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Re-shaping models of *E. coli* population dynamics in livestock faeces: increased bacterial risk to humans?

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Abstract

The aim of this study was to use a combined field and modelling approach to determine the importance of *Escherichia coli* growth in dung-pats when predicting faecal bacteria accumulation on grazed grassland. To do this an empirical model was developed to predict the dynamics of an *E. coli* reservoir within 1 ha plots each grazed by four beef steers for six months. Published first-order die-off coefficients were used within the model to describe the expected decline of *E. coli* in dung-pats. Modelled estimates using first-order kinetics led to an underestimation of the observed *E. coli* land reservoir, when using site specific die-off coefficients. A simultaneous experiment determined the die-off profiles of *E. coli* within fresh faeces of beef cattle under field-relevant conditions and suggested that faecal bacteria may experience growth and re-growth in the period post-defecation when exposed to a complex interaction of environmental drivers such as variable temperature, UV radiation and moisture levels. This growth phase in dung-pats is not accounted for in models based on first-order die-off coefficients. When the model was amended to incorporate the growth of *E. coli*, equivalent to that observed in the field study, the prediction of the *E. coli* reservoir was improved with respect to the observed data and produced a previously unquantified step-change improvement in model predictions of the accumulation of these faecal bacteria on grasslands. Results from this study suggest that the use of first-order kinetic equations for determining land-based reservoirs of faecal bacteria should be approached with caution and greater emphasis placed on accounting for actual survival patterns observed under field relevant conditions.

Keywords: cattle faeces, die-off, *E. coli*, grazing, growth, soil, uncertainty

Introduction

Livestock are an integral feature of the farmed landscape and a key component of the human food chain and rural economy. However, management of livestock and their manure must be undertaken with a view to ensure the sustainability of key ecosystem services, such as the provision of clean and safe recreational and drinking water (Pretty, 2008). Catchments dominated by agriculture have consistently been shown to generate high faecal indicator organism (FIO) pollutant concentrations in receiving waters (Sinclair *et al*., 2009; Kay *et al*., 2008a). Thus, microbial contamination of watercourses represents a critical component of diffuse water pollution (Kay *et al*., 2008b; Defra, 2007) and routine agricultural practices such as livestock grazing and manure spreading can introduce a range of bacterial, protozoan and viral contaminants to land via faecal material (Wilkes *et al*., 2009; Oliver *et al*., 2005). In particular, faeces excreted directly on pasture from grazing animals can contribute a significant burden of faecal microbes to agricultural land, often in excess of 10^{12} *E. coli* per hectare during each grazing season (Oliver *et al*., 2009). Importantly, dung-pats excreted by livestock undergo no microbial treatment phase (in contrast with stored manures) and so the microbiological content of faeces deposited directly to pasture is often high, though numbers vary with livestock type, diet and season (Chadwick *et al*., 2008, Donnison *et al*., 2008, Weaver *et al*., 2005). Dung-pats from livestock are therefore critical reservoirs of FIOs, such as *E. coli*, which are key regulatory determinands for assessing the microbiological quality of bathing and shellfish harvesting waters as specified in EU directives (CEC, 2006a, 2006b).

To reduce microbial contamination of watercourses and ultimately meet compliance requirements at designated bathing waters there is a need to target agricultural management options where they are likely to have most effect on mitigating FIO impact (e.g. Monaghan *et al*., 2009; Kay *et al*., 2007; Oliver *et al*., 2007). Monaghan *et al*. (2008) propose that the most effective mitigation strategies are those that target the

main *sources* of contaminants in farm systems. Being able to reliably predict FIO accumulation on grazed pastures would therefore prove useful in identifying potential microbial reservoirs, indicative of high risk critical source areas (CSAs) if combined with appropriate drivers such as rainfall events (Moriarty *et al*., 2008). The importance of on-farm microbial reservoirs has been reinforced at the international level with highest priority given to 'investigating the fate of faecal microbes on farms' in a recent and timely workshop to establish research priorities for coordinating management of food safety and water quality (Crohn and Bianchi, 2008). Predicting the balance between accumulation and depletion of *E. coli* within land-based reservoirs is thus crucial for understanding the dynamics of (or risk from) diffuse microbial pollution from agriculture.

Since the early 20th century (Bigelow, 1921) first-order kinetics have been used to describe the population decline of bacteria in research fields as diverse as medicine, food biotechnology and environmental microbiology (Peleg, 2003). When used to describe populations of faecal bacteria and pathogens in livestock faeces, these kinetics are commonly referred to as 'die-off', reflecting the generally held view that populations decline after faeces has been deposited. Consequently any potential risk of transfers to the wider environment and humans is thought to lessen with the passing of time after faeces deposition and thus models and policies reflect this. However, studies investigating naturally occurring bacterial survival in livestock faeces have tended to report on laboratory scale microcosm experiments (e.g. Echeverry *et al*., 2006; Himathongkham *et al*., 1999) which remove the complexity and heterogeneity of interacting natural processes. Fortunately, there has been a recent emergence of studies, particularly in North America and New Zealand, to investigate *field-relevant* bacterial die-off in faeces deposited on pasture (Muirhead *et al*., 2009; Soupir *et al*., 2008; Sinton *et al*., 2007; van Kessel *et al*., 2007; Meays *et al*., 2005; Muirhead *et al*., 2005). These studies have suggested that bacterial growth may be occurring, thus

questioning the suitability of approximations of FIO die-off in line with traditional first-order decline. If bacterial growth does occur then the potential underestimation resulting from first-order approximations can be theorised for a single dung-pat as shown in Fig 1;b. The aim of this study was to test the suitability of first-order inactivation curves by: (i) modelling *E. coli* dynamics on grassland grazed by cattle using a traditional first-order die-off equation and comparing the output with field data of *E. coli* accumulation within replicated 1-ha plots; (ii) determining an approximate growth value for *E. coli* in freshly deposited faeces via field experimentation in order to amend the first-order modelled predictions of *E. coli* dynamics on a grazed plot; and (iii) providing a first-approximation of the magnitude of potential error associated with adoption of first-order decline for predicting *E. coli* reservoirs on pasture whilst appreciating uncertainty within model parameters.

Materials and methods

Field monitored *E. coli* levels on pasture

A field study was undertaken to compare modelled predictions of *E. coli* burden to land with observed data. The field study reported here used the Rowden Research Platform (UK National Grid Reference (NGR): SX 650 995) reported previously in others studies (e.g. Oliver *et al*., 2005). Four replicated 1-ha plots were sampled at two week intervals throughout the May to November 2003 grazing season for *E. coli* and soil moisture content. Cattle were introduced onto the plots on May 9th and removed on November 5th, equivalent to Julian day 129 and 305, respectively. Each 1-ha plot was subdivided into a 6 x 6 grid and 12 soil cores (7 cm deep) were bulked from each sub-sector sampled. None of the plots had been grazed for over a year prior to 2002 because of the UK outbreak of Foot and Mouth Disease (FMD) in 2001 and pre-experiment soil concentrations of *E. coli* were below detection levels in 2003. To calculate the total reservoir of *E. coli* within each plot, mean cell concentrations per gram of dry weight soil

were multiplied by the estimated dry weight of soil in each 1 ha plot (to a depth of 7 cm, as per soil core depth).

Field monitored faecal deposits

An experiment was conducted to determine *E. coli* content in cattle faeces. This served two purposes. Firstly it provided a site-specific *E. coli* content for fresh dung-pats from beef cattle as model input. Secondly, given the potential for bacterial growth to impact on the model results, a field study of *E. coli* die-off was needed to determine a first approximation for the implementation of bacterial growth into the model; it was not conducted to provide a detailed account of FIO population increase in dung-pats or identify causal effects. Fresh dung-pats from beef cattle were collected during the grazing season from the experimental site. Dung-pats (n = 6) were monitored to assess changes in the number of *E. coli* within faeces over time. Six different animals each contributed a single faecal deposit to serve as a replicate dung-pat. The deposits were collected from cattle that had been allowed to graze for over two months, allowing the gut microbial community of livestock to develop from that of a housed diet to one typical of grazing animals. Each of the six dung-pats was collected from pasture within five minutes of excretion from each animal and all six dung-pats were collected within two hours of each other. The six fresh dung-pats were transferred, intact, to a grassland plot adjacent to the grazed plots because this: (i) prevented cattle treading through the excrement which may have resulted in destruction of a replicate deposit; and (ii) allowed for a more convenient and rapid sampling protocol as each dung-pat was placed in relatively close proximity to the next (~ 1 m spacing). Dung-pats were transferred using a sterilised spade (70% industrial methylated spirit [IMS], rinsed with sterile water). The dung-pats were not protected from rainfall, thus allowing a population change in accordance with field conditions.

Sample collection from dung-pats

Each of the dung-pats was repeatedly sampled on days 0, 1, 4, 8, 14, 28, 48 and 70 post defecation. This repeated strategy was adopted because a destructive sampling approach was deemed impractical due to the number of dung-pats required to be obtained for t = 0 days and also because of the limit on the number of dung-pats one animal makes per day. It was considered that the most dramatic changes in population numbers may occur during the early stages of voidance from the warm and moist gut environment because environmental conditions on the field surface become variable and not optimal (see also Wang *et al.*, 1996). Thus, sampling was skewed so as to obtain cell counts more regularly at the start of the experiment. Approximately 2 g of faeces was collected (0.5 g for bacteriological analysis, 1.5 g for dry weight analysis). Samples were randomly taken from the middle depth region of the dung-pat below the formed crust. Faecal material was removed with a sterile spatula (70% IMS, rinsed with sterile water) and placed into sterile MacCartney bottles (autoclaved at 121^{o}C for 15 minutes). Moisture content of faeces was determined by drying 1.5 g of faeces at 105^{o}C for 24 hours in an oven and then weighing the residual.

Microbiological analysis of samples

Soil analysis: All bacterial analysis of samples was conducted within 4 hr of collection. Briefly, fresh soil samples were crumbled and 10 g was added to 90 mL sterile water prior to mixing for 40 min on a rotary agitator. The resulting soil suspensions were serially diluted in sterile water then spread-plated onto MacConkey agar and incubated at 37^{o}C for 24 hr before enumeration of colony forming units (CFU). *Faecal analysis*: a mass of 0.5 g of faeces was added to 4.5 mL of sterile water in a sterile 15-mL centrifuge tube and shaken for 60 minutes on a rotary shaker, before being shaken vigorously on a Whirlpool mixer for a few additional minutes. Serial dilutions were then made in sterile water and 0.1 mL (or 0.2 mL in cases of low counts) spread-plated onto MacConkey agar (Oxoid) as per soil analysis. Those colonies characteristic of *E. coli* growing on MacConkey agar were enumerated and seven random isolates were used to

confirm their identity using both MicroPlate test panels (Biolog, Hayward, CA) and API 20E biochemical identification kits (bioMerieux Vitek, Hazelton, MO). Both these procedures rely on the biochemical profiles exhibited by the test isolates for confirmation of their identity through database comparison.

Modelling *E. coli* dynamics on grassland plots

An empirical model was established to estimate the accumulation of an *E. coli* reservoir on four replicated 1 ha paddocks grazed by four beef steers during a typical six month grazing season in the UK. This empirical model was constructed using biological parameters of die-off, faecal excretion and *E. coli* shedding rate and was informed by previous field experimentation reported in the literature. The model accounts dynamically for the accumulation and depletion of FIO burden to land at daily time-steps. The quantity of *E. coli* on a defined plot (Equation 1) was calculated as the sum of two terms (i) the daily fresh input of *E. coli* by all livestock; and (ii) the *E. coli* burden deposited on previous days and now declining as a result of first-order die-off:

$$E_{(x)} = Ein_{(x)} + E_{(x-1)}e^{-bx} \qquad (1)$$

Where E_x is the magnitude of the *E. coli* store on day x, Ein is the *E. coli* input of fresh deposits, e is a mathematical constant (base of natural log), b is the exponential die-off constant. Specifically, daily *E. coli* loading was calculated by multiplying the number of cattle (n = 4) by both the daily dry matter excreted per beef steer and a typical value for *E. coli* per gram of dry cattle faeces (see Table 1). Many literature values for *E. coli* content of livestock faeces exist and McDowell *et al* (2008) provide a succinct summary table from herds across the world. However, in order to constrain uncertainties for this specific study we analysed the *E. coli* content of dung-pats from beef steers grazing the field site used to evaluate the model to provide an average value for this parameter. Estimates of the error associated with dung-pat *E. coli* content were based on the

distribution of measured values from this study and on the range of existing literature values for cattle faeces (see Table 2).

Seasonal die-off profiles for *E. coli* under field conditions typical of the UK are sparse and only one study by Avery *et al*. (2004) provided appropriate data for use in the model outlined here (0.061 day^{-1}). Other laboratory based studies do exist but these were considered unsuitable to extrapolate to field conditions due to the degree of uncertainty in translating controlled experimental data to the field. Die-off coefficients from lowland areas of New Zealand (which can experience similar climatic conditions as the UK) provided first-order die-off rates of similar range (0.050-0.060 day^{-1}) for spring, summer, autumn and winter seasonal experiments (Sinton *et al*., 2007) to help constrain our estimates of the range of error associated with the die-off parameter. Given the scarcity of die-off data we allowed a +/- 33% error in this coefficient. The die-off data was used to determine the daily *E. coli* decline within all deposited faecal material for each successive day within a six month grazing period.

The model was run 500 times using randomly chosen parameter scenarios from the error ranges (Table 2). Each scenario was given a different weighting, based upon its deviation from the nominal parameter values (Table 1). Each scenario weighting was calculated using triangular fuzzy membership functions for each parameter, summed to give an overall weighting (e.g. see the approach of Page *et al*., 2004). When sampling the *E. coli* concentration distributions a day-to-day correlation of 0.7 was assumed as it is unlikely that cattle excrete exactly the same number of cells each day owing to biological variability and fluctuations reported in the literature (Robinson *et al*., 2009; Donnison *et al*., 2008). This allowed a general ‘drift’ in shedding rate, but did not allow large, unrealistic short-term fluctuations.

The model was modified to incorporate post-deposition growth using experimental results from this study (see above). This was achieved by taking the average growth observed in the present study for the six days after deposition. As fresh deposits were input on each grazing day this equates to a six-day moving-window of growth through the grazing season. The additional *E. coli* burden is discussed above and is included as specified by Equation 2.

$$E_{(x)} = Ein_{(x)} + E_{(x-1)}e^{-bx} + ER_{(x)} \qquad (2)$$

Where ER is the magnitude of *E. coli* growth for any given day.

The multiple parameter scenarios and associated fuzzy weightings provided a distribution of values for the *E. coli* reservoir at each time-step, expressed as percentiles of these distributions in the sections below.

Results

Measured *E. coli* in the faecal store (dung-pats)

Overall, *E. coli* was present on the day of excretion at a mean concentration of 7.12 $\log_{10}$ *E. coli* CFU g^{-1} dry faeces and showed fluctuation in population numbers, rather than first-order die-off kinetics, during the 70 day period of investigation. The average growth recorded in the first 10 days post defecation was approximately 0.5 $\log_{10}$ CFU g^{-1} dry faeces before cell numbers gradually declined to a level of 6.06 $\log_{10}$ CFU g^{-1} dry faeces by day 48. However, between day 48 and day 70 cell numbers recovered to a mean of 6.29 $\log_{10}$ CFU g^{-1} dry faeces; a level greater than observed 28 days after deposition. The individual die-off profiles for all six dung-pats are shown in Figure 2 along with rainfall and air temperature conditions recorded throughout the die-off study. It was not possible to use linear or non linear regression analysis on the replicate faecal deposits because the percentage variation accounted for by the model fittings was inappropriate

for all replicates, demonstrating poor applicability of both linear and non-linear model fits to the data points plotted. Deposit 1, 5 and 6 accommodated a final *E. coli* concentration similar to that of the initial concentration on day 0. Five of the six replicates experienced an increase in *E. coli* concentrations between day 48 and day 70. Changes in the dry matter content of the dung-pats during the experimental period are shown in Table 3.

Measured *E. coli* in the soil store

Bacterial analysis of the topsoil layers (0 - 7cm), which were sampled between dung-pats, detected *E. coli* numbers ranging between levels below detection (< 5 x10^2 CFU g^{-1} dry soil) through to 10^6 CFU g^{-1} dry soil. No water drained from the 1 ha plots during this grazing period because brief spells of rainfall (Figure 3) were insufficient to initiate drainage. On 10 of the 12 sampling dates the mean measured *E. coli* levels in the plots exceeded the *upper* levels predicted by the model. Only on Julian Days 133 (near the onset of grazing), 217 and 309 (end of grazing) were predicted *E. coli* values of the same order of magnitude as those predicted by the model using 1st order die-off coefficients. Time-series values (mean, 5th and 95th percentiles) of the measured *E. coli* within the four replicate plots are shown in Figure 3 by the vertical bars. A paired *t*-test to check for differences in mean *E. coli* stores within observed versus predicted datasets on the 12 sampling dates showed that the values within the measured dataset were significantly higher ($P < 0.05$) than those of the predicted dataset.

Model output

Predicted *E. coli* levels on pasture using first-order die-off coefficients and first-order die-off coefficients combined with the moving-window representation of growth are shown in Figures 3 and 4, respectively. The plots show 5th, 50th and 95th percentile of predicted *E. coli* values. Within approximately 50 days from the onset of grazing the rate of *E. coli* accumulation was seen to reach a near equilibrium (i.e. excreted *E. coli* and total die-off were approximately in balance) and during each successive day through to Day 305 (the

end of grazing), the accumulating *E. coli* deposition rate exceeded or equalled that of the combined die-off rate for all cells. Using first-order die-off coefficients the maximum mean potential *E. coli* reservoir was predicted to be approximately 3 x 10^{12} $\log_{10}$ *E. coli* on day 305. Incorporation of growth in the order of 0.5 log CFU g^{-1} dry faeces into the model increased the maximum value of predicted *E. coli* burden to approximately 8 x 10^{12} $\log_{10}$ *E. coli* (Figure 4) and reduced the sum of absolute errors between observed and predicted *E. coli* levels by 3 x 10^{13} CFU; a significant underestimation did, however, remain ($P < 0.05$). On day 305 (the end of grazing) no fresh faecal material was added to pasture and the *E. coli* reservoir declined following a first-order profile for both model scenarios.

Discussion

An empirical model governed by biological parameters of die-off, shedding and excretion rate is presented. Moriarty *et al*., (2008) have suggested that for the management and mitigation of bacterial pollution of watercourses, and for on-farm microbial risk assessment, it would be useful to model the size of faecal microbe reservoirs on pasture. To do this they propose that data is needed on the bacterial content of fresh faeces and associated die-off data. The research reported here offers a critical first step towards accounting for land-based reservoirs of *E. coli*.

A key finding of this study was reflected in the difficulty of extracting suitable die-off coefficients for the model. The lack of data informing reliable *E. coli* die-off estimates for faecal pats under farm conditions meant that we were restricted in our ability to parameterise the model with UK field relevant coefficients. An alternative approach would have been to use laboratory derived die-off coefficients but these are less representative of environmental conditions (e.g. McGechan and Vinten, 2003; Oliver *et al*., 2006). However, New Zealand derived data from Christchurch (Sinton *et al*., 2007), associated with similar meteorological conditions to the UK, provided a die-off rate

constant of almost equal value to that used in this study (previously reported by Avery *et al*., 2004) and within the margin of uncertainty embedded into the model structure. This provided reassurances with regard to the suitability of the die-off parameter used. This lack of field relevant data to parameterise basic fundamental models of faecal bacteria accumulation on land is alarming. Faecal bacteria such as *E. coli* represent basic microbial determinands for policy drivers such as the rBWD in Europe (CEC, 2006a) and TMDL assessments in the US (Chin *et al.*, 2009) and are key indicators of faecal contamination of water. However, critical data on the most basic behavioural traits of these bacteria – namely die-off under field relevant conditions – for UK climatic conditions is limited to one study (Avery *et al*., 2004). Unfortunately the aforementioned study only covered die-off profiles for faeces deposited in November and so no account of differential die-off profiles for differing months, or even seasons, was provided. As a result, in our model we used a step-change approach in die-off coefficients between seasons, whereas had sufficient data been available we could have developed a better understanding of die-off fluctuations over an annual time-course (e.g. used a sine wave approximation of seasonal die-off fluctuations). New Zealand-based research has appreciated the value in providing key month-by-month assessments of FIO decline in faeces (e.g. Muirhead, 2009) but in the UK there appears to be unfounded complacency about the survival of bacterial indicators, largely stemming from the fact the much work has been done in the laboratory to study *E. coli*. Muirhead (2009) detailed the first example of FIO decline in dung-pats deposited throughout each month of the year providing a much needed resource for researchers investigating bacterial pollution from agriculture. Muirhead (2009) therefore represents a key study which other geographic regions across the world should replicate to provide an equivalent and comparable dataset of faecal indicator population dynamics under field conditions.

The discrepancy in assumed first-order die-off and actual field persistence of FIOs was highlighted using our plot experiment and associated model. As noted by Beven (2007),

more information can often be learned from model rejection than acceptance, leading to inference of key processes, in this case the potential for *E. coli* growth. However, even when the model was amended with a growth phase the predictions still underestimated observed values on pasture. The accumulating observed total of *E. coli* within the plot soil should, in theory, have been lower than that of the maximum number of cells predicted by the model to be within the faecal reservoir. This is because first-order die-off would make it impossible to detect larger numbers of *E. coli* in the soil store (given that concentrations were below detection on day 0) than in the maximum predicted faecal store as first-order die-off implies a decline of cell numbers rather than an increase. The fact that soil *E. coli* levels exceeded that of the maximum predicted input levels on over 80% of sampling dates suggested that first-order die-off did not satisfactorily describe changes in *E. coli* populations within cattle faeces and that higher order approximations and complex growth patterns operate under field conditions. The argument is reinforced further given that a proportion of the FIO population may have entered a viable-but-non-culturable state yet detected numbers were still significantly higher than those predicted. This revealed a major underestimation of diffuse source bacterial risks from cattle to soil and water quality, with increased threats to public health that may worsen if combined with expected climate change outcomes (Hulme *et al*., 2002; Boxall *et al*., 2009).

Overall the study identified post defecation *E. coli* growth equivalent to a magnitude 0.5 $\log_{10}$ CFU g^{-1} dry faeces. It is possible that the discrepancy between the observed and modelled *E. coli* reservoir was a function of erroneous die-off coefficients but the complementary dung-pat die-off experiment would suggest that growth is a factor often ignored in faecal microbe fate models, and this is reinforced by other studies (Soupir *et al*., 2008; Muirhead, 2009). Both Sinton *et al*. (2007) and Van Kessel *et al*. (2007) recorded potential growth of up to 1.5 orders of magnitude, more than that observed in our die-off study. This difference may be related to the timing of the experiments, the UV

radiation intensity and variations in ambient temperature among other environmental factors experienced during the period of study but highlights that different levels of growth may take place during different periods of the year on pasture and may explain why our amended model still under predicted.

We appreciate that the die-off study reported here represents only a first step in what should be a continued integrated field and modelling research programme and as such it has provided data to help form a first approximation analysis of the impact of ignoring growth in model predictions. The repeated use of a single value for *E. coli* growth through time was somewhat limited because growth may in fact vary day-by-day for each deposited dung-pat depending on the complex array or interacting environmental variables. Soupir *et al.* (2008) observed differing levels of growth and times to reach growth peak for different seasons so the 'moving window' of growth used in the model is perhaps too regular but current knowledge prevented us from modifying this *E. coli* growth approximation.

Furthermore, the complementary *E. coli* die-off experiment using replicated dung-pats highlighted fluctuations of *E. coli* numbers within faeces over a 70 day period, including a growth phase not only in the immediate period post defecation, but also a secondary re-growth phase over 40 days after deposition. This was not accounted for in the original or the amended model. During brief periods of precipitation, cell numbers did not decline as might be expected due to wash-out from the faecal deposit. Instead, the resurgence of cells in the secondary re-growth phase appeared to coincide with rainfall and may be a function of the conditions brought about by rehydration of the faecal material and requires further investigation (c.f. Sinton *et al*., 2007).

It has been argued that first-order approximations do not account for *adaptation* of bacterial communities. In fact, *E. coli* are a notable example of bacterial cells capable of

adjusting their metabolism in response to stress in order to increase their survivability (Corradini and Peleg, 2009), and may explain the observed growth ignored in traditional log-linear models. A key objective is now to investigate field relevant die-off of *E. coli* for varying UV radiation and rainfall typologies to start to account for variable die-off rates and potential for growth as a function of fluctuating field conditions. As noted by Soupir *et al.* (2008), higher-order approximations and the inclusion of weather variables are likely to improve predictions of bacterial decline when compared to first-order approximations: this will however require an increase in high quality data to constrain model structures and parameter values. The current lack of representation of growth dynamics in models of bacterial die-off, and the general assumption of first-order decline equates to a bias or structural error that leads to potential underestimation of diffuse-source microbial risks to soil and water quality at the field and catchment scale.

Conclusion

The availability of fundamental field relevant data for *E. coli* population dynamics in faeces deposited onto pasture by grazing livestock is currently poor and the reliance on 1st-order 'die-off' approximations will in some instances significantly underestimate the size of *E. coli* populations on grassland. Information derived from laboratory experiments is not satisfactory to underpin the development of models of bacterial fate and dynamics at farm and catchment scales. Given that *E. coli* are monitored across the world, because of regulatory drivers and legislation criteria for water quality and human health, this emphasises the need to understand better the behaviour of faecal bacteria in the environment. This critical finding has wide-ranging implications that may be applicable to other faecally-derived bacteria and disease causing microorganisms such as *E. coli* O157:H7. It is therefore relevant to microbial ecologists, policy makers, agronomists and those working in soil and water science who can use this data to frame future evaluations of bacterial risks to public health, the human food chain and key ecosystem services such as the provision of clean and safe recreational and drinking water. The

potential of *E. coli* population increase under environmental conditions highlights that we should focus on understanding bacterial population dynamics and their ability to proliferate and persist in the environment rather than deriving traditional distinct coefficients that focus only on 'die-off', which are both misleading in terminology and erroneous in nature. This is especially pertinent given predicted changes in surface soil temperature and moisture under grasslands which may favour survival further and thus exacerbate human health risks in the future.

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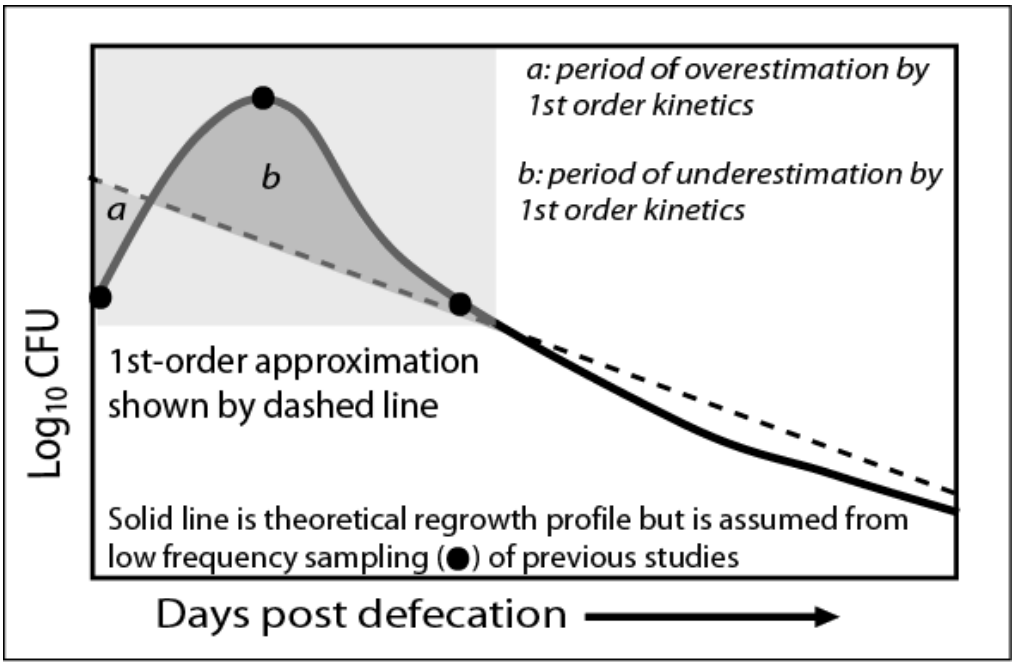
Figure Captions

Figure 1: Theorised dynamics of faecal bacterial re-growth and errors relative to first-order die-off approximation for a single dung-pat

Figure 2: Die-off patterns of *E. coli* within six dung-pats (FD1-6) under field conditions in Devon, UK. Average rainfall and temperature data are shown on the secondary y axis. Day 0 = July 31st 2003.

Figure 3: Modelled *E. coli* reservoir (mean, 5th and 95th percentile shown by solid and dashed lines respectively) on a 1 ha plot grazed by 4 beef steers over a 6 month grazing season. May 9th (day 129) and November 5th (day 305) represent the start and end of grazing, respectively. Bar-plots show actual soil *E. coli* levels measured in 1 ha plots (Horizontal dashes represent median and upper and lower values).

Figure 4: Modelled *E. coli* reservoir (mean, 5th and 95th percentile shown by solid and dashed lines respectively) on a 1 ha plot grazed by 4 beef steers over a 6 month grazing season with re-growth accounted for. May 9th (Day 129) and November 5th (Day 305) represent the start and end of grazing, respectively. Bar-plots show actual soil *E. coli* levels measured in 1 ha plots (Horizontal dashes represent median and upper and lower values). Faded data plot shows previous modelled output excluding re-growth.



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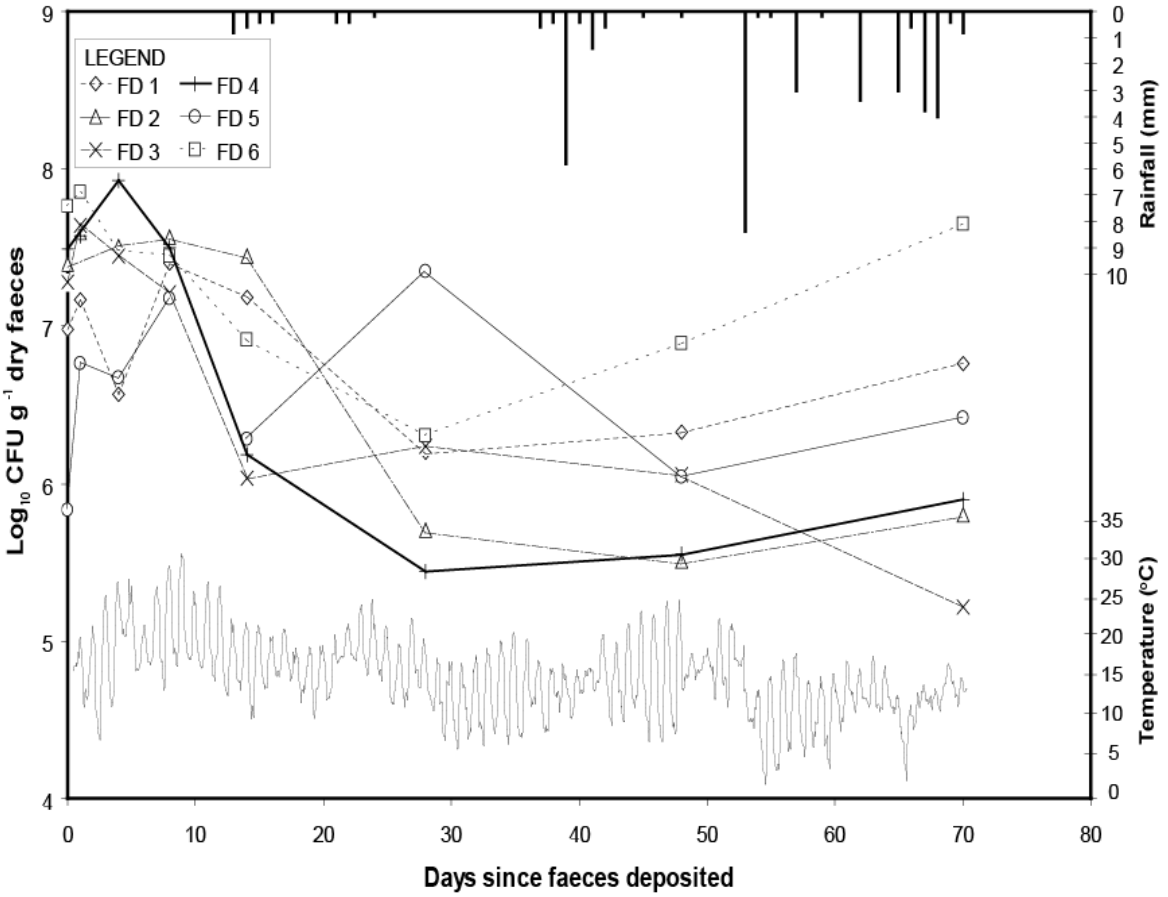

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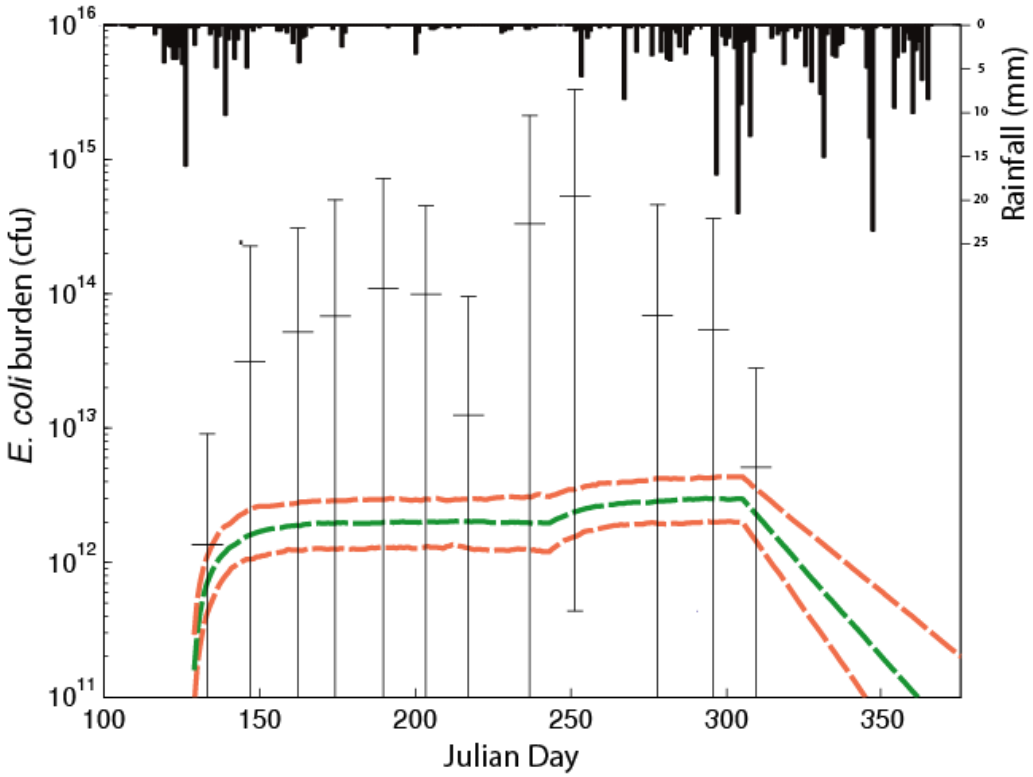

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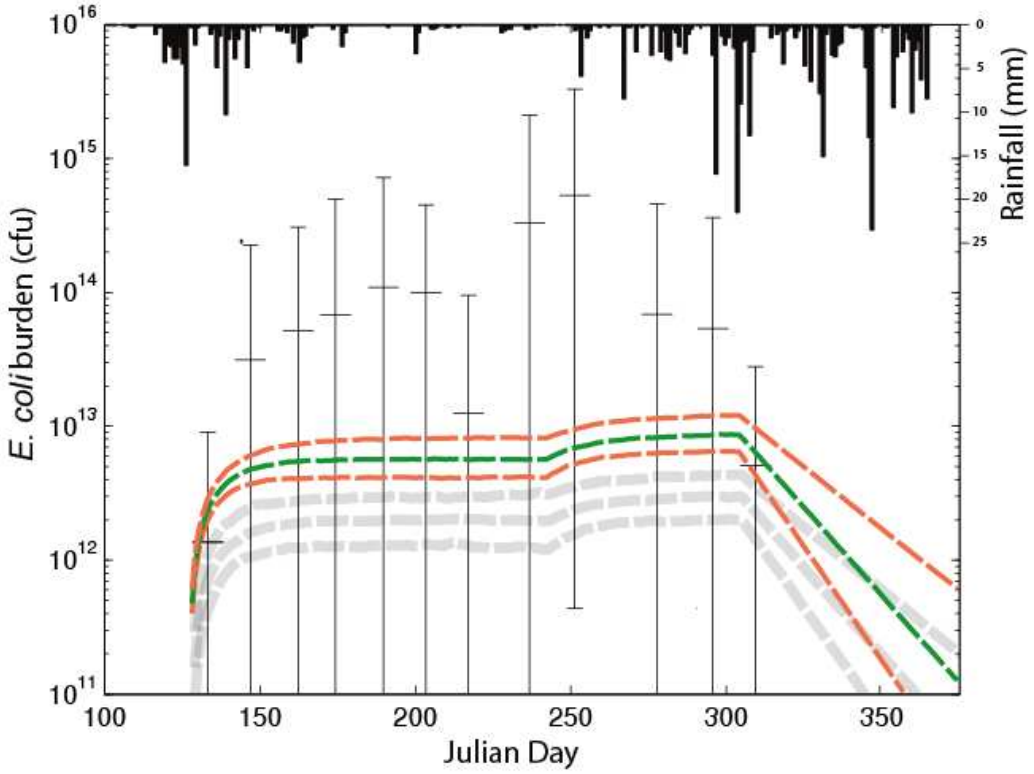

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