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Rainfall-induced release of microbes from manure: model development, parameter estimation, and uncertainty evaluation on small plots

Keewook Kim, Gene Whelan, Marirosa Molina, S. Thomas Purucker, Yakov Pachepsky, Andrey Guber, Michael J. Cyterski, Dorcas H. Franklin and Ryan A. Blaustein

ABSTRACT

A series of simulated rainfall-runoff experiments with applications of different manure types (cattle solid pats, poultry dry litter, swine slurry) was conducted across four seasons on a field containing 36 plots (0.75×2 m each), resulting in 144 rainfall-runoff events. Simulating time-varying release of *Escherichia coli*, enterococci, and fecal coliforms from manures applied at typical agronomic rates evaluated the efficacy of the Bradford–Schijven model modified by adding terms for release efficiency and transportation loss. Two complementary, parallel approaches were used to calibrate the model and estimate microbial release parameters. The first was a four-step sequential procedure using the inverse model PEST, which provides appropriate initial parameter values. The second utilized a PEST/bootstrap procedure to estimate average parameters across plots, manure age, and microbe, and to provide parameter distributions. The experiment determined that manure age, microbe, and season had no clear relationship to the release curve. Cattle solid pats released microbes at a different, slower rate than did poultry dry litter or swine slurry, which had very similar release patterns. These findings were consistent with other published results for both bench- and field-scale, suggesting the modified Bradford–Schijven model can be applied to microbial release from manure.

Key words | bootstrap, Bradford–Schijven release model, manure, microbe, PEST, quantitative microbial risk assessment (QMRA)

Keewook Kim (corresponding author)
Oak Ridge Institute for Science and Education,
US Department of Energy,
Oak Ridge, TN 37830,
USA
and
Idaho Falls Center for Higher Education,
University of Idaho,
Idaho Falls, ID 83402,
USA
E-mail: *kkeewook@uidaho.edu*

Keewook Kim
Gene Whelan
Marirosa Molina
S. Thomas Purucker
Michael J. Cyterski
National Exposure Research Laboratory,
Ecosystem Research Division,
US Environmental Protection Agency,
Athens, GA 30605,
USA

Yakov Pachepsky
Environmental Microbial and Food Safety Laboratory, Agricultural Research Service,
US Department of Agriculture,
Beltsville, MD 20705,
USA

Andrey Guber
Department of Plant, Soil and Microbial Sciences,
Michigan State University,
East Lancing, MI 48824,
USA

Dorcas H. Franklin
Agricultural Research Service,
US Department of Agriculture,
Watkinsville, GA 30677,
USA
and
Department of Crop and Soil Sciences,
University of Georgia,
Athens, GA 30602,
USA

Ryan A. Blaustein
Department of Environmental Science and Technology,
University of Maryland at College Park,
College Park, MD 20742,
USA

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INTRODUCTION

The United States Environmental Protection Agency (EPA 2002) reported that 35% of impaired rivers and streams (EPA 2012a) in the United States had elevated levels of fecal indicator bacteria (FIB) which exceed water quality standards; this condition signifies the potential presence of pathogens. With more than 10,300 water body impairments identified nationwide, FIB are the most commonly reported evidence of water pollution and the largest class of pollutants with specified total maximum daily loads, which represent the maximum amount of a pollutant a waterbody can receive and still meet water quality standards (EPA 2012b). Manure from farm animals and wildlife is a potential source of fecal contamination to water bodies and, therefore, a cause for public health concern; for example, poultry are responsible for 44% of the total feces production in the United States, followed by cattle (31%) and swine (24%) (Kellog *et al.* 2000).

There is evidence that release kinetics may differ by microbe and manure type (cattle solid pats, poultry dry litter, swine slurry), as some literature (Bradford & Schijven 2002; Schijven *et al.* 2004; Guber *et al.* 2013) suggests there may be differences in release patterns for different components (microbes, ions, etc.). These facts have motivated a desire to improve prediction of microbial release from manure by quantifying manure-borne release mechanisms and microbial die-off. Numerous studies have focused on estimating parameters for manure-borne release or microbial die-off (Bradford & Schijven 2002; Guber *et al.* 2006, 2013). Guber *et al.* (2006) compared three different models on results of release experiments conducted with bovine manure on eight vegetated plots with 20% slope, and recommended the Bradford–Schijven model as having more stable parameters related to irrigation rates and vegetation. In their review of 10 runoff-box and field runoff-plot experiments, Guber *et al.* (2013) concluded that the number and selection of manure components, along with experimental conditions (manure type, manure application, and irrigation rates and intensity, etc.), required additional study to quantify parameters describing the release of constituents from manure.

Parameters of microbial release models are typically estimated by solving the inverse problem (i.e., model calibration) on experimental data. Model calibration adjusts model parameters to a reference system (e.g., data) (Hofmann 2005) and has been shown to provide more information (e.g., standard error, confidence intervals) when combined with the bootstrapping statistical technique (Efron & Tibshirani 1993). Bootstrapping allows estimation of the sample parameter distribution using simple methods (Varian 2005), infers variability in an unknown distribution from which data are drawn by resampling, and assesses variable uncertainty by estimating confidence intervals (Felsenstein 1985). Kim *et al.* (2014) combined inverse modeling (Doherty 2005) with bootstrapping and Monte Carlo analysis to calibrate overland runoff parameters with a non-linear and highly parameterized model from available data.

In the present study, we modified the Bradford–Schijven model by adding modified release-efficiency and transportation-loss terms to estimate corresponding microbial release parameters using more plots in a natural setting, microbes, manure types, and aging times. The modified model was tested on 144 rainfall-runoff experiments on 36 plots including triplicates on the same field over four seasons (36×4), with three manure types (fresh cattle pats, dry poultry litter, swine slurry), aged over three time periods (0, 1, 2 weeks), with three FIBs (*Escherichia coli*, enterococci, fecal coliforms). Inherent difficulties in the process include reliance on a nonlinear model, parameters that are difficult to estimate, a highly parameterized model with respect to available data, and a lack of field-scale monitoring, all of which were illustrated by Kim *et al.* (2014).

MATERIALS AND METHODS

Field method

The study site was located in Watkinsville, Oconee County, Georgia, USA (33 °47′N, 83 °23′W, elevation 225 m). The experimental set-up was established on a Cecil coarse sandy loam soil (fine, kaolinitic, thermic, and Typic Kanhapludult) with mixed tall fescue–Bermuda grass vegetation on

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3–10% slopes (mean of 6%). Kim *et al.* (2014) and Butler *et al.* (2006) describe additional site characteristics.

Local, fresh cattle (solid) manure was collected immediately as it was shed from the cattle; poultry (dry litter) and swine (liquid manure) manures were separately collected from local farms. Each type was thoroughly mixed and stored at 4 °C overnight prior to runoff experiments. Manure applications followed a randomized split-plot design, and utilized agronomic rates following USDA Natural Resources Conservation Services guidelines (Midwest Plan Service 2004). Treatments consisted of fresh manure applications from cattle, poultry, and swine, and a control treatment (no manure application). Solid cattle manure was applied at a rate of 1.6 kg/m^2, poultry litter at 0.67 kg/m^2, and swine slurry at 0.67 to 0.73 L/m^2. Fresh pats were randomly deposited with an average number and area of 29 pats/plot and 64 cm^2/pat, respectively. Dry litter was evenly spread over the plot area, and swine slurry was stirred and poured evenly over the plot area to within 15 cm of the sides.

Three replicates (plots) were employed with each manure treatment, and all plots received applications during Week 0. For one-third of the plots, rainfall simulation began immediately following manure application (Week 0); one-third delayed the simulation for one week after application (Week 1); and one-third delayed for two weeks (Week 2). Plots containing aged manure (i.e., Weeks 1 and 2) were protected from rainfall with clear plastic, but were exposed to other environmental elements such as sunlight and wind. Prior to application, each manure source was analyzed via randomized composite samples for FIBs. Runoff was collected in 5 min increments over the 60 min runoff period, for 12 *in toto*. During the 5, 10, 15, 20, 30, and 60 min time intervals, microbial samples were collected and were used to derive time series of integrated, volume-weighted (i.e., average) densities. As confirmation, composite (i.e., combined runoff volume) microbial samples were also collected at 30 and 60 min, and their densities were compared to integrated 30 and 60 min densities from the individual time-interval samples (see Figure 3 in Soller *et al.* 2015). The Colilert procedure (Idexx Laboratories, Inc., Westbrook, ME, USA) was employed for *E. coli* and fecal coliform enumeration based on the most probable number approach and selective/differential growth media. Enterococci were quantified by membrane filtration using US EPA Method 1600 (EPA 2006).

Model description

Bradford & Schijven (2002) developed a mathematical model describing manure release to the aqueous phase:

$$\frac{M_{mr}(t)}{M_{m0}} = \left[1 - (1 + \alpha\beta t)^{-1/\beta}\right] \tag{1}$$

where $M_{mr}(t)$ is the cumulative released mass of manure at time t (Mass), M_{m0} is the initial mass of source manure at $t = 0$ (Mass), α (T^{-1}) and β are fitting parameters of the manure-release curve, and t is time (T). Guber *et al.* (2010, 2013) used the Bradford & Schijven (2002) model in modified form and adding a version of the release efficiency parameter (E_r) to Equation (1):

$$\frac{M_r(t)}{M_0} = E_r\left[1 - (1 + a\beta q t)^{-1/\beta}\right] \tag{2}$$

in which:

$$a = \alpha/q \tag{3}$$

where $M_r(t)$ is the cumulative amount of released manure components (e.g., microbes, organic carbon, water-extractable phosphate-P, total bioactive P, chloride) at time t (mass or cell number), M_0 is the initial amount of manure components in the source manure at $t = 0$ (mass or cell number), E_r is the maximum relative mass of a manure component that can be released into an aqueous phase and q is the rainfall intensity (L T^{-1}). E_r is considered a constant indicative of the entire time-varying release curve.

Equations (2) and (3) assume that all manure components are uniformly distributed in manure, and manure volume is exposed to rainfall; however, water content and microbial distributions in manure change over time, while manure is drying. Moreover, during rainfall events, surface layers protect the internal layers of the manure from direct impact of droplets. So, source manure is saturated from the outside in, and all microbes are not equally available for release to the aqueous phase. Length of time until saturation

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may vary with, for example, microbe, rainfall intensity, and manure source (e.g., bovine, poultry, swine, etc.); form (e.g., dry litter, pats, slurry, etc.); and condition (age). This may imply that E_r changes in time to reflect changes in manure properties. To account for such changes, E_r was further modified while ensuring the complete release of microbes from manure approaches unity as time approaches infinity:

$$E_r(t) = 1 - e^{-bt} \tag{4}$$

where $E_r(t)$ is the microorganism release efficiency as a function of time, and b is a fitting parameter that accounts for microbe and manure properties (e.g., age, type, and form). Note that Equation (4) has a similar form to HSPF (Hydrological simulation program – FORTRAN; Bicknell *et al.* 2001) in its description of time-varying release densities of fecal coliforms from manure, accounting for the susceptibility of fecal coliforms to be washed off the manure. Replacing E_r with $E_r(t)$ in Equation (2) and assuming that microbes are evenly distributed within source manure, the microbial release rate from the manure is as follows:

$$\frac{dM_r(t)}{dt} = aqM_0E_r(t)(1 + a\beta qt)^{-\frac{1}{\beta}-1} + be^{-bt}M_0\left[1 - (1 + a\beta qt)^{-\frac{1}{\beta}}\right] \tag{5}$$

where $M_r(t)$ is the cumulative released microbes at time t based on $E_r(t)$ (mass or cell number). The transient nature associated with the fraction of contaminated water ending as run-off can be accounted for through $E_t(t)$, assuming microbial concentrations in run-off and those in water remaining with the soil are the same (Kellog *et al.* 2000; Blaustein *et al.* 2015):

$$E_t(t) = \frac{Q_{run}(t)C(t)}{Q_{tot}(t)C(t)} = \frac{Q_{run}(t)}{Q_{tot}(t)} \tag{6}$$

where $E_t(t)$ is the fraction of microbes as runoff (i.e., microbial runoff to released microbes) at time t where $(1 - E_t(t))$ accounts for microbial loss to soil during run-off, $Q_{run}(t)$ is the runoff discharge at time t ($L^3\ T^{-1}$), $Q_{tot}(t)$ is the rainfall rate applied to the plot at time t ($L^3\ T^{-1}$), and $C(t)$ is the microbial density at time t in water remaining with the soil and in the runoff [(mass or cell number) L^{-3}]. For application to plots containing aged manure, the microbial release rate accounting for transport becomes:

$$\frac{dM_t(t)}{dt} = aqM_o^{\xi}E_r(t)E_t(t)(1 + a\beta qt)^{-\frac{1}{\beta}-1} + be^{-bt}M_o^{\xi}E_t(t)\left[1 - (1 + a\beta qt)^{-\frac{1}{\beta}}\right] \tag{7}$$

in which microbial die-off in manure after time t was accounted for, assuming linear first-order die-off according to Chick's law (Chick 1908; Crane & Moore 1986):

$$M_o^{\xi} = M_0\exp(-\lambda t_{lag}) \tag{8}$$

where $M_t(t)$ is the cumulative microbes in the runoff at time t (mass or cell number); M_o^{ξ} is the initial aged microbial mass (cell number in the manure) at time t_{lag}, aged for ξ weeks (i.e., 0, 1, or 2) (mass or cell number); and λ is the first-order rate coefficient for the net die-off of microbes (T^{-1}). Guber *et al.* (2009) derived an empirical equation for estimating α with rainfall rate, suggesting the following relationship:

$$\alpha = 0.0036 + 0.86\,I \tag{9}$$

where I is rainfall intensity (cm/h).

Assuming independence, four unknown parameters require calibration: α, β, λ, and b, where α and β vary by manure type (consistent with different microbes), and b varies with microbe and manure type and age. Based on previous research (Kellog *et al.* 2000; Schijven *et al.* 2004; Thurston-Enriquez *et al.* 2005), λ varies with microbe and manure type, and all parameters vary by season. Upper and lower bounds of calibrated parameters were determined based on values in the literature (Table 1).

Approach for analysis

Two complementary, parallel approaches, similar to those used by Kim *et al.* (2014), calibrated release parameters α, β, λ, and b, and evaluated the model applied to 36 Watkinsville plots over four seasons (Events A

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Table 1 | Upper and lower bounds of calibrated parameters used in the present study, based on values suggested or used in the literature

	Bound			
Parameter	**Upper**	**Lower**	**Values suggested or used in references**	**Reference**
b	1,000	0	–	–
α (1/h)	100	0	0.0223–0.0395 (cattle)	Bradford & Schijven (2002)
			0.006–0.0582 (cattle)	Schijven *et al.* (2004)
			3.745–7.323 (cattle)	Equation (9), Guber *et al.* (2009)
			0.840–15.156 (cattle)	Guber *et al.* (2013)
			0.294–1.728 (cattle)	Blaustein *et al.* (2015)
β	100	0	4.55–26.4 (cattle)	Bradford and Schijven (2002)
			1.4–40 (cattle)	Schijven *et al.* (2004)
			0.56–33.36 (cattle)	Guber *et al.* (2006)
			0.15 (cattle)	Guber *et al.* (2011)
			0.05–12.6 (cattle)	Guber *et al.* (2013)
			0.001–5.258 (cattle)	Blaustein *et al.* (2015)
λ (1/day)	10	0	0.102–0.287 (cattle, *E. coli*) (2.4–6.8 days for half life)	Crane & Moore (1986)
			0.11–0.32 (cattle, *E. coli*) (2.2–6.3 days for half life)	Wang *et al.* (2004)
			0.065–0.165 (cattle, *E. coli*) (4.2–10.7 days for half life)	Meals & Braun (2006)
			0.11 (cattle, *E. coli*)[a] (6.4 days for half life)	Gu *et al.* (2012)
			0.09 (poultry, *E. coli*)[a] (7.7 days for half life)	
			0.08 (swine, *E. coli*)[a] (9.0 days for half life)	
			0.009 (cattle, fecal coliforms)[a] (77.6 days for half life)	
			0.104–2.605 (in water, *E. coli*) (0.3–6.7 days for half life)	Blaustein *et al.* (2013)
			0.064–0.175 (cattle, *E. coli*) (4.0–10.8 days for half life)	Martinez *et al.* (2013)
			0.68 (cattle, *E. coli*, unshaded)[b] (1.0 days for half life)	Oladeinde *et al.* (2014)
			0.41 (cattle, *E. coli*, shaded)[b] (1.7 days for half life)	

[a]Computed from half-life.

[b]β_2 values in Table 2 in Oladeinde *et al.* (2014) converted from the regression-based decimal log to natural log λ values.

through D from 2009 to 2010; Butler *et al.* 2006; Kim *et al.* 2014) for 144 (36×4) plot experiments involving *E. coli*, enterococci, and fecal coliforms for 432 (144×3) cases. Approach 1 implemented a four-step sequential procedure using the inverse model PEST (Doherty 2005) in which parameters varied up to five indices: Event, microbe, plot, and manure type and age. Modeled outputs were averaged incrementally to define the most appropriate initial values for calibration parameters that produced the most representative values across plots. Parameter calibrations began by individual case (Event, plot, microbe, and manure type and age) and continued by combining cases across plots, microbes, and manure ages.

Approach 2 implemented a PEST/bootstrap procedure in which α, β, λ, and b values were averaged across plots by Event and manure type. α and β were also averaged by manure age and microbe, while λ was additionally averaged by manure age. For each iteration, cases were divided into sample (randomly selected with replacement) and test (unselected) cases. Based on distributions of each parameter

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obtained by PEST/bootstrap calibration, results were compared to observed microbial runoffs by Event and manure type (see Kim *et al.* (2014) for more detail). Nash–Sutcliffe (NS) model efficiency between observed and calculated microbial runoff was estimated for each microbe by Event and manure type as:

$$NS = 1 - \frac{\sum_{i=1}^{N}\sum_{t=1}^{T_i}\left(q_{o,i}(t) - q_{m,i}(t)\right)^2}{\sum_{i=1}^{N}\sum_{t=1}^{T_i}\left(q_{o,i}(t) - w(t)\overline{q_o}\right)^2} \quad (10)$$

where $q_{o,i}(t)$, $\overline{q_o}$, and $q_{m,i}(t)$ are observed microbial runoff from the i-th case during time step t, its average value throughout the cases, and modeled microbial runoff from the i-th case during time step t, respectively [(mass or cell number) T^{-1}]; $w(t)$ is the weight at time step t, based on the difference between time steps; T_i is the number of observation time steps on the i-th case; and N is the number of cases.

RESULTS

Field experiment

Of 432 cases (144 plots × 3 microbes), 25% (108) were controls, and 75% (324) were plots containing manure. Twenty-one percent were not quantifiable (e.g., too numerous to count or below the detection/quantification limit), and fewer than 6% had more counts in runoff than source. Therefore, 48% (208) of the cases were included in the assessment: 53, 45, 59, and 51 cases for Events A through D, respectively. The distribution of these cases is summarized in Table 2. The ratio of cumulative released cells to cells initially present in the manure at Week 0, as they vary by time, manure type and age, microbe, and Event (season), is shown in Figure 1. The 95% confidence intervals of the observed median number of cells in microbial runoff are presented. By comparison, Brooks *et al.* (2009) and Thurston-Enriquez *et al.* (2005) reported recoveries up to 16.7% (poultry manure) and 7.0%, (cow and swine manure), respectively, with both studies having rain events averaging 30 min in length.

PEST and bootstrapping approaches

Under Approach 1, of the 208 cases, 132 exhibited positive NS values ranging from 0.06 to 0.95, with a median of 0.75 in the first step (individual plot calibrations with typical results illustrated in Figure 2). Parameter calibration combined cases across plots and microbes; then by plots and manure age, and microbes and plots; and then all combined. By comparing simulated and observed results and presenting the fraction of positive NS, the high variability in Figure 1 is reflected in the modeling performances of Approach 1 in Figure 3 by Event, by manure type, and by microbe even in the same field. Approach 2 combined the inverse model PEST and bootstrap technique to estimate confidence intervals for calibrated parameters: errors were calculated between observations and model output, and uncertainty was measured through sampled distributions by Event and manure type.

Modeling parameters

First-order die-off rate

Die-off rates, λ, were determined across plots by Event, manure type, and microbe. Contrary to results suggested by Martinez *et al.* (2013), seasonal variations (i.e., across Events) in λ did not indicate a trend consistent with temperature variations (hottest to coldest through Events C, D, A, and B). Although they indicated λ varied from 0.064 d^{-1} at 10 °C to 0.175 d^{-1} at 35 °C for *E. coli* (based on natural log), consistent trends were not found in λ values directly estimated from individual studies referred to in Martinez *et al.* (2013) (see Table 2). Die-off rates were, therefore, combined across Events and as a function of microbes. Median values with quartiles for *E. coli*, enterococci, and fecal coliforms for cattle, poultry, and swine are reported in Table 3.

Shape of the release curve

The value of α controls the initial manure release rate, while β determines the shape of the manure release curve (Bradford & Schijven 2002). Based on Schijven *et al.* (2004), Guber *et al.* (2013) surmised that parameter 'a' (related to α, see Equation (3)) reflects the erodibility of manure, which is influenced by

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Table 2 | Summary of the average ratio of cells in runoff to cells initially present in the manure as a function of Event, and manure type and age (0, 1, and 2 weeks)

				Average microbial recoveries in runoff as compared to source (%)[a]								Average microbial recoveries in runoff as compared to source (%)[a]		
Event	**Manure type**	**Microbes**	**No. of cases**	**Week 0**	**Week 1**	**Week 2**	**Event**	**Manure type**	**Microbes**	**No. of cases**	**Week 0**	**Week 1**	**Week 2**
A	Cattle	EC	10	1.3×10^{-2}	3.7×10^{-2}	1.8×10^{-3}	C	Cattle	EC	9	2.6×10^{-2}	8.0×10^{-3}	3.3×10^{-3}
		En	10	5.9×10^{-1}	3.2	$1.1\times10^{+1}$			En	9	2.2	4.8×10^{-1}	1.1
		FC	4	4.0×10^{-4}	1.1×10^{-1}	–			FC	2	–	1.0×10^{-4}	–
	Poultry	EC	9	$1.1\times10^{+1}$	$2.7\times10^{+1}$	$2.7\times10^{+1}$		Poultry	EC	8	2.4×10^{-2}	1.5×10^{-2}	3.5×10^{-2}
		En	8	8.7	3.4	$3.5\times10^{+1}$			En	9	1.5	6.5×10^{-1}	8.6×10^{-1}
		FC	0	–	–	–			FC	1	–	–	–
	Swine	EC	9	2.2	1.6	2.4		Swine	EC	9	1.8×10^{-2}	2.2×10^{-3}	1.0×10^{-3}
		En	1	$2.5\times10^{+1}$	–	–			En	9	$5.8\times10^{+1}$	$2.0\times10^{+1}$	$3.3\times10^{+1}$
		FC	2	$8.2\times10^{+1}$	–	–			FC	3	1.4×10^{-2}	–	–
B	Cattle	EC	9	2.3×10^{-2}	1.5×10^{-2}	5.1×10^{-3}	D	Cattle	EC	8	8.2×10^{-3}	2.6×10^{-3}	1.0×10^{-4}
		En	9	2.0	1.8×10^{-1}	2.5×10^{-2}			En	8	1.5	5.2×10^{-1}	9.4×10^{-2}
		FC	4	–	–	7.0×10^{-4}			FC	1	–	–	–
	Poultry	EC	2	–	1.4×10^{-2}	3.5×10^{-1}		Poultry	EC	9	5.0×10^{-3}	2.1×10^{-3}	9.0×10^{-4}
		En	9	$2.2\times10^{+1}$	7.9	$1.5\times10^{+1}$			En	7	2.1	2.5	$1.6\times10^{+1}$
		FC	3	8.2×10^{-2}	2.3×10^{-3}	–			FC	0	–	–	–
	Swine	EC	4	2.2	5.1	2.0×10^{-1}		Swine	EC	9	5.9×10^{-2}	4.4×10^{-3}	5.2×10^{-3}
		En	1	5.6	–	–			En	8	$4.6\times10^{+1}$	$1.5\times10^{+1}$	$4.6\times10^{+1}$
		FC	4	–	2.5×10^{-2}	3.3			FC	1	8.1	–	–

EC = *E. coli*, En = enterococci, FC = fecal coliforms.

[a]Ratio of absolute number of microbes in runoff to those in manure source.

Note: any potential microbial cells in the soil were not factored into the recovery levels.

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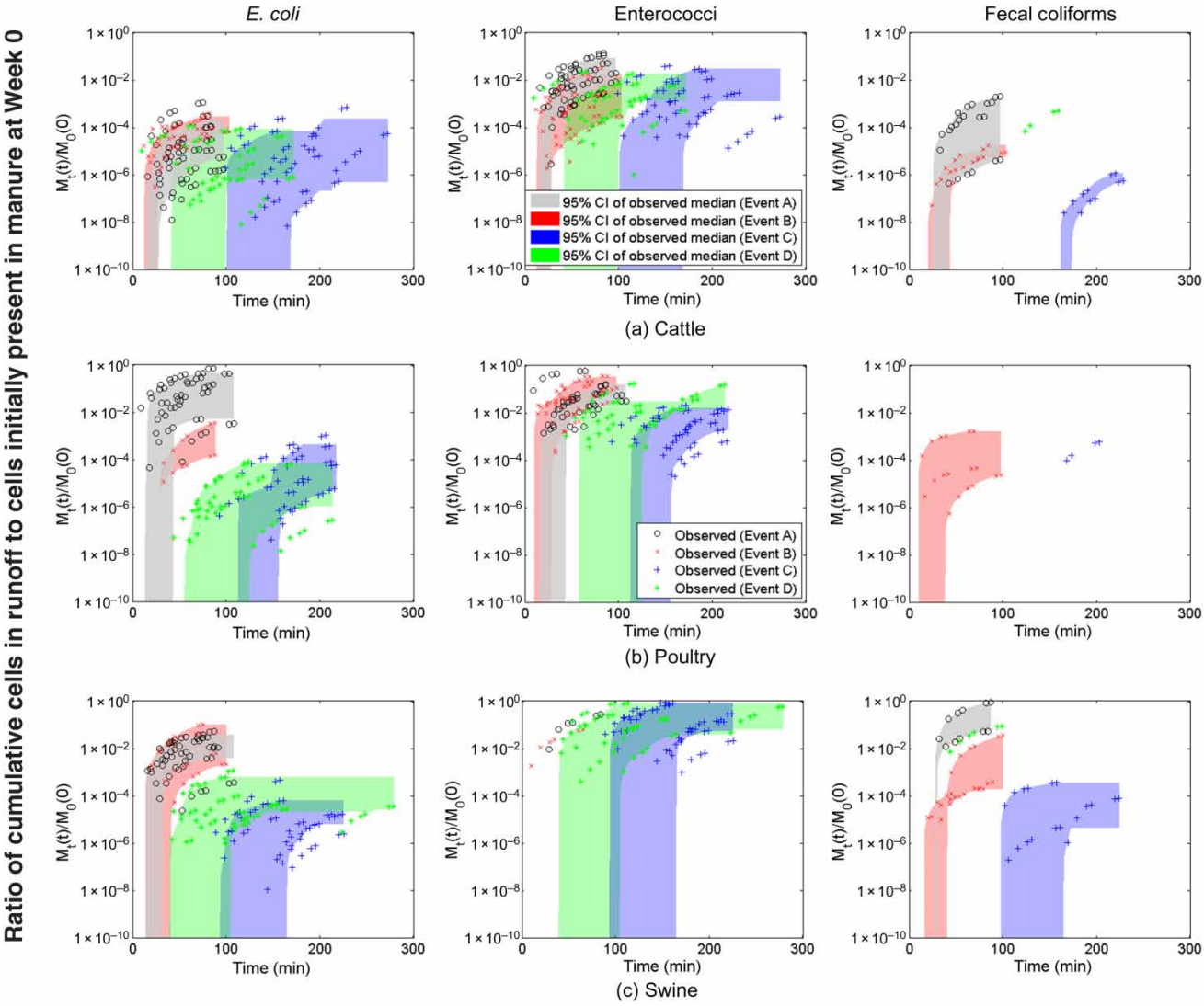

Figure 1 | Ratio of cumulative released cells to cells initially present in the manure at Week 0 as they vary by time, manure type and age, microbe, and Event (i.e., season). The 95% confidence intervals of the observed median number of cells in microbial runoff are shown as the shaded area.

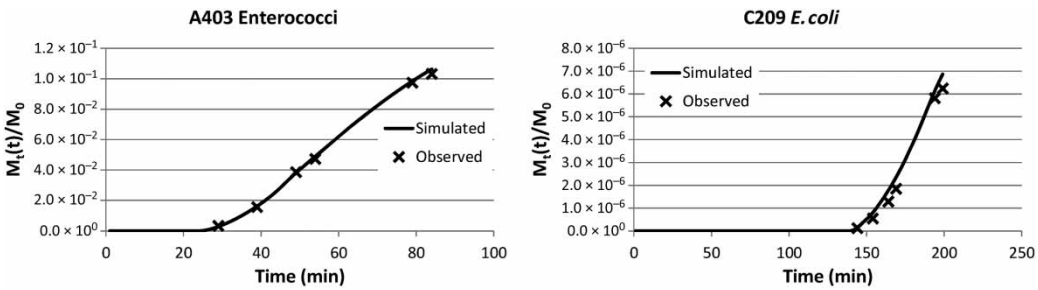

Figure 2 | Typical observed and simulated cumulative microbial runoff for Plots A403 and C209 with individual plot calibration.

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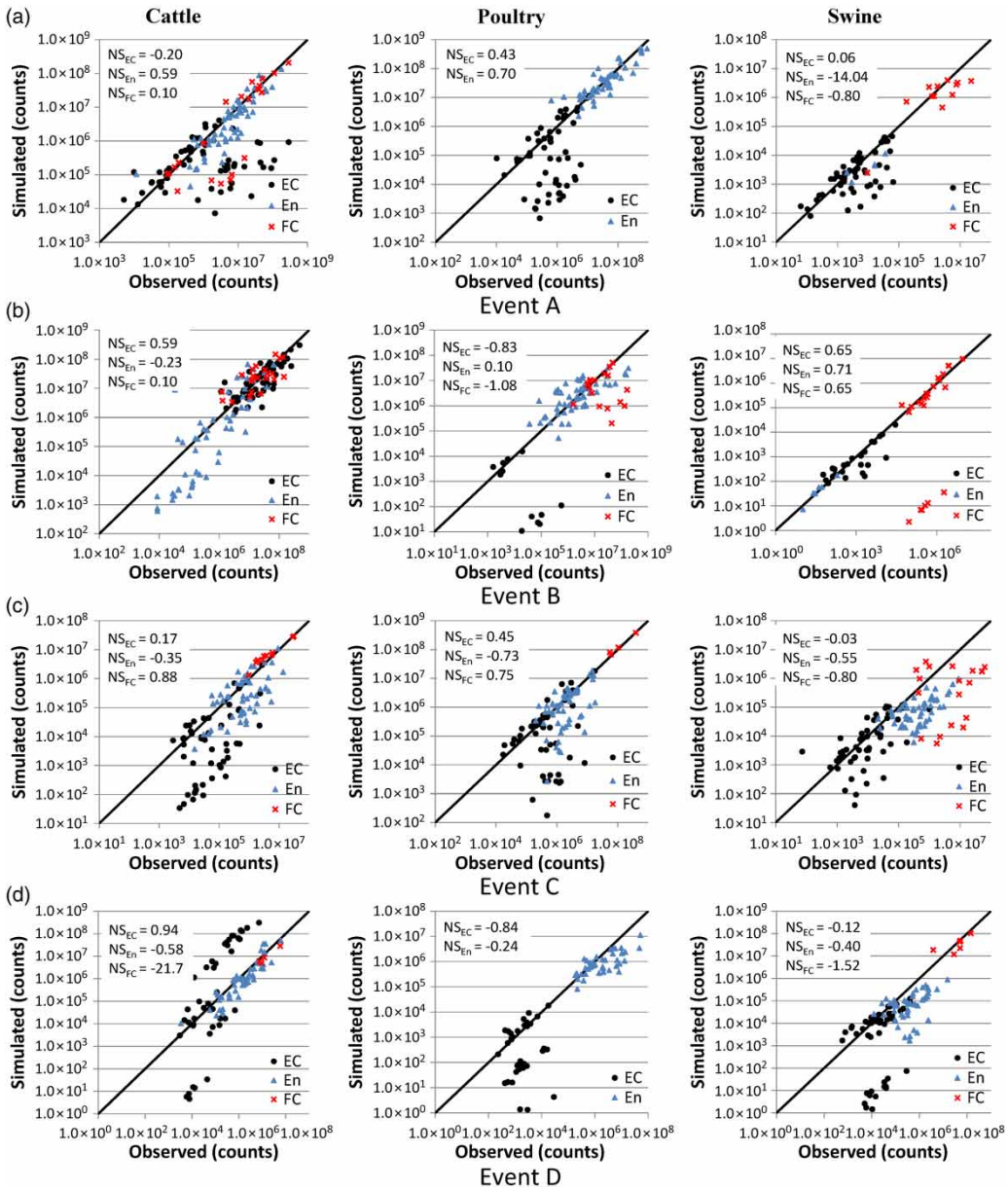

Figure 3 | Observed versus simulated microbial runoff associated with the Approach 1, adjusted for cumulative results by manure type and Event. Results accounted for counts associated with field monitoring time intervals described in Section 2.1 Field method. NS = Nash-Sutcliffe modeling efficiency, EC = *E. coli*, En = enterococci, FC = fecal coliforms.

manure properties, raindrop energy, and salinity of irrigation water; with larger 'a' values, applied manure depletes faster. Within the ranges of these experiments, modifications in α have only a slight impact on cumulative curves (shifting them up or down), while changes in β have a more profound impact. As α increases, applied manure depletes more quickly, and as β decreases significantly, the entire release curve increases. Although several third quartiles coincided with the upper limit (maximum α and β values of 100) described in Table 3, this is not critical because sensitivities of α and β decrease as their values increase.

Release efficiency from manure

Bradford & Schijven (2002) defined an E_{rp} as the microorganism release efficiency describing the partitioning

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Table 3 | Summary of b, α, β, and λ values used in defining the microbial release from manure by microbe, Week, and manure type

			Cattle solid pats			Poultry dry litter			Swine slurry		
Parameter	**Microbes**	**Week**	**1Q[a]**	**Median**	**3Q[a]**	**1Q[a]**	**Median**	**3Q[a]**	**1Q[a]**	**Median**	**3Q[a]**
b (1/h)	EC	0	6.8×10^{-5}	$\mathbf{2.0\times10^{-3}}$	14.78	0.26	11.81	20.90	3.7×10^{-3}	8.04	15.36
		1	1.6×10^{-3}	0.10	1.99	1.5×10^{-3}	0.04	2.78	0.01	0.96	13.98
		2	0.13	0.66	7.83	0.01	0.31	1.27	0.19	0.47	2.22
	En	0	0.02	0.40	4.15	0.67	2.05	42.36	0.38	0.57	1.28
		1	0.11	0.25	2.40	0.34	1.04	13.63	0.31	0.39	0.61
		2	0.19	0.90	84.52	0.47	1.89	7.11	0.26	0.35	0.56
	FC	0	7.2×10^{-6}	$\mathbf{4.0\times10^{-5}}$	1.47	7.3×10^{-5}	$\mathbf{2.7\times10^{-4}}$	3.5×10^{-3}	0.92	2.45	7.01
		1	4.9×10^{-5}	0.10	0.46	0.14	0.35	34.64	5.39	8.07	10.99
		2	0.20	1.55	7.03	-	-	-	2.3×10^{-3}	$\mathbf{2.8\times10^{-3}}$	0.33
	Combined		3.9×10^{-3}	0.31	6.20	0.09	0.85	14.00	0.23	0.68	7.06
α (1/h)			0.73	14.28	100.00	3.70	11.59	22.55	3.83	14.87	36.94
	β		3.87	19.66	100.00	1.24	3.70	8.40	0.38	6.10	28.17
λ (1/day)	EC		0.56	0.63	0.76	0.15	0.40	0.60	0.16	0.43	0.63
	En		0.00	0.05	0.21	0.00	0.01	0.08	0.00	0.00	0.00
	TC		0.04	0.42	0.50	0.50	0.57	0.96	0.43	0.64	0.78

[a]1Q: 1st quartile; 3Q: 3rd quartile.

behavior of (oo)cysts into water, relative to that of manure, depending on (oo)cyst size and charge as well as solution salinity. This implies that (oo)cysts can be released into the aqueous phase at different rates than manure (Schijven *et al.* 2004; Guber *et al.* 2013), and E_{rp} can have values less than or greater than unity (Bradford & Schijven 2002).

With the Guber *et al.* (2013) revision in Equation (2), the relative mass of the manure component asymptotically approaches E_r as time approaches infinity. E_r, therefore, represents the maximum possible relative mass of the manure component that can be released into the aqueous phase. Their experiments indicated that the length of the run-off experiments was not sufficient for complete release of manure components with large values of the recovered mass. Despite its high variability for the studied manure components, and consistent with such an interpretation of the parameter E_r, its values never exceeded unity, unlike E_{rp}.

The revision in the microbial release equation to include $E_r(t)$ assumes that not all microbes are equally available for release into the aqueous phase, and the time until saturation may vary with manure form (solid pat, dry litter, slurry), resulting in release time dependence. b in Equation (4) is a fitting parameter, and as it increases, more microbes are released sooner from manure over the same time interval, asymptotically approaching the original amount of viable microbes in manure. Microbial release is from the manure, not the plots, as microbes infiltrate, migrate, and are stored prior to reaching the end of the plots, and distribution of microbes in the environment is not the same on any plot since runoff and their start times varied greatly between plots (Kim *et al.* 2014).

Manure aging affects analyses for Weeks 1 and 2 because even when less runs off a plot, a higher fraction of viable microbes can still be released from the source, as reflected in higher b values. If fewer microbes are in the source due to die-off (i.e., Weeks 1 and 2) but a larger fraction of the source is released, the b value will be larger even though fewer microbes could be released. Manure aging results varied, as b sequentially increased over two weeks of manure aging in 23% of cases, decreased in 9%, and were mixed in 68%; b values for Week 2 were higher than Week 0 in 55% of cases. With respect to variations between microbes, Guber *et al.* (2007) noted substantial differences in release kinetics of *E. coli* and enterococci. They hypothesized that (1) *E. coli* resided in the liquid part of manure and was released as the liquid fraction was diluted and displaced by rainwater, and that (2) enterococci apparently were present in substantial numbers in less readily suspended, possibly solid, parts. To account for different release rates by microbe, b values were allowed to vary by microbe, manure type, and age, representing all plots; median values with quartiles for b are presented in Table 3. There were no apparent trends by seasons (Events A, B, C, D).

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Microbial release rates from manure

Values of λ, α, β, and b are documented in Table 3, whose values can be used in computing the cumulative microbial release curves normalized to the viable microbes remaining in the source after aging (Figure 4):

$$\frac{M_r^{\xi}(t)}{M_0^{\xi}} = E_r(t)\left[1-(1+a\beta qt)^{-\frac{1}{\beta}}\right] \tag{11}$$

Figure 4(a) curves account individually for microbe, Week (aging), and manure type. The smallest number of sampling points and farthest outliers (highlighted in bold in Table 3) are associated with fecal coliforms, whose results were restricted by having the most too-numerous-to-count samples. Figure 4(b) and 4(c) curves represent the 'Combined' data in Table 3 by solid pats, and dry litter and slurry, respectively. Bounds of the first and third quartiles associated with our results (data in Table 3, and gray and dashed areas in Figure 4) are also included on these plots.

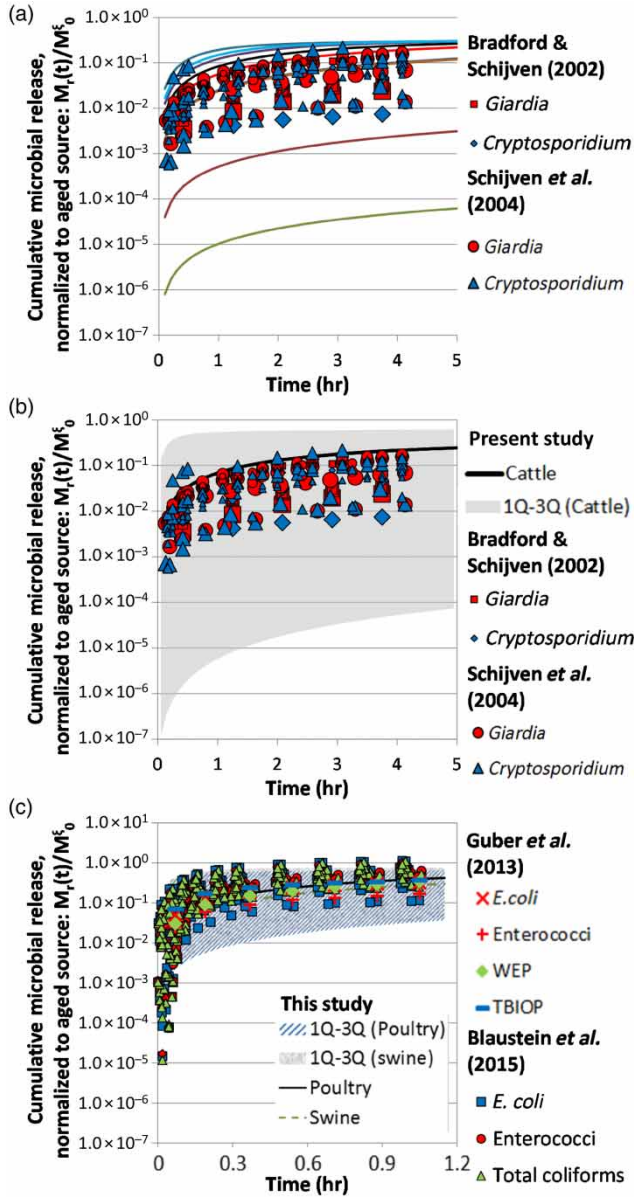

Figure 4 | Ratio of cumulative released cells/mass to cells/mass initially present in the aged manure by time and component (e.g., microbe) for solid manure (a) and (b), and amended, dry litter, and slurry manure (c). Solid lines (Equation (11) correspond to values in Table 3 for solid manure, and dry litter and slurry manure, respectively: (a) uses individual b values, and (b) and (c) use the combined values for b.

Bounds of first and third quartiles associated with the present study's results for cattle.

Bounds of first and third quartiles associated with the present study's results for poultry and swine. The full color versions of all figures are available in the online version of this paper, at http://dx.doi.org/10.2166/wh.2016.239.

DISCUSSION

Field experiment

In Figure 1, there are no clear seasonal trends; however, a larger portion of microbes are in flows associated with Events A and B which were relatively wet. A wide degree of variability – over eight orders of magnitude in microbial runoff associated with different plots across seasons – is also illustrated by Figure 1. The difficulty, then, is not the relative ease in recreating runoff and microbial release from manure from a single plot (see Kim *et al.* (2014) and Figure 2, respectively) but in mathematically describing the collective release from many plots.

PEST and bootstrapping approaches

The results of the individual plot calibration from selected plots in Figure 2 show good match of simulated runoff with observations. However, when the cases are combined across plots, microbe, and manure age (Figure 3), the comparison between simulated and observed microbial runoff illustrates high variability, resulting in poor performance in some cases. The highly variable results were not unexpected within these

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kinds of field experiments (see Figure 1; Guber *et al.* 2013; Kim *et al.* 2014). The plots reflected actual site conditions where plot preparation was minimal (i.e., natural setting). Although all plots were associated with the same field, vegetation cover, soil type, preparation procedure, and environmental conditions (e.g., antecedent moisture condition, temperature, soil saturation) varied greatly between plots and seasons (see Table 1 in Kim *et al.* (2014)).

Modeling parameters

First-order die-off rate

The λ values in Table 3 are within those estimated by Blaustein *et al.* (2013) (0.104–2.605 d^{-1} for *E. coli* in water). The median *E. coli* λ value of 0.63 d^{-1} is also very close to the first-order die-off rate of 0.68 d^{-1} reported in Oladeinde *et al.* (2014). The values are higher than those in a list of microbial die-off studies reported by Martinez *et al.* (2013) (Table 1). Although our study's λ values were higher than those reported by others (e.g., 0.064–0.32 d^{-1} for *E. coli* in cattle manure), this was not entirely unexpected, since we excluded plots where the microbial amount in runoff exceeded the amount in the source, meaning that only results with higher computed die-off rates were included. λ values estimated in the present study, therefore, may have been partially inflated.

Previous die-off studies indicated the microbial population in the manure could increase during the first few days or weeks (Soupir *et al.* 2008; Brooks *et al.* 2009; Martinez *et al.* 2013). Differences in die-off rates have been associated with the impact of environmental factors (Soupir *et al.* 2008), with differences becoming smaller when temperature was considered as an important factor (Pachepsky *et al.* 2014). As such, temperature can have a profound effect on inactivation in surface waters (Pachepsky *et al.* 2014), fermented meat (McQuestin *et al.* 2009), bovine feces (Gu *et al.* 2012), and manure-amended soils (Jamieson *et al.* 2002). In addition, changes in a manure's water content, temperature, and water activity can all affect levels of *E. coli* (Wang *et al.* 1996; Martinez *et al.* 2013) (and presumably other bacteria).

Microbial survival rates can be affected by the physiological characteristics. For example, Gram-positive bacteria (i.e., enterococci) typically have more robust survival rates in environmental matrices than Gram-negative bacteria (like *E. coli)*, suggesting enterococci are a better fecal indicator in more stressful environments such as marine waters (Mote *et al.* 2012). The same physiological differences allow enterococci to be more resistant to disinfection than fecal coliforms. The present study's results also confirm lower die-off rates of enterococci than *E.coli* and fecal coliforms in all manure types.

Shape of the release curve

Individual fits by plot and microbe generated highly variable values in the manure release parameters, consistent with even the high degree of variability reported for runoff (Kim *et al.* 2014) and with results reported by Blaustein *et al.* (2015) and Guber *et al.* (2013) for individual fits. Blaustein *et al.* (2015) reported high variability within replications for β, while 'a' values (i.e., α/q, see Equation (3)) were generally well replicated; neither parameter was significantly affected by rainfall intensity or manure surface slope. Guber *et al.* (2013) reported that grouping 'a' values across components (e.g., microbes) and release curves, and β for each runoff-box experiment, reduced the uncertainty of all parameters and improved model performance considerably. They also noted that variations in 'a' between experiments were most likely reflected in different vegetation conditions, although they did not investigate different manure types. Vegetation conditions were the same for all of our plots; therefore, α and β values were individually combined across microbes, Weeks, and Events, but allowed to vary by manure type. Median values with quartiles for α and β are presented in Table 3. By comparison, Figure 5 presents ranges in α and β values as published in or computed from the literature for bovine (dairy calf, cattle, or cow) manure (Bradford & Schijven 2002; Schijven *et al.* 2004; Guber *et al.* 2013; Blaustein *et al.* 2015; Equation (9)) and estimated by the present study. Estimated ranges of α and β complement those published in the literature, although the ranges and median values in the present study are larger (Figure 5). Groupings tended to produce higher values than individual simulations, and β values also tended to be higher when a single 'a' value was assigned (Guber *et al.* 2013).

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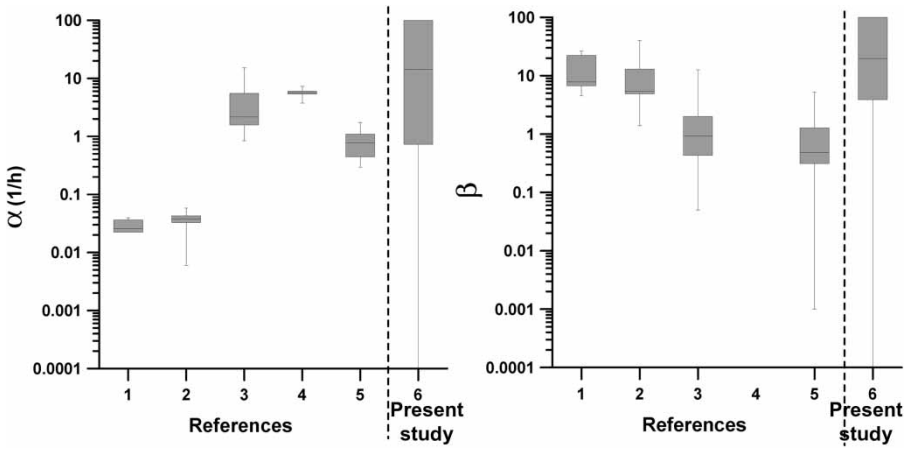

Figure 5 | Ranges in α and β values for bovine (dairy calf, cattle, or cow) manure, as published or computed from the literature: (1) Bradford & Schijven (2002), (2) Schijven *et al.* (2004), (3) Guber *et al.* (2013), (4) Equation (9), (5) Blaustein *et al.* (2015), and (6) estimated in the present study (PEST and bootstrap).

Release efficiency from manure

The lowest b values associated with solid pats were significantly smaller than for poultry or swine, across Weeks and microbes. For example, when b values are combined across microbes and Weeks, the combined median values for cattle, poultry, and swine are 0.312 h^{-1}, 0.845 h^{-1}, and 0.675 h^{-1}, respectively (see Table 3).

Guber *et al.* (2013) published E_r values for slurry ranging from 0.28 to 0.55 for enterococci and *E. coli*, respectively, and from 0.26 to 0.94 for all manure components for a rainfall duration of 1.5 h. Using the combined median b value for slurry of 0.675 h^{-1} (Table 3) and remembering that $E_r(t)$ is a function of time while E_r is a constant, $E_r(t)$ has values of 0.29 after 30 min and 0.64 after 1.5 h, similar to what Guber *et al.* (2013) reported. b values account for changes over the duration of the release with smaller $E_r(t)$ values earlier in the rainfall simulation.

Microbial release rates from manure

Blaustein *et al.* (2015) and Guber *et al.* (2007) suggested that manure structure may play an important role in the release of microbes. For cattle, b values are smaller and β are larger, compared to poultry and swine, indicating a slower microbial release and cumulative release curve. When comparing poultry to swine, b is slightly higher, but offset with slightly lower α and β values which result in very similar cumulative release curves. As such, Figure 4(a) and 4(b) present cumulative release curves and literature data for different microbes associated with cattle solid pats, and Figure 4(c) presents curves and literature data for different microbes associated with manure as a soil amendment, dry litter, and swine slurry. The curves in Figure 4(b) and 4(c) represent the combined effects of *E. coli*, enterococci, and fecal coliforms, while the other studies individually considered these microbes as well as *Giardia*, *Cryptosporidium*, and total coliforms. Given the relatively high surface area of surface applied manure (Blaustein *et al.* 2015) and dry litter compared to deposited solid pats, one might expect faster release of microbes from their surfaces. Likewise, liquid slurry does not contain an internal structure that prevents water from coming into contact with microbes, so there is a higher release potential of microbes compared to solid pats. Thurston-Enriquez *et al.* (2005) noted that numbers of *E. coli* recovered in runoff plots were highest for swine slurry and fresh cattle manure, although they did note that the liquid composition of fresh cattle and swine manures enabled a more homogeneous application, suggesting that cattle manure was not applied as individual pats but uniformly distributed. Given the liquid composition of the manures, it is unclear if the fresh cattle manure also happened to contain lower solids content than fresh pats.

Laboratory results from box and field plots published by Bradford & Schijven (2002), Schijven *et al.* (2004), Guber *et al.* (2013), and Blaustein *et al.* (2015) were overlaid on Figure 4 by manure form (solid, amended, slurry, and dry litter), not manure source (e.g., cattle, poultry, swine).

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Significantly lower microbial releases associated with the former two solid manure studies (Bradford & Schijven 2002; Schijven *et al.* 2004) are suggested by Figure 4(b) and 4(c). In those, fresh solid cattle manure was enclosed in cake pans or in 5-cm-diameter aluminum rings where the released suspension ran over the top, while the volume of the manure suspension was constantly maintained during the whole experiment (Guber *et al.* 2013). Alternatively, Blaustein *et al.* (2015) performed rainfall-release studies with an experimental design in which dairy manure was applied to a mesh screen in partitioning boxes (70 × 70 cm) and the manure could freely slough to runoff. The authors determined the effects of rainfall intensity and surface slope on bacterial release, and suggested that sloughing from the manure solid phases dominates over leaching from the manure liquids after the first few minutes of release (e.g., 10 min in their experiments).

In our study, fresh cattle pats were unprotected during the simulated rainfall event, allowing matrix disintegration and sloughing of the surface so that, unlike slurry, the manure was slowly eluted by rainfall and served as a long-term microbial source (Thurston-Enriquez *et al.* 2005). During their microbial salinity studies, Bradford & Schijven (2002) hypothesized that increases in manure stability account for decreases in release rate. As Guber *et al.* (2013) note, this explains the relatively fast decrease in concentrations of all manure components we observed, indicated by higher cumulative curves, especially for dry litter and slurry. Unlike Bradford & Schijven (2002), Schijven *et al.* (2004) and Guber *et al.* (2013) studied manure slurry for both runoff-box and -plot experiments, where manure was unrestrained. Their data are compared to our results in Figure 4(c).

Guber *et al.* (2007) reported differences in release kinetics of *E. coli* and enterococci from solid bovine manure, noting a bromide ion tracer had more similar release patterns to *E. coli* than enterococci, suggesting the former may be more associated with the aqueous phase. Similar results were reported by Blaustein *et al.* (2015) using a chloride tracer, who suggested the release of the ion and that of *E. coli* to be more associated with the release of the manure liquid phase. Likewise, Thurston-Enriquez *et al.* (2005) noted differences in *E. coli* and enterococci running off plots of different manure types: *E. coli* was highest in plots with swine slurry and fresh cattle manure, and enterococci were highest in plots with fresh and aged cattle manure. During microbial salinity studies, Bradford & Schijven (2002) hypothesized that differences between *Giardia* and *Cryptosporidium* (oo)cyst release rates are due to differences in organism size. If constituents had different release patterns from manure, b values for cattle manure in our study would be higher for *E. coli* than enterococci. We found no such trend; in fact, b values for enterococci were higher than *E. coli* (Table 3), but in a range that did not have significant impact on differences in release rates. The value of b captures the trend of more microbes appearing in the runoff due to more being released from the manure. The trend of median b values follows the microbial ratio in runoff from the source in only 20% of the total cases (Table 2), however, suggesting that b is not strongly correlated to the microbe; thus, b was combined across microbes and documented in Table 3.

Published data (Bradford & Schijven 2002; Schijven *et al.* 2004; Guber *et al.* 2013; Blaustein *et al.* 2015) were overlaid on the individual (Figure 4(a)) and combined (using median b values) cumulative release (Figure 4(b) and 4(c)) curves for solid pats (Figure 4(a) and 4(b)) and dry litter and swine slurry (Figure 4(c)). Bounds of the first and third quartiles associated with our results are also included on these plots, illustrating that all literature values fall within them. The combined cumulative release curve for solid manure (Figure 4(b)) represents an upper end of the laboratory experiments which could be partially explained by differences in experimental designs (e.g., unconfined versus confined solid manure). The cattle slurry results from Guber *et al.* (2013) and the cattle manure results from Blaustein *et al.* (2015), regardless of component, overlay and surround the combined cumulative release curves for poultry dry litter (black solid line) and swine slurry (dashed line) as illustrated in Figure 4(c). Blaustein *et al.* (2015) noted that differences between the release of microbes from manure at fine temporal and spatial scales suggest situation-specific release processes for different bacterial groups/species. As scale increases, it is possible that such differences in release become less discernible. Also, due to expected increases in variability of microbial concentrations in land-applied manure with an increase in spatial scale (Guber *et al.* 2006), one could expect that in a natural setting

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(i.e., on plots or field), there would be huge differences in microorganisms released, potentially resulting in a lack of noticeable differences in the release of different bacterial groups/species.

CONCLUSIONS

The monitored data from microbial rainfall runoff experiments with applications of different manure types (cattle pats, poultry dry litter, swine slurry) and controls, conducted over four seasons in 2009 and 2010 on a field containing 36 plots (0.75×2 m each), resulting in 144 runoff events containing 208 cases, were used for the application of a modified Bradford–Schijven model. The data showed a high degree of variability (up to six orders of magnitude by microbe and manure types within a season, and up to eight orders of magnitude across seasons) with simulation periods ranging from less than 2 hours to more than four. Microbial releases from manure associated with *E. coli*, enterococci, and fecal coliforms for each plot were simulated with the modified Bradford–Schijven model.

Under aging, the number of viable (culturable) microbes in the manure decreased significantly over one- and two-week periods after being exposed to all environmental conditions except precipitation. These results suggest the ratio of viable released microbes to the initial aged amount at the start of the rainfall simulation is similar, regardless of age. As the number of viable cells in the source decreased, the amount released also decreased, which has been described as a multi-stage release pattern by others such as Martinez *et al.* (2013).

Given variability in monitored runoff results, which propagated to variability on model parameters (Table 3) and simulated release curves (Figure 4), the manure matrix may be an important differentiator. Although there were no clear trends of definitive differences in release patterns by microbe within each manure form, release patterns from slurry, dry litter, and amended manures were similar, which were different from those of solid pats. Surface contact between water and manure, matrix disintegration, and sloughing of solid manure may play an important role by impacting release efficiency and patterns differently. Fresh pats with softer formation can be broken down by rainwater more easily, dry litter is distributed and has more surface area, and swine slurry infiltrates the soil more readily. That is not to say that all microbes behave the same when released from manure, because research suggests microbial release is impacted by different microbial properties such as mobility, size, surface properties, and shape (Bradford & Schijven 2002; Guber *et al.* 2013). Additional research may be required, since microbes more readily associated with the aqueous phase may release earlier from the matrix. Model parameters and their uncertainty can be efficiently estimated by PEST, coupled with a statistical method (i.e., bootstrapping). Modeling suggests that a single set of parameters – combining results for changes in season, manure age, and microbe – can reliably predict microbial release from manure. The model also appears to be robust and applicable to many components (microbes, etc.).

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