 2 and 4 (Fig. 1a). Batches 1, 2 and 4 tended to
230 cluster in the ordinations but were nonetheless significantly separated by PCs 1-3 (Fig. 1b).
231 Ordination of PC1 and PC2 for the PLFA profiles associated with each soil
232 independently showed concomitantly wide dispersion, with notable differences between the
233 ordinations in the four batches. Ordination of corresponding PLFA loadings in this case
234 showed that neither PC1 nor PC2 was dominated by particular PLFA types (Fig. 2a-h).
235
236 **3.2 Pathogen death rates**
237 Pathogens declined in an exponential manner in all instances (Fig. 3). There was visual
238 evidence to suggest different survival characteristics between soils in the form of notably
239 different gradients. This was confirmed by differences in pathogen-specific death rates (Table
240 3). Overall, there was an order of magnitude difference between highest and lowest death
241 rates, observed for *E. coli* Isolate 3 in Soil A and *L. monocytogenes* in Soil C, respectively.
242 The exponential decay function was a significant fit ($P<0.05$) for all pathogens within each
243 soil.
244 There was no association between death rate and PC score for PC1, with the exception
245 *E. coli* O157 which showed a significant linear relationship (Fig. 4a, $P<0.01$). A similar
246 relationship was observed between death rate and PC2 for *S.* Dublin (Fig. 4b, $P<0.005$) and *L.*
247 *monocytogenes* (Fig. 4c, $P<0.001$) where higher death rates were associated with greater

248 positive values in PC2. There was no association between death rate and PC2 for either *E. coli*
249 Isolate 3 or *E. coli* O157. There was no association between death rate and PC3 or PC4 for
250 any model pathogen tested.
251 Stepwise regression showed that variation in death rates between land-use treatments
252 was explained solely and significantly by phenotypic community structure according to PC
253 scores for all model pathogens, with the exception of *E. coli* Isolate 3 (Table 4). No other
254 physicochemical or biotic factor contributed to the stepwise regression model fitting
255 procedure.
256
257 **4. DISCUSSION**
258 A broad range of 39 soils were screened to prescribe biologically and physicochemically
259 contrasting soils for subsequent pathogen survival analysis. It was expected that soils from
260 different land-uses would provide the range of properties fit for purpose to test our
261 hypothesis, and this was investigated using a principal-component based screening approach.
262 It was shown that the soils possessed different physicochemical and community compositions,
263 such that an appropriate suite of 12 soils which showed a broad range of similarity and
264 difference across a range of biotic and physicochemical characteristics were selected, relating
265 to the main factors hypothesised to influence pathogen death rates.
266
267 **4.1 Soil community profiling**
268 Pathogens were inoculated into these 12 soils on a weekly basis in a series of pathogen-
269 specific batches. PC analysis of average PLFA profiles showed significant differences in
270 community composition between batches (Fig. 1). This indicates community composition
271 within soils was not entirely conserved during the inoculation period. This effectively means
272 that the respective pathogens were inoculated into subtly (but significantly) different

273 community contexts. Soil community shifts over storage time has been previously reported
274 (Petersen and Klug, 1994; Wu et al., 2009). However, the primary focus of this study was to
275 create different biological scenarios in order to compare the relative importance of biotic
276 versus physicochemical factors in regulating pathogen survival. Therefore these community
277 shifts did not impact on addressing the central hypothesis.

278 PC analysis also showed highly significant differences in community phenotypic
279 composition between soils, as anticipated (Fig. 1). Dispersal of soils within the PC trait space
280 indicates that a wide variety of community contexts were included in this study. The lack of a
281 significant soil-by-batch interaction is evidence that the *relative* differences between
282 communities were conserved over time when all batches were considered together, thus
283 providing evidence that communities were broadly congruent between batches, and allowing
284 similarities in pathogen behaviour to be tentatively evaluated.

285 The PC plots associated with each batch, representative of the range of soil
286 communities present at respective pathogen-specific T_0's (Fig. 1) depict the precise
287 community contexts to which the pathogens were exposed. PC analysis revealed significant
288 differences between soil communities within each batch. The PLFA loadings associated with
289 these PCs showed that differences in a range of PLFAs contributed to the significant
290 discrimination between communities associated with these 12 soils (Fig. 1). Therefore,
291 discrimination between communities associated with different land-uses was based on shifts
292 in the total microbial cohort in this case. This contrasts with other work that has looked at the
293 effect of different treatments on community configurations in soil. For example, Bossio et al.
294 (1998) found associations between fatty acid signatures and organic, low input and
295 conventional management, suggesting that particular groups were responsible for variation
296 between management regimes. Similarly Frostegård et al. (1997) showed distinct differences
297 in PLFAs associated with manure and those associated with soil, when investigating the

298 impact of manure hotspots on microbial community dynamics. The lack of dominant PLFAs
299 in this case may be due to comparison of a variety of soil communities, encompassing many
300 different land-use treatments and soil types.
301
302 **4.2 Pathogen death rates**
303 Death rates in the range of soils differed between pathogens (Fig. 3, Table 3). Greatest initial
304 decay was observed for both *E. coli* strains compared to *L. monocytogenes* and *Salmonella*
305 Dublin. Pathogen survival in soil is associated with initial inoculum density, cell physiology,
306 adaptability to new environments and capacity to utilise available substrate (van Veen et al.,
307 1997). These factors may have contributed to differential survival patterns across the suite of
308 pathogens used in this study. Fig. 3 also shows differences in overall persistence. Recovery of
309 *E. coli* Isolate 3 was highest at the end of the experimental period, which may have been
310 associated with its documented ability to persist and survive long-term within the soil matrix
311 (Brennan et al., 2010; Brennan et al., 2013). *L. monocytogenes* extraction at T_{110} was
312 unsuccessful, but a comparison of cell concentration at T_{64} shows that *L. monocytogenes* was
313 also strongly competitive across the range of soil treatments and persisted well. This is
314 consistent with previous findings that *L. monocytogenes* is a highly adaptable, saprophytic
315 organism which is ubiquitous in the soil environment (Weis and Seeliger, 1975; Freitag et al.,
316 2009).
317 Different pathogen death rates were also manifest within each soil. Death rate tended
318 to be greatest within grassland land-use class, and poorest in arable and wood land-use classes
319 for all pathogens. Regression analysis showed that PC scores representative of community
320 composition provided the best predictor of pathogen survival for 3 of 4 pathogens
321 investigated (Fig. 4, $P<0.01$). There was no significant correlation between survival and any

of the other physicochemical or biological factors tested – such terms were clearly excluded from the regression procedure (Table 4).

Communities associated with soils from the grassland land-use class, in particular Soil G, were more suppressive toward the pathogens than those associated with soils from arable or wood land-use classes. PLFA loadings show that the suppressive effect observed in this case was of general rather than specialist nature, caused by interactions with the total microbial consortium within these soils, rather than with specific microbial groups. Differential survival between grassland and arable soils has been shown previously in the context of the plant pathogen *Rhizoctonia solani* AG3. Greater microbial diversity in grassland as compared to arable soils, resulted in an enhanced suppressive effect and reduced spread of pathogenic fungal hyphae (van Elsas et al., 2002). It is possible that grassland represents intermediate disturbance levels, as compared to higher disturbance associated with arable and lower disturbance associated with woodland soils. Intermediate disturbance tends to promote diversification of the microbial community (Jangid *et al*., 2008), which could potentially account for greater suppression witnessed in grassland here. This diversification may have been more pronounced for Soil G, as this soil was particularly antagonistic towards the introduced pathogens. This suggests that the pathogen risk is higher when applying organic materials to arable soils relative to grasslands, as these soils may lack suppressive capacity associated with higher microbial diversity that tends to be promoted by intermediate disturbance regimes.

It was shown that pathogen survival was predominantly affected by the soil microbial community. Other work has found circumstantial evidence that antagonistic interactions with the soil community can regulate pathogen decline. For example, Jiang et al. (2002) compared survival of *E. coli* O157 in manure-amended autoclaved soil and unautoclaved soil, and noted rapid inactivation in unautoclaved soil. This response was attributed to the soil microbiota and

was contingent on other factors including temperature and manure:soil ratio. Similarly, *Salmonella enterica* serovar Newport showed greater initial population increase, slower rate of decline and longer survival periods in manure-amended sterile as compared to non-sterile soil. Again, this response was partially attributed to microbial antagonism (You et al., 2006). Further, work by Franz et al. (2008) investigated the main biotic and physicochemical factors influencing the persistence of *E. coli* O157 in a suite of manure-amended soils. They showed that in the presence of manure, pathogen survival was highly correlated with levels of dissolved organic carbon. In organic soils, a secondary correlation was identified with microbial diversity described by molecular techniques. These results suggested that pathogen survival times were mostly contingent on nutrient supply, and could be reduced by amending soil with high quality manure containing a comparatively lower and more complex nutrient load, in order to minimise nutrient availability to opportunistic pathogens. However, the soils that were used in our experiment did not receive any nutrient addition during the incubation period. Potentially, the role of soil biology in pathogen suppression becomes more apparent in the absence of nutrient input. Other work has shown that the competitive ability of *E. coli* O157, characterised by the quantity and rate of resource utilisation, was reduced in the presence of species-rich communities (van Elsas et al., 2012). More recently, Erickson et al. (2014) showed that physicochemical factors including moisture, texture, pH and electrical conductivity, affected *E. coli* and *Salmonella* differently, depending on levels of microbial diversity. Again, this provides further evidence for the important role played by soil microorganisms in regulating pathogen survival.

A recent study by Wang et al. (2014) showed that land-use factors including soil pH, organic matter and sand content significantly influenced the decay of *E. coli* O157; however the authors did not take account of the inherent soil biology associated with each land-use type. In contrast, we observed that none of the physico-chemical factors included in this study

could explain differences in pathogen survival between soils, when PC scores representing the community context were included in regression analysis (Table 4). The survival of all pathogens except *E. coli* Isolate 3 was significantly correlated with contrasting and unrelated communities associated with natural soils. Therefore these results support the hypothesis that soil biology, specifically microbial community structure, is more important than prevailing physicochemical conditions in regulating pathogen survival.

4.3 Differential response of *E. coli* Isolate 3

E. coli Isolate 3 did not respond to the community context in this experiment. This may be due to the fact that it is an environmentally-persistent isolate, which has been shown to form naturalised populations and persist in soil for more than 9 years (Brennan et al., 2010). Further, *E. coli* Isolate 3 has been shown to be metabolically flexible, and direct its proteome towards relatively fast growth, under low temperature conditions, thus demonstrating its environmental adaptability (Brennan et al., 2012). Other studies have also reported long-term growth and survival of *E. coli* in soil (Byappanahalli and Fujioka, 2004; Ishii et al., 2010). Therefore, *E. coli* Isolate 3 may not have been as susceptible to community interactions as other organisms used in this study. Alternatively, the lack of correlation for *E. coli* Isolate 3 could also be linked to the fact that the community context to which this organism was exposed differed to that of other pathogens, due to differential development in absolute community composition during the incubation period.

5. CONCLUSIONS AND FUTURE RESEARCH

This work has provided evidence to show that soil biology, specifically the *phenotypic community context*, determines pathogen survival behaviour and hence we accept our hypothesis. The phenotype is arguably the most relevant construct in this context since it

represents an integrated description of the literal manifestation of the microbial community which the introduced pathogens encountered. That a wide range of PLFAs appear to be implicated in these relationships suggests that the modulation of the pathogens operates at a scale well beyond one or two community members. However, as different microbial species can contain the same fatty acid signature, the phenotype does not provide information at species level. Thus it cannot be used to derive diversity indices, or draw conclusions on species evenness and abundance (Frostegård et al., 2010). Therefore, nucleic acid-based methods such as DGGE and terminal restriction length fragment polymorphism (T-RFLP), which offer more taxonomic resolution, may have added an extra dimension to this study (Zhang and Xu, 2008). For instance, genetic information could have been used to identify microorganisms within phenotypes associated with pathogen suppression. Linking phenotype with genotype and sequencing approaches on the viable community may offer a promising avenue for further research.

The precise nature of such survival may be associated with pathogen type. This suggests that the response of different organisms should be taken into account. This study used four model pathogens to illustrate the principles of soil biota affecting survival; however only single strains of *Listeria* and *Salmonella* were included and it is likely that inter-strain variability might also occur, which should be taken into account in subsequent studies.

Future work should focus on investigating survival characteristics following nutrient addition, as pathogens are typically introduced to soil in an organic carrier material such as manure or sewage sludge. Research should also seek to identify specific microbial configurations that are antagonistic towards human pathogens in soil, and to investigate means of managing the soil in such a way as to allow configurations appropriate to pathogen attenuation to be established. This would encourage more rapid death rates in soil, which

would reduce the risk of pathogen loss to water and crops, and thus break the cycle of infection, leading to better animal and public health protection.

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REFERENCES

ASTM Standard D422-63, 2007. Standard test method for particle-size analysis of soils. ASTM International, West Conshohocken, PA, 2007.

Benskin, C.M.H., Wilson, K., Jones, K., Hartley, I.R., 2009. Bacterial pathogens in wild birds: A review of the frequency and effects of infection. Biol. Rev. 84, 349-373.

Böhmer M.M., Remschmidt C., Wilking H., Deleré Y., an der Heiden M., Adlhoch C., Dreesman J., Ehlers J., Ethelberg S., Faber M., Frank C., Fricke G., Greiner M., Höhle M., Ivarsson S., Jark U., Kirchner M., Koch J., Krause G., Luber P., Rosner B., Stark K., Kühne M., 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. New England Journal of Medicine 365, 1763-1770.

444 Bolton, D.J., Byrne, C.M., Sheridan, J.J., McDowell, D.A., Blair, I.S., 1999. The survival
445 characteristics of a non-toxigenic strain of *Escherichia coli* O157:H7. J. Appl. Microbiol. 86,
446 407-411.
447
448 Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial
449 communities: Effects of agricultural management, season, and soil type on phospholipid fatty
450 acid profiles. Microb. Ecol. 36, 1-12.
451
452 Brennan, F.P., O'Flaherty, V., Kramers, G., Grant, J., Richards, K.G., 2010. Long-term
453 persistence and leaching of *Escherichia coli* in temperate maritime soils. Appl. Environ.
454 Microbiol. 76, 1449-1455.
455
456 Brennan, F.P., Grant, J., Botting, C.H., O'Flaherty, V., Richards, K.G., Abram, F., 2013.
457 Insights into the low-temperature adaptation and nutritional flexibility of a soil-persistent
458 *Escherichia coli*. FEMS Microbiol. Ecol. 84, 75-85.
459
460 Buckley, D., Schmidt, T., 2001. The structure of microbial communities in soil and the lasting
461 impact of cultivation. Microb. Ecol. 42, 11-21.
462
463 Byappanahalli, M., Fujioka, R., 2004. Indigenous soil bacteria and low moisture may limit but
464 allow faecal bacteria to multiply and become a minor population in tropical soils. Wat. Sci.
465 Tech. 50, 27-32
466
467 Cabanes, D., Dehoux, P., Dussurget, O., Frangeul, L., Cossart, P., 2002. Surface proteins and
468 the pathogenic potential of *Listeria monocytogenes*. Trends Microbiol. 10, 238-245.

469

470 Cools D., Merckx R., Vlassak K., Verhaegen J., 2001. Survival of *E. coli* and *Enterococcus*
471 spp. derived from pig slurry in soils of different texture. Appl. Soil Ecol. 17, 53-62.
472

473

474 Dahnke, W.C., 1990. Testing soils for available nitrogen, in: Westerman (Ed.), Soil Testing
475 and Plant Analysis, Soil Science Society of America Book Series 3. ASA, Madison WI, pp.
476 120-140.
477

478 Davies, B.E., 1973. Loss-on-ignition as an estimate of soil organic matter. Soil Sci. Soc. Am.
479 J. 38, 150-151.
480

481

482 Davis M.A., Cloud-Hansen K.A., Carpenter J., Hovde C.J., 2005. *Escherichia coli* O157:H7
483 in environments of culture-positive cattle. Appl. Environ. Microbiol., 71, 6816-6822.
484

485 DEFRA, 2010. Fertiliser recommendations for Agricultural and Horticultural Crops (RB209),
486 8th Ed., The Stationary Office, London, pp. 220.
487

488 Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M., 2000. Decreases in
489 organic C reserves in soils can reduce the catabolic diversity of soil microbial communities.
490 Soil Biol. Biochem. 32, 189-196.
491

492 England L.S., Lee H., Trevors J.T., 1993. Bacterial survival in soil: Effect of clays and
493 protozoa. Soil Biol. Biochem. 25, 525-531.

494

495 Erickson, M.C., Habteselassie, M.Y., Liao, J., Webb, C.C., Mantripragada, V., Davey, L.E.,
496 Doyle, M.P., 2014. Examination of factors for use as potential predictors of human enteric
497 pathogen survival in soil. J. Appl. Microbiol. 116(2), 335-349.

498

499 Franz, E., Semenov, A.V., Termorshuizen, A.J., De Vos, O.J., Bokhorst, J.G., van Bruggen
500 A.H.C., 2008. Manure-amended soil characteristics affecting the survival of *E. coli* O157:H7
501 in 36 Dutch soils. Environ. Microbiol. 10, 313-327.

502

503 Franz, E., van Hoek, A.H.A.M., Bouw, E., Aarts, H.J.M., 2011. *E. coli* O157 strain variability
504 in manure-amended soil survival in relation to strain origin, virulence profile and carbon
505 nutrition profile. Appl. Environ. Microbiol. 77, 8088-8096.

506

507 Freitag, N.E., Port, G.C., Miner, M.D., 2009. *Listeria monocytogenes* – from saprophyte to
508 intracellular pathogen. Nature Rev. Microbiol. 7, 623-628.

509

510 Frostegård, A., Petersen, S.O., Baath, E., Nielsen, T.H., 1997. Dynamics of a microbial
511 community associated with manure hot spots as revealed by phospholipid fatty acid analyses.
512 Appl. Environ. Microbiol. 63, 2224-2231.

513

514 Frostegård Å., Tunlid A., Bååth E., 2010. Use and misuse of PLFA measurements in soils.
515 Soil Biol. Biochem. 43, 1621-1625.

516

517 Garbeva, P., van Veen J.A., van Elsas, J.D., 2004. Microbial diversity in soil: Selection of
518 microbial populations by plant and soil type and implications for disease suppressiveness.
519 Annu. Rev. Phytopathol. 42, 243-270.
520
521 Hrudey S.E., Payment P., Huck P.M., Gillham R.W., Hrudey E.J., 2003. A fatal waterborne
522 disease epidemic in Walkerton, Ontario: Comparison with other waterborne outbreaks in the
523 developed world. Water Sci. Technol. 47, 7-14.
524
525 Hutchison M.L., Walters L.D., Moore A., Crookes K.M., Avery S.M., 2004b. Effect of length
526 of time before incorporation on survival of pathogenic bacteria present in livestock wastes
527 applied to agricultural soil. Appl. Environ. Microbiol. 70, 75111-5118.
528
529 Ibekwe, A.M., Papiernik, S.K., Grieve, C.M., Yang, C.H., 2010. Influence of fumigants on
530 soil microbial diversity and survival of *E. coli* O157:H7. J Environ Sci Health, Part B: Pestic.,
531 Food Contam. Agric. Wastes 45, 416-426.
532
533 Irikiin, Y., Nishiyama, M., Otsuka, S., Senoo, K., 2006. Rhizobacterial community-level, sole
534 carbon source utilization pattern affects the delay in the bacterial wilt of tomato grown in
535 rhizobacterial community model system. Appl. Soil Ecol. 34, 27-32.
536
537 Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalised
538 Escherichia coli in temperate soils from Lake Superior watersheds. Appl. Environ. Microbiol.
539 72, 612-621.
540

Jangid, K., Williams, M.A., Franzluebbers, A.J., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biol. Biochem. 40, 2843-2853.

Jiang, X., Morgan, J., Doyle, M.P., 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. Appl. Environ. Microbiol. 68, 2605-2609.

Jiang, S., Chu, W., Olson, B., He, J.W., Choi, S., Zhang, J., Le, J., Gedalanga, P., 2007. Microbial source tracking in a small southern California urban watershed indicates wild animals and growth as the source of fecal bacteria. Appl. Microbiol. Biotechnol. 76, 927-934.

Jones, K., 2001. Campylobacters in water, sewage and the environment. J. Appl. Microbiol. 90, 68S-79S.

Korajkic, A., Wanjugi, P., Harwood, V.J., 2013. Indigenous microbiota and habitat influence *Escherichia coli* survival more than sunlight in simulated aquatic environments. Appl. Environ, Microbiol. 79(17): 5329. DOI: 10.1128/AEM.01362-13.

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40, 2407-2415.

Ma, J., Ibekwe, M., Yang, C.H., 2013. Influence of bacterial communities based on 454-pyrosequencing on the survival of *Escherichia coli* O157:H7 in soils. FEMS Microbiol Ecol. DOI: 10.1111/1574-6941.12083.

566

567 Matos, A., Kerkhof, L., Garland, J.L., 2005. Effects of microbial community diversity on the
568 survival of *Pseudomonas aeruginosa* in the wheat rhizosphere. Microb. Ecol. 49, 257-264.
569

570 McLean, E.O., 1982. Soil pH and lime requirement, in: Page et al. (Eds.), Methods of Soil
571 Analysis, Part 2. Agronomy Monographs 9, 2nd edn. ASA and SSSA, Madison, WI, pp. 199-
572 223.
573

574 Mehlich, A., 1984. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractant.
575 Commun. Soil Sci. Plan. 15, 1409-1416.
576

577 Moynihan, E., Richards, K., Ritz, K., Tyrrel, S., Brennan, F., 2013. The impact of soil type,
578 biology and temperature on the survival of non-toxigenic *E. coli* O157. Biol. Environ. 113B,
579 1-6.
580

581 Mubiru, D.N., Coyne, M.S., Grove, J.H., 2000. Mortality of *Escherichia coli* O157:H7 in two
582 soils with different physical and chemical properties. J. Environ. Qual. 29, 1821-1825.
583

584 Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon and organic matter, in:
585 Bartels et al. (Eds.), Methods of Soil Analysis: Part 3 Chemical methods, 3rd edn. ASA and
586 SSSA Book Series 5, Madison, WI, pp. 961-1010.
587

588 Oliver, D.M., Haygarth, P.M., Clegg, C.D., Heathwaite, A.L., 2006. Differential *E. coli* die-
589 off patterns associated with agricultural matrices. Environ. Sci. Technol. 40, 5710-5716.
590

591 Olsen, S.R., Sommers, L.E., 1982. Phosphorus, in : Page et al. (Eds.), Methods of Soil
592 Analysis, Part 2. Agronomy Monographs 9, 2nd edn. ASA and SSSA, Madison, WI, pp. 403-
593 430.
594
595 Pagliai, M., Vignozzi, N., Pellegrini, S., 2004. Soil structure and the effects of management
596 practices. Soil Till. Res. 79, 131-143.
597
598 Petersen, S.O., Klug, M.J., 1994. Effects of sieving, storage, and incubation temperature on
599 the phospholipid fatty acid profile of a soil microbial community. Appl. Environ. Microbiol.
600 60, 2421-2430.
601
602 Rattray, E.A., Prosser, J.I., Killham, K., Glover, L.A., 1990. Luminescence-based
603 nonextractive technique for in situ detection of *Escherichia coli* in soil. Appl. Environ.
604 Microbiol. 56, 3368-3374.
605
606 Ross, D., 1995. Recommended soil tests for determining exchange capacity, in: Sims, J.T.,
607 Wolf, A. (Eds.), Recommended soil testing procedures for the northeastern United States.
608 Northeastern Regional Bulletin No. 493. Agricultural Experimental Station, University of
609 Delaware, Newark, DE, pp. 62-69.
610
611 Schulte, E.E., Hopkins, B.G., 1996. Estimation of soil organic matter by weight loss-on-
612 ignition, in: Magdoff, F.R., Tabatabi, M.A., Hanlon, E.A (Eds.), Soil Organic Matter:
613 Analysis and Interpretation. Special Publication No. 46. ASA and SSSA, Madison, WI, pp.
614 21-32.
615

616 Sjogren R.E., 1994. Prolonged survival of an environmental *Escherchia coli* in laboratory soil
617 microcosms. Water, Soil and Air Poll. 75, 389-403.
618
619 Sørensen, S.J., Schyberg, T., Rønn, R., 1999. Predation by protozoa on *Escherichia coli* K12
620 in soil and transfer of resistance plasmid RP4 to indigenous bacteria in soil. Appl. Soil Ecol.
621 11, 79-90.
622
623 Texier, S., Prigent-Combaret, C., Gourdon, M.H., Poirier, M.A., Faivre, P., Dorioz, J.M.,
624 Poulenard, J., Jocteur-Monrozier, L., Moënne-Loccoz, Y., Trevisan, D., 2008. Persistence of
625 culturable *Escherichia coli* fecal contaminants in dairy alpine grassland soils. J. Environ.
626 Qual. 37, 2299-2310.
627
628 Topp, E., Welsh, M., Tien, Y.C., Dang, A., Lazarovits, G., Conn, K., Zhu, H., 2003. Strain-
629 dependent variability in growth and survival of *Escherichia coli* in agricultural soil. FEMS
630 Microbiol. Ecol. 44, 303-308.
631
632 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil
633 microbial biomass C. Soil Biol. Biochem. 19, 703-707.
634
635 van Elsas, J.D., Garbeva, P., Salles, J., 2002. Effects of agronomical measures on the
636 microbial diversity of soils as related to the suppression of soil-borne plant pathogens.
637 Biodegradation 13, 29-40.
638

van Elsas, J.D., Hill, P., Chronakova, A., Grekova, M., Topalova, Y., Elhottova, D., Kristufek, V., 2007. Survival of genetically marked *Escherichia coli* O157:H7 in soil as affected by soil microbial community shifts. ISME J. 1, 204-214.

van Elsas, J.D., Semenov, A.V., Costa, R., Trevors, J.T., 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. ISME J. 5, 173-183.

van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottovā, D., Krištůfek, V., Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. P. Natl. Acad. Sci. USA 109, 1159-1164.

Vivant, A., Garmyn, D., Maron, P., Nowak, V., Piveteau., 2013. Microbial diversity and structure are drivers of the biological barrier against *Listeria monocytogenes* in soil. PLoS ONE 8(10): e76991.

Waldrop, M.P., Firestone, M.K., 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microb. Ecol. 52, 470-479.

Wang, H., Zhang, T., Wei, G., Wu, L., Wu, J., Xu, J., 2014. Survival of *Escherichia coli* O157: H7 in soils under different land-use types. Environ. Sci. Poll. Res. 21, 518-524.

Weis, J., Seeglier, H.P.R., 1975. Incidence of Listeria monocytogenes in nature. Appl. Microbiol. 30, 29-32.

663 Williams, A.P., Avery, L.M., Killham, K., Jones, D.L., 2007. Survival of *Escherichia coli*
664 O157:H7 in the rhizosphere of maize grown in waste-amended soil. J. Appl. Microbiol. 102,
665 319-326.
666
667 Winfield, M.D., Groisman, E.A., 2003. Role of nonhost environments in the lifestyles of
668 *Salmonella* and *Escherichia coli*. Appl. Environ. Microbiol. 69, 3687-3694.
669
670 Wu, Y., Ding, N., Wang, G., Xu, J., Wu, J., Brookes, P.C., 2009. Effects of different soil
671 weights, storage times and extraction methods on soil phospholipid fatty acid analyses.
672 Geoderma 150, 171-178.
673
674 Yao, Z., Wei, G., Wang, H., Wu, L., Wu, J., Xu, J. Survival of *Escherichia coli* O157:H7 in
675 soils from vegetable field under different cultivation patterns. Appl. Environ. Microbiol.
676 2013; DOI: 10.1128/AEM.03605-12.
677
678 You, Y., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D., Dou, Z., 2006. Survival of
679 *Salmonella enterica* serovar Newport in manure and manure-amended soils. Appl. Environ.
680 Microbiol. 72, 5777-5783.
681
682 Zhang L., Xu Z., 2008. Assessing bacterial diversity in soil. J. Soil Sed. 8, 379-388.
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686 **TABLES**

687 Table 1: Physicochemical and biomass properties of the 12 soils utilised for pathogen survival analysis experiments

Soil ID*	Site coordinates	Land use category	Specific land use	Total CEC (ME 100 g^{-1})	pH	% Organic matter	Olsen P (ppm)	% Organic C	% C	% N	C:N ratio	% Clay	% Silt	% Sand	Moisture (% field capacity)**	Biomass C (µg C g^{-1} dry soil)
A	52.17N, 6.31W	grass	grazing	11.7	6.2	6.1	46.7	3.3	3.75	0.36	10.42	13.6	34.1	52.3	46.3	356
B	52.17N, 6.31W	grass	grazing	13.2	5.6	6.4	54.7	3.6	3.80	0.36	10.56	12.9	31.6	55.5	48.3	384
C	52.52N, 6.55W	wood	forestry	26.4	7.5	5.2	8.0	4.1	4.55	0.25	17.97	25.2	33.0	41.8	41.1	230
D	52.51N, 6.54W	wood	willow	17.8	7.6	5.4	94.7	3.5	3.79	0.31	12.09	21.0	22.0	57.0	38.8	263
E	52.21N, 7.19W	arable	maize	10.0	6.4	3.0	70.3	1.5	1.89	0.21	8.84	19.4	32.6	48.1	43.0	81
F	52.21N, 7.18W	arable	cabbage	10.0	7.0	3.8	47.0	2.0	2.47	0.22	11.04	21.9	35.9	42.1	44.3	113
G	52.10N, 8.14W	grass	grazing	14.8	5.5	7.2	138.0	3.9	5.78	0.44	13.24	16.5	32.3	51.2	41.7	381
H	52.21N, 7.18W	grass	grazing	11.8	5.7	6.8	54.7	3.9	4.17	0.41	10.17	19.3	45.8	43.9	45.0	485
I	52.30N, 8.12W	grass	grazing	22.0	6.4	13.8	240.0	5.9	7.60	0.77	9.83	23.5	41.4	35.1	54.5	695
J	52.51N, 6.55W	arable	till, mustard cover	13.3	6.9	4.2	178.7	3.0	3.04	0.27	11.26	10.7	22.4	66.9	35.6	122
K	52.21N, 7.19W	grass	grazing	12.9	5.9	7.8	86.7	4.0	4.55	0.48	9.55	20.3	36.2	43.6	44.7	415
L	52.51N, 6.55W	arable	till, sprayed	12.9	6.9	4.2	145.0	2.8	2.84	0.28	10.27	8.8	21.7	69.5	36.0	115

*c.f. Figs. 2, 3, 4 and Supp. Fig. 1
**Moisture content at which samples were incubated

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694 Table 2: PLFA I.D and corresponding biomarkers (c.f. Fig. 2 and Supp. Fig. 1)

PLFA ID	Biomarker	PLFA ID	Biomarker
1	12:0	20	17:0br
2	14:0	21	17:1w8c
3	i15:0	22	cy17:0
4	a15:0	23	17:1w8t
5	15:0	24	17:1w7
6	2-OH 14:0	25	17:0 (12Me)
7	i16:1	26	18:2w6,9
8	16:1w11c	27	18:1w9c
9	3-OH 14:0	28	18:1w7t
10	i16:0	29	18:1w13
11	16:1w11t	30	18:1w10/11
12	16:1w7c	31	18:0
13	16:1w7t	32	18:0 (10Me)
14	16:1w5	33	19:0cy
15	16:0	34	19:0
16	Me17:0 isomer	35	20:4
17	Me17:0 isomer2	36	20:5w3
18	i17:0	37	20:0
19	ai17:0		

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697 Table 3: Death rates of pathogens introduced into soils from different land-uses (n=3)

	K-values (days^{-1})*			
Soil ID	*S.* Dublin	*L. monocytogenes*	*E. coli* LYS 9	*E. coli* O157
A	0.22±0.02	0.13±0.03	0.89±0.29	0.13±0.05
B	0.10±0.03	0.07±0.02	0.11±0.02	0.09±0.03
C	0.09±0.01	0.07±0.02	0.42±0.11	0.09±0.03
D	0.12±0.01	0.07±0.02	0.08±0.01	0.09±0.03
E	0.12±0.03	0.10±0.02	0.17±0.04	0.17±0.03
F	0.13±0.03	0.12±0.02	0.55±0.11	0.09±0.03
G	0.24±0.05	0.40±0.08	0.59±0.12	0.51±0.07
H	0.25±0.03	0.22±0.05	0.71±0.15	0.22±0.06
I	0.13±0.04	0.10±0.02	0.28±0.05	0.14±0.03
J	0.13±0.01	0.10±0.01	0.19±0.04	0.13±0.04
K	0.23±0.03	0.21±0.06	0.30±0.05	0.16±0.02
L	0.13±0.02	0.14±0.01	0.22±0.04	0.24±0.06

*Exponential decay model significantly fit curves for all pathogens and treatments ($P<0.05$)

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Table 4: Stepwise multiple regression results involving prediction of pathogen death rates versus physico-chemical and biological parameters (see text)

	Adjusted r^2	SS	df	MS model	SS model	df residual	MS residual	F	p
E. coli O157 PC1	0.455	0.077	1	0.077	0.076	10	0.008	10.2	0.010
S. Dublin PC2	0.519	0.023	1	0.023	0.018	10	0.002	12.9	0.005
L. monocytogenes PC2	0.667	0.066	1	0.066	0.028	10	0.003	23.1	0.001
E. coli Isolate 3	No fit								

723 **FIGURE LEGENDS**

724 Figure 1: Ordinations of (a) first and second principal and (b) third and fourth PCs derived
725 from average PLFA profiles in soils according to the pathogen batch with which they were
726 inoculated (points show means ± standard error (n=36).

727

728 Figure 2: Ordination of soils according to first and second PCs derived from individual PLFA
729 profiles and corresponding loadings plots for each pathogen batch at respective T_0's for soils
730 inoculated with (a, b) *S*. Dublin, (c, d) *L. monocytogenes*, (e, f) *E. coli* Isolate 3 and (g, h) *E.*
731 *coli* O157. Data represent PC scores ± standard error (n=3). Soil identification codes are in
732 Table 1.

733

734 Figure 3: Decay curves for (a) *S*. Dublin, (b) *L. monocytogenes*, (c) *E. coli* Isolate 3 and (d) *E.*
735 *coli* O157 following inoculation to soil microcosms. Data represent average $\log_{10}$ CFU g^{-1} soil
736 (dry weight) ± standard error (n=3). Soil abbreviation codes are as in Table 1.

737

738 Figure 4: Relationship between death rates of (a) *E. coli* O157, (b) *S.* Dublin and (c) *L.*
739 *monocytogenes* and community structure represented by PC scores derived from average
740 PLFA profiles associated with each batch. Data represent average values ± standard error
741 (n=3). See Table 1 for soil identification.

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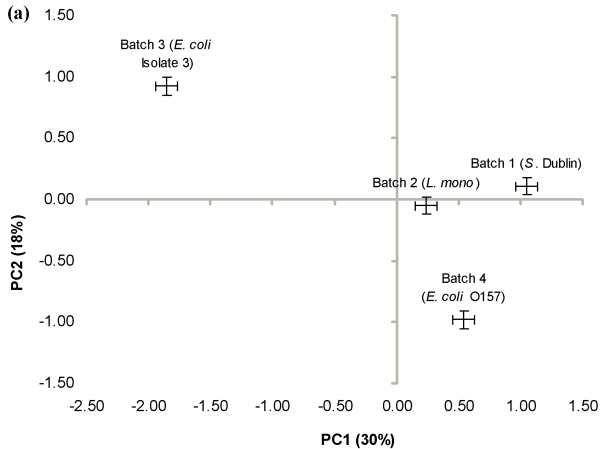
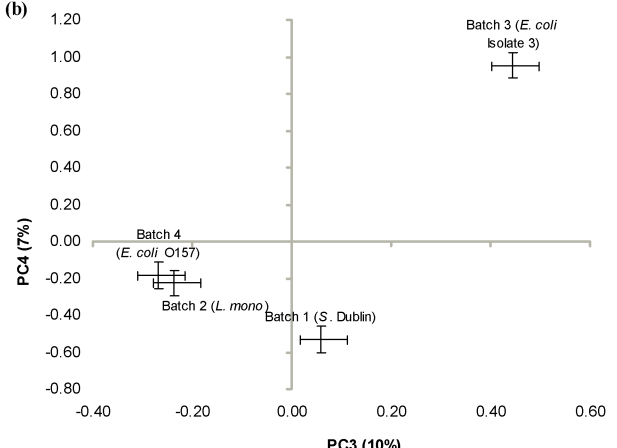
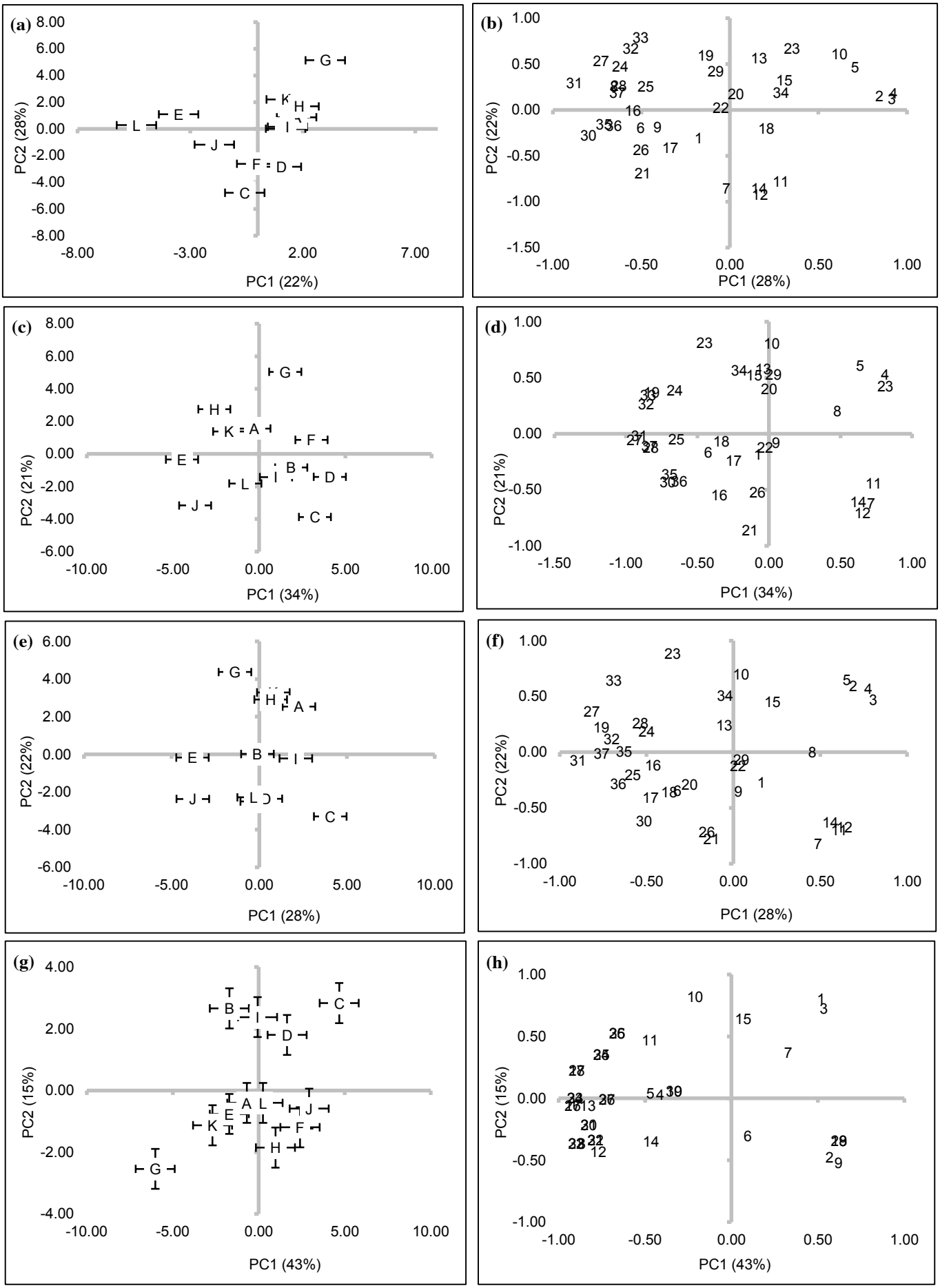
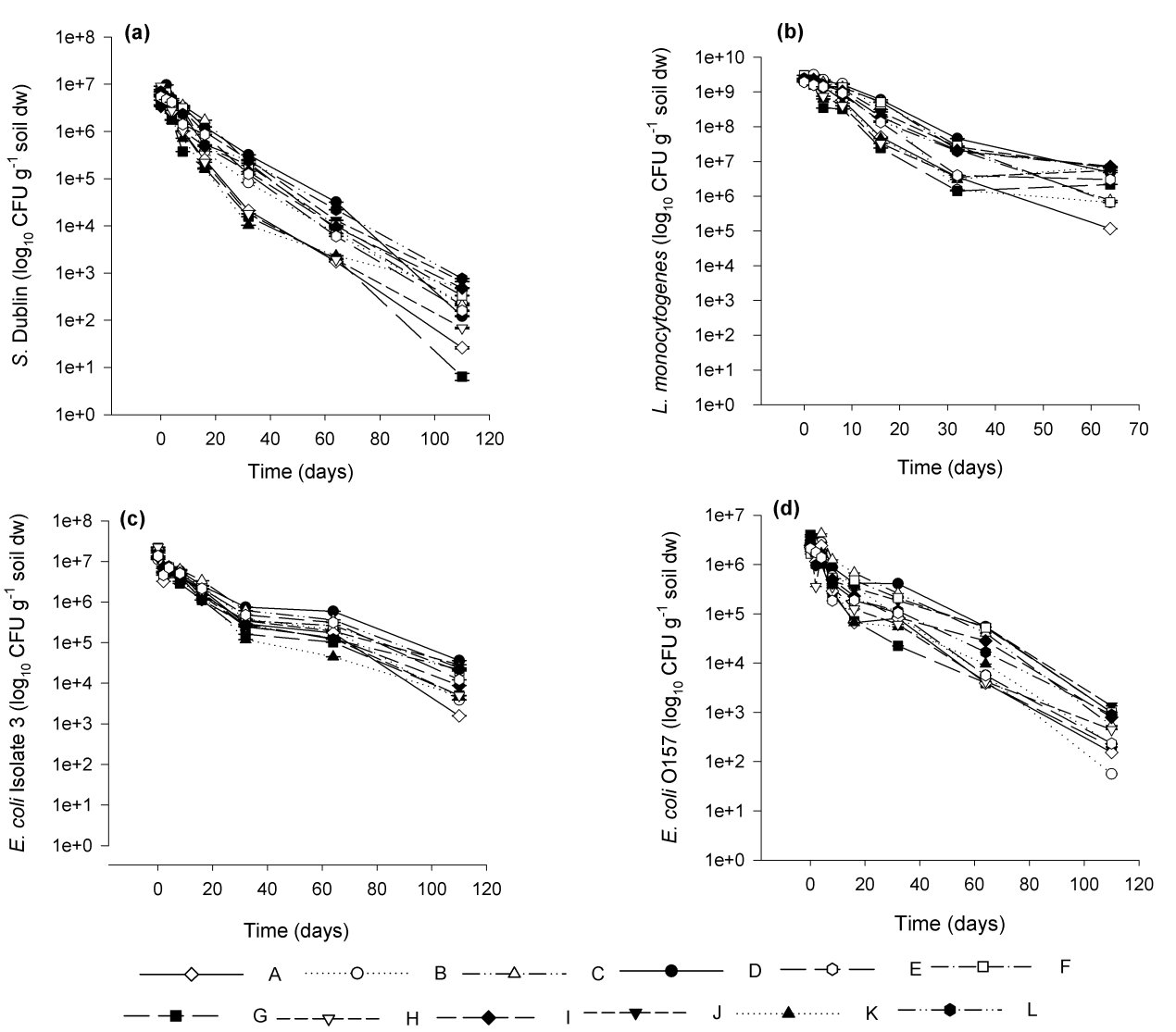
SUPPLEMENTARY INFORMATION

Supplementary Figure 1: First and second PCs derived from PLFA profiles of 39 soils according to (a) soil ID – letter codes A-L relate to final 12 soils selected for pathogen survival analysis, alphanumeric codes relate to other soils profiled, (b) land-use, (c) texture, (d) pH and (e) % OM (n=3). Encircled data-points represent final 12 soils selected for pathogen survival analysis in all graph panels.

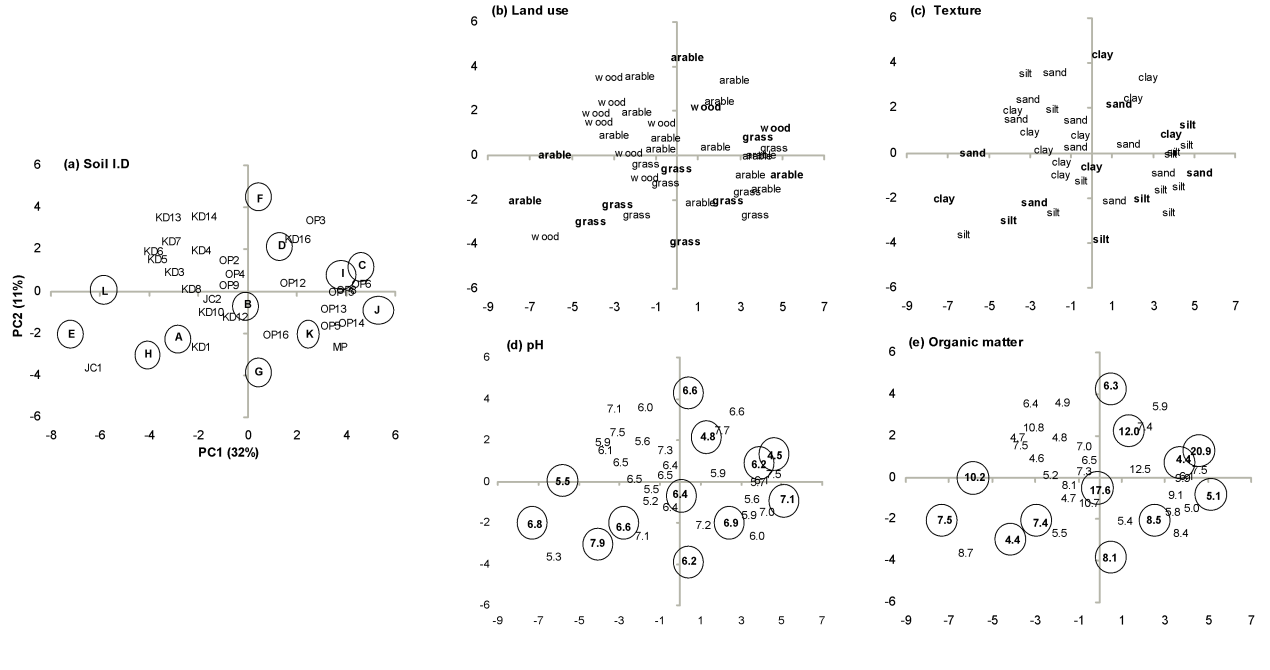

(a)
Batch 3 (E. coli Isolate 3)
Batch 1 (S. Dublin)
Batch 2 (L. mono)
Batch 4 (E. coli O157)
PC2 (18%)
PC1 (30%)
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50


(b)
Batch 3 (E. coli Isolate 3)
Batch 4 (E. coli O157)
Batch 2 (L. mono)
Batch 1 (S. Dublin)
PC4 (7%)
PC3 (10%)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
-0.40
-0.20
0.00
0.20
0.40
0.60


(a)
PC2 (28%)
PC1 (22%)
(b)
PC2 (22%)
PC1 (28%)
(c)
PC2 (21%)
PC1 (34%)
(d)
PC2 (21%)
PC1 (34%)
(e)
PC2 (22%)
PC1 (28%)
(f)
PC2 (22%)
PC1 (28%)
(g)
PC2 (15%)
PC1 (43%)
(h)
PC2 (15%)
PC1 (43%)


(a)
S. Dublin (log10 CFU g-1 soil dw)
Time (days)
(b)
L. monocytogenes (log10 CFU g-1 soil dw)
Time (days)
(c)
E. coli Isolate 3 (log10 CFU g-1 soil dw)
Time (days)
(d)
E. coli O157 (log10 CFU g-1 soil dw)
Time (days)
A
B
C
D
E
F
G
H
I
J
K
L

(a)
y = -0.0294x + 0.1713
R² = 0.5045
P<0.01
Death rate (k)
PC1 (43%)
(b)
y = 0.0173x + 0.1579
R² = 0.5625
P<0.005
Death rate (k)
PC2 (22%)
(c)
y = 0.0304x + 0.1482
R² = 0.6976
P<0.001
Death rate (k)
PC2 (21%)


(a) Soil I.D
PC2 (11%)
PC1 (32%)
F
D
C
I
L
B
J
E
A
K
H
G
KD13
KD14
OP3
KD7
KD16
KD6
KD4
KD5
OP2
KD3
OP4
OP9
OP12
KD8
JC2
KD10
KD12
OP13
OP14
OP16
OP15
KD1
MP
JC1
(b) Land use
arable
wood
grass
(c) Texture
clay
silt
sand
(d) pH
6.6
4.8
4.5
6.2
5.5
6.4
7.1
6.8
6.6
7.9
6.9
7.4
6.2
(e) Organic matter
6.3
12.0
20.9
4.4
10.2
17.6
5.1
7.5
7.4
8.5
4.4
8.1