Remobilization of Fecal Indicator Bacteria in Dune Sands During Transients in Water Flow

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Abstract

The processes controlling remobilization of bacteria in the unsaturated zone under the influence of transients in water flow have not often been studied and are poorly understood. Fecal indicator bacteria, *Enterococci*, have been observed in water collected after infiltration and soil passage in sand dunes, leading to questions about the capacity of dune sands in general and the unsaturated zone in particular, to filter and retain bacteria from the infiltrating water. This study measured the release of two fecal indicator bacteria, *E. coli* and *E. moraviensis*, in dune sands as a result of transients in flow. The release was measured in column experiments with either heavy rainfall events or fluctuations in the groundwater level as the forcing mechanisms. Subsequently, microbial release was modeled with HYDRUS-1D, using either a one- or two-site kinetic attachment/detachment model to account for retention and release of the bacteria in the subsurface. The model was fitted to the experimental data. Experimental results reveal that imbibition dominates the release of *E. coli*, where drainage is the controlling process for the release of *E. moraviensis*. Furthermore, *E. moraviensis* are more easily attached and less easily detached from soil grains than *E. coli*. Hysteresis and air entrapment occur during the unsaturated experiments and are thought to have a large effect on microbial transport. Agreement between measured and modeled concentrations of bacteria under influence of rainfall is generally good and the attachment and detachment parameters that control the kinetics of bacteria (re)mobilization vary in a recognizable manner with changes in bacteria species and transient flow scenario. The one- and two-kinetic site model simulations of groundwater level fluctuations fitted poorly with the measured effluent concentrations. More than 99% of the bacteria applied to the sand column during inoculation is retained in the sand. However, the large number of bacteria contained in animal feces as well as accumulation of bacteria in the subsurface over time, can results in significant contamination of the groundwater. The results of this study indicate that under certain unfavorable conditions, additional steps need to be taken in drinking water production after dune filtration in order to ensure the required drinking water quality. Further research is needed to fully distinguish the factors controlling remobilization of bacteria in the unsaturated zone in dune sands.

Introduction

Clean drinking water is one of the most important resources in modern times, with the demand for water increasing with the global population growth. Groundwater is an important source of drinking water globally; at least 1.5 billion people depend on groundwater as their sole source of drinking water (World Resources Institute, 2000). Pathogenic microorganisms, derived from for example wastewater, animal manure and leaking sewage pipes, are a significant group of contaminants that pose a threat to groundwater quality. Dune filtration has been proven to be an efficient treatment step in drinking water production and its capacity to remove microorganisms is generally seen as sufficient (Schijven et al., 1998). In the Netherlands, dune infiltration is an important step in drinking water production for large parts of the country and accounts for approximately 14% of the total drinking water production (Schijven et al., 1999). The dunes used for drinking water production are generally large nature reserves that also host animals such as cattle, sheep and geese, that can introduce harmful bacteria to the groundwater. For example, Taučer-Kapteijn (2017) reported an average of 3.48×10^5 CFU/g of *Enterococci* in goose feces measured at the Castricum dune filtration site (The Netherlands) and Ervin et al. (2013) measured $10^{4.9}$ – $10^{5.0}$ CFU/g of *E. coli* in cow feces. Thus, these animal feces can be a source of large bacterial contaminations in the subsurface under the influence of rainfall, especially in the summer. In relation, *Enterococci* have occasionally been observed in large samples (100 L) of water collected after infiltration and soil passage in the Castricum dune infiltration area (Taucer-Kapteijn et al., 2016). This is an indication that the influx of bacteria can exceed the capacity of the dunes in general and the unsaturated zone in particular, to remove microorganisms from infiltrating groundwater.

The unsaturated zone is a natural barrier between groundwater reserves and contaminants typically found at the soil surface. Numerous steady-state microbial transport studies have shown that retention of microbes occurs in the subsurface, especially under unsaturated conditions (e.g. Schijven and Hassanizadeh, 2000; Jin et al., 2000). The prevailing idea is that this is due to retention of the microbes on the interface between grains and water (SWI), the interfaces between air and water (AWI) in the matrix and/or the triple point between air, water and solid grains (AWS) and that the immobilized bacteria die off in time. This makes the vadose zone a suitable barrier that can be used for removing microbes. However, as mentioned above, bacteria are occasionally observed in water collected after dune filtration. Secondly, several studies suggest that some microbes can survive for extended periods of time of more than 6 months in the subsurface (Staley et al., 2016; Hornstra and Cirkel, 2018, Nautiyal et al., 2010), allowing them to be possibly remobilized by changes in water content (Engström et al., 2015; Cheng and Saiers, 2009). Staley et al. (2016) and Hornstra and Cirkel (2018) studied *E. coli* and *Enterococci* while Nautiyal et al. (2010) studied only *E. coli*. Transients (i.e. variations) in water content occur commonly due to infiltration, evapotranspiration and groundwater level changes, amongst others. A better understanding of the processes that play a role in the retention and release of microorganisms in the unsaturated zone is required in order to protect groundwater resources and thereby safeguard the drinking water supply.

Many column-scale experimental and modeling studies have been conducted to study bacteria, virus and colloid transport in the subsurface, focusing on the saturated zone (Engström et al., 2015). The hydrological processes in the saturated zone are more straightforward than in the unsaturated zone, due to for example, the presence of air in partially saturated soils. As such, more is known on the subject, making it easier to research and model. Pathogen removal is generally higher under unsaturated conditions due to generally lower flow rates, a larger air water interface, smaller distances between the microorganisms and soil particles, and more microbe decay by aerobic organisms (e.g. Torkzaban et al., 2006; Wan and Wilson, 1994). Moreover, pathogen removal is high in fine-textured and heterogeneous soils at a low infiltration rates (Nicosia et al., 2001) as lower flow rates result in more time for adsorption of microbial particles to soil grains.

Pore scale studies have shown that colloid release is influenced by transients in water content and that these colloids are retained on the SWI and AWI (Zhang et al., 2013). However, there are very few studies that incorporate release of colloids from the SWI to the AWI, retention of colloids on the AWI and release of colloids from the AWI to the aqueous phase (Engström et al., 2015; Wang et al., 2014). Furthermore, the relative importance of imbibition as opposed to drainage and vice-versa is still unclear (Wang et al., 2014) as some studies report colloid release only through imbibition (Russell et al., 2012) while other studies found

that release takes place during both imbibition and drainage (Zhang et al., 2012). Many studies have been conducted into the retention and release of abiotic colloids in the unsaturated zone, however the differences in removal behavior between these abiotic colloids and microorganisms such as pathogenic bacteria have not been fully investigated (Engstrom et al, 2015; Ohshima, 1995)

During drainage the AWI increases as air enters the pore spaces, starting in the larger pores with a lower capillary force, followed by the smaller ones. Water films cover drained pore spaces, decreasing in thickness as drainage continues (Wang et al., 2014). A number of forces act on microorganisms retained in the soil including van der Waals and electrostatic interactions between the microorganisms or colloids and the SWI and AWI, and capillary forces from the water films (Wang et al., 2014; Saiers and Lenhart, 2003).

During imbibition the AWI decreases as water fills the pore space, starting with the smaller pores, then the larger ones. The AWS (air-water-solid) contact line moves in the direction of water flow. Water films expand and destruction of the AWI and AWS contact points can result in the release of microbes from the AWI and AWS to the aqueous phase (Cheng and Saiers, 2009). The amount of microorganisms or colloids released during imbibition depends on a number of factors including particle geometry (Aramrak et al., 2013), water film thickness (Wan and Tokunaga, 1997) and flow velocity (Saiers and Lenhart, 2003).

Schijven and Simunek (2002) modeled bacteriophage transport using a two-site kinetic model allowing for reversible adsorption to two sites in saturated porous media. Bradford et al. (2003) pose that colloid retention in saturated porous media is the result of a combination of colloid attachment and irreversible straining. Later studies modeled experimental results of virus transport in the saturated and unsaturated zones, taking into account irreversible attachment of the viruses to the AWI (Torkzaban et al., 2006). Cheng and Saiers (2009) and Russel et al. (2012) modeled colloid release taking into account only transfer of colloids from the SWI to the aqueous phase. These studies did not consider interactions between the SWI and AWI, or release of colloids from the AWI to the aqueous phase.

The aim of this research is twofold: firstly, to gain further understanding of the processes that play a role in the retention and release of microorganisms in the unsaturated zone in an applied manner. Secondly, to quantify the potential for release of bacteria initially retained in the unsaturated zone in dune sands under the influence of changes in water content and therefore the potential for contamination of groundwater used in drinking water production. This was done by carrying out a number of column experiments under unsaturated conditions involving cycles of precipitation events and/or groundwater level changes, varying the water content. The experimental setup is designed to reflect a field situation where pathogens (mainly from animal feces) are washed out and infiltrate into the soil through rainwater and are transported to the groundwater though the unsaturated zone. Groundwater level rise is relevant in almost all natural environs but is additionally important in areas managed for drinking water production where the groundwater level can easily be varied by switching on or off certain wells. The hydrology of the groundwater level variation scenario is expected to be much more complex than that of the rainfall scenario which makes it worthwhile but difficult to research. Furthermore, a model was developed which was fitted to the experimental data. The aim of the model is to be able to quantify and predict bacteria retention and release under different external forcing scenarios. Parameter values obtained are generally situation specific; however, the underlying processes are expected to be applicable to retention and release of bacteria in the unsaturated zone in general.

Materials & Methods

Experimental outline

E. coli and *E. moraviensis* retention and release is investigated in packed sand columns during varying cycles of imbibition through rainfall or groundwater level rise, and subsequent draining. Each experimental run consisted of a salt tracer, introduction of bacteria and cycles of imbibition through either rainfall or groundwater level rise, and drainage.

Two different scenarios are simulated in the sand column experiments. The first scenario is a series of three heavy rainfall events of 4 hours in duration each, separated by 24 hours. The rainfall experiment was carried out in quadruplicate and these are referred to as RAIN1, RAIN2, RAIN3 and RAIN4. The fourth rainfall experiment, RAIN4, experienced a failure in the airtight bottom seal resulting in free drainage of the column, as opposed to the intended bottom suction pressure. Therefore, this experiment was no longer comparable to the other rainfall experiments. Results of this experiment run can be found in the appendix. The second scenario simulates 3 cycles of groundwater level rise from a depth of 50 cm initially, to the sand surface, followed by drainage as the groundwater level is returned to 50 cm depth. One cycle is executed per day, for the duration of 3 days. This experiment was carried out in duplicate and is referred to as GWL1 and GWL2. The experimental runs and their associated imbibition and drainage conditions are listed in Table 1. A rainfall flux of 1.2 cm/hour was chosen for the inoculation and rainfall events as it reflects a heavy rainfall event with a return period of 50 years (Smits et al., 2004; Buishand and Wijngaard, 2007). Drainage was conducted with no flow at the column surface and a bottom pressure of -30 cm at the bottom of the column. This setup reflects the soil column as the top 25 cm of an unsaturated zone with a total depth of 0.5m.

Table 1: List of experimental runs and associated boundary conditions

Experimental run	Rainfall flux [cm/hr]	Groundwater level [cm below surface]
RAIN(1, 2, 3)	1.2	-30
GWL(1, 2)	0	-30 to 0

Bacteria

The fecal indicator bacteria *Escherichia coli* (PWN 831804-1, 24-12-14) and *Enterococcus moraviensis* (PWN) are used in the experiments. *E. coli* (PWN 831804-1) and *E. moraviensis* (PWN) were selected for this study because they have been previously isolated from water in the study area and are known to be able to survive under the experimental conditions. Furthermore, *E. coli* and *E. moraviensis* are fecal indicator bacteria used in the analysis of drinking water production. Fecal indicator bacteria (FIB) are generally not harmful to humans but are indicators of fecal pollution which might include harmful pathogens (Myers et al., 2007). *E. coli* is a member of the family of Enterobacteriaceae and are Gram-negative, facultatively anaerobic, non-sporing rods that are often motile (LMB-042, 2019; Jiang et al., 2007). Because *E. coli* are negatively charged they generally attach unfavorably in soil and could therefore potentially be suitable for predictive modeling. *Enterococcus moraviensis* is a member of the *Enterococcus* genus and are Gram-positive, ovoid, non-motile cells that can occur in pairs, short chains or small groups (Svec et al., 2001).

The concentrations of *E. coli* and *E. moraviensis* in the influent and effluent samples were determined according to the KWR microbiology lab protocol for microbial analysis (LMB-042, 2019; LMB-044, 2016). The concentrations in each sample are determined for the undiluted sample as well as 4 dilutions. The dilutions are respectively 1:10, 1:100, 1:1000 and 1:10000. Of each dilution, as well as the undiluted sample, 0.1 ml. of diluted is plated on a petri dishe containing lauryl sulphate agar (LSA) or Slanetz and Bartley medium (S&B-medium) for *E. coli* or *E. moraviensis* respectively. Additionally, 1, 10 and 100 mL of each sample are filtered through a cellulose nitrate (CN) or GN-6 Metricel membrane filter and plated to detect very low concentrations of *E. coli* or *E. moraviensis* respectively. The dishes for *E. coli* determination are then

incubated at 25˚C for 5 hours followed by 14 hours at 36˚C. For *E. moraviensis* determination, the plates are incubated at 36˚C for 44 hours. After incubation, the bacteria are counted per colony-forming unit (CFU).

Concentrations of both strains in the stock suspensions were determined before each experiment, using the method described above. The decay rates of the bacteria were determined from soil samples mixed with the bacteria stock solutions and kept at the same conditions as the sand column experiments. The soil samples were kept in a beaker placed in an anaerobic jar (OXOID) kept at a constant temperature of 15 degrees Celsius. The jar contained a standing water level of approximately 2 cm to ensure 100% air humidity and had a lid fitted with an O-ring and clamp in order to prevent exchange of oxygen with the outside air. The sand in the container was sampled at regular intervals in order to determine the microbial decay rates.

Column experiment setup

The column experiments were carried out in transparent PVC cylinders of 25cm height and with an inside diameter of 15.2 cm. Two experiments were carried out simultaneously in two columns in a climate room with an average temperature of 15 degrees Celsius reflecting soil temperatures during summer. Ceramic tip tensiometers (RhizoInstruments) were inserted at 6 and 14 cm from the upper sand surface. Air entry valves were inserted on the opposite side of the columns to the tensiometers to accommodate air entry and exit during imbibition and drainage. Under saturated conditions, these tubes were closed. Each column was permanently rested on a scale (AllScales Europe) in order to measure changes in the average water content. The initial bacteria suspension as well as rainfall simulations were delivered to the column via a rainfall simulation head situated atop the column, attached to a peristaltic pump (Cole-Parmer Instrument Company). A polyamide woven filter cloth with a nominal pore size of 20 µm (SEFAR) was placed at the bottom of the column. Based on nominal pore size the bubble pressure was estimated to be ca.100 cm. The membrane retained soil particles but no bacteria. The suction pressure at the bottom of the column could be adjusted through a hanging water column and was set to a default value of -30 cm.

Dune sand from managed aquifer recharge (MAR) site ICAS, operated by PWN in the vicinity of Castricum was used in the column experiments. For this study sand from depths between 60 and 80cm below ground surface was collected. Samples taken from the columns after completion of one of the experiments were characterized using the Sandbox method (van der Harst and Stakman, 1965) to construct a pF curve and Loss on Ignition (LOI) method (Heiri et al., 2001; Salehi et al., 2011) to determine organic matter and CaCO3 content. Soil organic matter content can influence microbial attachment to sand grains (Schijven and Hassanizadeh, 2000). The pF curve was subsequently fitted using the RetC program (van Genuchten et al., 1991) to obtain estimates for the porosity and the empirical parameters alpha and n used in the van Genuchten – Mualem (van Genuchten, 1980) hydraulic model.

The application of bacteria to the sand column was carried out via the rainfall simulator at an intensity of 12.3 mm/hour for the duration of 4 hours. Approximately 1.5 pore volumes of stock solution were flushed through the column. The column effluent during inoculation is collected in a single fraction and analysed for *E. coli* and *E. moraviensis* concentration. The volume of and concentration of bacteria in the inoculation solution and effluent are known, making it possible to calculate the absolute number of bacteria retained in the sand column directly after inoculation.

The effluent from each experiment was collected in fractions: 5 fractions per event for RAIN1, GLW1 and GWL2, 8 fractions per event for RAIN2 and RAIN3. The fraction volume and its corresponding drainage time depends on the conditions of the experiment and the drainage discharge. The microbial analysis of the samples yields the average concentration of bacteria in CFU/ml, over the entire effluent fraction. As the volume of each fraction is known, the total number of *E. coli* and *E. moraviensis* in each fraction can be calculated using the concentrations. Thus, the total number of bacteria released per event is also known.

A core was taken of the uppermost 17 to 20 cm of the sand column at the end of each experiment to determine the distribution of bacteria entrained in the soil. The lowest 2 to 5 cm of sand was not sampled in

order to maintain filter cloth integrity. The bacteria concentration in the lowest section of the column was extrapolated using a trendline fitted to the measured data.

Soil characterization

The pF curve obtained from the sandbox method and the subsequent fit in the RetC program yielded parameter estimates for the saturated conductivity and the alpha and n parameters used in the van Genuchten – Mualem hydraulic model. These results are displayed in Table 1 alongside the results from the LOI method.

Table 2: Soil characterization and RetC results

		95% Confidence limits	
Variable	Value	Lower	Upper
θ_S	0.44527	0.3897	0.5008
α	0.02247	0.0143	0.0306
n	3.62304	1.5247	5.7214
K_{sat} [cm/hr]	0.81		
LOI_{330} (Bulk organic matter)	0.78%		
LOI_{550} (Organic matter)	1.05%		
LOI_{1000} (CaCO3)	1.50%		

Each column was packed to a height of 22 cm by pouring discrete amounts of field moist soil into the column in a layer by layer manner. Layers were packed by gently tapping with a ram to ensure homogeneous compaction (Lewis and Sjöstrom, 2010; Lima and Sleep, 2007; Gilbert et al., 2014). Bulk density ranging between 1.51 and 1.65 g/cm^3, measured on undisturbed samples taken at the end of the experiment (Table 3) confirm that bulk densities of the packed columns reflect conditions in Dutch dune soils (Ritsema and Dekker, 1994). A small spread is measured in the bulk densities of the different experimental runs with the largest difference being 0.14 g/cm^3 (140 g/L). Assuming a constant soil particle density between experiments, it is assumed that these differences in bulk density are caused by differences in compaction during column packing. This could result in small differences in water flow between experiments.

Table 3: Measured bulk density of packed column per experiment

Experiment run	Bulk density [g/cm^3]	Porosity (-)
RAIN1	1.51	0.45
RAIN2	1.63	0.42
RAIN3	1.59	0.44
GWL1	1.53	0.45
GWL2	1.65	0.42

Hysteresis

Many experimental setups and models assume unique functions for the water content and pressure head. However, hysteresis in dune sands is a widely reported phenomenon in general (Toride et al., 2003) and in Dutch dune soils (e.g. Ritsema and Dekker, 1994). Hysteresis in soil hydraulic properties causes the water content at a given pressure head to vary depending on whether the soil is in a wetting or a drying phase.

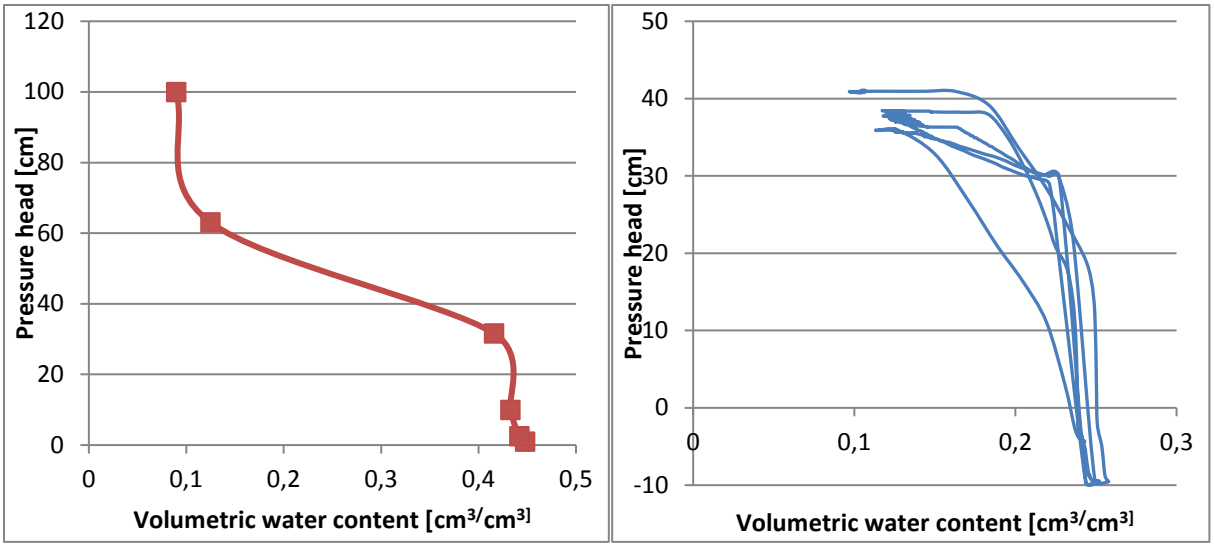


Figure 1: A) Fitted pF curve of column soil samples; B) Measured water retention and pressure head during GWL1 showing hysteresis

The fitted water retention curve of Figure 1 is the main drying curve of the soil sample. In order to measure possible hysteric effects, the average water content and the pressure head in the sand column were compared to the water retention curve.

Tracer experiments

A salt tracer experiment was carried out before each experiment to estimate soil hydraulic and solute transport parameters using the breakthrough curve. A solution of 8.56 mM NaCl was applied to the top of the column via the rainfall simulator at an intensity of 50 mm/hour for the duration of 4 hours, followed by rainfall of drinking water for an additional 24 hours with a bottom pressure head of -30 cm. The electrical conductivity of the effluent was measured for this duration. Salt tracer results and bulk densities from the different column experiments show that the column packing procedure was adequately replicable (Appendix A). The breakthrough curves from the experiments were sufficiently symmetrical to indicate that dead-end pores and immobile water did not play a significant role in the transport of the solute (Torkzaban et al. 2006). The low or absent tailing in salt concentrations point to advection being the dominant transport mechanism.

Mathematical model

A HYDRUS-1D version 4.16.0110 (Simunek et al., 2005) model is used in order to simulate bacteria retention and release using inverse modeling. The soil hydraulic and solute transport parameters necessary to achieve that aim are fitted to the experimental results using parameter estimation based on the Marquardt-Levenberg optimization algorithm. In total, three separate models are developed (Figure 2). First, a hydrological model is developed, simulating only water flow. The pressure head and water content results of the initial flushing of the column during column setup as well during the salt tracer experiment are used as input data for the inverse modeling. The parameters Θsat, n and α, resulting from the soil characterization are used as known parameters and are therefore not fitted. The modified van Genuchten equation (van Genuchten, 1980) is used coupled with the hysteresis model of Lenhard et al. (1991) and Lenhard and Parker (1992) to account for hysteresis and air entrapment. Secondly, solute transport is introduced in the model. This model includes the transport of a conservative salt tracer applied to the top of the soil column in a step pulse, as is done during the tracer experiment explained above. This is done to obtain a value for the longitudinal dispersivity. An average dispersivity parameter value of 0.55 cm was obtained and is used for all subsequent microbial transport modeling. The obtained hydrological and dispersion parameter estimates are then used to simulate bacteria retention and release under varying conditions. The main processes included in the microbial

transport model are advection, attachment and detachment. Diffusion in packed columns is usually negligible (Schijven and Hassanizadeh, 2000) and is therefore not taken into account.

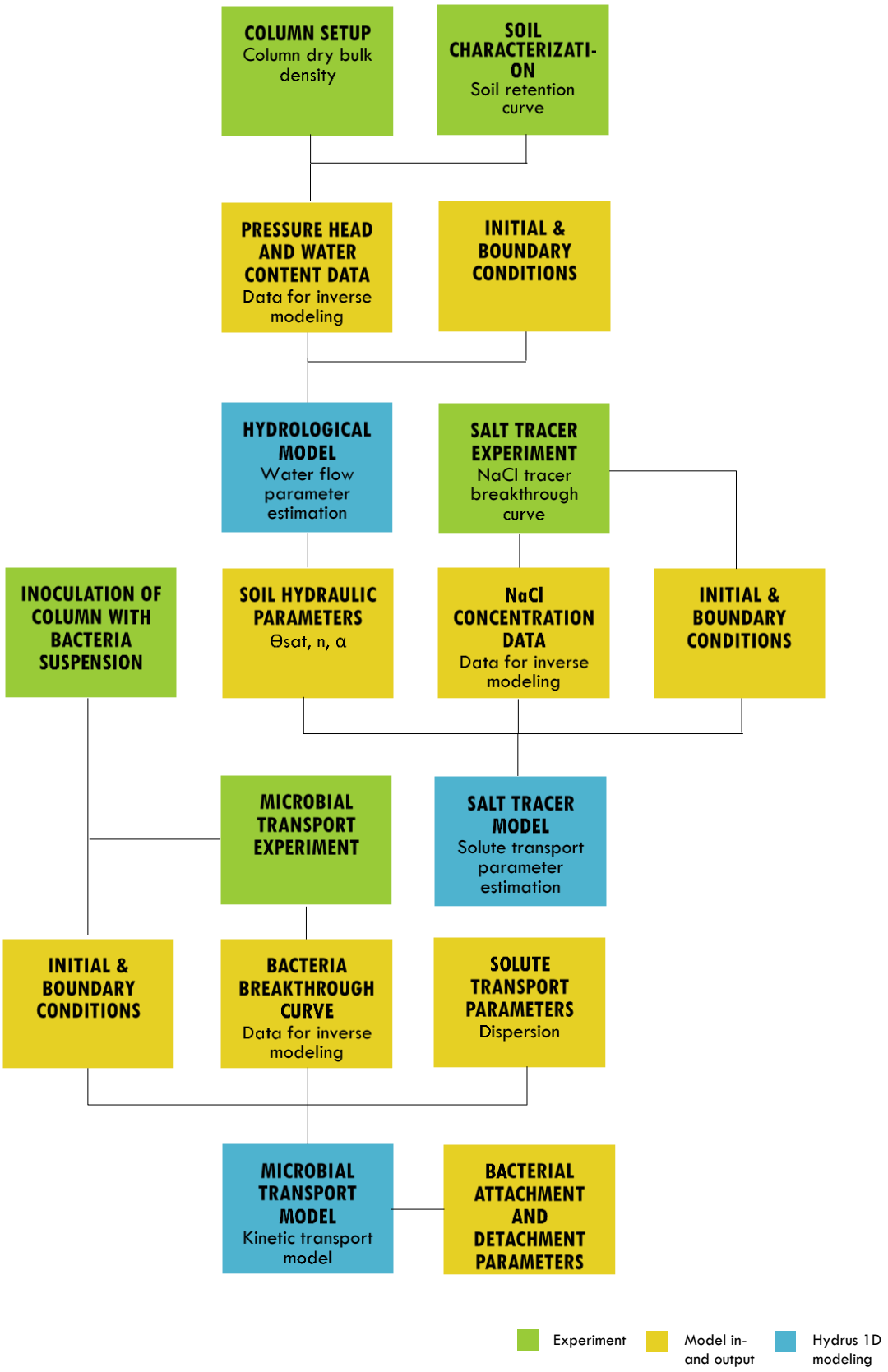


Figure 2: Flowchart displaying the steps taken in the inverse modeling of microbial transport.

Each microbial transport experiment (e.g. RAIN1, RAIN2, GWL1) is modeled individually, in its entirety with the column after inoculation as the initial condition. That is to say, each experiment consisting of three rainfall events or groundwater level cycles applied to the sand column containing bacteria is modeled as a continuous whole. The column effluent during inoculation is collected in one fraction. Therefore, a breakthrough curve of the bacteria during inoculation cannot be constructed. However, as the volume of the effluent fraction and its bacteria concentration are known, the number of bacteria retained in the column at the start of the rainfall and groundwater events is known and can be used as the initial condition for the model. Therefore, microbial transport parameter estimates resulting from the fitting of experimental data apply to the entire experiment including transients in water flow. Rainfall events are implemented in the HYDRUS model as a step pulse of rain by applying a boundary flux of 1.2 cm/hour to the soil surface for the duration of the event (4 hours). In-between events there is no flux applied to the soil surface. Groundwater level variations were modeled by varying the groundwater level option in HYDRUS, according to the position of the hanging water column in the experimental setup. The onset of each subsequent event is 24 hours after the previous event. The measured concentrations of bacteria in the column effluent are used as input data for the fitting equation.

Both one-site and two-site kinetic attachment-detachment models are used to simulate bacteria retention and release, after the example of Schijven & Simunek (2002) and Bradford et al. (2004). For the one-site model, one site represents the sum of the sorption processes where for the two-site model the sites represents sorption to two distinct sites or phases, such as the soil water interface and the air water interface. The results of the inverse modeling using one-site and two-site models are then compared. The governing equations of the attachment/detachment model are shown in Equations 1 and 2.

Equation 1)

$$\theta \frac{\partial C}{\partial t} + \rho_b \frac{\partial S_1}{\partial t} + \rho_b \frac{\partial S_2}{\partial t} = D\theta \frac{\partial^2 C}{\partial x^2} - \theta v \frac{\partial C}{\partial x} - \mu_1 \theta C - \mu_{s1} \rho_b S_1 - \mu_{s2} \rho_b S_2$$

The mass balance for the two kinetic site model is defined in Equation 1 where C is the bacteria concentration in the aqueous phase [$N_c L^{-3}$], Θ is the water content [-], v (=q/ Θ) is the pore water velocity, [LT^{-1}], D is the effective dispersion coefficient [L^2T^{-1}], t is the time [T], S1 and S2 are the concentrations of attached bacteria on two different kinetic sites [$N_c M^{-1}$], ρ_b is the bulk density [$M\ L^{-3}$], μ_1 and μ_2 represent growth/death of the free and attached bacteria in two different kinetic sites [$N_c L^{-3} T^{-1}$] and x is the distance in the vertical direction [L] (Simunek et al., 2013). Mass transfer between the aqueous phase and the kinetic sites is described by Equation 2:

Equation 2)

$$\rho_b \frac{\partial S}{\partial t} = \rho_b \frac{\partial (S_1 + S_2)}{\partial t} = \theta k_{att} C - \rho_b k_{\mathrm{det}} S$$

where k_{att} is the first-order attachment coefficient [T^{-1}], k_{det} is the first-order detachment coefficient [T^{-1}]. The subscripts have been dropped in Equation 2 to make it a general equation for each kinetic site. Equations 1 and 2 are formulated for two kinetic sites but can be applied to a single kinetic site by setting the attachment and detachment coefficients of the second site to zero.

The one- and two site models are compared to concentration measurements of the column effluent, hereby fitting the attachment and detachment parameters. The water flow parameters are kept constant during the simulation of microbial transport and are set to the values resulting from the salt tracer experiments, taking hysteresis into account. Comparison of the 1- and 2-site models is done using the Akaike Information Criterion (AIC). The Akaike Information Criterion (AIC) evaluates the quality of the model taking into account the fit with the experimental data and applying a penalty for the number of fitting parameters. When comparing the 1- and 2-site models fit to a single set of data points, the model with a lower AIC is deemed the better model.

Microbial decay rate

The decay rate of both bacteria strains was determined simultaneously with the column experiments, under the same conditions. The measured decay rates for both *E. coli* and *E. moraviensis* are consistently higher than those measured in an earlier study under similar conditions with the same bacterial strains (Hornstra and Cirkel, 2018). Hornstra and Cirkel measured decay rates of 0.184 $\log_{10}$CFU/day and 0.07 $\log_{10}$CFU/day for *E. coli* and *E. moraviensis* respectively, observed after one week, similar to the duration of the experiments in this study. However, as microbial decay is a non-linear process, in natural environments, the bacterial decay rate is thought to decrease significantly over time. Hornstra and Cirkel reported decay rates of 0.029 $\log_{10}$CFU/day and 0.069 $\log_{10}$CFU/day for *E. coli* and *E. moraviensis* respectively, measured over a period of 150 days. This means bacteria can survive for much longer periods of time than might be concluded based on the decay rates measured in this study (Table 4).

Table 4: Measured microbial decay rates

Experiment run	*E. coli* decay rate [$\log_{10}$CFU/day]	*Enterococci* decay rate [$\log_{10}$CFU/day]
RAIN1	0.207	0.072
GWL1/GWL2	0.272	0.103
RAIN2/RAIN3	0.2532	0.1119

Results

Inoculation

Table 5 shows the absolute number of bacteria in the inoculation influent suspension and the inoculation effluent. The difference between these two is the number of bacteria retained in the column directly after inoculation. During inoculation, more than 99% (approximately 2 $\log_{10}$) of the total bacteria concentration was retained in the sand columns for both strains in all experiments. A higher percentage of *E. moraviensis* is retained in the column during inoculation as compared to *E. coli*.

Table 5: Concentrations and number of bacteria in the inoculation solution and retained in the column directly after inoculation for each experiment

Experimental run		Inoculation influent concentration	Number of bacteria in inoculation	Inoculation Effluent concentration	Number of bacteria in inoculation effluent	Number of bacteria in column after inoculation	Number of bacteria in column after inoculation	$\log_{10}$(Nin-Neff)/Ninit))
		[CFU/ml]	[CFU]	[CFU/ml]	[CFU]	[CFU]	[$\log_{10}$ CFU]	[$\log_{10}$]
E. coli	RAIN1	6.90E+07	1.01E+11	2.90E+04	5.15E+07	1.01E+11	11.01	2
	RAIN2	6.70E+07	1.00E+11	2.58E+03	4.03E+06	1.00E+11	11.00	2
	RAIN3	4.00E+07	5.98E+10	1.02E+03	1.62E+06	5.98E+10	10.78	2
	GWL1	4.50E+07	6.52E+10	3.30E+03	4.82E+06	6.52E+10	10.81	2
	GWL2	4.50E+07	6.52E+10	3.50E+02	4.91E+05	8.96E+10	10.95	2
E. moraviensis	RAIN1	5.50E+06	8.07E+09	5.10E+01	9.06E+04	8.07E+09	9.91	2
	RAIN2	6.30E+05	9.42E+08	1.08E-01	1.68E+02	9.42E+08	8.97	2
	RAIN3	7.20E+05	1.08E+09	1.08E-01	1.71E+02	1.08E+09	9.03	2
	GWL1	4.90E+06	7.10E+09	1.60E+00	2.34E+03	7.10E+09	9.85	2
	GWL2	4.90E+06	7.10E+09	3.40E-01	4.77E+02	7.81E+09	9.89	2

Rainfall experiments

Hydrological data

Pressure head and discharge data in Figure 3 and total effluent volumes in Figure 5 show similar hydrological conditions between the different rainfall experiments. The pressure head and discharge data indicate that the different sand columns react to the rainfall flux in the same way in terms of hydrology and are therefore an indication of the reproducibility of the column packing and rainfall experiment boundary conditions.

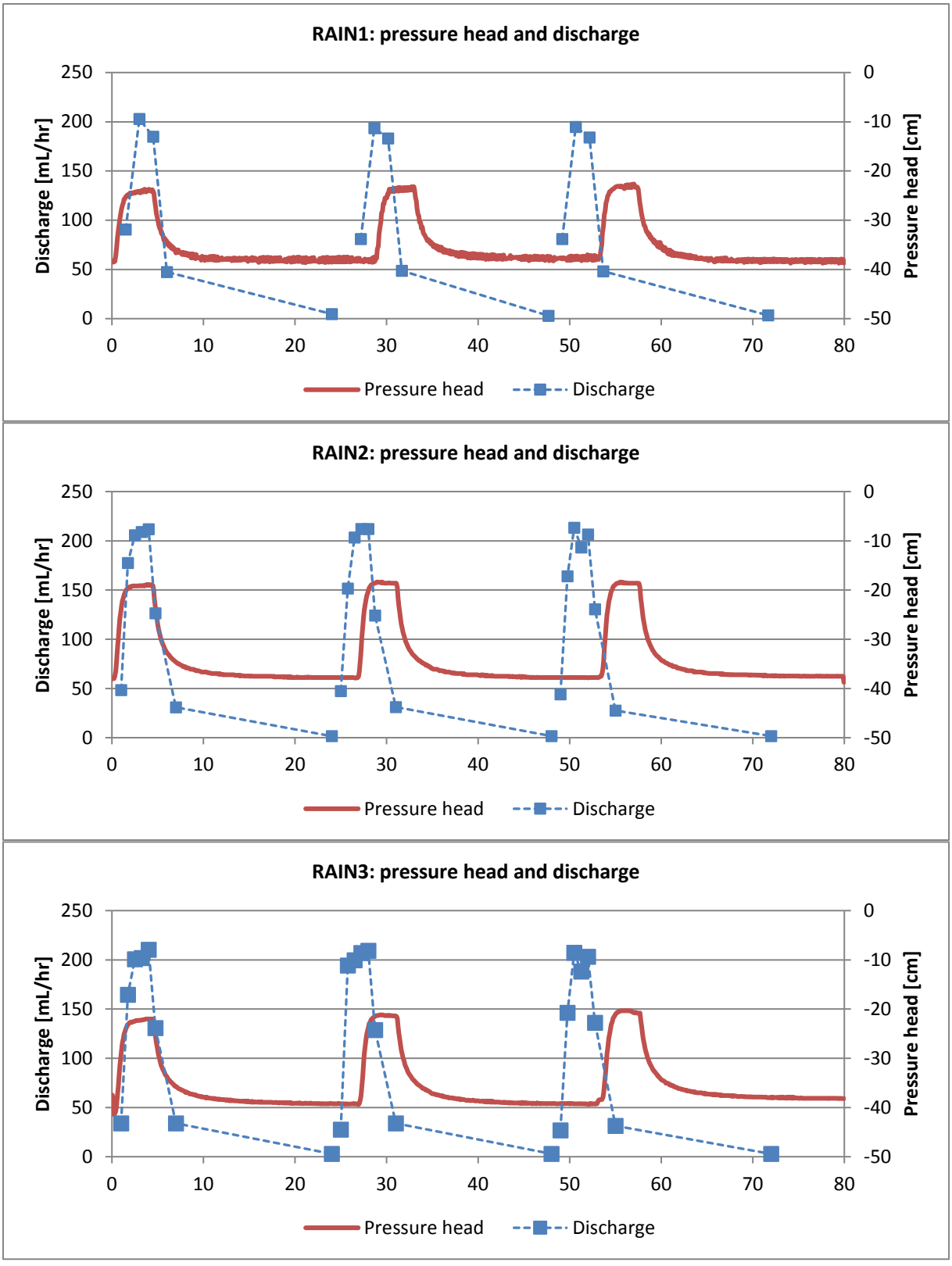


Figure 3: Pressure head and discharge measurements of A) RAIN1, B) RAIN2 and C) RAIN3

Average water content data of the rainfall experiments show that the average water content in the columns varies between 0.11 and 0.19 during drainage and at the peak of a rainfall event, respectively (Figure 3). At the measured pressure heads during peak rainfall the water content is expected to be 0.4, close to the

theoretical Θs of 0.44, based on the fitted pF curve (Figure 1). These results indicate hysteresis in the soil hydraulic properties. Furthermore, Figure 4 shows that the surface of the sand column is wetter than the bottom during the rainfall event but drier when there is no rainfall flux.

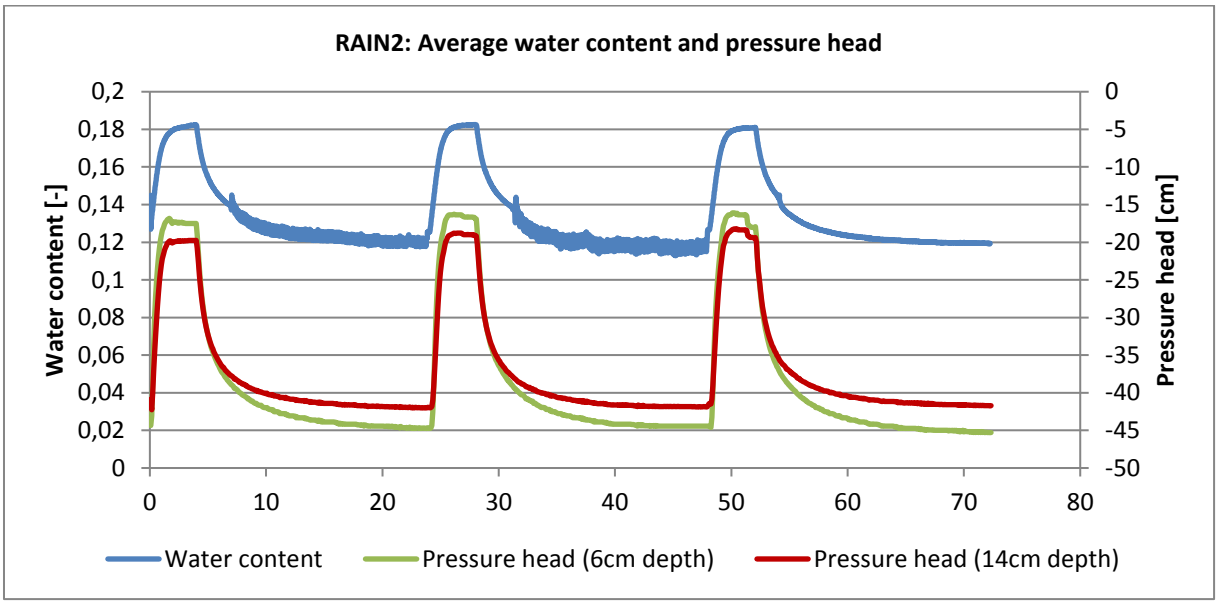


Figure 4: Average water content and pressure head measured at 11 cm depth for RAIN2

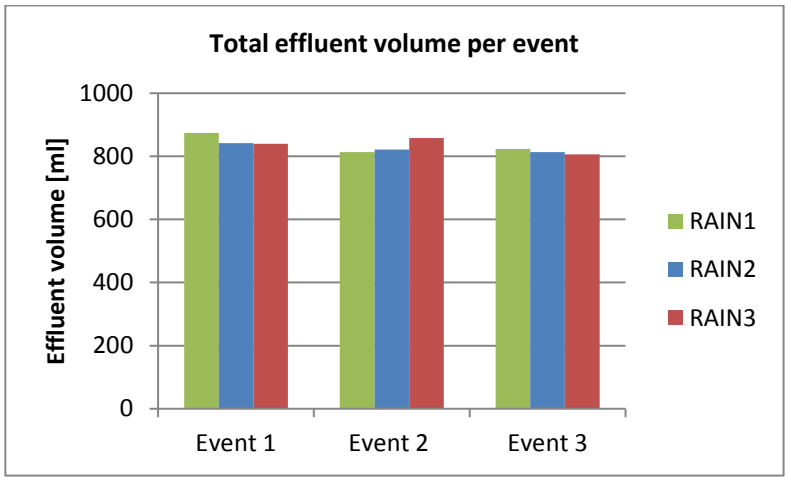


Figure 5: Total effluent volume per event for all rainfall experiments

The total effluent volume varies between experimental runs and rainfall events as a result of the soil hydrological properties as well as slight variations in the volume of water in each rainfall event caused by variations in the discharge of the peristaltic pump in the order of 20ml per rainfall event.

Microbial transport

Figure 6 expresses the ratio between the number of *E. coli* and *E. moraviensis* released per event, and that retained in the sand column at the end of each experiment. It is evident that the amount of bacteria released is four orders of magnitude lower than that retained in the column. Furthermore, Figure 6 shows that the release of *E. moraviensis* decreases with each following rainfall event. During RAIN1, this also applies to the release of *E. coli*. Thirdly, release of *E. coli* is higher than that of *E. moraviensis*.

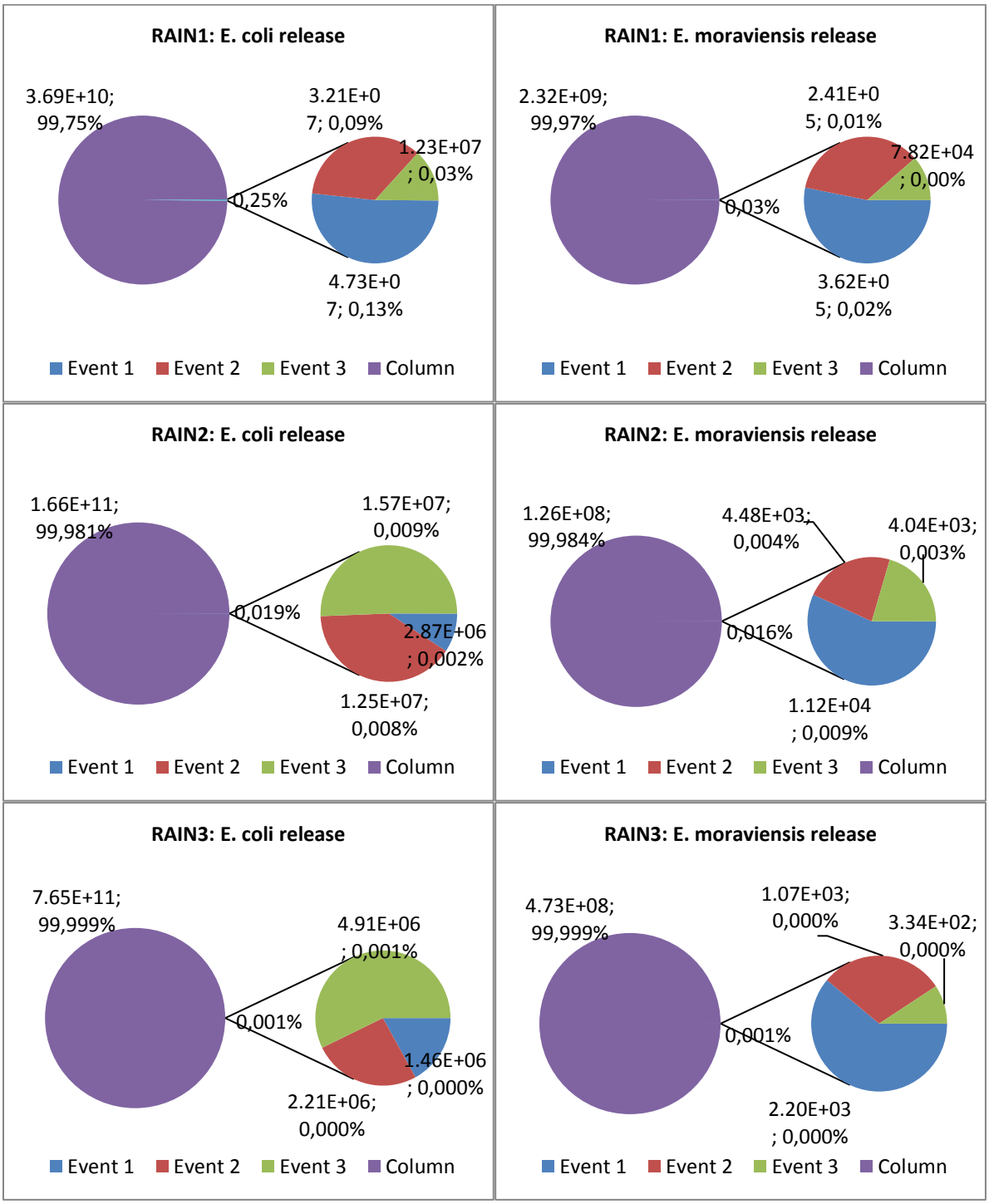


Figure 6: Absolute number of *E. coli* and *E. moraviensis* measured per event and in the sand column at the end of the experiment, as a percentage of the total bacteria measured in the sand and the effluents of the three events.

Table 6: Number of bacteria released during each event as a percentage of the initial number of bacteria retained in the column directly after inoculation. The log_{10} ratio (log_{10}(Nout/Ninit*100)) is given in parentheses. All values are corrected for microbial decay.

		Number of bacteria released in i_{th} event as a percentage (log10 difference) of initial CFU in column after inoculation			
		Event 1	Event 2	Event 3	Total bacteria release after 3 events
E. coli	RAIN1	**0.047** (-3.331)	**0.032** (-3.498)	**0.012** (-3.915)	**0.091** (-3.043)
	RAIN2	**0.003** (-4.543)	**0.012** (-3.905)	**0.016** (-3.804)	**0.031** (-3.508)
	RAIN3	**0.002** (-4.614)	**0.004** (-4.433)	**0.008** (-4.086)	**0.014** (-3.844)
E. moraviensis	RAIN1	**4.48E-03** (-4.349)	**2.98E-03** (-4.526)	**9.69E-04** (-5.014)	**8.43E-03** (-4.074)
	RAIN2	**1.19E-03** (-4.925)	**4.76E-04** (-5.322)	**4.29E-04** (-5.368)	**2.09E-03** (-4.679)
	RAIN3	**2.04E-04** (-5.690)	**9.93E-05** (-6.003)	**3.10E-05** (-6.509)	**3.35E-04** (-5.475)

The number of bacteria released with each rainfall event is expressed as a percentage of the initial number of bacteria retained in the sand column directly after inoculation (Table 6). Release of both *E. coli* and *E. moraviensis* is consistently higher during RAIN1 than the other rainfall experiments. Release of *E. moraviensis* is lower than that of *E. coli* in all rainfall experiments. The amount of *E. moraviensis* released decreases steadily with each successive event for all experiments. During RAIN1, this also holds for the amount of *E. coli* released. RAIN2 and RAIN3 show increasing amounts of *E. coli* released with each successive rainfall event.

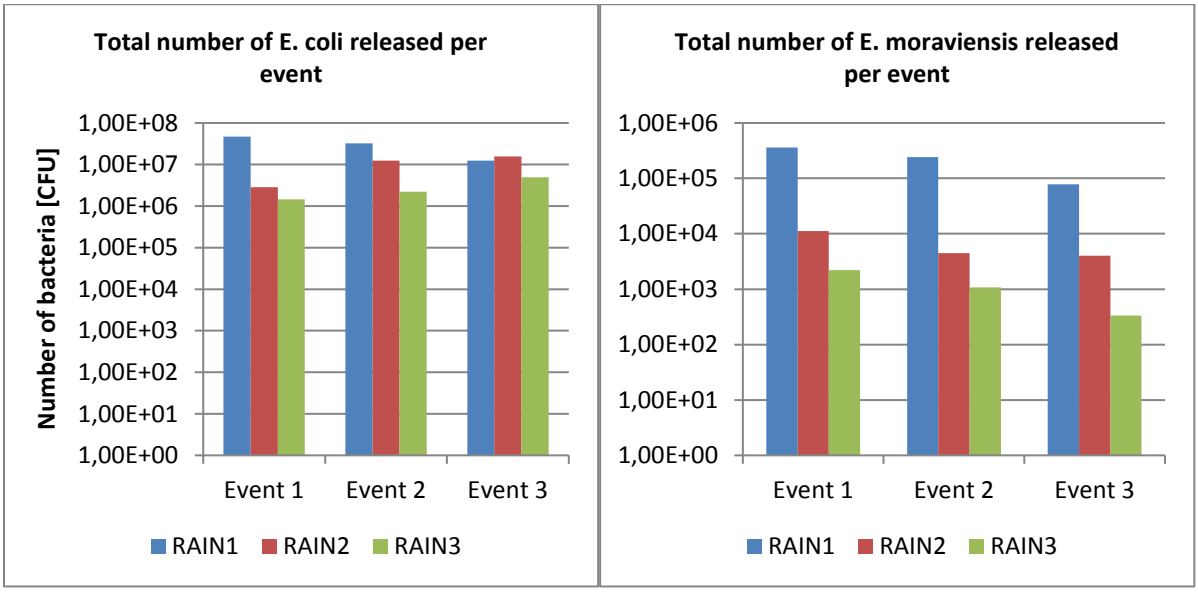


Figure 7: A) Total number of *E. coli* released during an event for the rainfall experiments; B) Total number of *E. moraviensis* released during an event for the rainfall experiments. Note the logarithmic scale on the vertical axis.

Figure 8, 9 and 10 show a clear correlation between the discharge of the sand columns in ml/hour and the concentration of both bacteria species in the effluent. The figures also show a slight delay between the peak discharge and the peak concentrations of *E. moraviensis* in the effluent, most evident in the first and second events. During rainfall events, the peak in effluent discharge occurs between 1.5 and 3 hours after the start of the rainfall (Figure 8, 9 and 10). This interval coincides with the highest concentration of *E. coli* per ml of effluent, resulting in the highest transport of absolute number of *E. coli* in that interval. For *E. moraviensis*, the highest concentrations in the effluent occur between 3 and 4.5 hours after the start of rainfall. This indicates that transport of *E. coli* through sand takes place earlier than that of *E. moraviensis*. This might indicate that different processes play a role in the transport of the different bacteria.

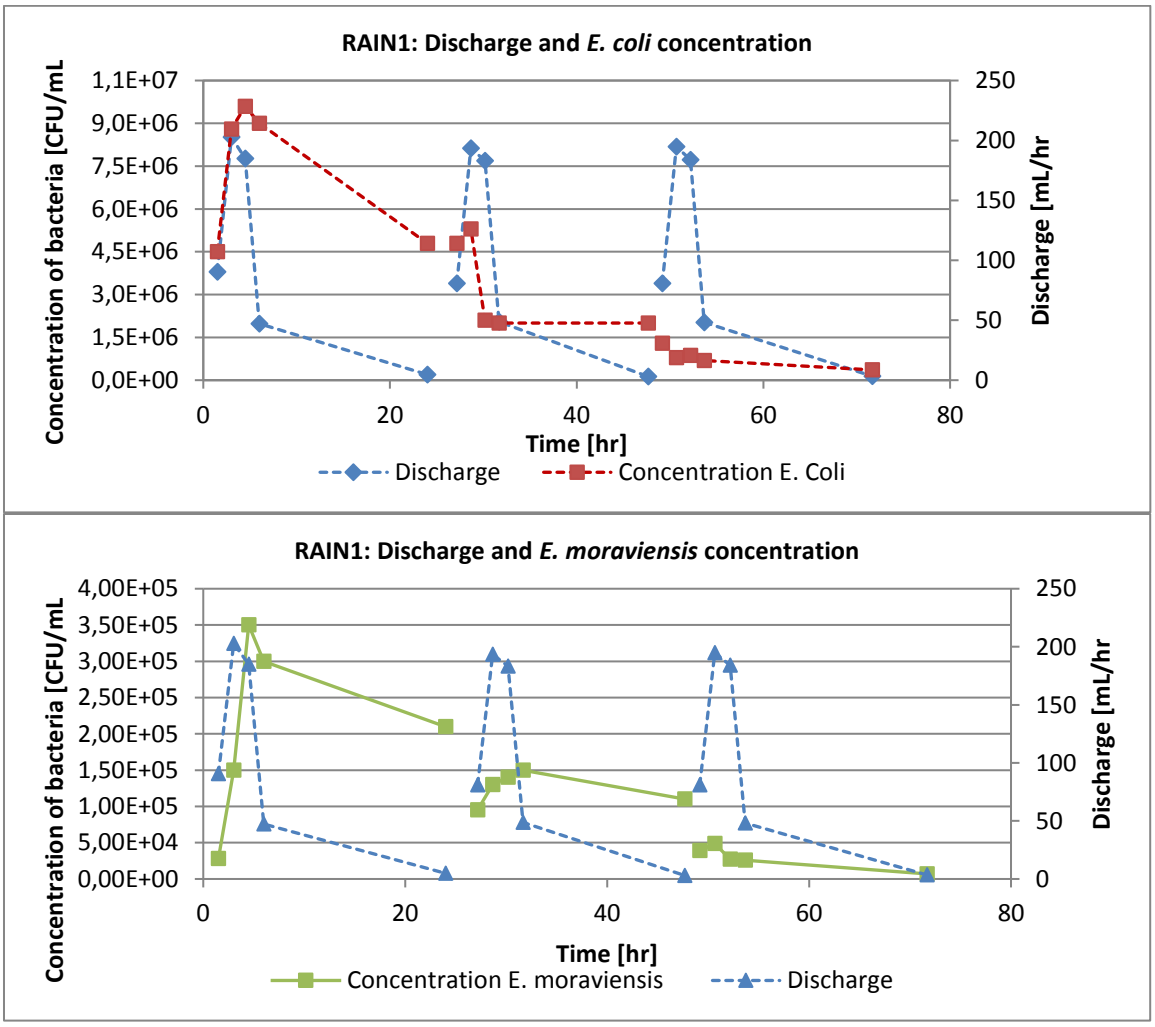


Figure 8: Discharge and concentrations of E. coli and E. moraviensis of Rain1.

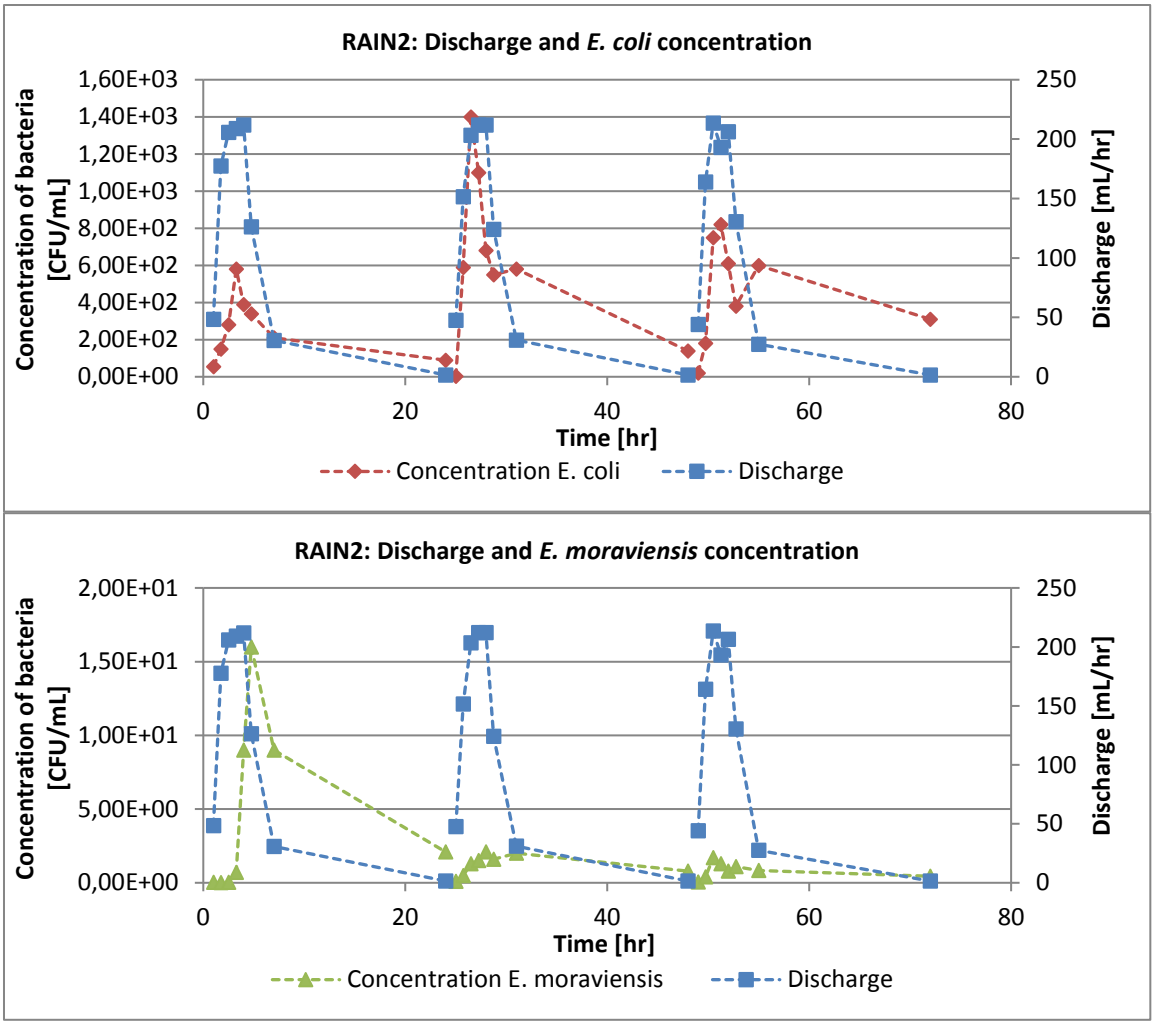


Figure 9: Discharge and concentrations of E. coli and E. moraviensis of the Rain2.

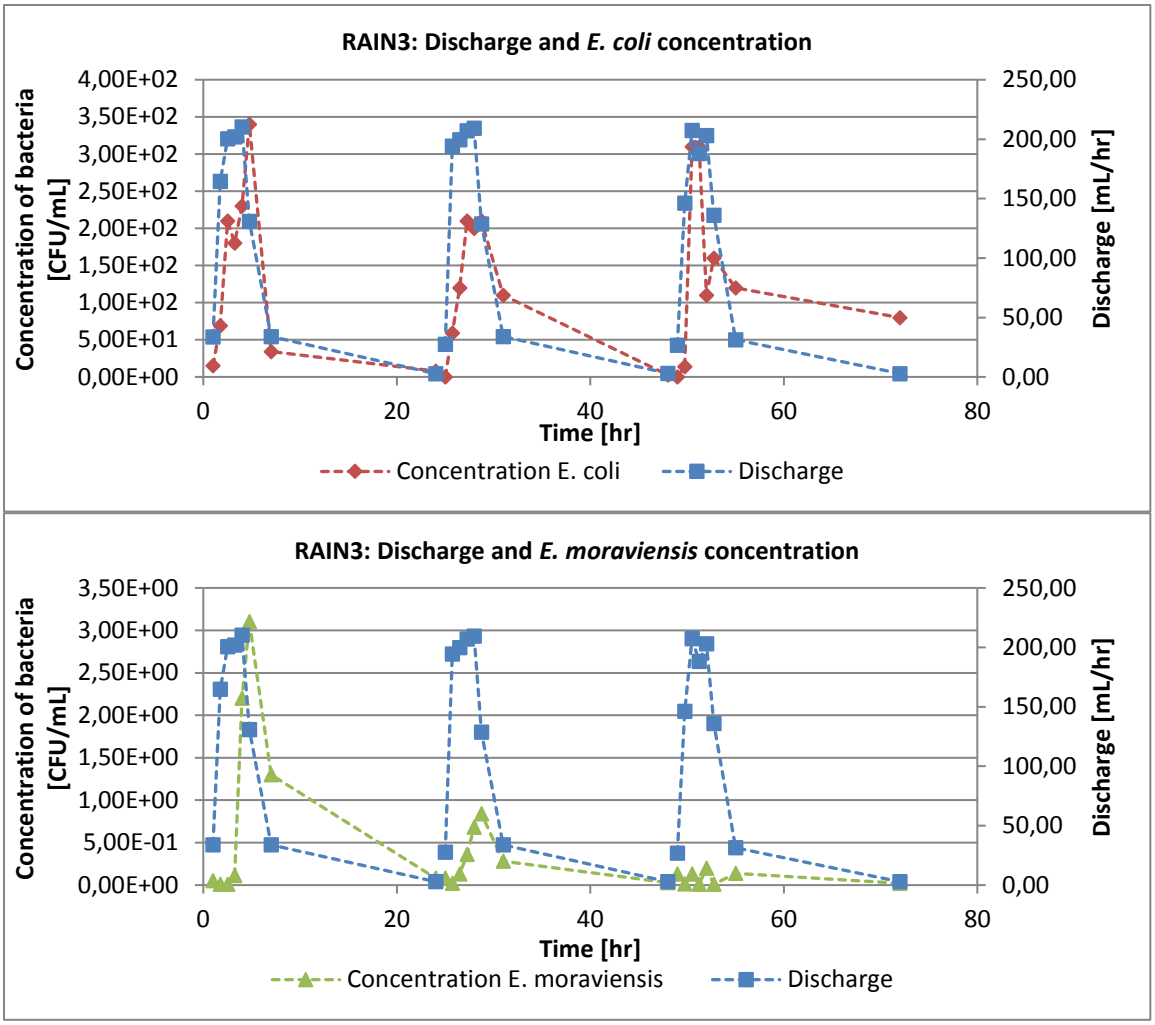


Figure 10: Discharge and concentrations of *E. coli* and *E. moraviensis* of the Rain3.

After three rainfall events, between 0.14% and 0.09% of the *E. coli* and 0.0003% to 0.0084% of the *E. moraviensis* retained in the sand after inoculation was released and flushed out of the sand column (Table 6). The remainder of the bacteria are retained in the sand, with the highest concentrations found at the surface of the column and the concentrations decreasing with depth (Figure 11). This is caused by the initial inoculation with bacteria, where more bacteria are retained in the upper regions of the sand column as the bacteria are applied through a simulated rainfall event on the sand surface. Furthermore, the water content gradient plays an important role, with the highest water contents found at the bottom of the column and lowest at the sand surface (Figure 4). The drier surface sand has a larger AWI and thinner water films surrounding the sand grains thereby retaining more bacteria than the wetter lower regions where the bacteria can be released into the aqueous phase and flushed out of the column.

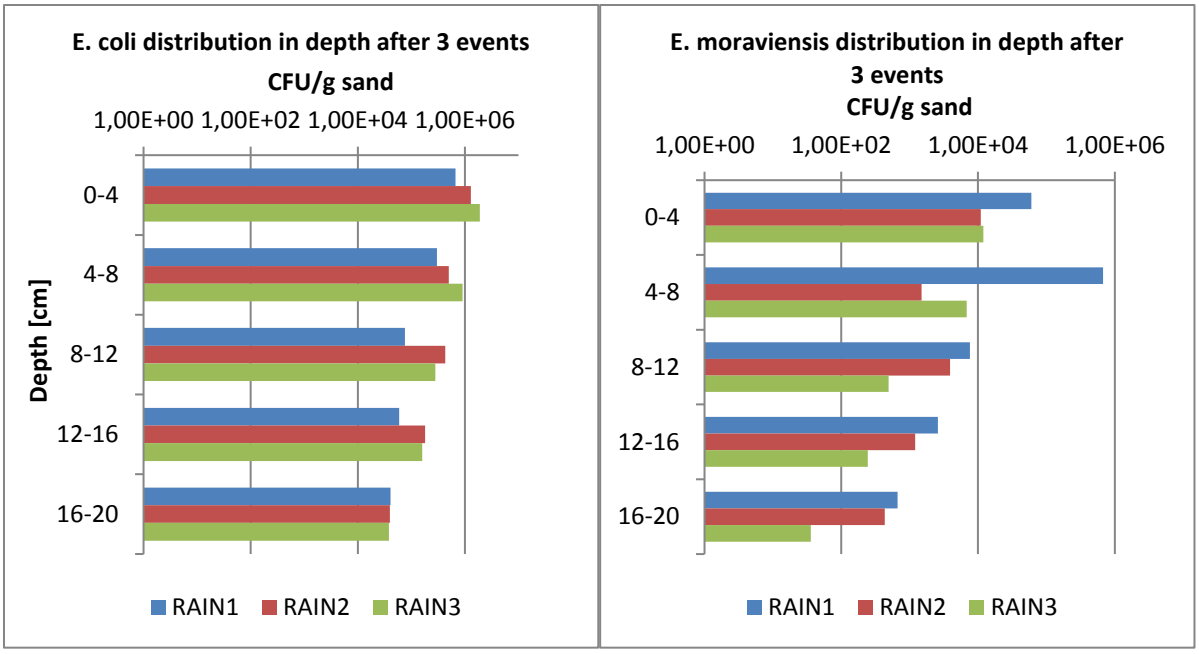


Figure 11: Concentration profile of *E. coli* (A) and *E. moraviensis* (B) in the sand column after 3 consecutive rainfall events. All concentrations in CFU/g dry sand.

Groundwater level experiments

Hydrological results

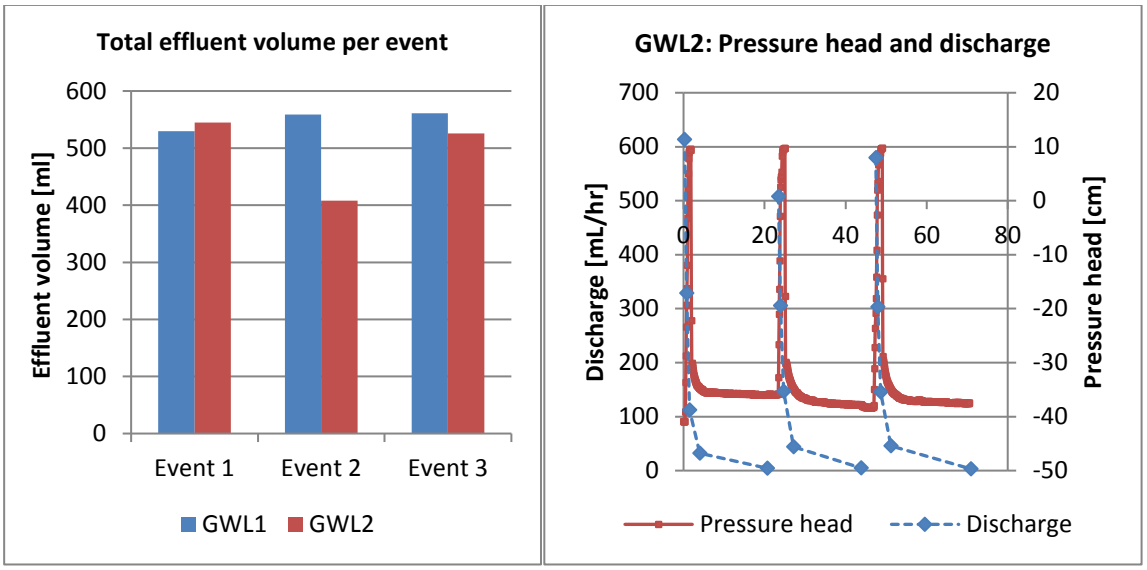


Figure 12: A) Total effluent volume per event for all groundwater level experiments; B) Discharge and pressure head of GWL2

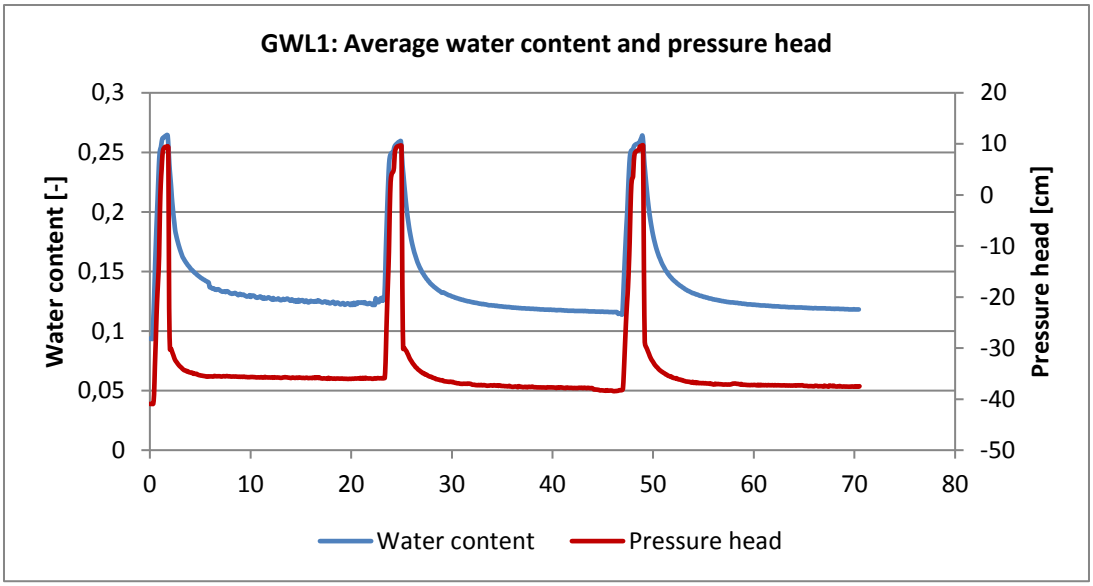


Figure 13: Pressure head and average water content measurements of GWL1

Figure 12A shows that the total effluent volume increases slightly with each event for GWL1. The total effluent of the second event of GWL2 is 140 ml less than the previous event which is expected to have an effect on the total and peak microbial release during the second and third event.

Tensiometer and water content data indicate that the sand columns do not reach complete saturation during cycles of groundwater rise and fall despite visible ponding on the sand surface. Hysteric effects combined with air entrapment result in a lower effective maximum water content of approximately 0.32 as opposed to the expected Θs of 0.44. Furthermore, the time spent at raised groundwater level was not sufficient to allow equilibration and thus complete saturation. The hysteric effects also prevent the sand from draining fully when the groundwater level is lowered, likely combined with gas clogging of the membrane underneath the sand column. Air bubbles were observed beneath the membrane, giving reason to believe that the discharge of effluent could have been deterred by the pressure of the air bubbles on the membrane. The air bubbles most likely originated from degassing of the water in the sand column as a result of constant high suction on the bottom of the column. Furthermore, suspected algal growth could be observed on the filter cloth after each experiment, possibly causing additional fouling and clogging.

Microbial transport

Figure 14 expresses the ratio between the number of *E. coli* and *E. moraviensis* released per event, and that retained in the sand column at the end of each experiment. Similar to the rainfall experiments, the amount of *E. coli* released is 3 to 4 orders of magnitude lower than that retained in the column (Table 7). For *E. moraviensis*, the difference between bacteria released and that in the column is 5 orders of magnitude. Furthermore, Figure 14 shows that the number of bacteria released during GWL1 decreases with each following groundwater level event. This is not the case for GWL2, which can be explained by the low effluent volume during the second event. Thirdly, release of *E. coli* is much higher than that of *E. moraviensis*.

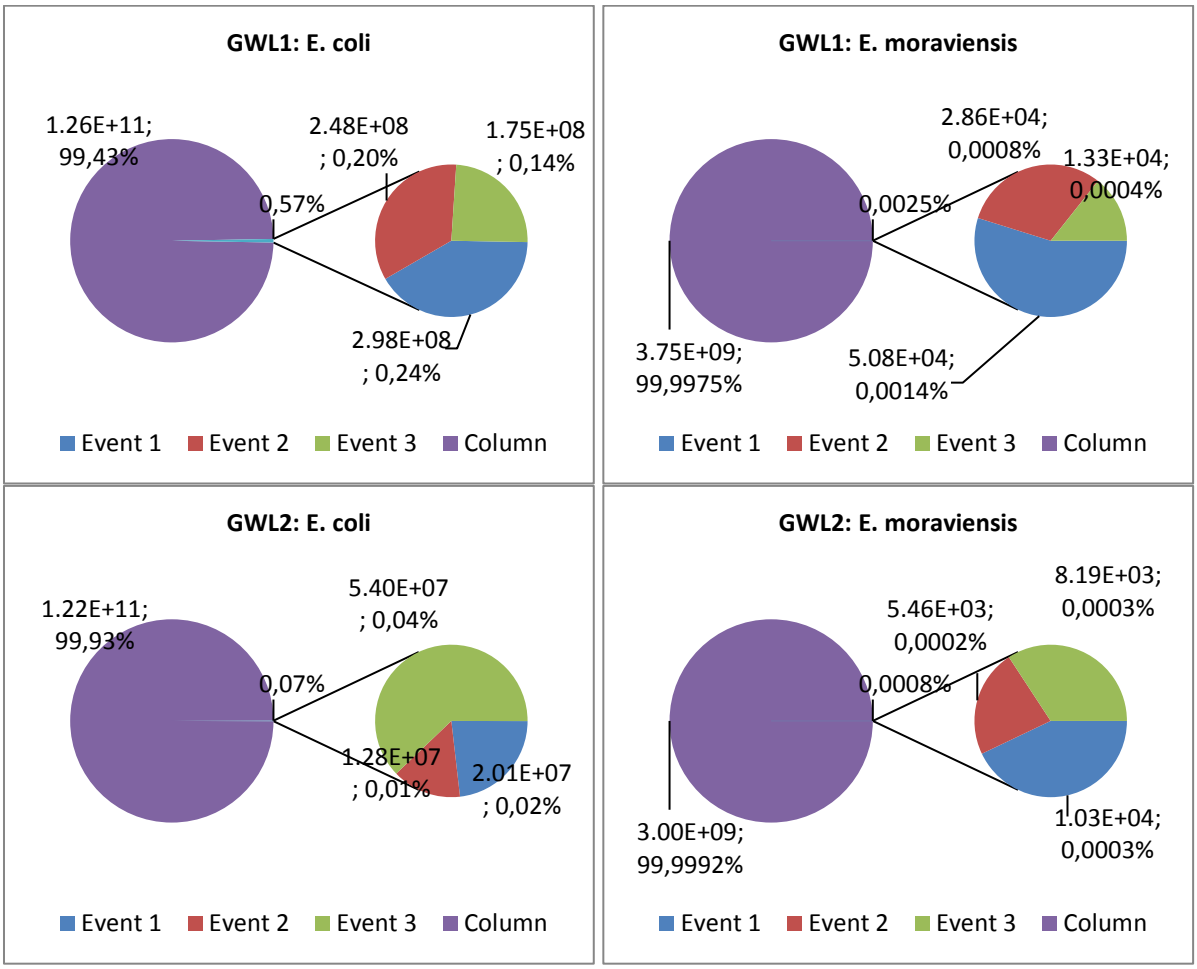


Figure 14: Absolute number of *E. coli* and *E. moraviensis* measured per event, and in the sand column at the end of the experiment, as a percentage of the total bacteria measured in the sand and the effluents of the three events.

Table 7: Number of bacteria released during each event as a percentage of the initial number of bacteria retained in the column directly after inoculation. The log_{10} ratio (log_{10}(Nout/Ninit*100)) is given in parentheses. All values are corrected for microbial decay.

		Number of bacteria released in i_{th} event as a **percentage** (log10 difference) of initial CFU in column after inoculation			
		Event 1	Event 2	Event 3	Total bacteria release after 3 events
E. coli	GWL1	**0.458** (-2.339)	**0.381** (-2.420)	**0.268** (-2.571)	**1.107** (-1.956)
	GWL2	**0.031** (-3.511)	**0.020** (-3.708)	**0.083** (-3.082)	**0.133** (-2.875)
E. moraviensis	GWL1	**7.16E-04** (-5.145)	**4.03E-04** (-5.395)	**1.87E-04** (-5.728)	**1.31E-03** (-4.884)
	GWL2	**1.45E-04** (-5.840)	**7.70E-05** (-6.114)	**1.15E-04** (-5.938)	**3.37E-04** (-5.472)

Release of both *E. coli* and *E. moraviensis* is consistently higher during GWL1 than GWL2 (Table 7). Release of *E. moraviensis* is lower than that of *E. coli* in both experiments. The amount of *E. coli* and *E. moraviensis* released decreases steadily with each successive event for GWL1. During GWL2, the amount of both bacteria released is lowest in the second event and increases again during the last event.

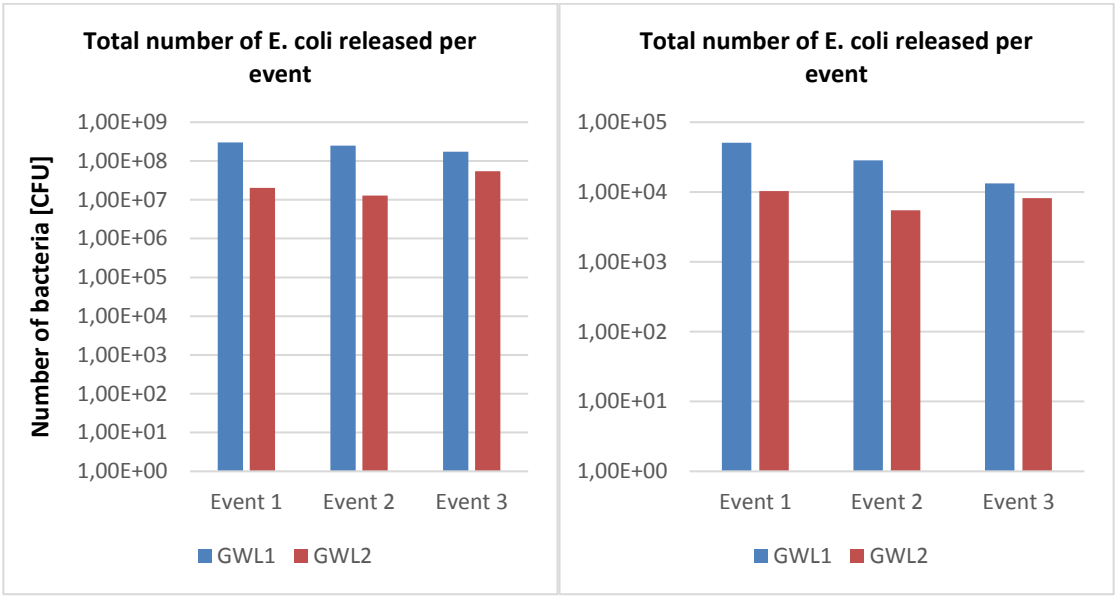
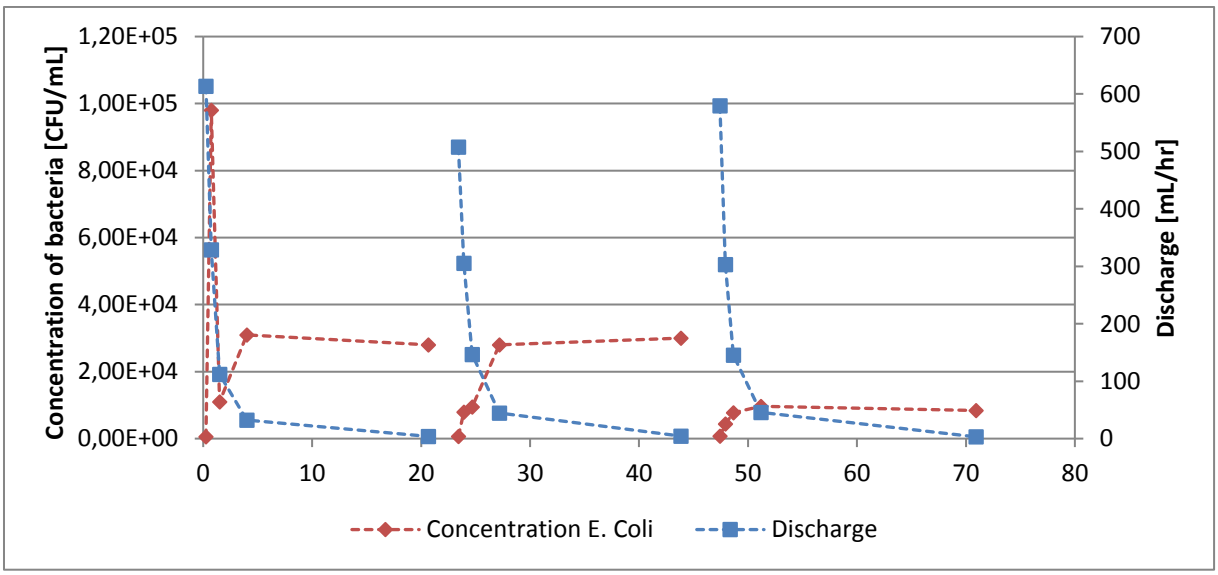


Figure 15: A) Total number of *E. coli* released during an event for the groundwater level experiments; B) Total number of *E. moraviensis* released during an event for the groundwater level experiments. Note the logarithmic scale on the vertical axis.

The release of *E. coli* increases during the course of each drainage cycle after initial groundwater rise, with the highest concentrations generally found in the tail of the groundwater level experiment (Figure 16). This is in contrast to the release profile observed in the rainfall experiments. The *E. moraviensis* release profile is similar to that observed in the rainfall experiments, with the highest amounts released in the first effluent fractions and concentrations decreasing afterwards. Figure 17 shows that the peaks in *E. coli* concentration in the effluent coincide with the minima in soil water content, whereas for *E. moraviensis*, the inverse occurs. This indicates that the lower sections of the column are relatively depleted in *E. coli* at the beginning of drainage, causing the first effluent fractions to contain low concentrations. This does not apply to the *Enterococci* distribution.



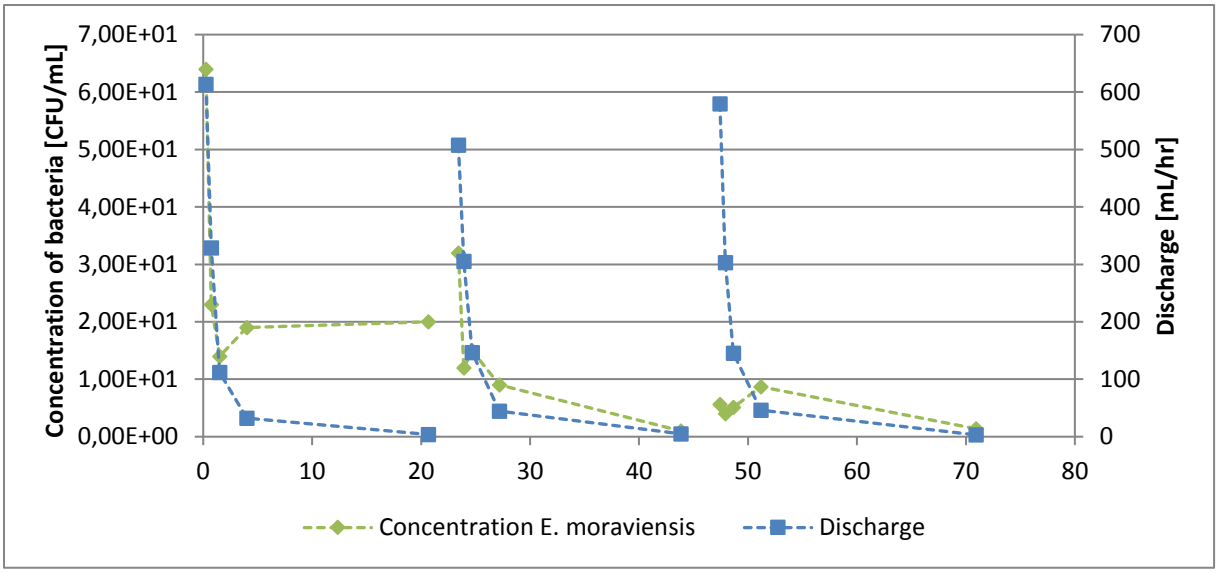


Figure 16: Concentration [CFU/ml] of *E. coli* (A) and *E. moraviensis* (B) plotted with the discharge [ml/hr].

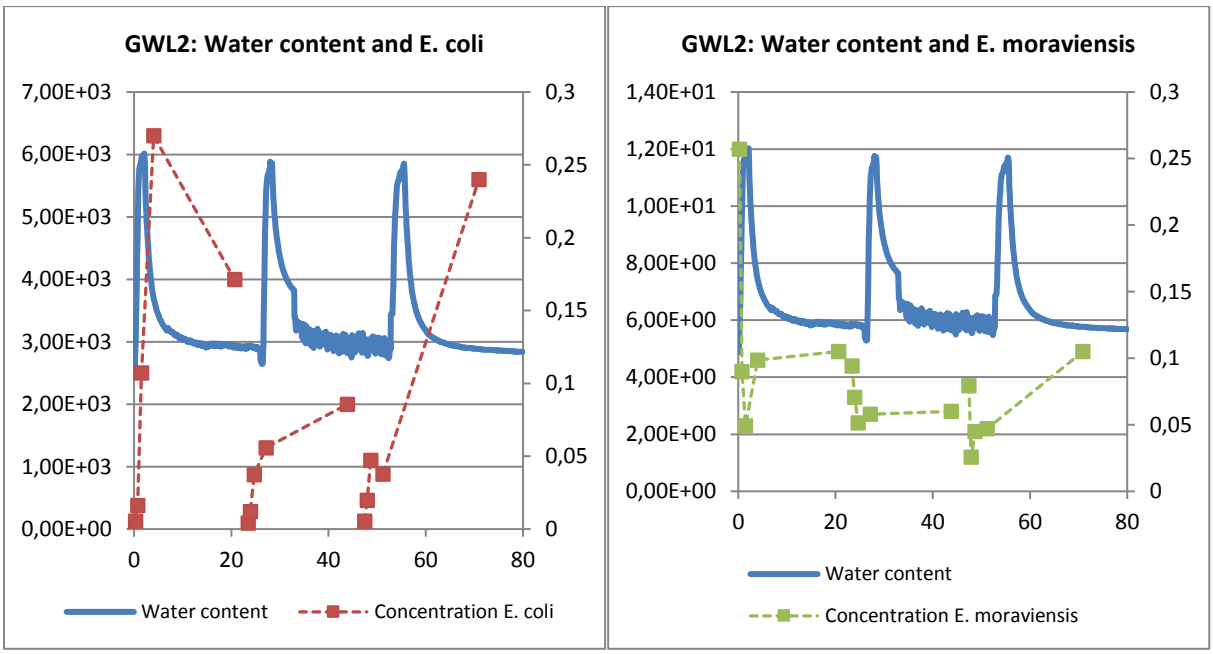


Figure 17: Water content and *E. coli* (A) and *E. moraviensis* (B) concentrations during GWL2.

The bacterial concentration retained in the sand column after three cycles of groundwater level fluctuation also shows a contrast between *E. coli* and *E. moraviensis* (Figure 18). The *Enterococci* have a distribution similar to that observed in the rainfall experiments, with the highest amounts found in the surface layer and concentrations rapidly decreasing towards the bottom. The *E. coli* concentrations are highest at a depth of 6.8 to 10.2 cm below the sand surface. Above that, the concentrations are highest at the surface and decrease towards the 6.8 cm mark. Note that a logarithmic scale is used for the *Enterococci* in order to display the very low values measured in the lower parts of the sand column.

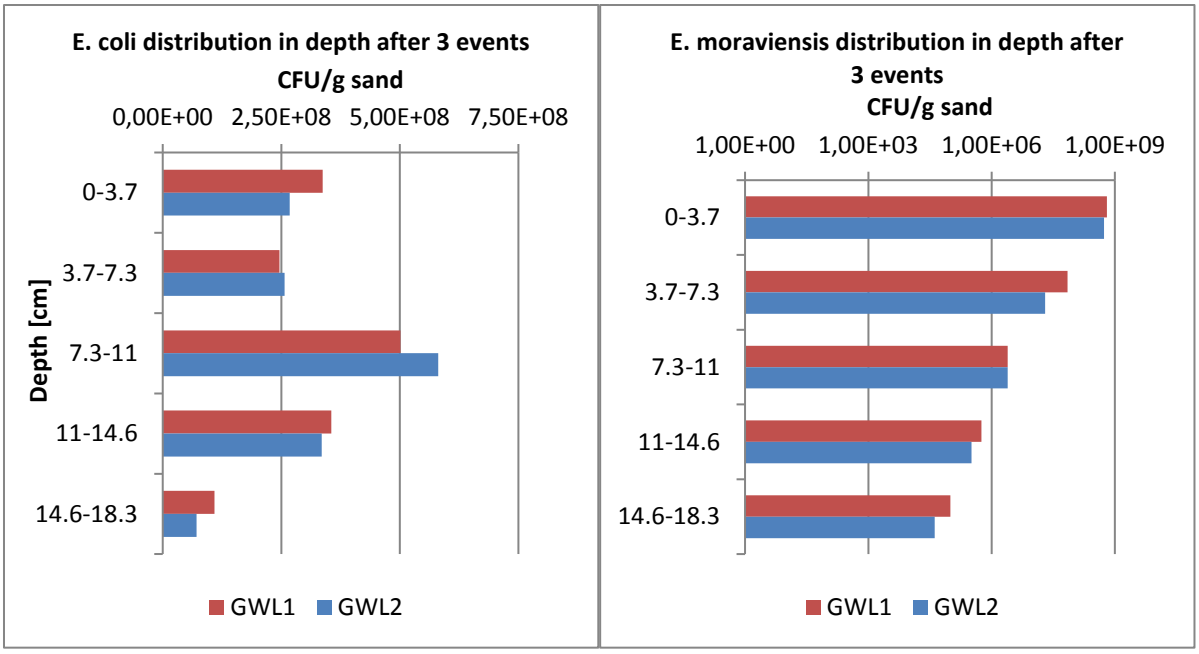


Figure 18: Concentration profile of *E. coli* (A) and *E. moraviensis* (B) in the sand column after 3 consecutive groundwater level variation events. All concentrations in CFU/g dry sand. Note the logarithmic scale used for *E. moraviensis* concentrations.

Modeling results

The one-site and two-site kinetic models used both adequately describe the trends of the experimental data of the rainfall experiments in most situations (Figure 19 to 24). The model with a better AIC-score is indicated in the figures with an asterisk. The initial peak in concentration as a result of the onset of the first rainfall event is expressed by the models for all scenarios. For the *E. coli* breakthrough curve of RAIN1, the two-site model maintains a relatively high concentration after the first peak. There is no second peak at the onset of the second rainfall event, but rather a decrease in concentration after the event has passed. Both the one- and two-site models of the *Enterococci* release of RAIN2 introduce a sinusoidal at the onset of the second rainfall event (Figure 22). This oscillating front is thought to be caused by numerical dispersion in the results of the fitting function. The difference between the one- and two-site models for the release of *E. moraviensis* is the timing and height of the peak concentrations as well as the onset of release. The peak concentrations are much higher for the two-site model and the onset and peak occur later than with the one-site model, resulting in a much better fit of the experimental data.

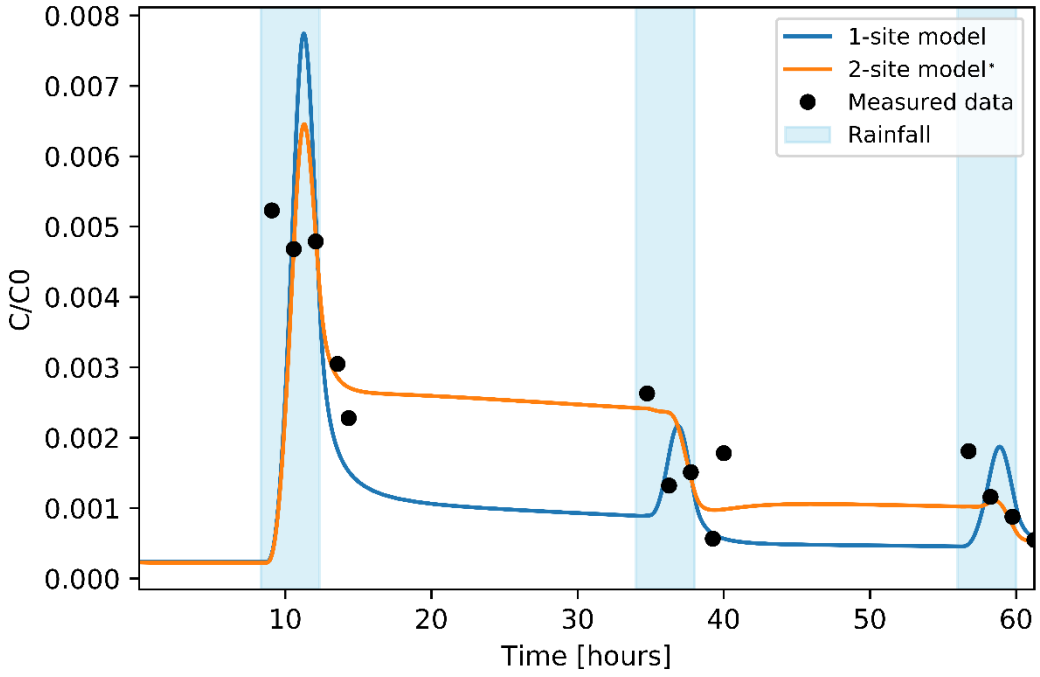


Figure 19: Fitted 1- and 2-site model of *E. coli* during RAIN1

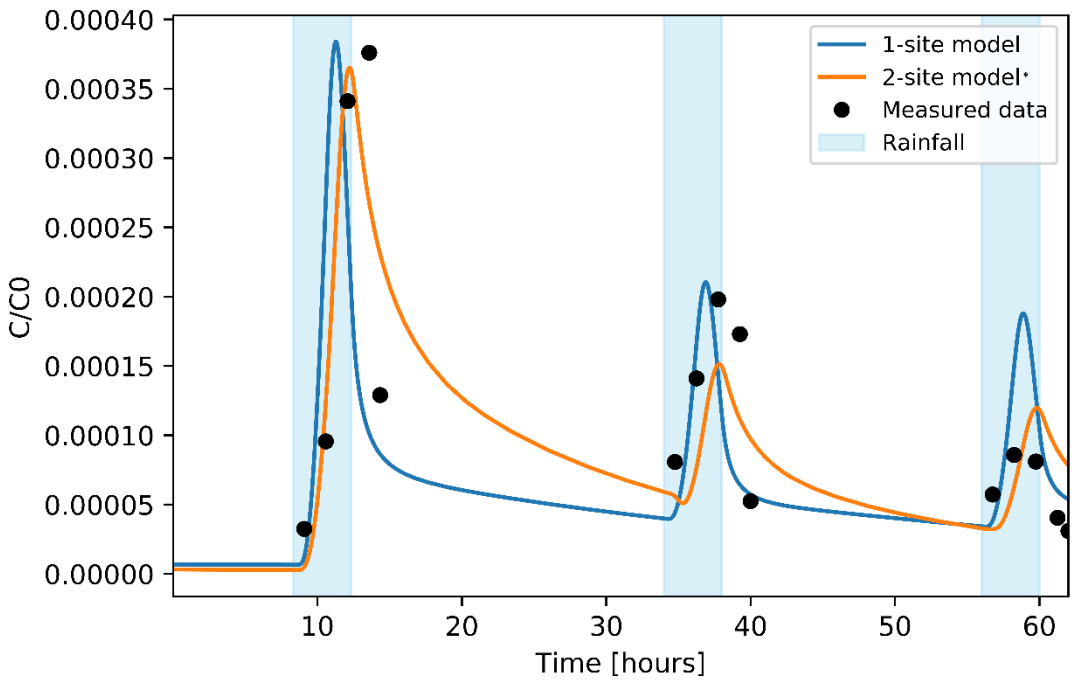


Figure 20: Fitted 1- and 2-site model of *E. moraviensis* during RAIN1

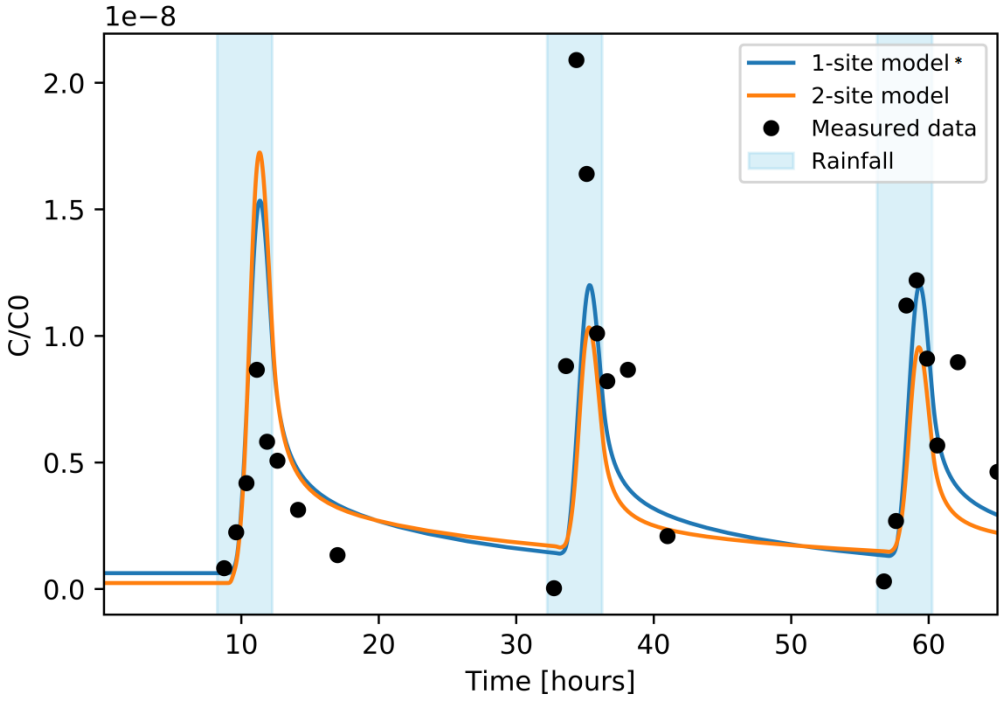


Figure 21: Fitted 1- and 2-site model of *E. coli* during RAIN2

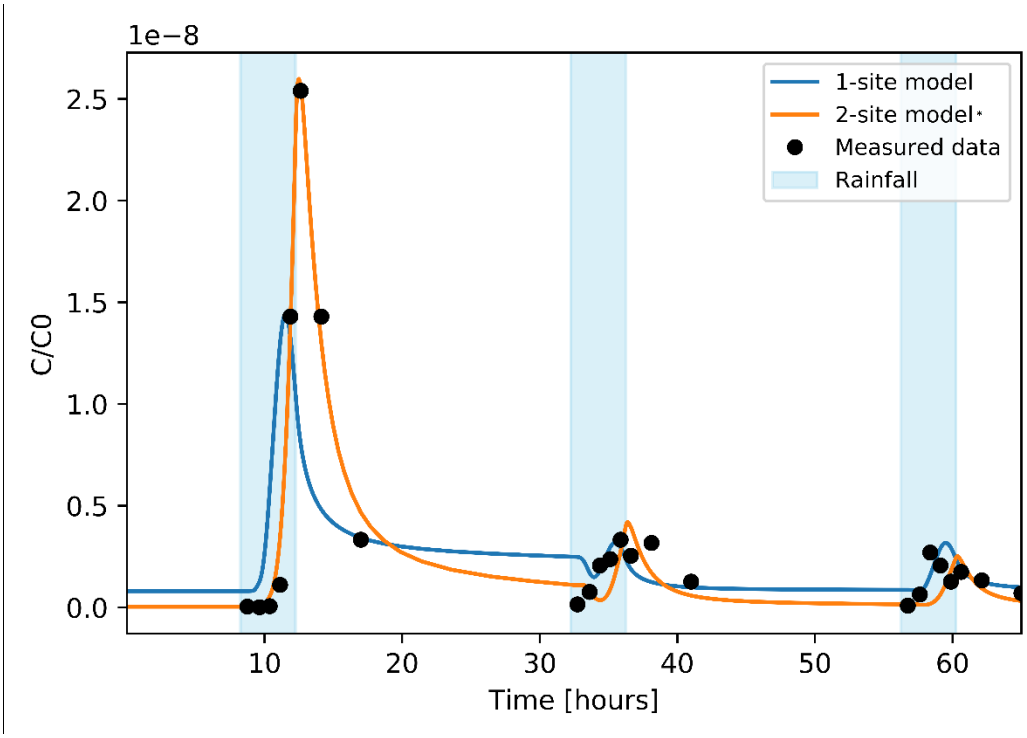


Figure 22: Fitted 1- and 2-site model of *E. moraviensis* during RAIN2

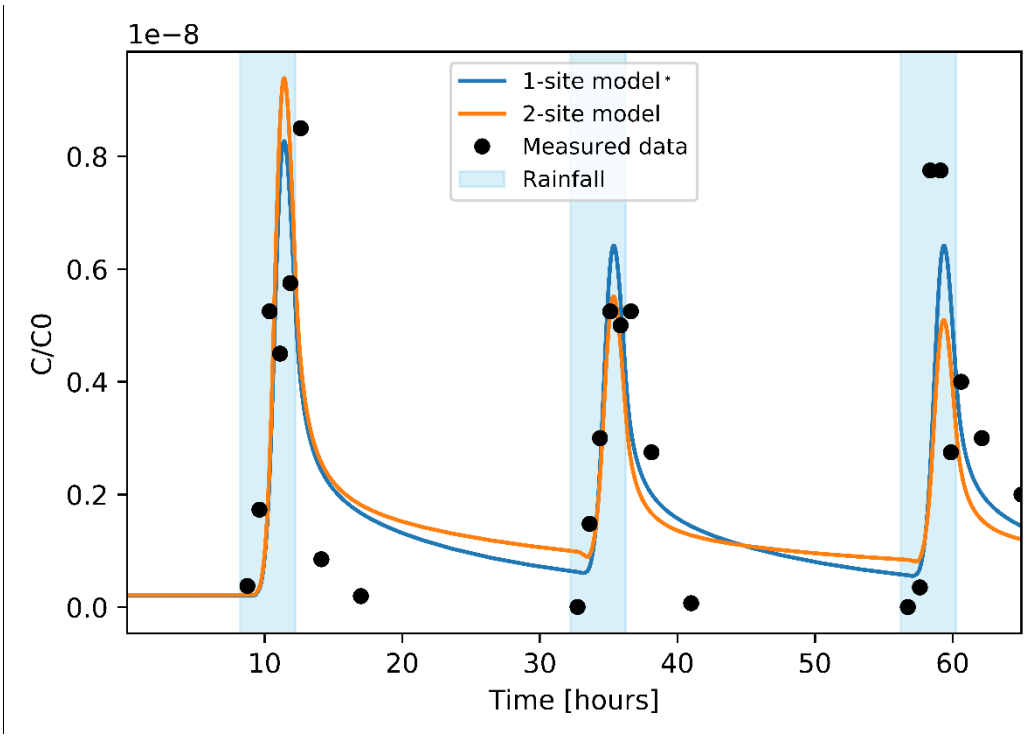


Figure 23: Fitted 1- and 2-site model of *E. coli* during RAIN3

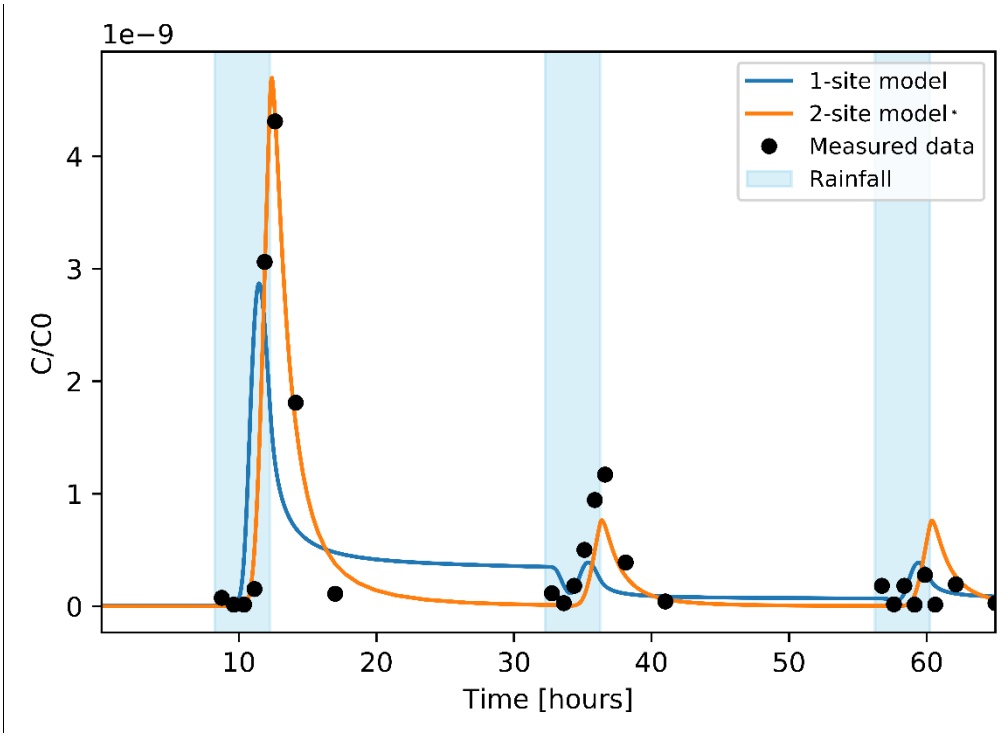


Figure 24: Fitted 1- and 2-site model of *E. moraviensis* during RAIN3

When comparing the Akaike information criterion of the one-site versus the two-site models, the one-site models for E.*coli* transport generally have a better score, indicating that one kinetic site adequately describes the attachment and detachment processes occurring between *E. coli* and the sand (Table 8). The two-site models for *E. moraviensis* transport generally score higher than the one-site models. The R^2 and AIC values of the *Enterococci* two-site models for the rainfall experiments indicate exceptionally good fits of the experimental data.

Table 8: Summary of the inverse modeling results of the rainfall experiments. Model yielding the best fit and AIC score is indicated with an asterisk (*).

Exp run	Model	Bacteria	Fitted: 2-site attach-detach parameters				Quality of fit	
			k_att1	k_det1	k_att2	k_det2	R2	AIC
RAIN1	1-site	*E. coli*	2.71E-02	7.42E-06			0.31	-3.5E+01
RAIN2*	1-site	*E. coli*	7.05E-02	9.22E-11			0.30	-7.8E+01
RAIN3*	1-site	*E. coli*	7.28E-02	2.10E-11			0.45	-8.5E+01
RAIN1*	2-site	*E. coli*	1.47E-05	3.97E-09	3.90E-01	4.22E-01	0.86	-6.0E+01
RAIN2	2-site	*E. coli*	4.51E-02	5.38E-03	9.66E-04	7.85E-13	0.18	-6.7E+01
RAIN3	2-site	*E. coli*	4.52E-02	5.40E-03	9.66E-04	3.00E-13	0.38	-7.7E+01
RAIN1	1-site	*E. morav.*	2.73E-02	7.47E-07			0.27	-3.8E+01
RAIN2	1-site	*E. morav.*	1.14E-02	1.62E-10			0.30	-8.1E+01
RAIN3	1-site	*E. morav.*	6.80E-03	1.67E-11			0.32	-8.1E+01
RAIN1*	2-site	*E. morav.*	3.71E+01	9.37E+01	4.08E-02	7.75E-07	0.82	-5.8E+01
RAIN2*	2-site	*E. morav.*	1.71E+03	1.26E+03	1.25E-01	7.62E-11	0.97	-1.5E+02
RAIN3*	2-site	*E. morav.*	8.91E+02	1.37E+03	2.88E-01	1.05E-11	0.94	-1.4E+02

The one- and two-site kinetic models generally yield poor fits to the groundwater level experimental data. The release of *E. coli* during GWL2 (Figure 27) is well described by both the one- and two-site models where the rest of the experimental data could not be properly fitted. The quick changes in groundwater level at the onset of the groundwater rise results in a sharp drop and immediate rise in concentrations at the start of each event. Notably, the two-site models yield the same results as the one-site models and show no improvement of the fit as compared to the one-site models.

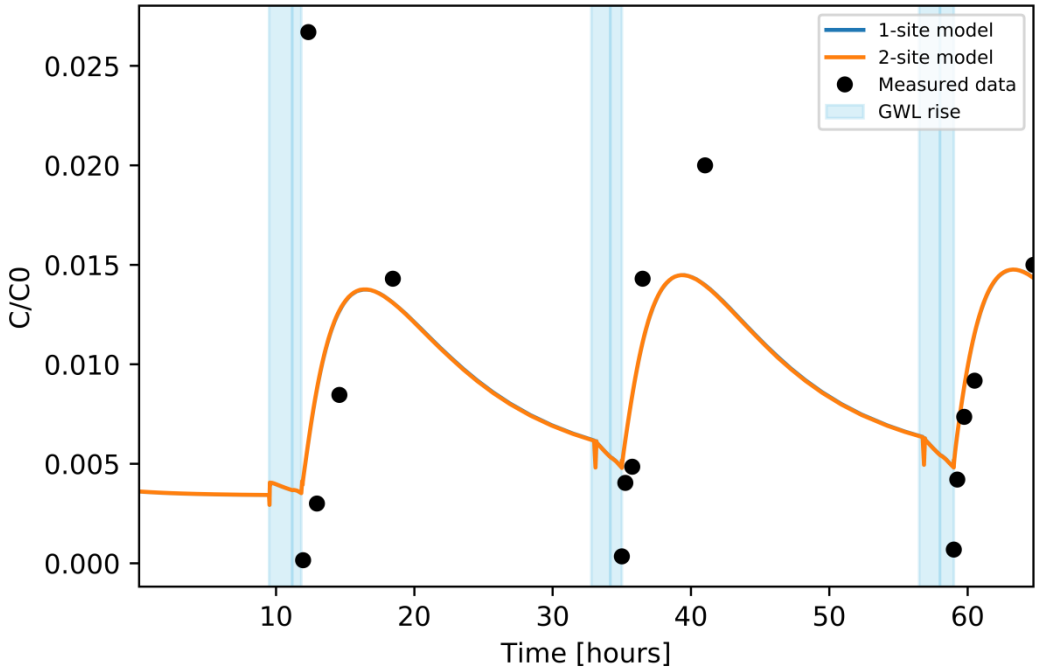


Figure 25: Fitted 1- and 2-site model of *E. coli* during GWL1

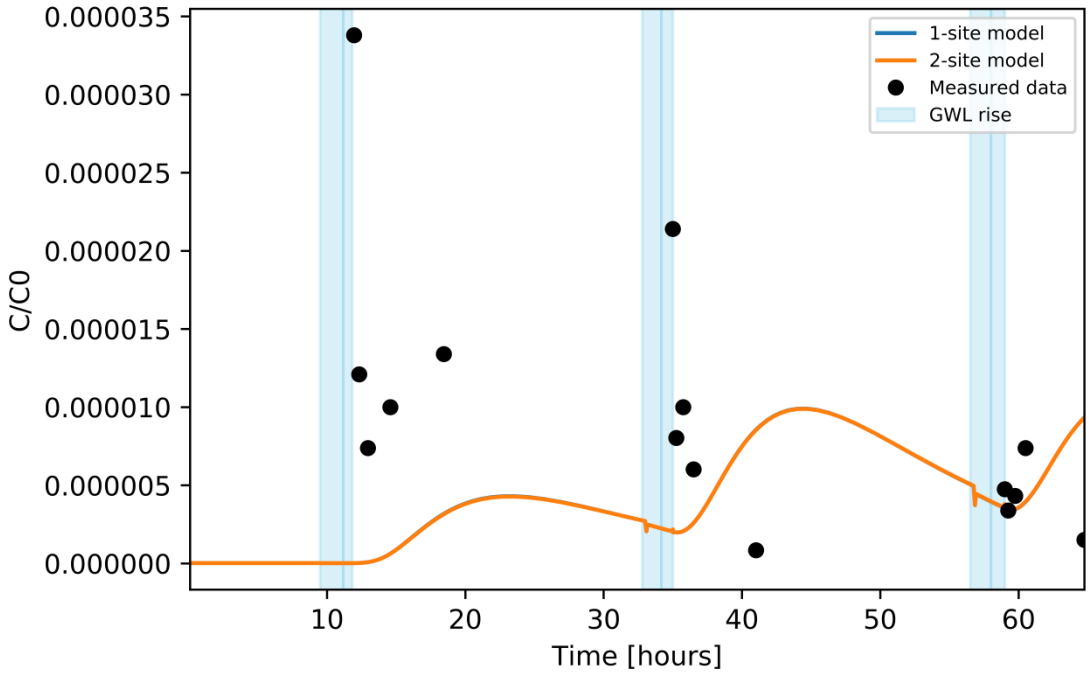


Figure 26: Fitted 1- and 2-site model of *E. moraviensis* during GWL1

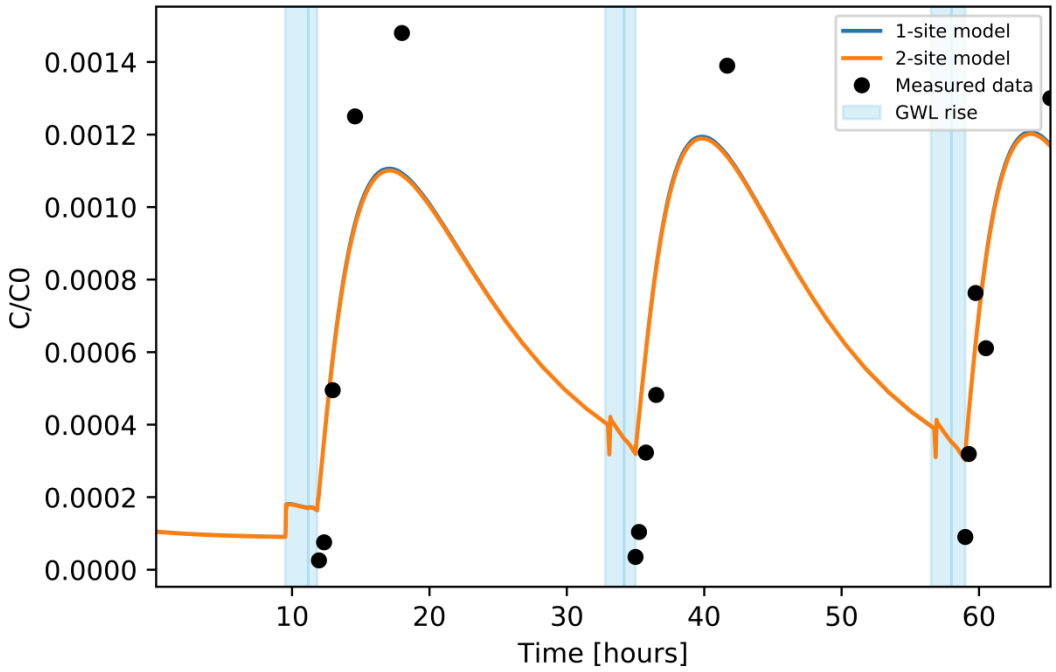


Figure 27: Fitted 1- and 2-site model of *E. coli* during GWL2

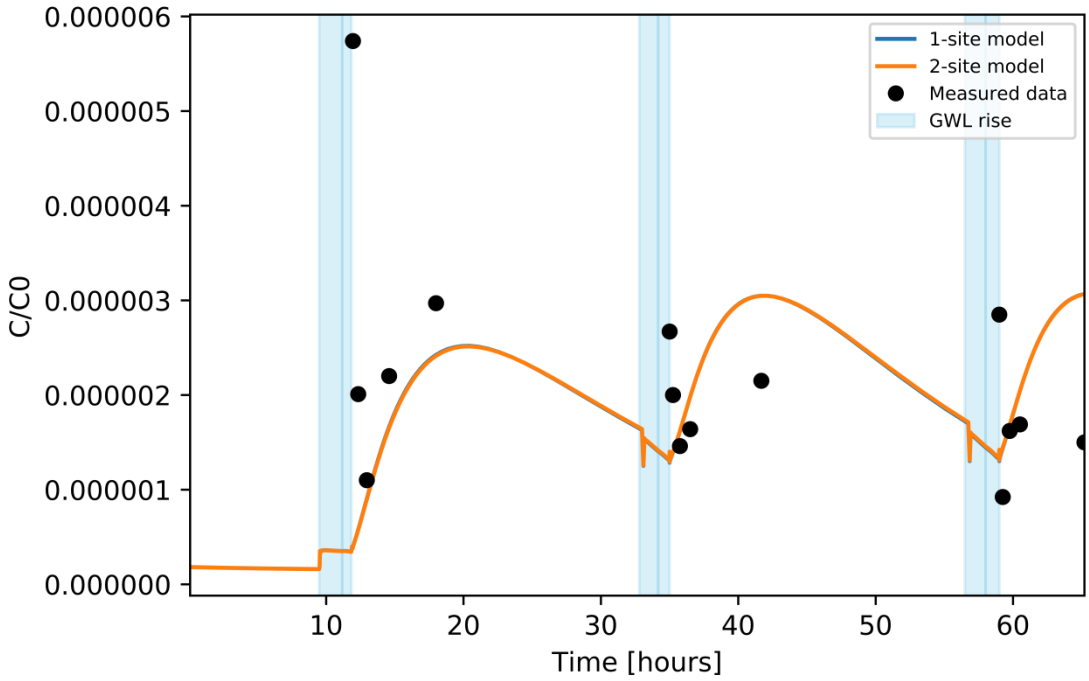


Figure 28: Fitted 1- and 2-site model of *E. moraviensis* during GWL2

Table 9: Summary of the inverse modeling results of the groundwater level experiments. Model yielding the best fit and AIC score is indicated with an asterisk (*).

Exp run	Model	Bacteria	Fitted: 2-site attach-detach parameters				Quality of fit	
			k_att1	k_det1	k_att2	k_det2	R2	AIC
GWL1*	1-site	*E. coli*	2.90E-01	2.24E-03			0.29	-4.2E+01
GWL2*	1-site	*E. coli*	2.27E-01	1.59E-04			0.89	-5.8E+01
GWL1	2-site	*E. coli*	2.93E-01	2.26E-03	2.80E-10	1.25E-08	0.29	-3.8E+01
GWL2	2-site	*E. coli*	2.26E-01	1.89E-04	1.50E-05	6.33E-08	0.89	-5.4E+01
GWL1*	1-site	*E. morav.*	1.10E-01	2.60E-05			0.34	-2.5E+01
GWL2*	1-site	*E. morav.*	1.08E-01	2.24E-07			0.11	-2.6E+01
GWL1	2-site	*E. morav.*	1.10E-01	2.64E-05	5.51E-07	4.16E-09	0.34	-2.1E+01
GWL2	2-site	*E. morav.*	1.07E-01	2.23E-06	1.44E-05	3.29E-11	0.11	-2.2E+01

Within experimental scenarios, there is generally a good match of the fitted attachment and detachment parameters of *E. coli* and *E. moraviensis* between the different runs. As an example, the experimental data of E. moraviensis concentrations of RAIN2 and RAIN3 were combined and fitted using both a 1- and 2-site model (Figure 29) which results in a reasonably good fit. The fitted parameters for *E. coli* also show a higher transferability between experiments than *E. moraviensis*. The two-site attachment and detachment coefficients obtained from inverse modeling of *E. coli* release in RAIN2 were used to directly model release of *E. coli* during RAIN3. Figure 30 shows the result of this direct modeling as well as the inverse modeling results of RAIN3.

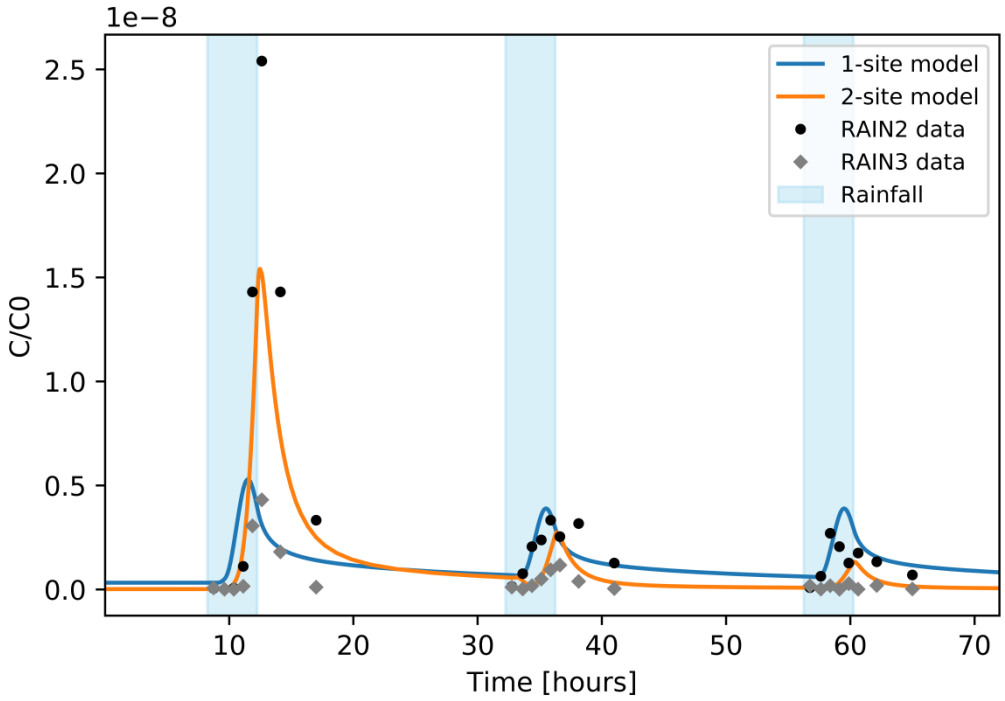


Figure 29: 1- and 2-site models fitted to combined RAIN2 and RAIN3 *E. moraviensis* release data. R^2 values are 0.10 and 0.57 for 1- and 2- site models respectively.

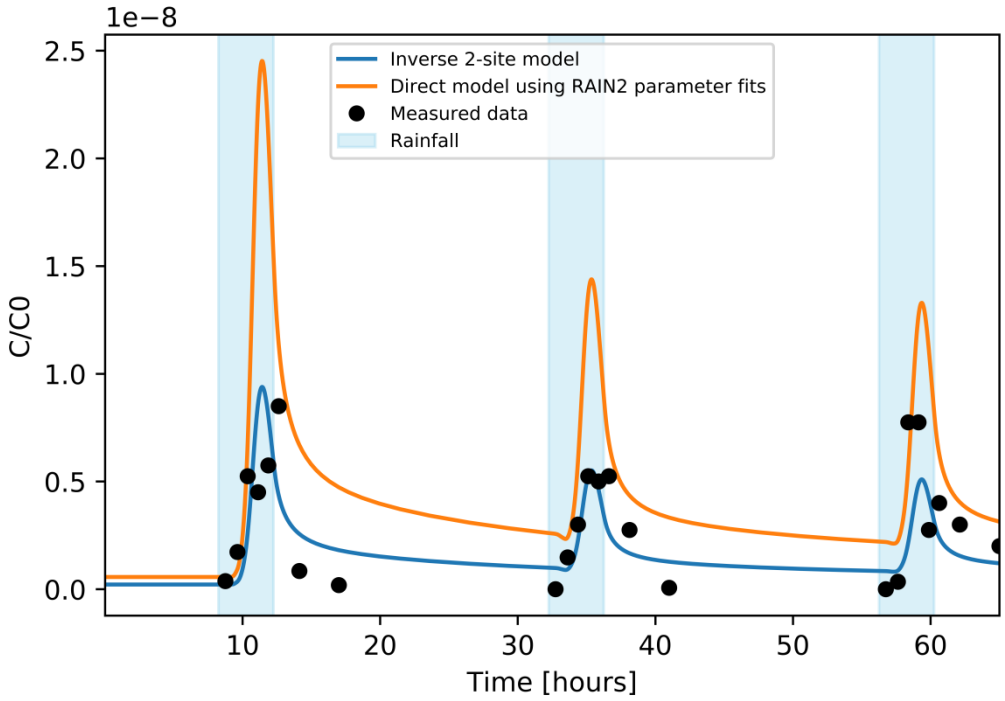


Figure 30: RAIN3 *E. coli* release measurements with inverse 2-site model (blue line) and direct 2-site model (orange line) using parameter fits of RAIN2 inverse modeling (R^2 both = 0.38).

Discussion

Experimental setup

The hydrological and bacterial results of the duplicate experiments indicate that the experimental setup was suitable for measuring the transport processes that are the focus of this study. However, uniform packing in depth within a single column and identical packing over the different columns proved difficult to achieve, with small variations in bulk densities as a result. Furthermore, the hanging water column used in the groundwater level experiments introduces a small dilution effect due to post-column mixing. Finally, it is possible that the inserted tensiometers might influence the flow of water in the column.

Hydrology

Air entrapment and hysteresis

Comparison of the water content and tensiometer data of both the rainfall and groundwater level experiments show that the measured water contents and corresponding pressure heads do not fit along the pF curve fitted with RetC. During both imbibition and drainage, the water content does not reach its maximum (0.44) or minimum values (0.05), respectively. Thirdly, during GWL1, ponding on the sand surface was visible, indicating maximum saturation of the sand column. The measured average saturation at this point was 0.32. These observations point to hysteresis in the soil hydraulic properties during all experiments, as well as the presence of entrapped air during the groundwater level experiments. This results in a decrease in the effective water content at the measured pressure heads as compared to the water retention curve of Figure 1.

Hysteresis of soil hydraulic characteristics is known to be of great influence on water flow and solute transport in unsaturated soils (Abdelkabir et al., 2004, Gillham et al., 1979, Vereecken et al., 1995). Transitions in soil texture as a result of layering in the soil can cause hysteresis in soil characteristics. As the columns in this study are packed by hand in a layer-wise manner, it is quite plausible that such layering was introduced in the soil profile despite precautions being taken to prevent this. The difference between the effective water content and the theoretical or expected water content is largest in the groundwater level experiment due to hysteresis being particularly relevant in situations involving reversal of flow (Aziz and Settari, 1979), coupled with the high level of saturation being reached. Hysteresis and entrapped air were taken into account during modeling by applying the hysteresis option in HYDRUS. The hysteresis model of Lenhard et al. (1991) and Lenhard and Parker (1992) is used that incorporates hysteresis without pumping in order to simulate air entrapment, while keeping the hysteresis loops in physically realistic parts of the retention function.

Air entrapment (Peck, 1965; Wang et al., 1998) and variations in soil properties between soil layers (Ma et al., 2009) can cause flow of water and solutes along preferential pathways, commonly referred to as fingered flow. The possibility of layering in the column profile and variations in soil hydraulic properties between these layers has been discussed above. Furthermore, indications of air entrapment were observed during the groundwater level experiments. The column setup used in this study is air confining, meaning that air was generally confined ahead of the wetting front and could only escape through the soil surface or the two air escape valves. An uneven distribution of air pressure can cause variations in infiltration rate and groundwater level locally. Consequently, the occurrence of fingered flow during the groundwater level experiments should be considered likely. Both macropore and fingered flow are reported to occur in packed column studies (Wilson et al., 1995).

Microbial transport

Inoculation

The inoculation of the sand columns with bacteria resulted in more than 99% of the bacteria being retained in the sand. This demonstrates that the unsaturated zone is an effective barrier between microbial

contamination and the saturated zone under these conditions. The inoculation effluent was captured and analyzed in a single fraction, therefore there is no breakthrough curve of the bacteria inoculation. This was done because the focus of this study lies on transport as a result of transients after inoculation, as well as to minimize costs. Measurement of the bacteria concentration in the inoculation effluent in multiple fractions would have allowed inverse modeling of the initial breakthrough. This would have yielded an additional fit of attachment and detachment parameters and thus more certainty, but does not otherwise affect the result of the inverse modeling results.

The attachment of bacteria to sand grains is highly sensitive to the water content (Wang et al., 2014). At low water content, the water films around sand grains are thinner, decreasing the distance between these grains and the bacteria and therefore increasing the strength of the adhesive forces as compared to at higher water content. The efficiency of attachment during inoculation is therefore strongly dependent on the saturation of the sand column at the time of inoculation. Another factor that influences the removal of bacteria from the inoculation influent is the bulk density of the sand column. As the same sand was used for all columns, it is assumed that variations in the bulk density are the result of the column packing procedure. Therefore, at a higher bulk density, there are more sand grains in a given volume and thus more grain surfaces accessible for bacterial attachment. Secondly, pore space, pore throat and macropore size is reduced by compaction (Lewis and Sjöstrom, 2010; Oliviera et al., 1996), further reducing the distance between sand grains and bacteria, thereby facilitating bacterial attachment. Macropore flow is especially of importance at and close to saturation (Jarvis and Dubois, 2006). Weight measurements of the columns showed small variations in bulk density between experiments on the scale of 50 grams per liter. The concentration profile of both bacteria strains in the column after inoculation shows that the top 4 cm of sand contains the highest concentrations of bacteria and that the concentrations quickly decrease with depth. This distribution is the result of the concentration of the inoculation influent decreasing as it travels through the sand as bacteria are retained to the sand grains. The downward moving pore water becomes depleted of bacteria, therefore the number of bacteria available for attachment to lower parts of the column decreases. Additionally, the abovementioned effect of water content on the attachment of bacteria causes attachment to be more effective in the drier upper parts of the column than in the more saturated lower parts of the sand column.

E. coli and E. moraviensis

Based on the inoculation results, *E. moraviensis* are more easily bound to the sand grains than *E. coli*. The percentage of bacteria retained in the column is consistently higher for *E. moraviensis* than *E. coli* during all experiments. Furthermore, the percentage of *E. coli* released with each rainfall and groundwater level event is consistently higher for *E. coli* than *E. moraviensis*, indicating stronger adhesive forces between *E. moraviensis* and the sand grains than for *E. coli*. This is reflected in the results of the kinetic attachment/detachment model: the attachment and detachment parameters of the *E. moraviensis* are generally higher and lower than those of the *E. coli*, respectively. This might be caused by differences in size, as *Enterococci* are known to cluster, forming pairs (diplococci) short chains or groups (Svec et al., 2001). This increase in size could cause more retention in sand through straining or bridging mechanisms (Engström et al., 2015). The decay rates of *E. coli* are more than twice as high as of *E. moraviensis*, which is in contrast to the results of Hornstra and Cirkel (2018). Hornstra and Cirkel report higher decay rates for *E. moraviensis* (0.07 $\log_{10}$CFU/day) than for *E. coli* (0.184 $\log_{10}$CFU/day). The *E. moraviensis* decay rates measured in this study (average 0.096 $\log_{10}$ CFU/day) approach that of Hornstra and Cirkel while the *E. coli* decay rates measured (average 0.244 $\log_{10}$ CFU/day)are slightly higher than that of Hornstra and Cirkel. This differencd is well within the margin of error.

Experimental results show that the release of *E. moraviensis* is slightly retarded as compared to that of *E. coli* in the rainfall experiments. The peak in *E. coli* concentrations coincide with peak discharge when the volumetric water content in the column is at its highest. Besides the peak concentrations, the very low concentrations of *Enterococci* in the first fractions of the rainfall experiments, during imbibition, indicate that different processes play a role in the release of *E. moraviensis* as compared to *E. coli*. The *Enterococci* concentrations start to increase approximately 3:15 hours after the start of rainfall where for *E. coli* this increase is visible after 1 hour. This indicates that imbibition is the dominant process in the transport of *E.*

coli whereas for *E. moraviensis*, drainage is of more importance. As the AWI decreases during imbibition, the dominance of imbibition in the release of *E. coli* could indicate that *E. coli* have a relatively high ratio of attachment to the AWI as compared to the SWI. The inverse could be stated for *E. moraviensis*, as the passing AWI during drainage liberates bacteria attached to the SWI.

Under the conditions simulated here, *E. coli* is better removed through groundwater level changes than rainfall and *E. moraviensis* is more efficiently removed by rainfall when taking into account correction for microbial decay. It should be noted that the two scenarios are difficult to compare here, as the amounts of water passing through the sand columns are not the same for the groundwater level and rainfall experiments. Furthermore, it should be taken into account that the microbial decay rates are not equal for the rainfall and groundwater level experiments; decay rates for RAIN1 are lower than for GWL1. *E. coli* decay rates of RAIN2 and RAIN3 are lower than that of GWL1 and GWL2, but the *Enterococci* decay rates are higher. The variations in die-off rates between experiments can be caused by variations in temperature, water content or local differences in initial microbial content in the unsterilized sand (Staley et al., 2016; Feng et al., 2010).

Release during rainfall

The results of RAIN1 show that the total amount of bacteria released decrease with each rainfall event. During each rainfall cycle, large amounts of bacteria are released from the soil and flushed out of the column. The number of bacteria released during an event, relative to the number of bacteria in the column at the start of that event, also decreases with each rainfall event during RAIN1. In the case of the release rate (i.e. the net sum of attachment and detachment) remaining constant over cycles, the total amount of cell release would decrease after each cycle because the initial adsorbed concentration of bacteria is lower than in the previous cycle. Furthermore, it seems likely that the cells that are bound to easily accessible locations on soil grains are more easily removed during imbibition and drainage than bacteria attached to less accessible regions. Therefore after each rainfall cycle, the relative amount of bacteria released decreases as well. The results of the second and third rainfall experiment do not match this pattern. During the course of RAIN2 and RAIN3, the total released bacteria increases with each rainfall event. Secondly, the concentrations of *E. coli* and *E. moraviensis* in the effluents of inoculation and of each rainfall event are an order of magnitude lower than that of RAIN1, despite comparable inoculation influent concentrations. This can be explained in part by higher bulk densities of the sand column in the second and third rainfall experiments (Table 3). Miles et al. (1988) show that, after air entry, sand samples with lower dry bulk densities have lower hydraulic conductivities, which is the reverse of the situation under saturated conditions. Additionally, more water is retained at high suctions with increasing bulk density (Ritsema and Dekker, 1994). Therefore, it is likely that the bulk density influenced the effects of hysteresis and that the sand columns with a higher bulk density were drained to a lesser extent, therefore retaining more bacteria, causing the observed differences in microbial transport behavior. This hypothesis cannot be confirmed due to the lack of weight data for the first rainfall experiment. After three consecutive cycles of rainfall, the bulk of the bacteria are still retained in the surface of the sand column; however, transport of bacteria in a downwards direction results in a less steep gradient in concentrations with depth.

Results show a strong correlation between discharge, i.e. flux, and water content one hand, and concentrations of bacteria in the column effluent on the other. This indicates that use of the kinetic attachment/detachment model, that relates release or detachment of bacteria from the solid surfaces to the water flux and relative water content, is valid.

Release during groundwater level change

The complex hydrology of the groundwater level experiments complicates interpretation and subsequent modeling of the results. The water flow is directed upwards during imbibition and downwards during drainage. At the time that reversal of flow takes place, it is likely that for a short period of time, the upper

section of the column experiences upwards flow, while the lower section experiences downwards flow. Fingered flow enhances the transport of water and solutes vertically in the unsaturated zone (de Rooij. 1999). Flow velocity is much higher during fingered flow, resulting in more release and less retention of bacteria from and to the sand grains. Furthermore, at and close to saturation, macropores can dominate flow. It is quite likely that macropores are introduced by the packing procedure. The breakthrough curve of bacteria in the column effluent therefore may not represent the column draining uniformly in depth through time. Bypass flow may locally cause pore water from higher in the column with a high bacterial concentration, to be drained from the column at an earlier time than pore water from a lower part of the column.

The initial distribution of bacteria in the soil profile is expected to show the highest concentrations at the surface, decreasing towards the bottom of the column. During the rainfall simulation, the infiltrating water front moves from the surface to the bottom, carrying along a fraction of the microbes. Conversely, during groundwater level rise, the saturation front moves upwards from the bottom of the column. During this movement, some of the bacteria on the sand grain surfaces in the lower section of the sand column are released into the aqueous phase or onto the AWI. This applies mostly to *E. coli* as they are easily mobilized by transients in groundwater as seen in the results of the rainfall experiments. This reduces the already low concentrations of *E. coli* in the lower sections of the sand column. In the case of release from the AWI, these bacteria are transported upwards with the advancing saturation front. In the case of release to the aqueous phase, these bacteria are transported upwards with the advancing pore water. In the subsequent drainage phase, it is thought that a fraction of the bacteria on the AWI and in the aqueous phase attach to the sand grains, causing higher concentrations of bacteria in the soil profile near the location of the saturation front at the time of reversal of flow. During the subsequent drainage, the *E. coli* concentrations remain low in the first effluent fractions because the bottom of the column is relatively depleted. The highest concentrations of *E. coli* are found in the last effluent fractions as those fractions contain the water drained from the upper section of the column. *E. moraviensis* are less easily mobilized by the advancing saturation front and therefore retain their initial distribution profile. During drainage the *Enterococci* profile in the effluent fractions therefore show a similar distribution to the rainfall simulation experiment. As the bacteria concentrations in the column cannot be measured during the experiment with the current setup, this hypothesis cannot be tested.

Furthermore, the large amount of air entrapment in the sand column during the GWL experiments causes the water saturation to be higher in the lower and middle sections of the column than in the upper parts. Tensiometer data shows that the bottom of the column remains wetter than the top at all times during the groundwater level experiment. Therefore, it is likely that the before mentioned process of release and reattachment that occurs within a single cycle of groundwater level change, plays a larger role in the lower and middle sections of the column than at the soil surface due to a higher relative water saturation. A slower groundwater level rise could prevent or limit fingered flow and air entrapment, resulting in a more uniform saturation front. This would likely result in a smoother breakthrough curve which is more representable for bacteria release in depth.

After three consecutive cycles of groundwater level fluctuations, the distribution of *E. coli* in depth has been changed thoroughly. The concentration of *E. coli* is more equal over depth than before, and the peak concentration is now found between 7.3 and 11 cm depth. The distribution of *E. moraviensis* after three groundwater level variations resembles that after rainfall.

Kinetic attachment/detachment model

The one- and two-site kinetic models employed in this study were used to approach microbe retention and mobilization as a kinetic process dependent of water content and water flux. This approach yields a good fit with the experimental data of the rainfall experiments, considering the complexity of the processes at hand. The quality of the model fits, despite fitting experimental results during transients in water content indicates that the attachment and detachment parameters in microbial transport are not as dependent of water content as is commonly thought (e.g. Bradford et al, 2015). The one- and two-site models were unable to adequately describe the results of the groundwater level experiments with the exception of *E. coli* transport during

GWL2. The breakthrough curve of *E. coli* during GWL2 deviates from the remainder of the GWL data in that it expresses a trend of clear increase in concentration with time during each event. The complexity of the hydrological conditions during the groundwater level experiments is likely the cause of the poor inverse modeling results. In the fitting of the rainfall experimental data, the variations in flux applied to the sand surface corresponding with the rainfall events results in peaks in concentration. The timing of the peaks is successfully reproduced. The initial peak in the breakthrough curves of both *E. coli* and *E. moraviensis* is modeled well and subsequent peaks decrease in concentration for each successive event. The model therefore captures the expected effect of the accessible bacteria concentration in the sand decreasing after each rainfall event.

In literature, the relative importance assigned to imbibition and drainage in the release of microbes varies per study (Wang et al., 2014). It is thought that the timing of the onset and peak in concentrations could give an indication of the relative importance of imbibition versus drainage in the remobilization of bacteria. However, the importance of imbibition or drainage on the release of microbes depends on the initial water content and the history of drainage and imbibition. For example, microbes retained on sand grains are more accessible to a moving AWI at lower water content. Thus, if the sand had been drained to a lower water content in a previous cycle, the following imbibition would yield much higher release of bacteria than drainage. That being said, under the conditions of this study, imbibition appears to be the dominant process in the release of *E. coli*, whereas drainage is of more importance for the release of *E. moraviensis*.

The one-site model produces an equally good fit to the rainfall experimental data as the two-site model for the release of *E. coli*. The one-site models for *E. coli* transport generally score higher on the AIC than the two-site models. For *Enterococci*, the two-site models provide a much better fit and have a higher AIC score. Based on the modeling results, it cannot be concluded nor confirmed that attachment to two different sorption sites takes place in the transport of *E. coli*. In the transport of *E. moraviensis*, the added quality in model fit provided by the addition of the second kinetic site indicates that adsorption to two distinguishable sites plays a role. Processes of attachment to two different sites, such as the SWI and AWI, may take place and may influence microbial (re)mobilization in sand; however, the nature of these different processes cannot be distinguished from each other based on the experimental and modeling results of this study.

A good match is observed between attachment and detachment parameters of a single bacteria strain between experiments. For example, the fitted K_{att} and K_{det} parameters of the one-site model describing *E. coli* release in RAIN2 can moderately describe *E. coli* release in the RAIN3. When comparing these parameters between all rainfall experiments, the parameters are within the same order of magnitude. It is reasonable to suggest that reiteration of the experiments and subsequent model fitting can lead to general attachment and detachment parameters for each bacteria species, allowing forward modeling in the same transient flow scenario, but under different conditions such as multiple consecutive rainfall events or with for example different initial conditions.

In general, the experimental results can be improved by continuously measuring bacteria concentrations in the column effluent instead of in fractions, as was done in this study. This would provide smoother breakthrough curves with a much higher resolution which would also improve the inverse modeling, especially for the groundwater level experiments. The narrow spread of effluent fractions in the first 3 hours of the GWL experiments is made necessary by the high effluent discharge at the onset of drainage. As a result, not much information is obtained on the tail of the bacteria breakthrough curves. Slower saturation of the columns during the groundwater experiment is likely to yield breakthrough curves with a smother trend, which would further benefit the model fit.

Conclusions

The unsaturated zone is an effective barrier between microbial contamination and the saturated zone. The inoculation of the sand columns with bacteria resulted in more than 99% of the bacteria being retained in the sand. However, accumulation of bacteria in the unsaturated zone can build up large reserves that can potentially be released to the saturated zone with transients in water flow. The attachment of bacteria to sand grains is highly sensitive to the water content (Wang et al., 2014). At low water content, the water films around sand grains are thinner, decreasing the distance between these grains and the bacteria and therefore increasing the strength of the adhesive forces as compared to at higher water content. Hysteresis in the soil hydraulic properties and air entrapment affect the effective soil water content and thereby have a large influence on the microbial transport in the soil. After inoculation, most of the bacteria in the sand column can be found in the upper 4 cm with the concentration rapidly decreasing with depth. The results of the rainfall experiments show that the number of bacteria released generally decreases with each successive rainfall event. This is due to the decreasing number of bacteria in the soil at the start of each next rainfall event and possibly to the most accessible bacteria being released first, making it progressively more difficult to release bacteria with each event. After three consecutive rainfall events, between 0.014% and 0091% of the *E. coli* initially retained in the sand after inoculation is released and washed out of the column. For *E. moraviensis* the release after three rainfall events is between 3.35E-04% and 8.43E-03%. Three consecutive cycles of groundwater level variations result in 0.13% to 1.1% and 3.37E-04% to 1.31E-03% release of *E. coli* and *E. moraviensis*, respectively, relative to the number of bacteria retained in the column after inoculation. E. *coli* are easily mobilized by upwards flow of groundwater as a result of groundwater level rise. This applies to *E. moraviensis* to a lesser extent. This upwards flow of bacteria results in less release than rainfall and redistributes the bacteria over the sand column, changing the profile of bacteria with depth. After three consecutive cycles of rainfall, the bulk of the bacteria are still retained in the surface of the sand column; however, transport of bacteria in a downwards direction results in a less steep gradient in concentrations with depth. After three consecutive cycles of groundwater level fluctuations, the distribution of *E. coli* in depth has been changed thoroughly. The concentration of *E. coli* is more equal over depth than before, and the peak concentration is now found between 7.3 and 11 cm depth. The distribution of *E. moraviensis* after three groundwater level variations resembles that after rainfall.

E. moraviensis is more easily attached to sand grains and once attached, is not as easily detached as *E. coli*. *E. coli* is relatively mobile in the unsaturated zone and is easily transported in groundwater by both rainfall and groundwater level changes. These findings are reflected in the attachment and detachment parameters of the one- and two-site models used to describe microbial transport in this study. The timing of the onset and peak concentrations of *E. coli* and *E. moraviensis*, compared to the soil water content, indicate that imbibition is the dominant process for *E. coli* release, where drainage is more important for *E. moraviensis*.

Both the one- and two-site kinetic attachment/detachment models adequately describe the concentrations of *E. coli* and *E. moraviensis* in the effluent of the sand columns for the rainfall experiments. The experimental results of the groundwater level scenario yielded poor model fits. The two-site model generally provides a significantly better fit of the experimental data than the one-site model. Therefore it is likely that attachment to two different sorption sites takes place. Processes of attachment to two different sites, such as the SWI and AWI, may take place and may influence microbial (re)mobilization in sand; however, these different processes cannot be distinguished from each other based on the experimental and modeling results of this study. Further experiments and accompanying modeling are necessary in order to fully distinguish the factors controlling remobilization of bacteria and to derive general attachment and detachment parameters for *E. coli* and *E. moraviensis* that can be used in forward modeling.

References

Adamczyk, Z., Siwek, B., Zembala, M., & Belouschek, P. (1994). Kinetics of localized adsorption of colloid particles. Advances in Colloid and Interface Science, 48, 151-280.

Bai, H., Cochet, N., Pauss, A., & Lamy, E. (2016). Bacteria cell properties and grain size impact on bacteria transport and deposition in porous media. Colloids and Surfaces B: Biointerfaces, 139, 148-155.

Bai, H., Cochet, N., Pauss, A., & Lamy, E. (2017). DLVO, hydrophobic, capillary and hydrodynamic forces acting on bacteria at solid-air-water interfaces: Their relative impact on bacteria deposition mechanisms in unsaturated porous media. Colloids and Surfaces B: Biointerfaces, 150, 41-49.

Bekhit, H. M., El-Kordy, M. A., & Hassan, A. E. (2009). Contaminant transport in groundwater in the presence of colloids and bacteria: Model development and verification. Journal of contaminant hydrology, 108(3-4), 152-167.

Bradford, S. A., Torkzaban, S., Leij, F., & Simunek, J. (2015). Equilibrium and kinetic models for colloid release under transient solution chemistry conditions. Journal of contaminant hydrology, 181, 141-152.

Bradford, S. A., & Torkzaban, S. (2008). Colloid transport and retention in unsaturated porous media: A review of interface-, collector-, and pore-scale processes and models. Vadose Zone Journal, 7(2), 667-681.

Bradford, S. A., Wang, Y., Torkzaban, S., & Šimůnek, J. (2015). Modeling the release of E. coli D21g with transients in water content. Water Resources Research, 51(5), 3303-3316.

Bradford, S. A., Simunek, J., Bettahar, M., van Genuchten, M. T., & Yates, S. R. (2003). Modeling colloid attachment, straining, and exclusion in saturated porous media. Environmental science & technology, 37(10), 2242-2250.

Buishand, T.A. & J.B. Wijngaard, 2007. Statistiek van extreme neerslag voor korte neerslagduren. KNMI Technisch Report, TR-295, Koninklijk Nederlands Meteorologisch Instituut, De Bilt, pp. 19.

Chen, G. (2008). Bacterial interactions and transport in unsaturated porous media. Colloids and Surfaces B: Biointerfaces, 67(2), 265-271.

De Rooij, G. H. (2000). Modeling fingered flow of water in soils owing to wetting front instability: a review. Journal of hydrology, 231, 277-294.

DeNovio, N. M., Saiers, J. E., & Ryan, J. N. (2004). Colloid movement in unsaturated porous media. Vadose Zone Journal, 3(2), 338-351.

Engström, E., Thunvik, R., Kulabako, R., & Balfors, B. (2015). Water transport, retention, and survival of Escherichia coli in unsaturated porous media: a comprehensive review of processes, models, and factors. Critical reviews in environmental science and technology, 45(1), 1-100.

Ervin, J. S., Russell, T. L., Layton, B. A., Yamahara, K. M., Wang, D., Sassoubre, L. M., ... & Holden, P. A. (2013). Characterization of fecal concentrations in human and other animal sources by physical, culture-based, and quantitative real-time PCR methods. Water research, 47(18), 6873-6882.

Feng, F., Goto, D., & Yan, T. (2010). Effects of autochthonous microbial community on the die-off of fecal indicators in tropical beach sand. FEMS microbiology ecology, 74(1), 214-225.

Gargiulo, G., Bradford, S., Šimůnek, J., Ustohal, P., Vereecken, H., & Klumpp, E. (2007). Bacteria transport and deposition under unsaturated conditions: The role of the matrix grain size and the bacteria surface protein. Journal of contaminant hydrology, 92(3-4), 255-273.

Gargiulo, G., Bradford, S. A., Simunek, J., Ustohal, P., Vereecken, H., & Klumpp, E. (2008). Bacteria transport and deposition under unsaturated flow conditions: The role of water content and bacteria surface hydrophobicity. Vadose Zone Journal, 7(2), 406-419.

Gargiulo, G., Bradford, S. A., Šimůnek, J., Ustohal, P., Vereecken, H., & Klumpp, E. (2007). Transport and deposition of metabolically active and stationary phase Deinococcus radiodurans in unsaturated porous media. Environmental science & technology, 41(4), 1265-1271.

Gilbert, O., Hernández, M., Vilanova, E., & Cornellà, O. (2014). Guidelining protocol for soil-column experiments assessing fate and transport of trace organics. Demeau, European Union, 54.

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of paleolimnology, 25(1), 101-110.

Hornstra, L.M., and Cirkel, D. G., (2018). Overleving van Escherichia coli en Enterococcus moraviensis in duinzand. KWR rapport KWR2018.005, KWR Nieuwegein.

Inoue, M., Šimůnek, J., Shiozawa, S., & Hopmans, J. W. (2000). Simultaneous estimation of soil hydraulic and solute transport parameters from transient infiltration experiments. Advances in water resources, 23(7), 677-688.

Jarvis, N. J., and Dubois, I. G. (2006). State-of-the-art review of preferential flow. Report DL#6 of the FP6 EU-funded Functional Tools for Pesticide Risk Assessment and Management project.

Jiang, G., Noonan, M. J., Buchan, G. D., & Smith, N. (2007). Transport of Escherichia coli through variably saturated sand columns and modeling approaches. Journal of Contaminant Hydrology, 93(1-4), 2-20.

Lewis, J., & Sjöstrom, J. (2010). Optimizing the experimental design of soil columns in saturated and unsaturated transport experiments. Journal of contaminant hydrology, 115(1-4), 1-13.

Lima, G. D. P., & Sleep, B. E. (2007). The spatial distribution of eubacteria and archaea in sand–clay columns degrading carbon tetrachloride and methanol. Journal of contaminant hydrology, 94(1-2), 34-48.

LMB-042 (KWR, Nieuwegein). (2019). Onderzoek met behulp van membraanfiltratie naar de aanwezigheid en het aantal kolonievormende eenheden (kve) van Escherichia coli (E. coli) en bacteriën van de coligroep in water en suspensies.

LMB-044 (KWR, Nieuwegein). (2016). Onderzoek met behulp van membraanfiltratie naar de aanwezigheid en het aantal kolonievormende eenheden (kve) van enterococcen in water en suspensies.

Ma, K. C., Tan, Y. C., & Chen, C. H. (2009). Effect of hysteresis and infiltration rate on finger dynamics. Eurasian Soil Science, 42(13), 1504-1514.

Myers, D. N., Stoeckel, D. M., Bushon, R. N., Francy, D. S., & Brady, A. M. G. (2007). Fecal indicator bacteria: US Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.1 (version 2.0).

Nautiyal, C. S., Rehman, A., & Chauhan, P. S. (2010). Environmental Escherichia coli occur as natural plant growth-promoting soil bacterium. Archives of microbiology, 192(3), 185-193.

Oliviera, I. B., Demond, A. H., & Salehzadeh, A. (1996). Packing of sands for the production of homogeneous porous media. Soil Science Society of America Journal, 60(1), 49-53.

Pang, L. (2009). Microbial removal rates in subsurface media estimated from published studies of field experiments and large intact soil cores. Journal of Environmental Quality, 38(4), 1531-1559.

Passmore, J. M., Rudolph, D. L., Mesquita, M. M., Cey, E. E., & Emelko, M. B. (2010). The utility of microspheres as surrogates for the transport of E. coli RS2g in partially saturated agricultural soil. water research, 44(4), 1235-1245.

Ritsema, C. J., & Dekker, L. W. (1994). Soil moisture and dry bulk density patterns in bare dune sands. Journal of Hydrology, 154(1-4), 107-131.

Russell, T. L., Yamahara, K. M., & Boehm, A. B. (2012). Mobilization and transport of naturally occurring enterococci in beach sands subject to transient infiltration of seawater. Environmental science & technology, 46(11), 5988-5996.

Saiers, J. E., & Lenhart, J. J. (2003). Colloid mobilization and transport within unsaturated porous media under transient-flow conditions. Water Resources Research, 39(1).

Saiers, J. E., & Lenhart, J. J. (2003). Ionic-strength effects on colloid transport and interfacial reactions in partially saturated porous media. Water Resources Research, 39(9).

Salehi, M. H., Beni, O. H., Harchegani, H. B., Borujeni, I. E., & Motaghian, H. R. (2011). Refining soil organic matter determination by loss-on-ignition. Pedosphere, 21(4), 473-482.

Sasidharan, S., Bradford, S. A., Torkzaban, S., Ye, X., Vanderzalm, J., Du, X., & Page, D. (2017). Unraveling the complexities of the velocity dependency of E. coli retention and release parameters in saturated porous media. Science of the Total Environment, 603, 406-415.

Sasidharan, S., Torkzaban, S., Bradford, S. A., Cook, P. G., & Gupta, V. V. (2017). Temperature dependency of virus and nanoparticle transport and retention in saturated porous media. Journal of contaminant hydrology, 196, 10-20.

Schijven J. F., Hoogenboezem W., Nobel P. J., Medema G. J. & Stakelbeek A. 1998 Reduction of FRNA-bacteriophages and faecal indicator bacteria by dune infiltration and estimation of sticking efficiencies. Water Science and Technology, 38 (12), 127-131.

Schijven, J. F., Hoogenboezem, W., Hassanizadeh, M., & Peters, J. H. (1999). Modeling removal of bacteriophages MS2 and PRD1 by dune recharge at Castricum, Netherlands. Water Resources Research, 35(4), 1101-1111.

Schijven, J. F., & Hassanizadeh, S. M. (2000). Removal of viruses by soil passage: Overview of modeling, processes, and parameters. Critical reviews in environmental science and technology, 30(1), 49-127.

Schijven, J. F., & Šimůnek, J. (2002). Kinetic modeling of virus transport at the field scale. Journal of Contaminant Hydrology, 55(1-2), 113-135.

Sepehrnia, N., Memarianfard, L., Moosavi, A. A., Bachmann, J., Guggenberger, G., & Rezanezhad, F. (2017). Bacterial mobilization and transport through manure enriched soils: Experiment and modeling. Journal of environmental management, 201, 388-396.

Sepehrnia, N., Bachmann, J., Hajabbasi, M. A., Afyuni, M., & Horn, M. A. (2018). Modeling Escherichia coli and Rhodococcus erythropolis transport through wettable and water repellent porous media. Colloids and Surfaces B: Biointerfaces, 172, 280-287.

Simunek, J., van Genuchten, M. T., & Sejna, M. (2005). The HYDRUS-1D software package for simulating the movement of water, heat, and multiple solutes in variably saturated media, version 3.0, HYDRUS software series 1. Department of Environmental Sciences, University of California Riverside, Riverside.

Šimůnek, J., Šejna, M., Saito, H., Sakai, M. & Van Genuchten, M. T. (2013). The HYDRUS-1D Software Package for Simulating the One-Dimensional Movement of Water, Heat, and Multiple Solutes in Variably-Saturated Media. Technical manual, version, 4.16.

Šimunek, J., Van Genuchten, M. T., & Šejna, M. (2012). HYDRUS: Model use, calibration, and validation. Transactions of the ASABE, 55(4), 1263-1274.

Šimůnek, J., Van Genuchten, M. T., & Šejna, M. (2016). Recent developments and applications of the HYDRUS computer software packages. Vadose Zone Journal, 15(7).

Smits, I., Wijngaard, J.B., Versteeg, R. & Kok, M. (2004). (STOWA, 2004). Statistiek van extreme neerslag in Nederland. Rapport nr. 26, STOWA, Amersfoort.

Staley, Z. R., Robinson, C., & Edge, T. A. (2016). Comparison of the occurrence and survival of fecal indicator bacteria in recreational sand between urban beach, playground and sandbox settings in Toronto, Ontario. Science of The Total Environment, 541, 520-527.

Svec, P., Devriese, L. A., Sedlácek, I., Baele, M., Vancanneyt, M., Haesebrouck, F., ... & Doskar, J. (2001). Enterococcus haemoperoxidus sp. nov. and Enterococcus moraviensis sp. nov., isolated from water. International journal of systematic and evolutionary microbiology, 51(4), 1567-1574.

Taučer-Kapteijn, M., Hoogenboezem, W., & Medema, G. (2016). Environmental growth of the faecal indicator Enterococcus moraviensis. Water Science and Technology: Water Supply, 16(4), 971-979.

Taučer-Kapteijn, M., Hoogenboezem, W., Hoogenboezem, R., De Haas, S., & Medema, G. (2017). Source tracking of Enterococcus moraviensis and E. haemoperoxidus. Journal of water and health, 15(1), 41-49.

Torkzaban, S., Bradford, S. A., Vanderzalm, J. L., Patterson, B. M., Harris, B., & Prommer, H. (2015). Colloid release and clogging in porous media: Effects of solution ionic strength and flow velocity. Journal of contaminant hydrology, 181, 161-171.

Torkzaban, S., & Bradford, S. A. (2016). Critical role of surface roughness on colloid retention and release in porous media. Water research, 88, 274-284.

Torkzaban, S., Bradford, S. A., van Genuchten, M. T., & Walker, S. L. (2008). Colloid transport in unsaturated porous media: The role of water content and ionic strength on particle straining. Journal of Contaminant Hydrology, 96(1-4), 113-127.

Torkzaban, S., Hassanizadeh, S. M., Schijven, J. F., & Van Den Berg, H. H. J. L. (2006). Role of air-water interfaces on retention of viruses under unsaturated conditions. Water Resources Research, 42(12).

Torkzaban, S., Hassanizadeh, S. M., Schijven, J. F., De Bruin, H. A. M., & de Roda Husman, A. M. (2006). Virus transport in saturated and unsaturated sand columns. Vadose Zone Journal, 5(3), 877-885.

Touch, N., Hibino, T., Takata, H., & Yamaji, S. (2017). Loss on ignition-based indices for evaluating organic matter characteristics of littoral sediments. Pedosphere, 27(5), 978-984.

Tufenkji, N. (2007). Modeling microbial transport in porous media: Traditional approaches and recent developments. Advances in water resources, 30(6-7), 1455-1469.

Van der Harst, G. G., & Stakman, W. P. (1965). Soil Moisture Retention Curves. Pt. 2. Directions for the Use of the Sandbox Apparatus Range Pf 0 to 2.7. Institute for Land and Water Management Research.

Van Genuchten, M. T. (1980). A closed-form equation for predicting the hydraulic conductivity of unsaturated soils 1. Soil science society of America journal, 44(5), 892-898.

Van Genuchten, M. V., Leij, F. J., & Yates, S. R. (1991). The RETC code for quantifying the hydraulic functions of unsaturated soils.

Wang, Y., Bradford, S. A., & Simunek, J. (2014). Release of E. coli D21g with transients in water content. Environmental science & technology, 48(16), 9349-9357.

Wang, Z., Feyen, J., van Genuchten, M. T., & Nielsen, D. R. (1998). Air entrapment effects on infiltration rate and flow instability. Water Resources Research, 34(2), 213-222.

Wilson, L.G., Everett, L.G., Cullen, S.J., 1995. Handbook of Vadose Zone Characterization and Monitoring. Lewis, Boca Raton, Fla. xii,730p. pp.

World Resources Institute (Washington, DC). (2000). A Guide to World Resources, 2000-2001, People and Ecosystems: the Fraying Web of Life.

Xing, J., Wang, H., Brookes, P. C., Salles, J. F., & Xu, J. (2019). Soil pH and microbial diversity constrain the survival of E. coli in soil. Soil Biology and Biochemistry, 128, 139-149.

Zhang, Q., Hassanizadeh, S. M., Raoof, A., van Genuchten, M. T., & Roels, S. M. (2012). Modeling virus transport and remobilization during transient partially saturated flow. Vadose Zone Journal, 11(2).

Zhang, Q., Hassanizadeh, S. M., Karadimitriou, N. K., Raoof, A., Liu, B., Kleingeld, P. J., & Imhof, A. (2013). Retention and remobilization of colloids during steady-state and transient two-phase flow. Water Resources Research, 49(12), 8005-8016.

Zhuang, J., Tyner, J. S., & Perfect, E. (2009). Colloid transport and remobilization in porous media during infiltration and drainage. Journal of hydrology, 377(1-2), 112-119.