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Title page

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

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Running Title: Soil microbial community affects pathogen survival

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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 relative impact of biotic, specifically microbial community structure, and physico-chemical properties associated with soils derived from 12 contrasting land-uses on enteropathogen survival. Phenotypic profiles of microbial communities (via phospholipid fatty acid (PLFA) profiling), and total biomass (by fumigation-extraction), in the soils were determined, as well as a range of physicochemical properties. The persistence of *Salmonella* Dublin, *Listeria monocytogenes,* and *Escherichia coli* was measured over 110 days within soil microcosms. Physicochemical and biotic data were used in stepwise regression analysis to determine the predominant factor related to pathogen-specific death rates. Phenotypic structure, associated with a diverse range of constituent PLFAs, was identified as the most significant factor in pathogen decay for *S.* Dublin, *L. monocytogenes,* non-toxigenic *E. coli* O157 but not for environmentally-persistent *E. coli*. This demonstrates the importance of entire community-scale interactions in pathogen suppression, and that such interactions are context-specific.

1. INTRODUCTION

Microbial enteropathogens are released in faecal waste of both animals and humans, and enter the soil environment either directly via faecal shedding, or indirectly via the application of slurry, manure and sewage sludge. In addition, wild animals and birds contribute to enteropathogen load in the environment (Jones, 2001; Jiang et al., 2007; Benskin et al., 2009), and there is evidence to suggest that potentially pathogenic enteric bacteria can exist as naturalised populations within the soil matrix (Texier et al., 2008; Ishii et al., 2006; Brennan et al., 2010). Enteropathogens can pose a serious public health risk, contingent on survivability within the soil environment. Viable pathogens may be transmitted to humans by direct contact with contaminated surfaces and accidental ingestion of faeces or contaminated soil particles (Davis et al., 2005). Pathogens can also be transported via overland or subsurface flow to surface and groundwaters, and infection may arise via ingestion of contaminated water, e.g. Walkerton Outbreak, Ontario in 2000 (Hrudey et al., 2003). It is also possible that pathogens could be present on the crop surface following manure application. In this case, a person may become infected if they consume the contaminated produce, as demonstrated by the 2011 *E. coli* O104 outbreak in Germany, associated with consumption of contaminated beansprouts (Böhmer et al., 2011).

To date, enteropathogen survival in soil has been mostly investigated in relation to prevailing physicochemical conditions. Factors known to affect pathogen survival include moisture, temperature, texture, pH, cation exchange capacity (CEC), UV irradiation, organic matter (OM) and soil nutrient status (summarised by van Elsas et al., 2011). For example, persistence is favoured by cool moist conditions (Cools et al., 2001), where exposure to UV is limited (Hutchison et al., 2004b). Typically, the survival of enteric bacteria is reduced at low pH, and tends to increase when approaching a neutral to alkaline state (Sjogren, 1994). Fine textured soils with well-developed microstructure and high clay content offer habitat, water and nutrients, which can sustain pathogens introduced via manure application (England et al., 1993).

Soil biology also plays an important function in regulating pathogen survival; however research on interactions with the soil community has been comparatively limited. Pertinent biotic interactions include predation (Sørensen et al., 1999), antagonism from indigenous microorganisms (Garbeva et al., 2004) and competition for resources (Irikiin et al., 2006). It has been found that bacteria introduced into soil decline more rapidly when other microbes are present. This decline is apparently accelerated when the indigenous microbial community is increasingly diverse. A range of experimental approaches have been used to manipulate microbial diversity

with a view to investigating the relationship between diversity and *E. coli* survivability (Vivant et al, 2013; Yao et al., 2013; Ma et al., 2013; Korajkic et al., 2013; Erickson et al., 2013). All of these studies showed an inverse relationship between community complexity and pathogen survival, attributed to progressively increasing competition for resources and antagonistic interactions associated with greater diversity.

The soil microbial community is typically sensitive to changing environmental conditions (Waldrop and Firestone, 2006), and consequent shifts in community structure could influence the survival behaviour of introduced enteric pathogens. Land-use and management has been implicated in shaping the microbial community by modulating the physicochemical environment (Lauber et al., 2008). It has been shown that intensity of land-use (Jangid et al., 2008), length of time under a particular management (Buckley and Schmidt, 2001), substrate addition (Degens et al., 2000) and the presence of a plant rhizosphere (Garbeva et al., 2004) can contribute to defining microbial community structure. Some work has been carried out to demonstrate the effects of land-use and management on pathogen suppression (van Elsas et al., 2002; Williams et al., 2007; Franz et al., 2008; Yao et al., 2013). However, the pathogen survival response is often variable and difficult to predict within a framework of complex interactions between site-specific factors, including current and historical

land-use, the physicochemical environment, predominant management strategies and resultant impact on community composition. In addition, these studies focused solely on a single pathogen, namely *E. coli* O157, despite the fact that survival and behavioural profiles within soil are species, and even strain-specific (Topp et al., 2003). This is because enteropathogens have different physiological properties and life cycles which will influence survivability within the soil matrix (Winfield and Groisman, 2003).

It is therefore unclear whether physicochemical or biotic factors play a dominant role in governing pathogen survival, particularly as few studies have considered both in a coherent manner. Therefore, the aim of this study was to investigate pathogen survival in relation to naturally-contrasting community phenotypes derived from 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, and conducted a controlled microcosm-based study to test this in the context of four model pathogenic bacteria. 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, comprising mainly cambisols, gleysols and stagnosols (Table 1), 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 samples were also assayed for microbial biomass carbon, according to the method described by Vance et al., 1987. Average PLFA profiles for the initial 39 soils were compared via principal component (PC) analysis. First and second PC scores were ordinated to visualise the relative distribution of soils according to community composition, and were labelled according to soil ID, land-use, texture, pH and % OM (Supp. Fig. 1a-e, respectively). By comparing these ordinations, it was possible to visualise community differences with respect to physicochemical properties and thus prescribe a suitably broad range of naturally-derived contexts to subsequently characterise pathogen survival. Twelve soils were duly prescribed from this population, representing the gamut of community structures and soil physico-chemical properties (encircled in Supp. Fig 1).

2.2 Microcosm establishment

The water holding capacity (WHC) for each prescribed soil was determined by the method described in Franz et al. (2011). Moisture content was then adjusted so that soils exhibited similar cohesiveness to achieve standard friability between different soil types, by wetting-up or restricted slow drying on the bench as appropriate. Following adjustment, soil moisture was measured by oven-drying at 105^{o}C for 24 hours, and expressed as a percentage of WHC. Microcosms designed to

quantify pathogen survival were established by weighing out aliquots of 5 g soil into sterile 40 ml polypropylene tubes. The tubes were covered with Parafilm to prevent moisture loss during incubation. Caps were then loosely replaced to allow for gas exchange, whilst minimising the risk of contamination. All tubes were stored at 10^{o}C until inoculated with the pathogen suspension. This temperature was selected as it reflects the average annual topsoil soil (0-10 cm) temperature in Ireland. Pathogen inoculation was staggered over a 4-week period, with exactly one week between each inoculation. Therefore the precise community configuration to which each pathogen was exposed was determined by undertaking PLFA analysis at the outset of each inoculation, in order to capture any microbial changes associated with storage and physical alteration, including sieving and moisture adjustment and ensure that the precise configuration of the microbial community was determined in each instance.

2.3 Pathogen inoculation and enumeration

Four model pathogens were selected to investigate community interactions, namely an environmentally-persistent *E. coli* (Brennan et al., 2013), *Salmonella* Dublin (NCTC 9676), *Listeria monocytogenes* (Strain no. 1778) and non-toxigenic lux-marked *E. coli* O157 (Strain no. 3704), which has been shown to be a representative proxy for the toxigenic O157

strain of clinical importance (Bolton et al., 1999). These organisms were prescribed since they were considered relevant in terms of public health significance, and also represented contrasting cellular structures and growth strategies (Winfield and Groisman, 2003).

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

Enumeration was carried out by suspending the soil in 10 ml of ¼ strength Ringer's solution, vortexing briefly and shaking on an end-over-end shaker for 15 minutes. These suspensions were then used to create serial dilutions, which

were then spread-plated onto Sorbitol MacConkey, XLD or Oxford agars (Oxoid) for *E. coli* spp., *S.* Dublin and *L. monocytogenes*, respectively. All plates were incubated at 37^{o}C for 24 hours, with the exception of *L. monocytogenes* – these were incubated at 37^{o}C for 48 hours. All soils were screened for bacterial targets prior to the experiment, to ensure background levels were negligible. *L. monocytogenes* could not be quantified at T_{110}, due to excessive growth of background microflora on Oxford agar plates. Therefore, survival data for this organism are only presented to T_{64}.

2.5 Data analysis

Pathogen survival data were collected by counting characteristic colonies. Triplicate counts for each soil treatment were averaged and were plotted as CFU g^{-1} (dry weight). These data were used to fit exponential decay curves and calculate the average death rate for each pathogen within the context of each soil treatment, according to the following equation: $y=a+b*e^{-kt}$, where y represents the population (CFU g^{-1} soil dry weight) at a given time t, a+b denotes the apparent starting concentration of cells (i.e. intercept with the y-axis), a denotes the asymptote of the final population concentration, and k denotes the death rate (d^{-1}). This function has been used previously to estimate pathogen death rates (Mubiru et al., 2000; Oliver et al., 2006). PLFA profiles were analysed by principal components (PC)

analysis, and relative PLFA abundances were ordinated for each soil independently for each batch. PC analysis was also applied to the entire dataset across the four batches, and the relative PLFA abundances were ordinated accordingly. Physicochemical, community and k-values were averaged per soil, and entered into a forward stepwise regression model (Statistica v. 11) to investigate the predominant influential factor regulating pathogen death rates across the 12 soil types.

3. RESULTS

3.1 Soil community profiling

The prescribed 12 soils were labelled alphabetically (Table 1) and PLFAs were labelled numerically (Table 2) to aid visualisation during PC analysis. When PLFA profiles were analysed collectively across all four batches, there was a highly significant effect of batch ($P<0.001$) and soil ($P<0.001$), but no significant interaction between these terms ($P=0.2$-0.5) for any of PC1-4, which accounted for 66% of the variability between soils. Ordination of mean scores for each batch showed significant separation of all four circumstances, with Batch 3 being notably separated by PC1, 2 and 4 (Fig. 1a). Batches 1, 2 and 4 tended to cluster in the ordinations but were nonetheless significantly separated by PCs 1-3 (Fig. 1b). Ordination of PC1

and PC2 for the PLFA profiles associated with each soil independently showed concomitantly wide dispersion, with notable differences between the ordinations in the four batches. Ordination of corresponding PLFA loadings in this case showed that neither PC1 nor PC2 was dominated by particular PLFA types (Fig. 2a-h).

3.2 Pathogen death rates

Pathogens declined in an exponential manner in all instances (Fig. 3). There was visual evidence to suggest different survival characteristics between soils in the form of notably different gradients. This was confirmed by differences in pathogen-specific death rates (Table 3). Overall, there was an order of magnitude difference between highest and lowest death rates, observed for *E. coli* Isolate 3 in Soil A and *L. monocytogenes* in Soil C, respectively. The exponential decay function was a significant fit ($P<0.05$) for all pathogens within each soil. Stepwise regression showed that variation in death rates between land-use treatments was explained solely and significantly (P always <0.01) by phenotypic community structure according to PC scores for all model pathogens, with the exception of *E. coli* Isolate 3 (Table 4). No other physicochemical or biotic factor contributed to the stepwise regression model fitting procedure. There was no association between death rate and PC score for PC1, with the exception *E.*

coli O157 which showed a significant linear relationship (Fig. 4a, $P<0.01$). A similar relationship was observed between death rate and PC2 for *S.* Dublin (Fig. 4b, $P<0.005$) and *L. monocytogenes* (Fig. 4c, $P<0.001$) where higher death rates were associated with greater positive values in PC2. There was no association between death rate and PC2 for either *E. coli* Isolate 3 or *E. coli* O157. There was no association between death rate and PC3 or PC4 for any model pathogen tested.

4. DISCUSSION

The anticipation that the population of soils from different land-uses would provide the range of properties fit for purpose to test our hypothesis, duly investigated using a principal-component based screening approach was confirmed. It was shown that the soils possessed different physicochemical and community compositions, such that an appropriate suite of 12 soils which showed a broad range of similarity and difference across a range of biotic and physicochemical characteristics were selected, relating to the main factors hypothesised to influence pathogen death rates.

4.1 Soil community profiling

Pathogens were inoculated into these 12 soils on a weekly basis in a series of pathogen-specific batches. PC analysis of average

PLFA profiles showed significant differences in community composition between batches (Fig. 1). This indicates community composition within soils was not entirely conserved during the storage period. This effectively means that the respective pathogens were inoculated into subtly (albeit significantly) different community contexts. Soil community shifts over storage time has been previously reported (Petersen and Klug, 1994; Wu et al., 2009). However, the primary focus of this study was to create *different* biological scenarios in order to compare the relative importance of biotic versus physicochemical factors in regulating pathogen survival. Therefore these community shifts did not impact on addressing our central hypothesis.

PC analysis also showed highly significant differences in community phenotypic composition between soils, as anticipated from the first-phase screening (Fig. 2). Dispersal of soils within the phenotypic 'trait space' (visualised via the PC ordinations) indicates that a wide variety of community contexts were included in this study. The lack of a significant soil-by-batch interaction is evidence that the *relative* differences between communities were conserved over time when all batches were considered together, thus providing evidence that communities were broadly congruent between batches, and allowing similarities in pathogen behaviour to be tentatively evaluated.

The PC plots associated with each batch, representative of the range of soil communities present at respective pathogen-specific T_0's (Fig. 1) depict the precise community contexts to which the pathogens were exposed. PC analysis revealed significant differences between soil communities within each batch. The PLFA loadings associated with these PCs showed that differences in a range of PLFAs contributed to the significant discrimination between communities associated with these 12 soils (Fig. 1). Therefore, discrimination between communities associated with different land-uses was based on shifts in the total microbial cohort in this case. This contrasts with other work that has looked at the effect of different treatments on community configurations in soil. For example, Bossio et al. (1998) found associations between fatty acid signatures and organic, low input and conventional management, suggesting that particular groups were responsible for variation between management regimes. Similarly Frostegård et al. (1997) showed distinct differences in PLFAs associated with manure and those associated with soil, when investigating the impact of manure hotspots on microbial community dynamics. The lack of dominant PLFAs in this case may be due to comparison of a wide variety of soil communities, encompassing many different land-use treatments and soil types. This makes the community-scale context of our

study, central to our hypothesis, rather robust since no single PLFA type dominated discrimination between soils.

4.2 Pathogen death rates

Death rates in the range of soils differed between pathogens (Fig. 3, Table 3). Greatest initial decay was observed for both *E. coli* strains compared to *L. monocytogenes* and *Salmonella* Dublin. Pathogen survival in soil is associated with initial inoculum density, cell physiology, adaptability to new environments and capacity to utilise available substrate (van Veen et al., 1997). These factors may have contributed to differential survival patterns across the suite of pathogens used in this study. Fig. 3 also shows differences in overall persistence. Recovery of *E. coli* Isolate 3 was highest at the end of the experimental period, which may have been associated with its documented ability to persist and survive long-term within the soil matrix (Brennan et al., 2010; Brennan et al., 2013). *L. monocytogenes* extraction at T_{110} was unsuccessful, but a comparison of cell concentration at T_{64} shows that *L. monocytogenes* was also strongly competitive across the range of soil treatments and persisted well. This is consistent with previous findings that *L. monocytogenes* is a highly adaptable, saprophytic organism which is ubiquitous in the soil environment (Weis and Seeliger, 1975; Freitag et al., 2009).

Different pathogen death rates were also manifest within each soil. Death rate tended to be greatest within grassland land-use class, and poorest in arable and wood land-use classes for all pathogens. Regression analysis showed that PC scores representative of community composition provided by far the best predictor of pathogen survival for 3 of 4 pathogens investigated (Fig. 4, $P<0.01$). There was no significant relation 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 a general community-scale basis rather than specialist nature, caused by interactions with the total microbial consortium within these soils, rather than with specific microbial groups, which would be indicated by a few dominant PLFAs in the loadings. 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, can be 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

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 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 appeared 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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1 **TABLES**

2 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)	WRB soil classification
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	Haplic cambisol
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	Stagnic cambisol
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	Ferralic cambisol
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	Ferralic cambisol
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	Luvic gleysol
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	Leptic cambisol
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	Haplic cambisol
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	Haplic cambisol
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	Haplic cambisoil
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	Haplic cambisol
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	Haplic cambisol
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	Haplic stagnosol

*c.f. Figs. 2, 3, 4 and Supp. Fig. 1

**Moisture content at which samples were incubated

3

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9 Table 2: PLFA I.D and corresponding biomarkers (c.f. Fig. 2

10 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		

11

12

13 Table 3: Death rates of pathogens introduced into soils from

14 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 ($P<0.05$)

15

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								

FIGURE LEGENDS

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

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

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

Figure 4: Relationship between death rates of (a) *E. coli* O157, (b) *S.* Dublin and (c) *L. monocytogenes* and community structure represented by principal component (PC) scores

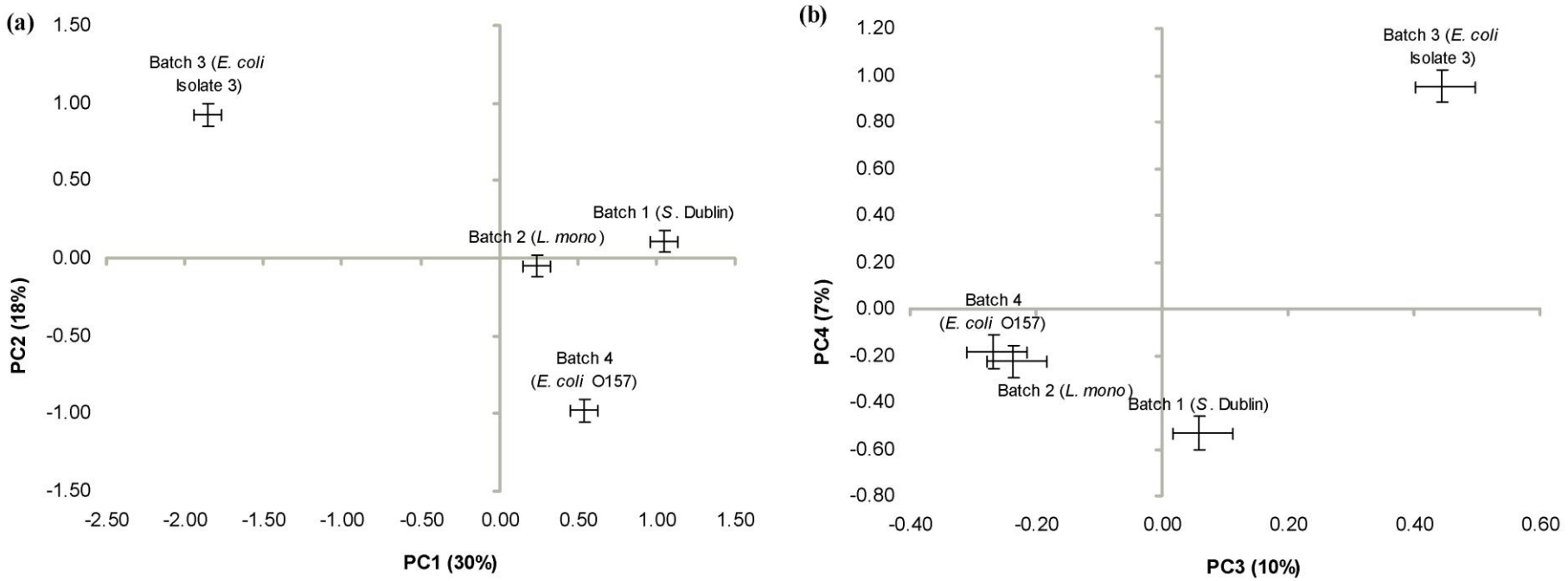
derived from average PLFA profiles associated with each batch. Data represent average values ± standard error (n=3). See Table 1 for soil identification.

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.

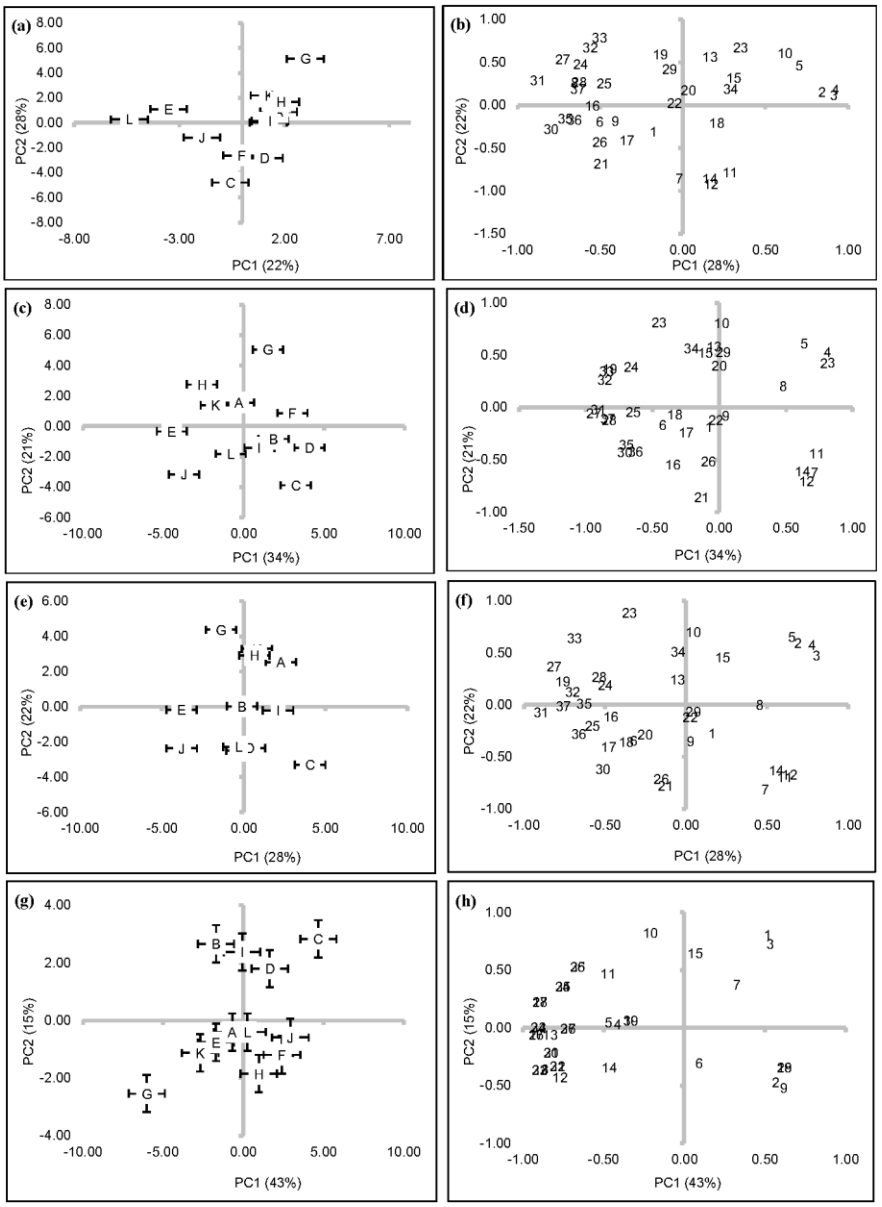
70 Fig. 1

71


(a)
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
PC2 (18%)
Batch 3 (E. coli Isolate 3)
Batch 1 (S. Dublin)
Batch 2 (L. mono)
Batch 4 (E. coli O157)
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
PC1 (30%)
(b)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
PC4 (7%)
Batch 3 (E. coli Isolate 3)
Batch 4 (E. coli O157)
Batch 2 (L. mono)
Batch 1 (S. Dublin)
-0.40
-0.20
0.00
0.20
0.40
0.60
PC3 (10%)

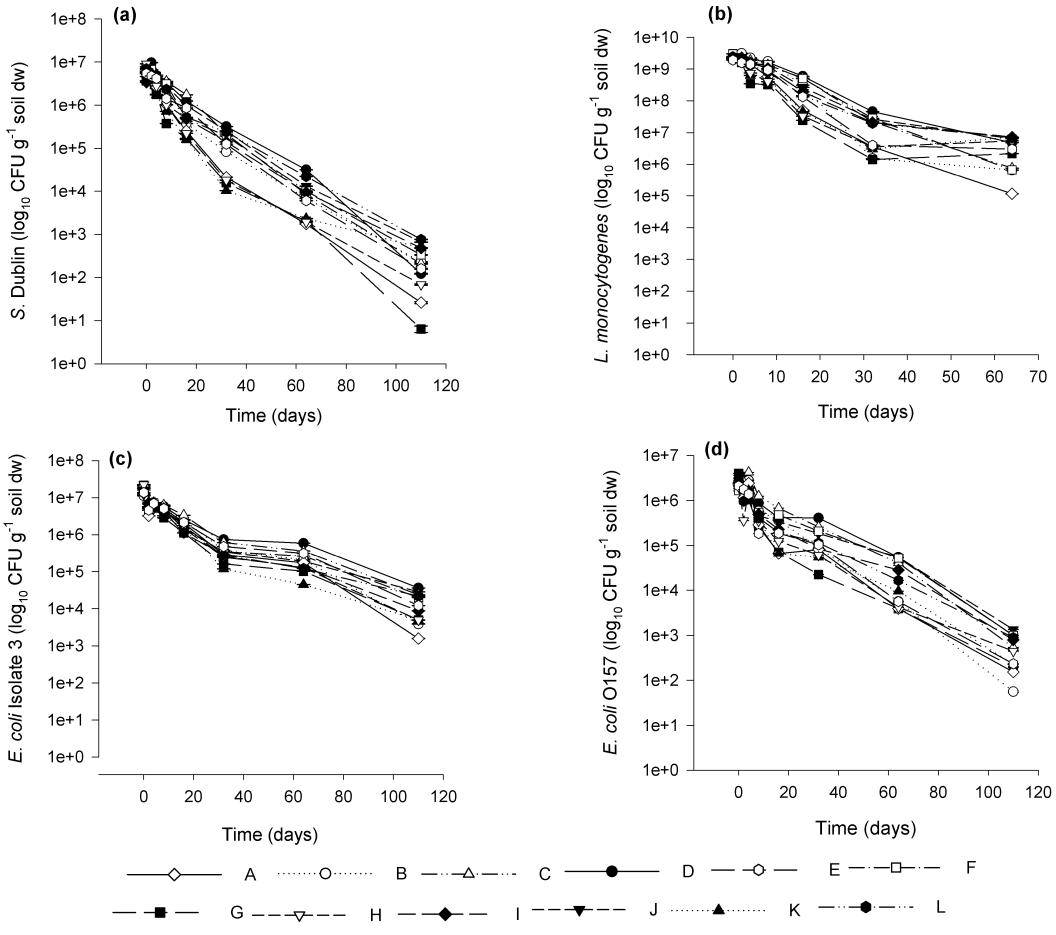
72

73 Fig. 2


(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%)

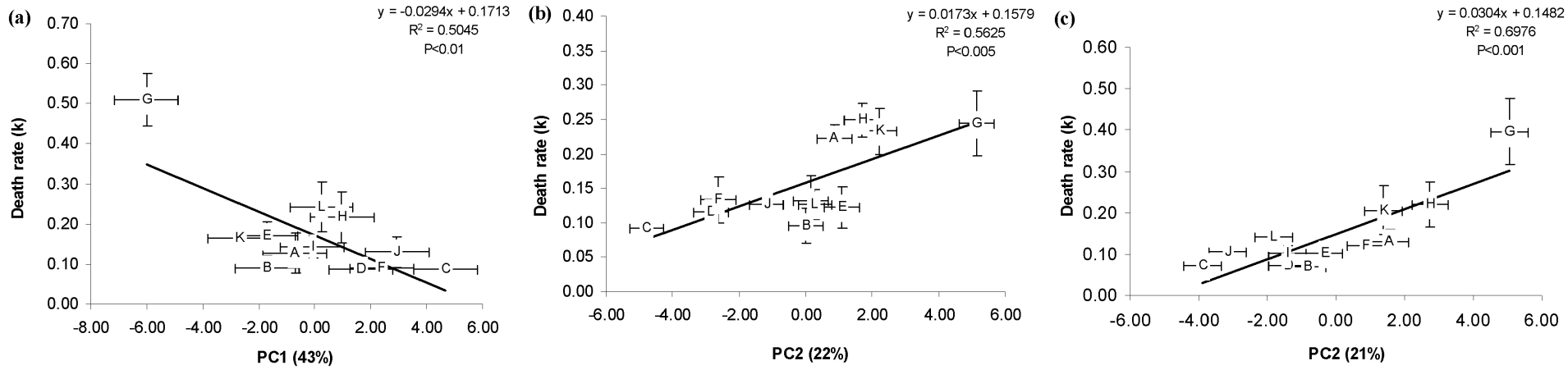
74

75 Fig. 3


(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

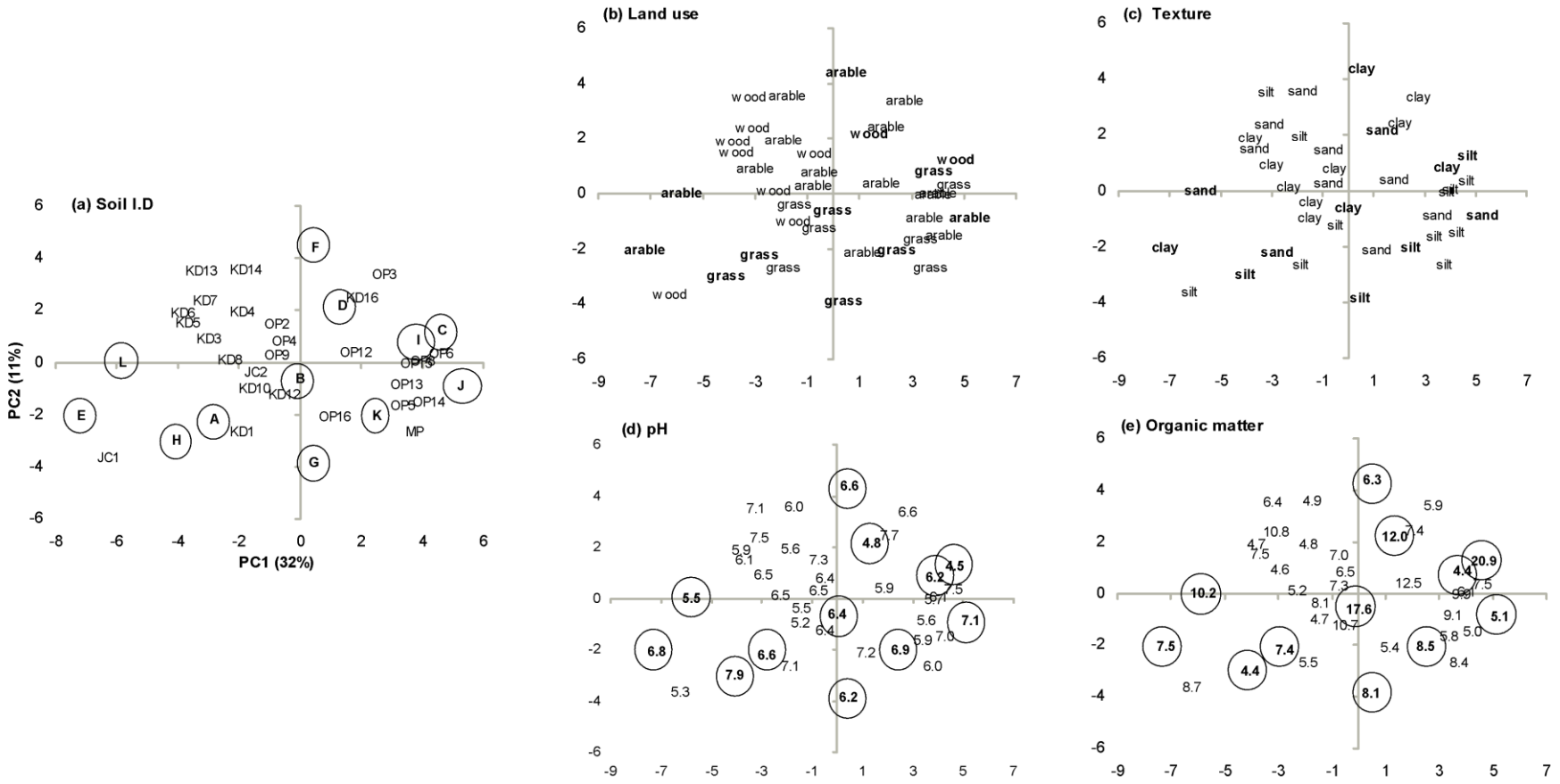
76

77 Fig. 4


(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%)

78

79 Supp. Fig.1


(a) Soil I.D
PC2 (11%)
PC1 (32%)
(b) Land use
(c) Texture
(d) pH
(e) Organic matter