

1 **Modeling *E. coli* fate and transport in and around a cattle pond**

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11 **Abstract** Contamination of surface water is a concern for public health. Lands used for animal production are sources of fecal
12 microorganisms that can reach water bodies, impact their quality, and adversely affect their potential uses. Understanding the
13 mechanisms of microbial transport through surface/subsurface flow is imperative to predict surface water contamination and
14 to assign management strategies for enhanced water quality. The aim of this work was to develop and test a mechanistic
15 numerical model to simulate watershed-scale surface/subsurface water flow, bacteria release from cow manure, and their fate,
16 as well as transport to a cattle pond. The integrated surface-subsurface hydrological platform HydroGeoSphere (HGS) was the
17 basis for the site-specific model. The pond and its environs were monitored for 15 months for *Escherichia coli* (*E. coli*)
18 concentrations, which remained relatively high throughout the study. The model was applied to simulate *E. coli* bacterial
19 transport in a grassed drainage basin grazed by a permanent herd of approximately 50 cattle. Most model parameter values
20 were adopted from the literature. The model explicitly accounted for cow excretion to the pond as a source of microbial
21 contamination. The latter was estimated from the time spent by cows in the pond, which in turn was estimated from imagery
22 obtained with eight trail cameras installed to cover the pond surface. Images were obtained every 15 min. Simulations for two
23 years showed that the non-calibrated model replicated spatiotemporal patterns and peak *E. coli* concentration reasonably well.
24 The *E. coli* cumulative flux loaded by cattle excretion directly to the pond was around two orders of magnitude greater than
25 that with the surface flow. The results demonstrate that mechanistic watershed-scale modeling combined with observational
26 data on cattle behavior can provide useful predictions of microbial contamination in cattle ponds using only readily available
27 data.

28

29 **1. Introduction**

30 Lands used for animal production, such as cow pastures, are sources of fecal microorganisms that can reach water
31 bodies, impact their quality, and adversely affect their potential uses. Water runoff during and after rainfall events is an
32 essential factor causing microbial transport from animal waste on pastures to water sources used for irrigation and recreation.
33 Public health concerns the fate and transport of pathogenic microorganisms and organisms used as indicators of microbial
34 pollution, such as *Escherichia coli* (*E. coli*).

35 Mechanistic mathematical modeling has provided essential tools for predicting surface water quality and assessing various
36 sources causing environmental contamination. Models represented by a compartmental setup use the mass balance, empirical,

37 and semi-empirical equations (Bradford et al., 2013; Cho et al., 2016; De Brauwere et al., 2014; van der Meulen et al., 2024).
38 The mechanistic models are based on the mathematical description of the momentum and mass balance equations. They
39 account for the physicochemical and biological processes via constitutive relations or sub-models and various sources and
40 sinks internally or through boundary conditions. One of the most popular models of this type is the Soil and Water Assessment
41 Tool (SWAT), which has often been used to simulate the fate and transport of *E. coli* in streams (Sowah et al., 2020; Kondo
42 et al., 2021; Iqbal et al., 2019). Both point and non-point microbial pollution have been simulated. For example, Kuang et al.
43 (2024) used the SWAT model to evaluate *E. coli* concentrations in surface water from domestic sewage and manure in China's
44 Three Gorges Reservoir region. Such models often simulate fecal contamination in rivers, estuaries, and coastal areas (Gao et
45 al. 2015; Wolska et al. 2022). Microbial transport has commonly been approximated as a one-dimensional process. Much less
46 work has been done to simulate 3D flow and transport in environmental settings. Curre et al. (2024) gave an example and
47 developed a model for simulating reactive microbial transport in river-groundwater systems. The model was implemented in
48 the integrated surface-subsurface hydrological platform HydroGeoSphere (HGS) (Therrien et al., 2010). The authors produced
49 a synthetic example emphasizing reactive microbial transport in riverbank filtration settings, aiming to quantify microbial
50 water quality in the aquifer with the pumping wells, which is crucial to improve drinking water management. The HGS
51 software can be applied to various water bodies, including ponds.

52 Ponds are important sources of agricultural water in rural environments. From 2.6 to 9 million ponds are used for irrigation,
53 recreation providing water to the livestock, and habitats for wildlife in the United States (Renwick et al., 2006). Little attention
54 has been paid to modeling microbial water quality in agricultural ponds. Vazquez et al. (2021) developed a mechanistic, runoff-
55 driven bacterial transport model to simulate peak bacterial concentration events for two highly variable irrigation ponds in
56 West Central Florida. The authors assumed that surface runoff driven by rainfall events is the primary mechanism driving
57 microbial contamination in these ponds. The calibrated model predicted *E. coli* peak events relatively well, but did not consider
58 the spatial distribution of pathogens in and around the ponds. Stocker et al. (2020) utilized the EFDC software to simulate the
59 fate and transport of *E. coli* in irrigation pond during the water extraction. These authors did not account for sources of
60 microbial contamination around the pond.

61 The Georgia Coastal plain, USA, has more than 13,000 farm ponds with typical surface area from one to four hectares
62 (Yao et al., 2024). Many of them are used as cattle ponds, given that the average high summer temperature is around 32 °C. It
63 is common for agricultural producers to impound water by constructing earthen dams across small streams, thereby capturing
64 and storing surface water. Additional water is often pumped from deeper aquifers to supplement the water supply (Albright et
65 al., accepted). These ponds tend to be relatively small (~2 ha) and shallow (< 3 m) and may be used for more than one purpose,
66 including irrigation, recreation, aquaculture, and a source of water for livestock, or "cattle ponds." Cattle ponds are farm ponds
67 used by cattle and other livestock animals, providing a perennial supply of available water for drinking and cooling on hot
68 days. Animals stocked in pastures are typically given free access to cattle ponds within the enclosed areas to wander and stay
69 at will. An essential feature of cattle ponds is the direct input of organic matter and enteric microorganisms into the aquatic
70 system when the animals eliminate waste. The microbiological quality of water is an essential issue because these waterbodies

71 are used as a source of drinking water for animals and crop irrigation. This raises concerns regarding microbial contamination
72 of water that may be used for consumption, either by animals or as irrigation inputs. However, to our knowledge, the microbial
73 quality of water in cattle ponds and the factors that influence it are poorly understood.

74 The farmers typically lack the resources to monitor their ponds. That limits opportunities for model calibration. Therefore,
75 it may be beneficial to apply modeling and to determine the accuracy of simulating the microbial quality of water that can be
76 achieved without model calibration.

77 The overarching objective of this work was to advance the current understanding of microbial contamination in cattle
78 ponds by integrating watershed-scale hydrological modelling, field monitoring of *E. coli* concentrations, and observational
79 quantification of cattle presence in the pond. We aimed to develop a modelling framework that would explicitly represent
80 both hydrologic transport pathways and direct microbial loading by livestock, providing new insight into the dominant
81 mechanisms controlling microbial water quality in agricultural ponds. The specific objectives of this work were to (a) carry
82 out spatiotemporal monitoring of the *E. coli* concentrations in a typical farm pond in Georgia where cattle grazed on the
83 surrounding land had uninterrupted access to water to drink and cool off; (b) monitor and quantify the presence of cattle in the
84 pond, and (c) develop an *E. coli* fate and transport hydrologic model that would include transport of manure borne *E. coli* to
85 the pond, direct deposition of animal waste to the pond, and mixing within the pond.

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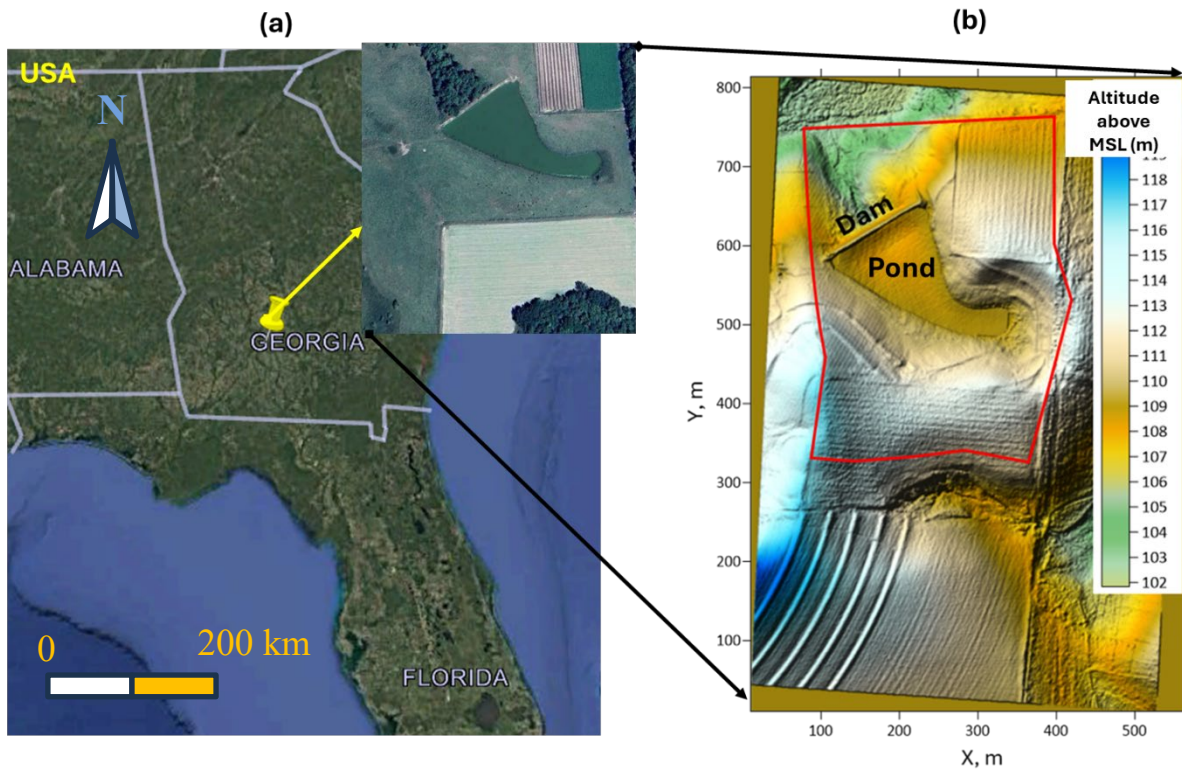
87 **2. Materials and Methods**

88 **2.1. Study area and environment**

89 The study area is a small watershed (area ~0.45 km²) with a pond within a larger, fenced pasture, located on a privately owned
90 crop-livestock integrated farm in the southern Coastal Plain of Georgia, USA (Figure 1a). The farm is referred to as the Sumner
91 Cooperator Farm (SCF). Currently, the area is used for cow grazing. The climate is humid subtropical, with a mean annual
92 temperature of 18.8 °C and mean annual precipitation of 1174 mm.

93 The land surface's Digital Elevation Model of 1 m resolution was downloaded from the Open Topography portal
94 (<https://portal.opentopography.org/>). The altitude of the land surface varies from 101.8 to 119.7 m (Figure 1b).

95



96

97

98 **Figure 1. Location from Google Earth(© Google Earth) images (a) and land topography of the study area (b). The red line represents**
 99 **the boundary of the simulation domain,**

100

101 Soil cover at the site consists of a loamy sand (average of 85% sand, 9% silt, and 7% clay) to depths of 60-80 cm,
 102 underlain by sandy clay loam (average of 62% sand, 8% silt, and 30% clay) containing plinthite, a low permeability layer of
 103 hard iron-rich soil restricting hydraulic connectivity between surface and groundwater (Blume et al., 1987). The latter soil was
 104 used to build the bottom of the pond and the dam.

105

106 Weather conditions were monitored by the USDA-ARS Southeast Watershed Research Laboratory (SEWRL) at the
 107 site to measure air temperature and humidity, wind speed, solar radiation, and soil and water temperature. Data were collected
 108 from a climate station located at the SCF noted as "Rain Gage 80" in the SEWRL public data website
 109 (<https://radio.tiftonars.org/rg80.htm>). Specifications for instrumentation follow the configurations for the Little River
 Experimental Watershed climate stations described in Bosch et al. (2007).

110 2.2 Quantifying pond use and bacterial concentrations in cattle manure

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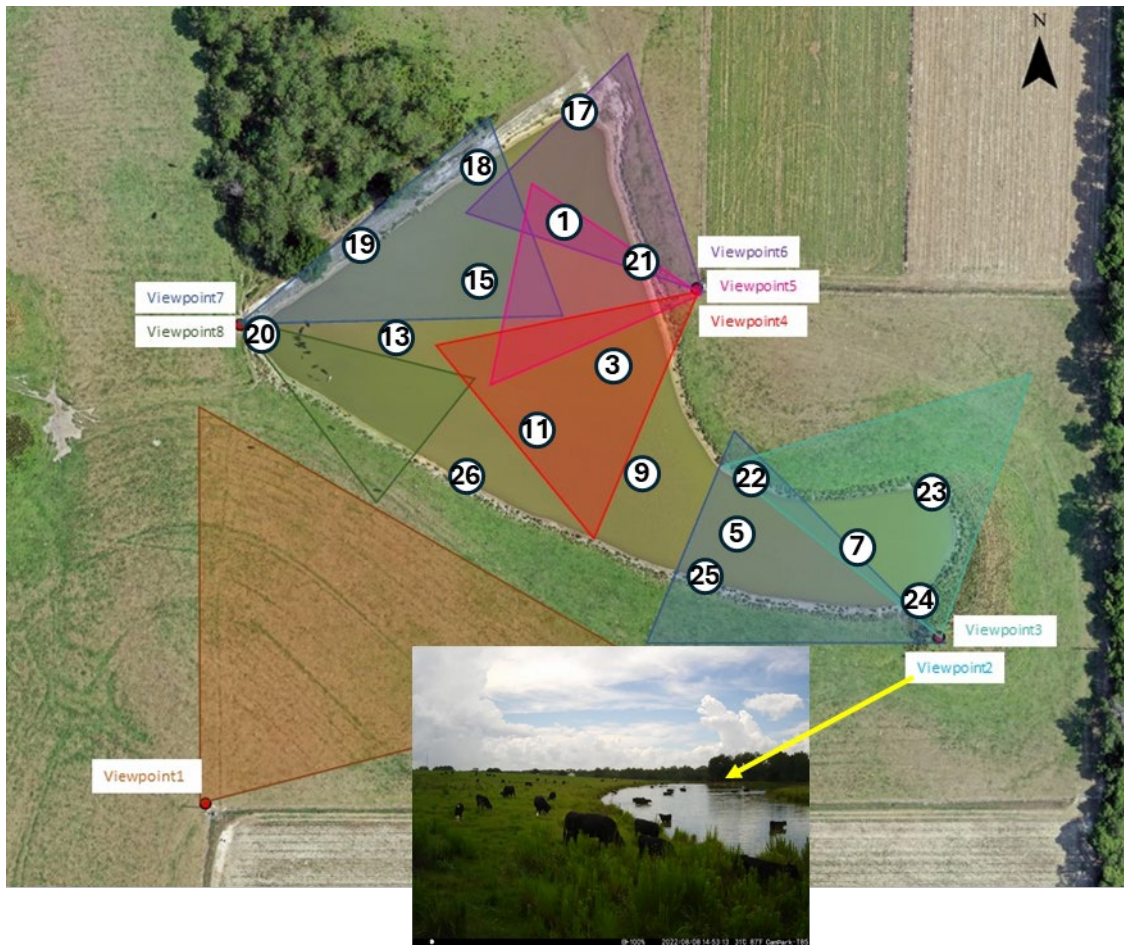
112 Cattle use of the pond and the pasture area draining into the pond was evaluated using automated trail cameras. Eight cameras
113 were fixed to solid structures (e.g., fence posts, trees) and their fields of view (FOVs) captured overlapping images of the pond
114 at regular intervals. Three comparable camera models were used for the study, including 3 Bushnell 24MP Prime Low Glow
115 (Model 119932C), 3 CamPark, and 2 Coleman (Model CHD400W) cameras. Cattle in the pond were imaged from July 2022
116 to December 2023. Secure digital (SD) removable media cards were used to record the imagery and were replaced every 6 to
117 10 weeks for the duration of the study. Imagery was downloaded and stored on a file server for subsequent analysis. The
118 imagery was visually assessed for pond use by cattle by a single observer for all days. Images were reviewed from each camera,
119 and the number of cows in the pond was counted in each image. Tallied viewpoint counts were summed according to the model
120 finite element mesh (FEM) nodes segmentation of the shoreline described below, multiplied by the time interval of the camera
121 setting (5 to 30 minutes), and divided by 1440 minutes per day. The resulting values were summed for each shoreline segment
122 to produce a daily value of "cow days" (Cex). To evaluate the accuracy of the visual assessment, two additional independent
123 observers conducted validation counts of a sample of the imagery following the same protocol, and agreement among the three
124 observers (OBS1, OBS2, and OBS3) was evaluated (Table S1).

125 Between February and May 2023, 18 fresh cow manure samples were collected from the area around the pond.
126 Samples were collected into 50-mL conical tubes using a sterilized tongue depressor and were then placed on ice for storage
127 and transport to the laboratory. Laboratory processing of manure samples occurred within 24 hours of sample collection.
128 Briefly, 2g of manure was blended with 200 mL of sterile, deionized water for 2 min on the highest setting. Mixed samples
129 were then allowed to settle for 15 minutes before aliquots were used to prepare serial dilutions of the manure mixture. Diluted
130 manure solutions were processed in duplicate using the Colilert method (IDEXX, Westbrook, Maine), which produced a most-
131 probable-number (MPN) of *E. coli* in each sample. An MPN was then calculated per mass of manure (MPN *E. coli* kg⁻¹) using
132 information from a dry weight analysis of the manure.

133 **2.3. Water sampling**

134 Figure 2 shows the locations of the water sampling points for measurements of *E. coli* concentration and of the camera
135 viewpoints for monitoring cattle. For the water sampling points, locations with even numbers (2, 4, 6, 8, 10, 12, 14, 16, not
136 shown) have coordinates the same as locations with odd numbers (1, 3, 5, 7, 9, 11, 13, and 15), respectively. However, locations
137 with odd numbers were sampled at the pond surface, whereas locations with even numbers were sampled at a depth of 50 cm
138 using a peristaltic pump. Locations 17 to 26 were sampled at the surface near the banks. All samples were taken between 10:00
139 and 12:00 in the absence of cows.

140



141

142

143 **Figure 2. Locations of water sampling points (1-26) and cattle monitoring cameras (Viewpoints 1-8). The insert shows an example**
 144 **of an image taken at monitoring viewpoint 2. Orthoimage from July 7, 2022 by USDA-ARS, Southeast Watershed Research**
 145 **Laboratory, Remote Sensing and Mapping Group, Tifton, Georgia, USA. The imagery was collected with a Hasselblad L1d-20C**
 146 **20mp camera using a DJI Mavic 2 Pro L1P drone, and created using Pix4D Mapper image processing software.**

147

148

149 **2.4 Mathematical model**

150 Accounting for the complexity of hydrogeological and hydrochemical processes, we choose the HGS (Therrien et al., 2010)
 151 as a basis for the model. In HGS, the flow of water is simulated in a fully integrated mode; water derived from rainfall inputs
 152 is partitioned into components such as overland and stream flow, evaporation, infiltration, recharge, and subsurface discharge
 153 into surface water features such as lakes, streams, and wetlands in a natural, physics-based fashion. It employs a fully coupled

154 numerical approach, allowing the simultaneous solution of both the surface and variably saturated subsurface flow, solute
155 transport, and heat transfer.

156 The mathematical model of the watershed flow and transport comprises the following components. The Richards equation
157 simulates three-dimensional transient subsurface flow in a variably saturated porous medium (PM domain). The van Genuchten
158 (1980) relations are used to calculate the pressure-saturation relationship and hydraulic conductivity. The two-dimensional
159 depth-averaged diffusive wave equation describes overland water flow (OVL domain). The subsurface and surface flow
160 equations are fully coupled. Evapotranspiration affects surface and subsurface flow domains and is modeled as a combination
161 of transpiration from vegetation and evaporation. Microbial transport is described by 3D and 2D coupled advection-dispersion
162 equations in the subsurface and surface, respectively. We chose the linear Henry isotherm for simulating *E. coli* sorption. A
163 first-order decay reaction describes the bacteria's die-off. The present model does not explicitly simulate soil and manure
164 erosion, suspended sediment transport, settling, or resuspension. As a result, bacterial transport is represented primarily through
165 direct runoff and overland flow pathways, without accounting for attachment to or release from suspended or bed sediments.
166 However, the model accounts for the release of bacteria from cowpats uploaded onto the soil surface.

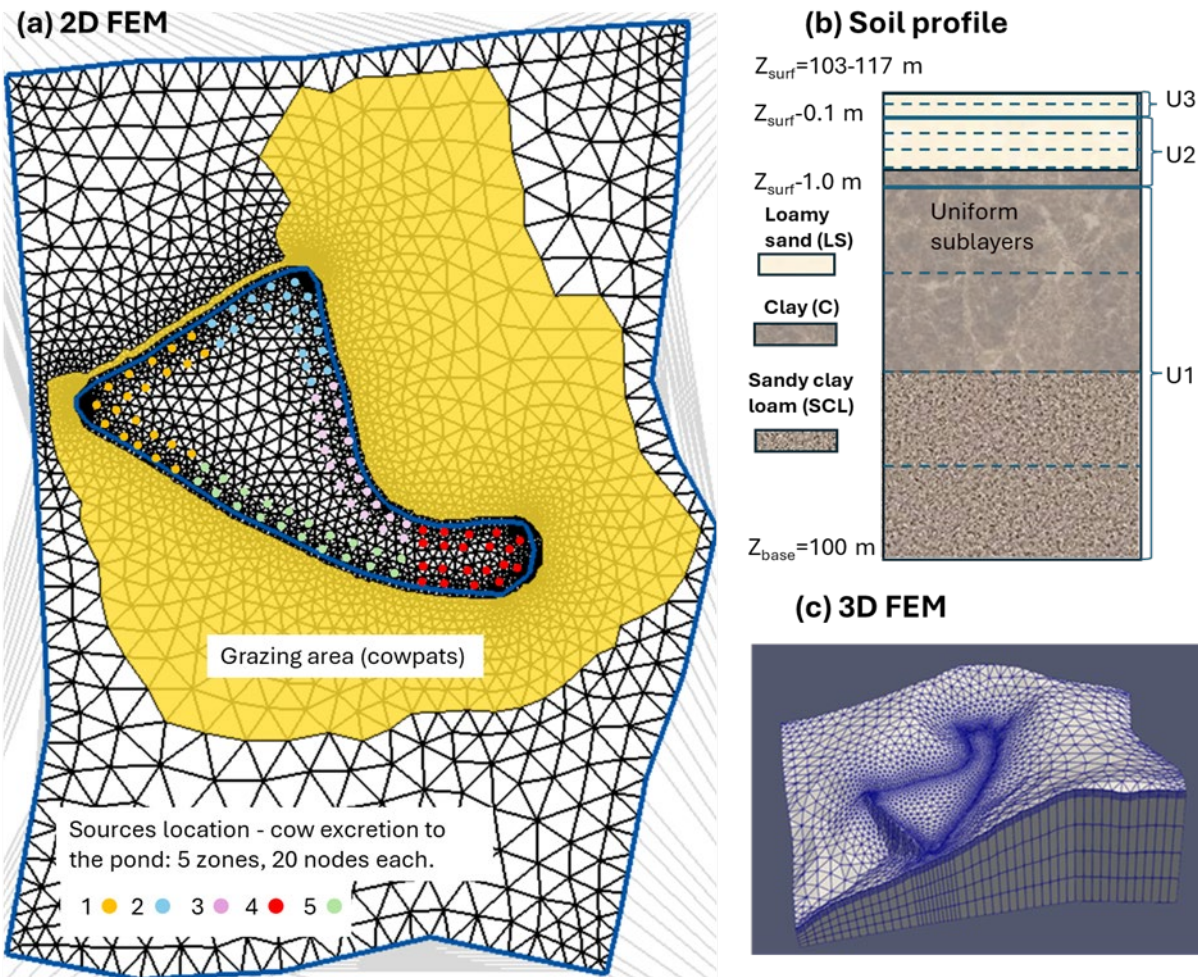
167 The numerical solution of partial differential equations and the initial and boundary conditions are done by the control volume
168 finite element approach.

169

170 2.4.1. Finite element mesh

171 The numerical setup of the simulation domain is shown in Figure 3. The lateral boundary was chosen to cover most of the area
172 where runoff water flows towards the pond. The 2D triangular finite element mesh (FEM) was created using AlgoMesh
173 software (www.hydroalgorithemics.com/software/algomesh) (Figure 3a). It consists of 2882 nodes and 5595 triangular
174 elements. The mesh has the highest density of nodes and elements near the pond boundary.

175 The base of the simulated profile is located at the depth corresponding to an altitude of 100 m. The thickness between the base
176 and soil surface was divided into three layers (U1-U3, Figure 3b), and each layer was split into 2 to 4 sublayers. The floors of
177 the layers U2 and U1 are located at depths of 0.1 and 1.0 below the land surface, respectively, thus mimicking the surface
178 topography. In the model, each sublayer is considered an independent layer defining the height of triangular prisms, which
179 presents the vertical extension of the 2D plane mesh within each layer (Figure 3c).



180
 181 **Figure 3. Numerical model setup:** (a) The 2D triangular finite element mesh in the simulation domain. Cowpats (a flat, round piece of cow dung) are
 182 located in the yellow color area. Colored circles represent the location of sources to simulate cattle excretion in the pond. (b) Schematic representation
 183 of numerical mesh layers in the simulated profile. Solid and dashed horizontal lines represent the boundaries between layers and sublayers,
 184 respectively. (c) 3D finite element mesh (FEM).
 185

186 2.4.2. Initial conditions

187 Initial conditions were obtained by running multi-year simulations. For the subsurface flow, a constant head of -2 m in each
 188 node of the finite element mesh was used. For the surface domain, the initial water depth was 10^{-7} m in each node, i.e., the
 189 pond was assumed to be empty. These initial conditions were corrected during simulations: 1) For two years, a constant
 190 precipitation rate (flux at the soil surface of 0.01 m/day) was simulated to "fill in" the pond with water and establish a steady-
 191 state flow regime in the simulation domain; 2) the resulting distribution of heads was taken as a new initial condition and the

192 model was run with varying precipitation and evapotranspiration for 6 years to establish a quasi-stationary flow regime; 3)
193 Finally, the initial conditions were taken from the results obtained at the end of the simulations.

194 Initially, the prescribed concentration of bacteria was equal to 0 everywhere in the domain. Then, during multi-year
195 simulations, a concentration distribution was developed in the simulation domain, exhibiting cyclic behavior with time, and it
196 was accepted as the initial condition for January 1, 2022.

197

198 **2.4.3. Boundary conditions and internal source terms**

199 In the study area, the groundwater level of a regional artesian aquifer was detected at around 70 meters above mean sea level
200 (mamsl) (Watson, 1976), which is 30 m below the bottom (100 m) of the simulated domain. Therefore, the groundwater is not
201 included in simulations. The free drainage boundary condition at the bottom and no flux at the lateral boundaries were
202 prescribed.

203 At the soil surface, time-variable precipitation rate and evapotranspiration were prescribed. The latter is calculated from the
204 potential evapotranspiration, computed using the Penman-Monteith equation (Monteith, 1981; Danielescu, 2022).

205 The critical depth boundary condition at the watershed boundary was set to the surface flow domain. The rainfall and
206 evaporation rates are prescribed as volumetric flow fluxes per unit area.

207 For the bacteria transport, zero-gradient and zero-concentration conditions were set in the subsurface domain along the inflow
208 and outflow lateral boundaries, respectively. The third-type (Cauchy) boundary condition was set at the surface, expressing
209 the balance between bacteria's advective flux at the soil surface and their advective and dispersive fluxes below. The land
210 surface is divided into a grazing area with cowpats and the rest (Figure 3a). Cowpats were assumed to be uniformly distributed
211 over the grazing area. The boundary concentration (and mass flux) equals zero in the area free from cowpats. For the grazing
212 area, a sub-model was developed to calculate the boundary concentration of bacteria. The sub-model simulates the daily
213 evolution of concentration depending on the load of cowpats and the initial concentration of bacteria (see Appendix A for the
214 equations). On each specific day, the fate of bacteria concentration in cowpats that were loaded during that, and every previous
215 day was calculated using the Q10 model (Martinez et al., 2013). The latter computes the bacteria's die-off/survival rate
216 depending on weather conditions. The concentrations of released microorganisms are calculated as a function of precipitation
217 according to Bradford and Schijven's (2002) equations for each cowpat's load, accounting for the remaining mass of bacteria
218 during the current day. The resulting boundary concentration is assessed as a sum of those concentrations.

219 Cattle excretion to the pond was simulated by introducing the internal source terms in the FEM nodes (Figure 3a). The
220 nearshore pond area, where bathing cattle were observed, was divided into five zones. Each zone includes twenty FEM nodes.
221 These nodes do not coincide with the water sampling locations. The source rates were calculated from the number of cattle
222 and the time they spent in the pond (Section 3.1). The time-variable boundary conditions and source/sink terms were prescribed
223 on a daily scale.

224

225 **2.5. Model parameters**

226 We adopted the values of model parameters from different sources or estimated based on existing experimental data or during
 227 simulations. Tables 1 and 2 present parameter values used in the simulations.

228 **Table 1. The model parameter values for the subsurface domain.**

Parameter	Soil			Source
	Loamy sand	Sandy clay loam	Clay	
Hydraulic conductivity K_s , m d ⁻¹	1.2(1.28)	0.35(0.36)	0.0001-0.01	Using Rosetta & PSD ¹
Porosity q_s	0.39(0.36)	0.45	0.48 (0.46)	Using Rosetta & PSD
Residual water content q_r	0.049(0.046)	0.08	0.067(0.098)	Using Rosetta & PSD
van Genuchten α , m ⁻¹	3.12(3.6)	2.35(2.29)	2.0 (1.5)	Using Rosetta & PSD
van Genuchten β	1.48(2.02)	1.36(1.35)	1.41(1.25)	Using Rosetta & PSD
Longitudinal dispersivity a_L , m	12.5			Assessed by trial-and-error
Transverse dispersivity a_T , m	2.5			Assigned $a_T=0.2 a_L$
<i>E. coli</i> distribution coeff. k_D , L kg ⁻¹	14.5			Mankin et al. (2007)
<i>E. coli</i> die-off rate, $k_{s,m,2}(20)$, d ⁻¹	0.042(cowpats), 0.111(soil)			Martinez et.al. (2013)
Parameter $Q_{10,m}$	1.48(cowpats), 1.65(soil),			Park et. al. (2016)

229 ¹PSD – particle size distribution

230

231 The geology of the subsurface is not well known. The following composition of soil layers was accepted, based on available
 232 information: loamy sand to a depth of 0.5 m, below which a clay layer of variable thickness extends down to the upper half of
 233 layer U1 (Figure 3b). The lower half of U1 is presented by sandy clay loam. The pond bottom (to 0.5 m depth) and the dam
 234 are built from clay with the hydraulic conductivity of 0.0002 m/day.

235

236 **Table 2. The model parameter values for the surface and evapotranspiration domains.**

Parameter	Value	Source
Manning X friction factor S_{fx} , m ^{-1/3} s	0.3 (grassland), 0.03 (pond)	HGS Introductory Manual
Manning Y friction factor S_{fy} , m ^{-1/3} s	0.3 (grassland), 0.03 (pond)	HGS Introductory Manual
Rill storage height, m	0.05 (grassland), 0.01 (pond)	HGS Introductory Manual
Coupling length l_{etch} , m	0.01	HGS Introductory Manual
Longitudinal dispersivity a_L , m	5-15	Assessed by trial-and-error
Transverse dispersivity, a_T m	1-3	Assigned $a_T=0.2 a_L$
<i>E. coli</i> die off rate in water, $k_{s,m,2}(20)$, d ⁻¹	0.056	Blaustein et. al (2013)

Parameter $Q_{10,m}$	1.415	Blaustein et. al (2013)
Evaporation depth, m	0.3	HGS Introductory Manual
Root depth, m	1.8	
Leaf area index (LAI)	2.08	HGS Introductory Manual
Transpiration fitting parameters: C1, C2, C3	0.1, 0.05, 2.0	HGS Introductory Manual
Wilting point pressure head, m	-150	HGS Introductory Manual
Field capacity pressure head, m	-3.8	HGS Introductory Manual
Evaporation limiting pressure heads	Min: -1.5, Max: -0.5	HGS Introductory Manual
Canopy storage parameter	0.	HGS Introductory Manual

237

238 *E. coli* fate and transport simulations were performed for 2022-2023. To progress the numerical convergence and reduce
239 simulation time, we use relative concentrations $C_r = C/C_{\max}$, where $C_{\max} = 1.4 \cdot 10^{10}$ MPN·m⁻³ is the maximum value of boundary
240 concentration at the soil surface. The value of the longitudinal dispersivity values for the porous media domain was chosen to
241 be 10 m, considering the scale problem (Neuman, 1990). There is no data concerning longitudinal dispersivity for the overland
242 flow domain. Therefore, we tested a few values of this parameter from 5 to 15 m. Longitudinal dispersivity equal to 12.5 m
243 produced a better agreement between simulated and observed concentrations. The transverse dispersivities were 1/5 of the
244 longitudinal ones for porous media and overland flow domains.

245 *E. coli* die-off/inactivation rates exhibit considerable variability depending on environmental conditions, e.g.,
246 temperature, moisture, pH, organic matter content, attachment to particles, solar radiation, predation, and matrix type such as
247 manure, soil, runoff, or pond water (Lim and Flint, 1989; Soupir, 2007; Ravva and Korn, 2007; Muirhead, and Littlejohn,
248 2009; Oliver et al., 2010; Tran et al., 2020). The *E. coli* die-off parameters in Table 1 and 2 were taken as average over the
249 parameters for datasets presented in published databases. The Q10 model parameters of *E. coli* survival in water were averages
250 over 16 survivals in wastewater datasets presented in the work of Blaustein et al (2013), and the Q10 model parameters of *E.*
251 *coli* survival in manure were averages over seven experimental datasets with bovine manure published by Martinez et al.
252 (2013). The *E. coli* temperature-dependent die-off in manure, soil, and water was simulated. The current version of HGS does
253 not allow the die-off rate in the surface flow domain to be prescribed as a function of temperature. Therefore, this parameter
254 (Tables 1 and 2) was recalculated for each three-month-long season using mean temperature values and used piecewise by
255 restarting simulations for each time interval.

256 **3. Results and discussion**

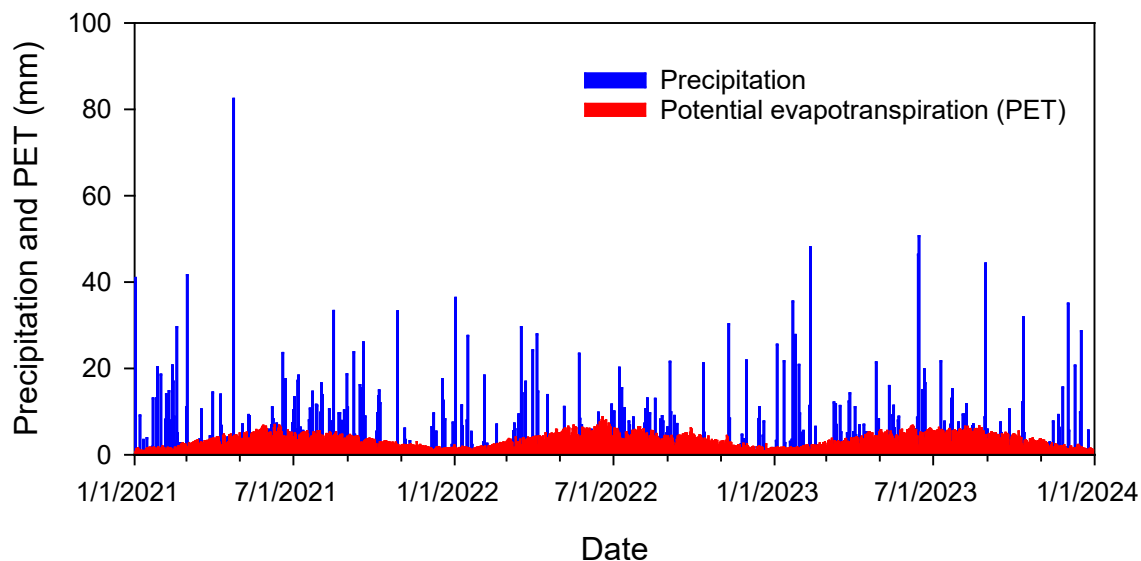
257 **3.1. *E. coli* boundary conditions and internal source terms**

258 All simulations were carried out using available weather data for a time interval from January 1, 2021, to December 31, 2023.

259 Annual precipitation was 1375, 998, and 1167 mm in 2021, 2022, and 2023, respectively. Figure 4 shows rainfall and calculated

260 potential evapotranspiration in the study area in 2021-2023.

261



262

263 **Figure 4. Precipitation and calculated potential evapotranspiration.**

264

265 The surface Cauchy-type boundary concentration at the grazing area (Figure 3a) was calculated using the equations presented

266 in Appendix A, as described above in section 2.3.3. We assume that 50 cattle (the maximum number of animals that appeared

267 in monitoring camera photos) were grazing in the watershed. The total grassland area of around 60000 m² was estimated based

268 on recent aerial imagery provided in Google Earth (version Pro 7.3). Nennich et al. (2005) estimated the average daily load of

269 manure of 38.6 and 66.3 kg·day⁻¹·cow⁻¹ for dry and lactating cows, respectively. The ratio of urine to feces in dairy cows varies,

270 but on average, slightly less than one-third of manure is urine. Thus, we estimate average daily feces excretion as

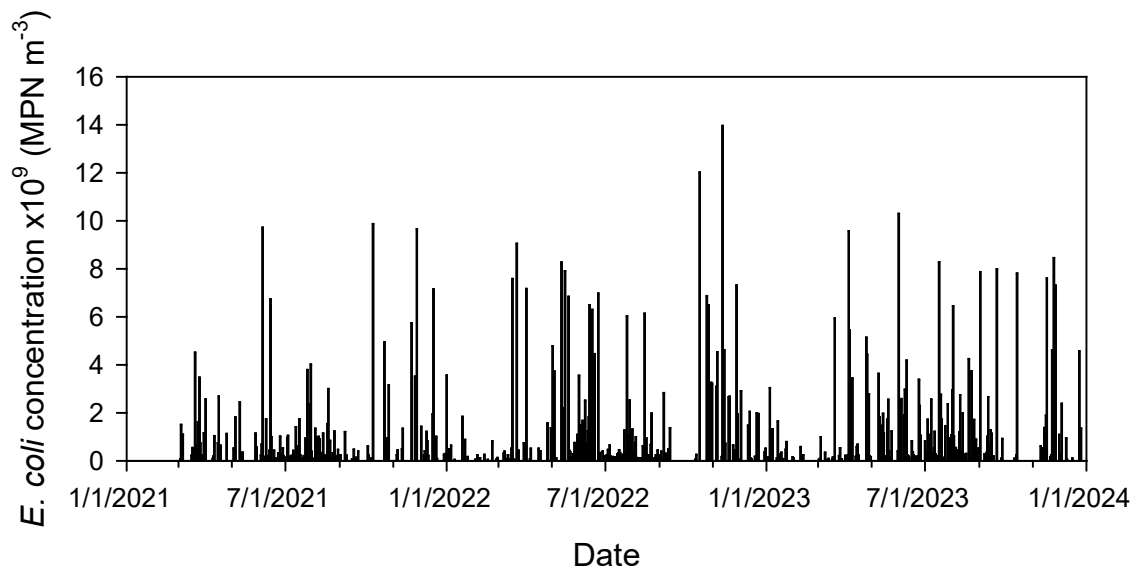
271 $0.5 \cdot (38.6 + 66.3) \cdot 0.667 = 35 \text{ kg} \cdot \text{day}^{-1} \cdot \text{cow}^{-1}$. Monitoring shows that during the day, cows usually graze for at least 12 hours.

272 Therefore, we estimate solid manure load $M_0 = \text{kg} \cdot \text{day}^{-1} \cdot \text{cow}^{-1} \cdot 12 \text{h} / 24 \text{h} \cdot 50 \text{ cows} / 60000 \text{ m}^2 = 0.0146 \text{ kg} \cdot \text{m}^{-2}$. The initial

273 concentration of *E. coli* in fresh cowpats, $m_{i0} = 7.38 \cdot 10^8 \text{ MPN} \cdot \text{kg}^{-1}$, was calculated as an average in 16 samples collected in

274 2023. Other parameters in equations A3-A4 were $\alpha_m = 23.375 \text{ h}^{-1}$, $\beta_m = 1.732$, and $E_r = 1$ (Stoker et al., 2018). No cattle

275 were observed in the field from the beginning of December to the end of February. Figure 5 shows the calculated boundary
276 concentrations.

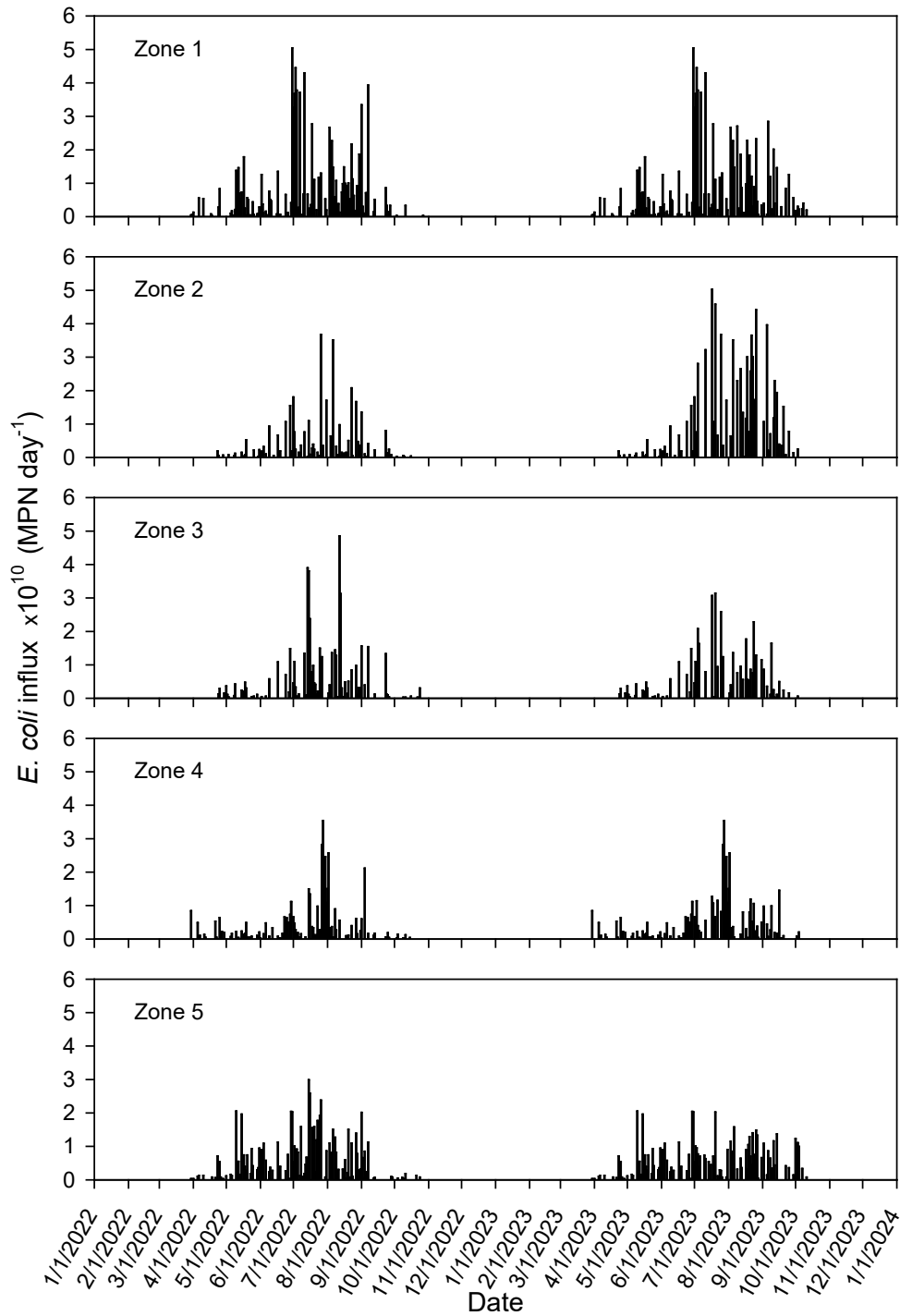


277

278 **Figure 5. Simulated temporal variation of *E. coli* surface boundary concentration at the grazing land.**

279

280 The influx of source terms representing *E. coli* loading from direct cattle excretion into the pond was calculated as described
281 in Section 2.4.3. Validation of the visual assessment of trail camera imagery to derive Cex yielded strong correlations among
282 the three observers (OBS1 v OBS2, n = 64, R² = 0.93; OBS1 v OBS3, n = 46, R² = 0.90; OBS2 v OBS3, n = 18, R² = 0.99). It
283 was assumed that all bacteria were immediately released from manure in the pond. Accounting for the daily cow excretion (as
284 explained above for the surface boundary concentration) and *E. coli* concentration in cowpats, we calculated the daily source
285 rate for each pond zone (Figure 6) as a portion of the daily manure excretion. The rate for each node is one-tenth of the zone
286 rate. Cattle use monitoring started in July 2022. Therefore, in simulations for the period January-June 2022, where there were
287 no observations, the calculated rates for 2023 were used.



288

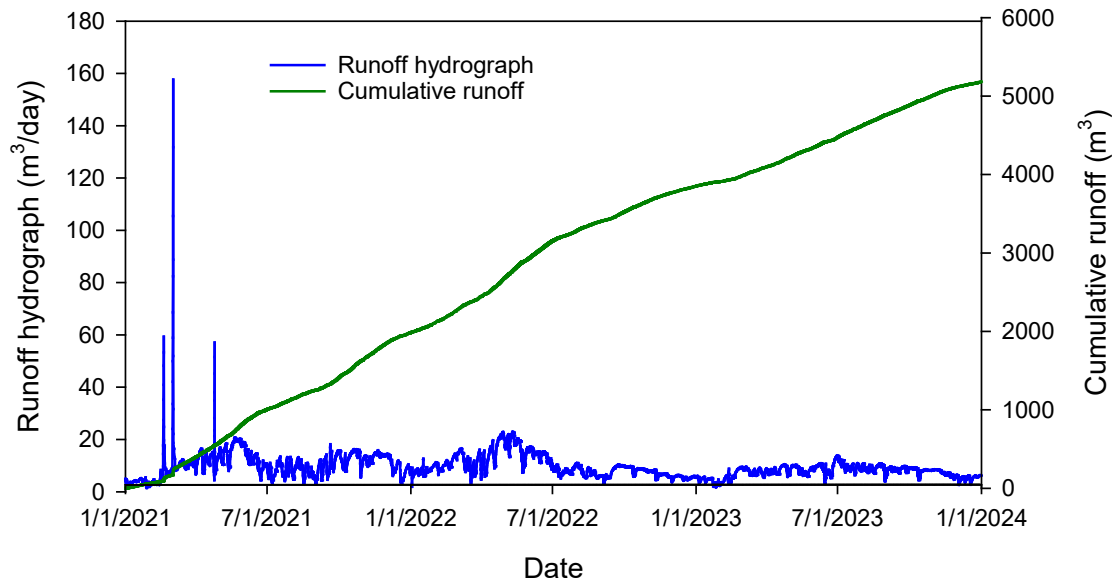
289 **Figure 6. Calculated *E. coli* inputs to the pond through cattle excretion for the five zones.**

291 **3.2. Flow simulation results**

292 Water flow simulations were carried out for 2021-2023. The simulated water level in the pond is controlled by a balance of
 293 inflows (precipitation and overland runoff) and outflows (evaporation, infiltration through the pond bottom and dam). To
 294 ensure realistic pond persistence during the multi-year simulation period, while preventing unrealistic complete drying while
 295 avoiding excessive overflow, we fitted the hydraulic conductivity of the clay liner at the pond bottom to a value of 0.0002
 296 m/day. Increasing this parameter above 0.0002 m/day resulted in excessive seepage losses, leading to a significant and
 297 unrealistic decline in the simulated pond water level over the simulation period, which was inconsistent with observed or
 298 expected pond behaviour in the study area.

299 The rest of the parameters are presented in Table 1. During periods with high precipitation, perched water was developed
 300 above the clay layer (around 0.5 m below the land surface, not shown). Daniels et al. (1978) reported that soil horizons having
 301 10% of platy plinthite will perch water.

302 Runoff is rarely observed in the area. The HGS model calculates fluid fluxes across the pond boundary. Water fluxes are
 303 computed between active nodes (located on the pond boundary—dark blue dots in Figure 3a) and contributing nodes (located
 304 just outside the pond boundary). The calculated surface water flux represents the simulated runoff to the pond (Figure 7).



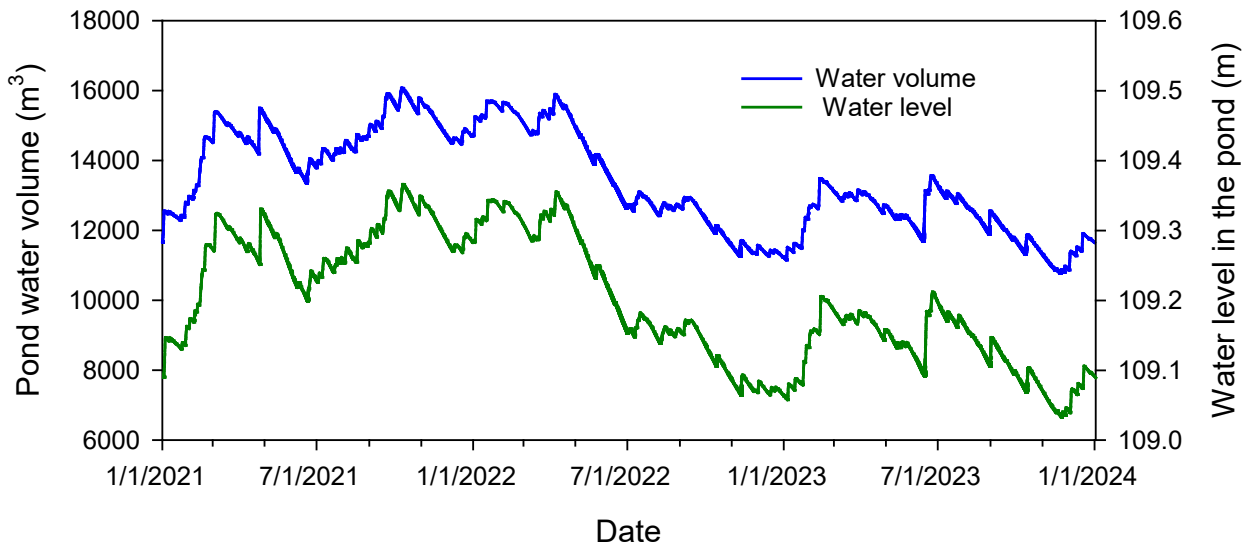
305

306 **Figure 7. Computed runoff to the pond.**

307 At the beginning of 2021, three significant flow events occurred after intensive 30, 50, and 82 mm rainfalls on February 18,
 308 Mar 1-2, and April 24, respectively. During the rest of the time, the average computed surface flow rate to the pond is around

309 4.4 m³·day⁻¹. The latter is a relatively small value given a 650 m-long pond perimeter. This small but persistent surface water
310 inflow to the pond during dry periods (no precipitation), attributable to slow drainage of shallow subsurface lateral flow
311 (interflow or return flow) from upslope areas that daylight and reaches the pond via surface pathways. This is distinct from
312 precipitation-driven overland flow/surface runoff, which occurs only during or immediately following rainfall events
313 (Tarboton, 2003).

314 Simulated changes to pond water volume (m³) and water level (mamsl) were tracked over the study period (Figure 8). The
315 simulated minimal and maximal water levels were 109.03 and 109.37 mamsl. A rise in water level by 0.34 m causes an increase
316 in water volume from 10770 to 16075 m³.



317

318 **Figure 8. Simulated temporal variation of water volume and level in the pond (above mean sea level).**

319

320

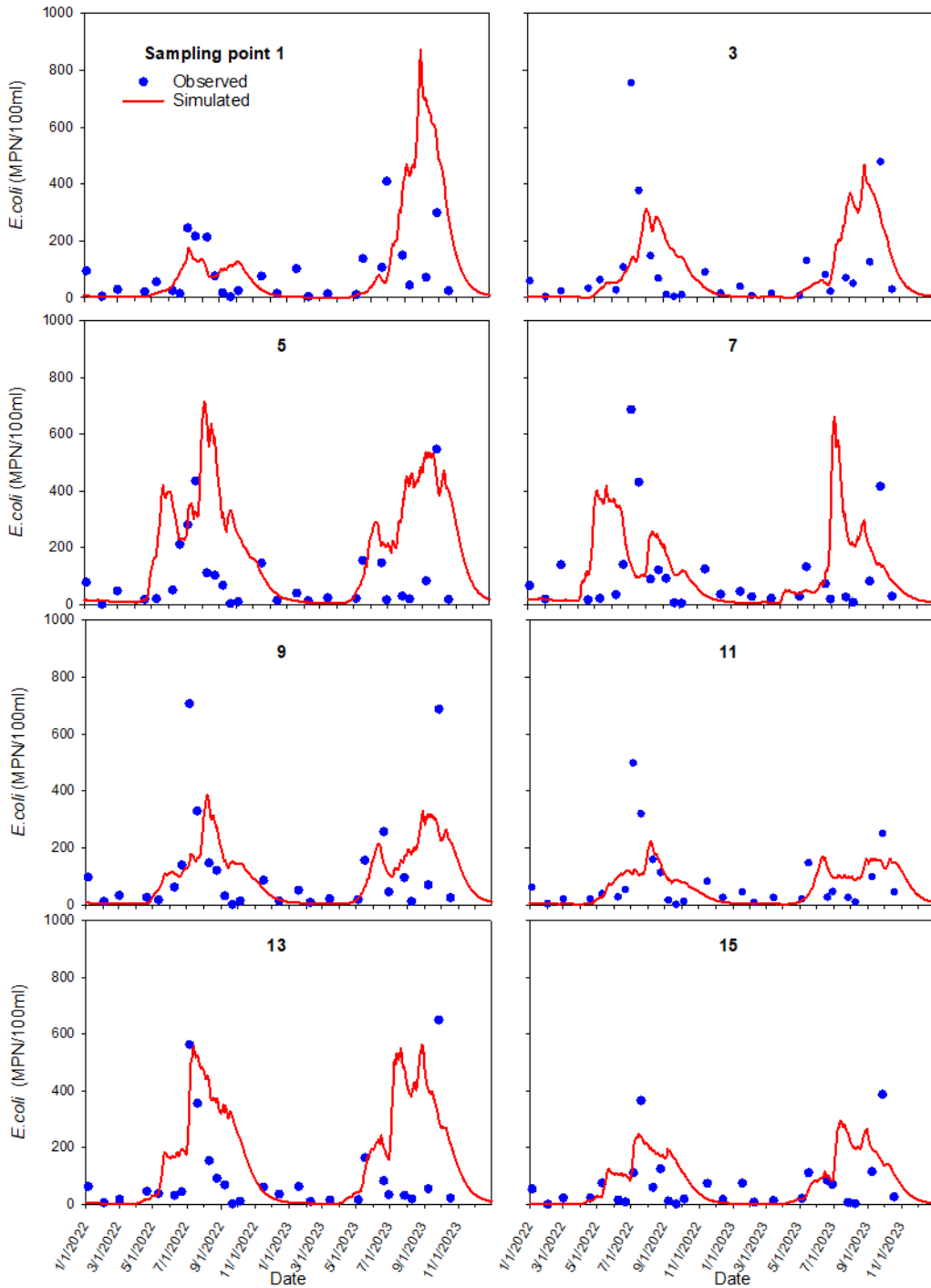
321 **3.3. Bacteria fate and transport simulations**

322 **3.3.1. Results for individual locations**

323

324 Observed and simulated *E. coli* concentrations in the interior and nearshore pond sampling locations were compared for each
325 sample location (Figures 9 and 10). The non-calibrated model tolerably mimicked *E. coli* concentration patterns and times of
326 peak events in many of the pond's sampling locations. Concentrations increased during summer and decreased during winter
327 months.

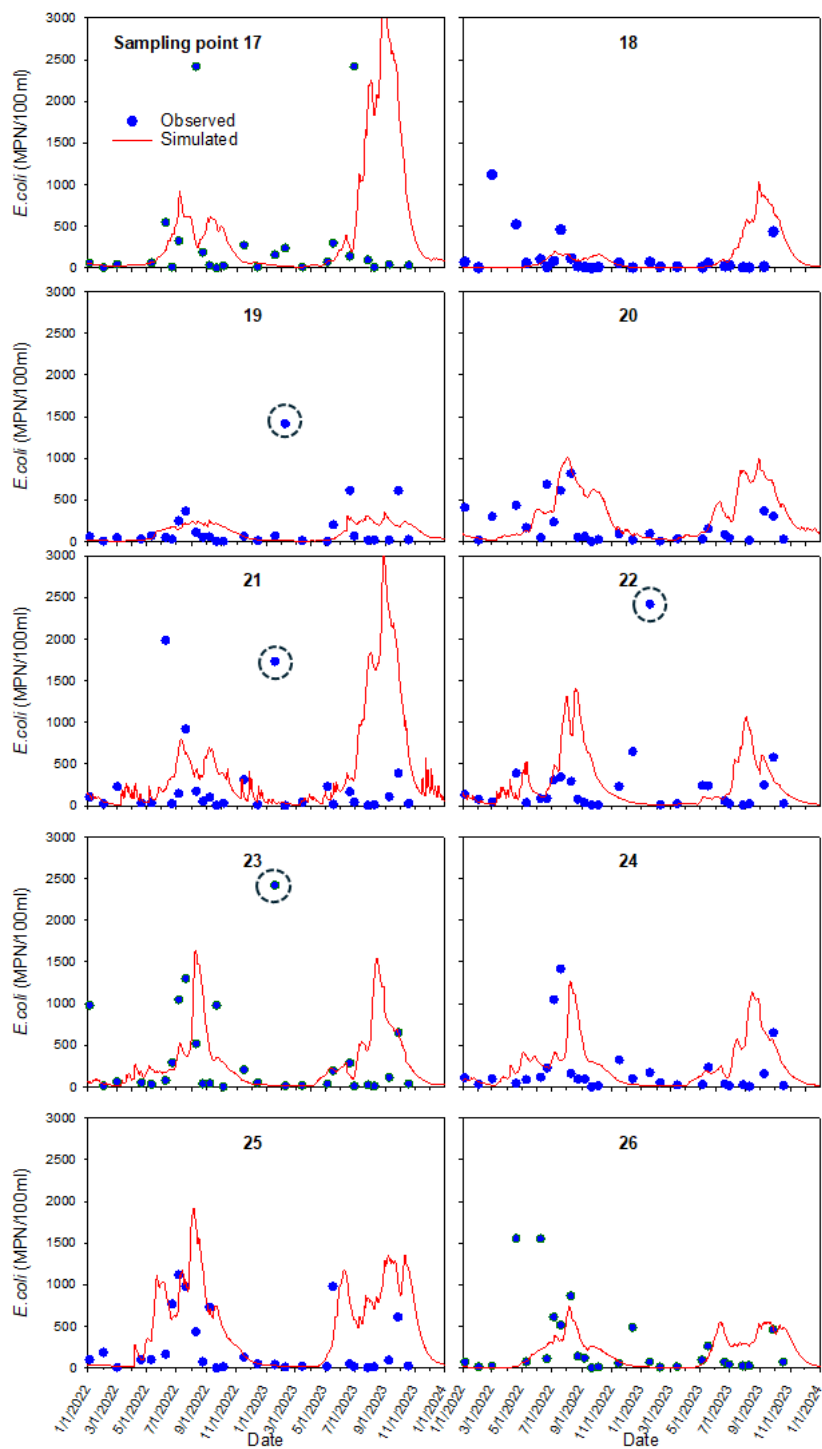
328 For the internal sampling locations 1 to 15, correlation coefficients between the monitored and simulated *E. coli*
329 concentrations varied between 0.24 and 0.44. An exception was location 7, where the correlation coefficient was -0.01. For
330 the near-shore sampling locations 17 to 26, the correlation coefficient varied between -0.15 and 0.32.
331



332

333 **Figure 9. Observed (blue dots) and simulated (red line) *E. coli* concentrations at interior sampling locations 1-15.**

334



336 **Figure 10. Observed (blue dots) and simulated (red line) *E. coli* concentrations at nearshore sampling locations 17-26. Dashed circles**
337 **indicate winter increases in concentrations on January 18, 2023.**

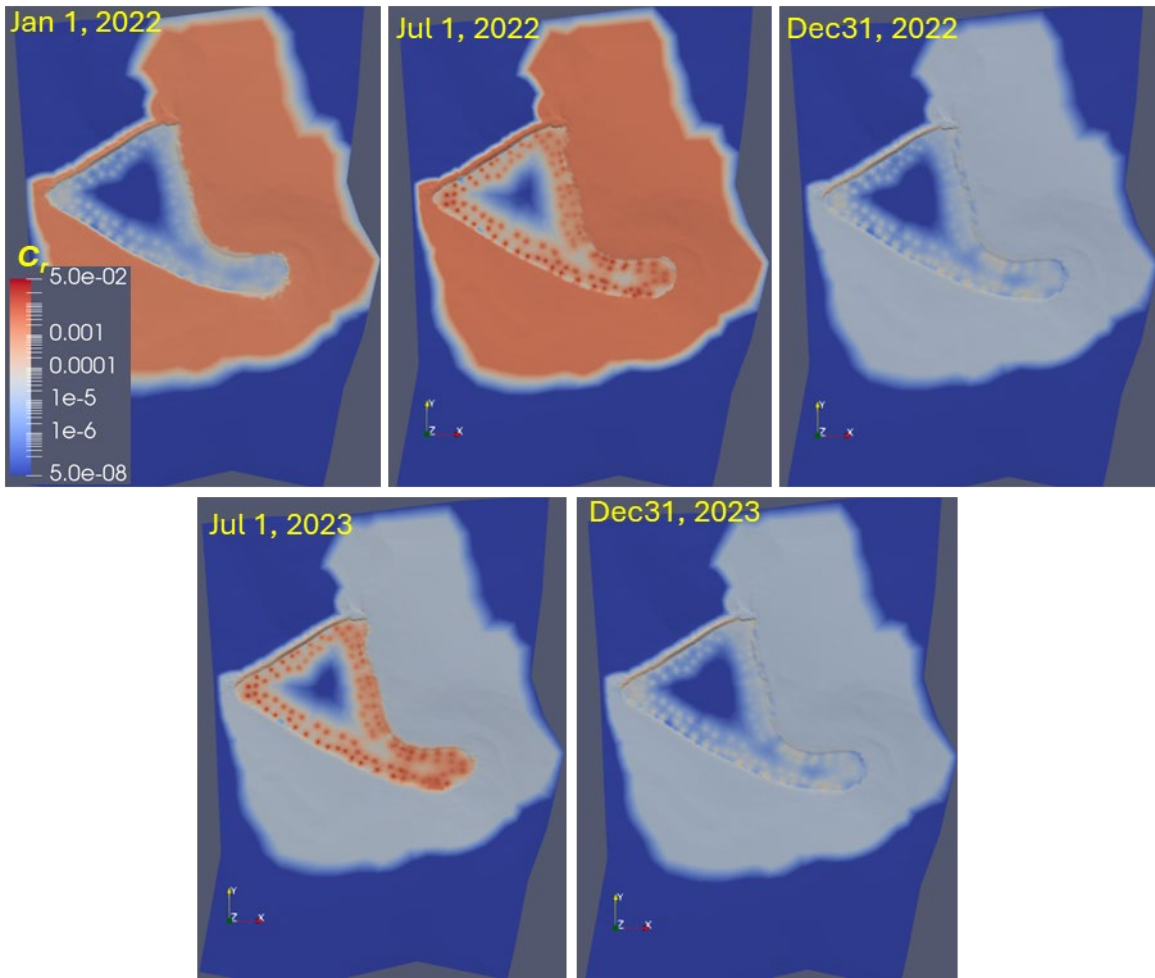
338

339 The observed differences between simulated and measured concentrations in individual sampling locations might
340 originate from the model setup and computational features. The simulated concentration peaks strongly depended on the
341 proximity of the sampling points to the internal source locations, which were constant in time. Cattle frequently moved across
342 the pond in different directions, which affected the concentration distribution. Most assigned locations for the pond's internal
343 source were 10-20 m from the nearest sampling point.

344 The reproduction of mixing in the pond might be locally unsatisfactory. Figure 11 shows the simulated relative *E.*
345 *coli* concentration distribution at the surface for different dates. Concentration in the source locations, simulating cattle
346 excretion in the pond (Figure 3a), rises during summer and dissipates in the winter. Simulations show a low *E. coli*
347 concentration zone in the middle of the pond during the entire simulation period. For example, the model underpredicts peak
348 concentration at sampling location 15 (Figure 9) in summer 2022 and autumn 2023. This indicates that mixing in the pond was
349 stronger than the dispersion mechanism suggests. Lateral transport is often dependent on persistent wind-forced circulation.
350 Henderson et al. (2024) describe wind-forced processes responsible for ponds' vertical mixing or lateral transport. Additional
351 mixing resulting from induced eddies may cause more rapid cross-pond mixing and potentially affect pond ecology and
352 biogeochemistry. At sampling location 23, the increase in concentration could be due to the occasional resuspension of bacteria
353 from the bottom sediments during sampling.

354

355



356

357

358 **Figure 11.** Simulated spatiotemporal distribution of the relative *E. coli* concentration at the surface. $C_r=C/C_{\max}$, $C_{\max}=1.4 \cdot 10^{10}$

359 $\text{MPN}\cdot\text{m}^{-3}$, the legend is on a log scale.

360

361

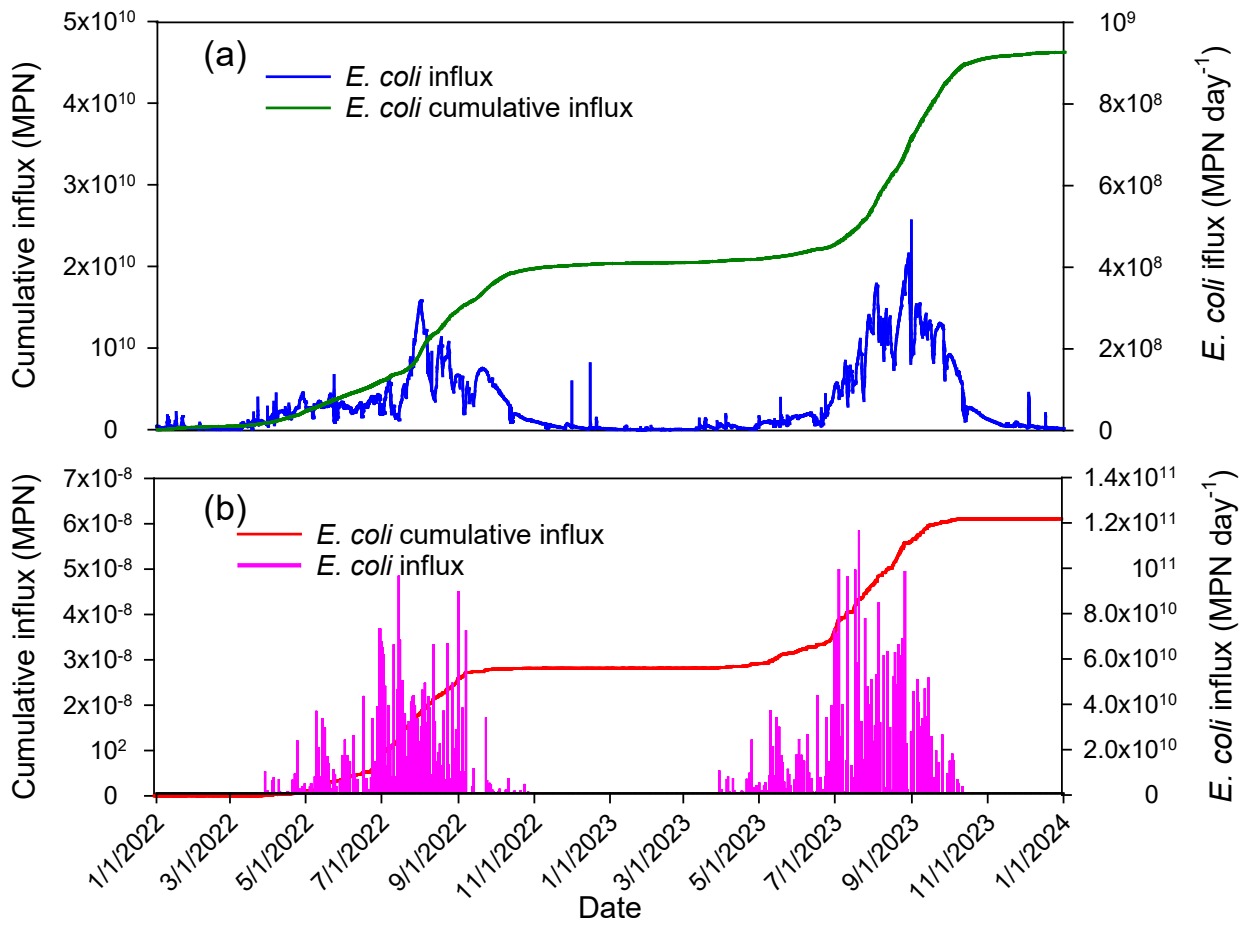
362

363 More insight could be expected from considering the specifics of *E. coli* survival in waters rich in organic matter and
364 nutrients (Cho et al., 2021). Blaustein et al (2013) analyzed the database on *E. coli* survival in various surface waters. They
365 found that the logarithm of *E. coli* remained approximately constant or even grew for some lag period time after the inactivation
366 experiments in wastewaters with high content of organic matter and nutrients. After the lag period. The dependence of log C
367 on time changed to a linear decrease. No model has been proposed to estimate the duration of the lag period in wastewater so
368 far.

369 A sudden rise in *E. coli* concentration was observed on January 18, 2023, at sampling locations 19, 21, 22, and 23
370 (Figure 9, dashed circles). The model does not reproduce this increase. The monitoring camera photos show flocks of birds in
371 the pond near the first three locations 1 to 2 days before the sampling date. At the same time, the model's internal sources on
372 that day were equal to zero since there were no cattle in the pond. We hypothesized that excretion by birds was a reason for
373 elevated *E. coli* concentrations. During the fall and winter, Georgia's inland freshwaters become populated with waterfowl
374 such as ducks, Canada geese, and migratory birds (Balkcom et al., 2025). One duck generates on average $3.8 \cdot 10^{10}$ *E. coli* CFU
375 per day (Moriarty et al., 2011), which is similar to the daily *E. coli* output from one cow ($4 \cdot 10^{10}$ MPN (g wet feces)⁻¹ day⁻¹
376 in this work). It appears that the contribution of waterfowl can be very substantial in comparison with cattle contributions (see
377 Figure 5). To our knowledge, the fraction of waterfowl excreta that enters water has not been reported in the literature. Overall,
378 we concur with Vasquez et al. (2021) who emphasized the need to collect more data on the fecal contamination inputs of the
379 ponds.

380 **3.4. Results for the pond as a whole**

381 The HGS computed temporal *E. coli* fluxes entering the pond with surface water. The influxes of *E. coli* to the pond were
382 visualized along with their calculated cumulative numbers over time for both sources of runoff (Figure 12a) and direct
383 excretion of feces (Figure 12b). At the end of simulations, the number of bacteria entering the pond by manure excretion was
384 around 130 times greater than by surface runoff. Simulations show that water flowed mainly from the pond into the subsoil,
385 so that *E. coli* concentrations in the subsoil did not affect the water quality in the pond. .



386
 387 **Figure 12. Simulated *E. coli* fluxes to the pond: (a) with runoff; (b) excreted into the pond.**
 388

389

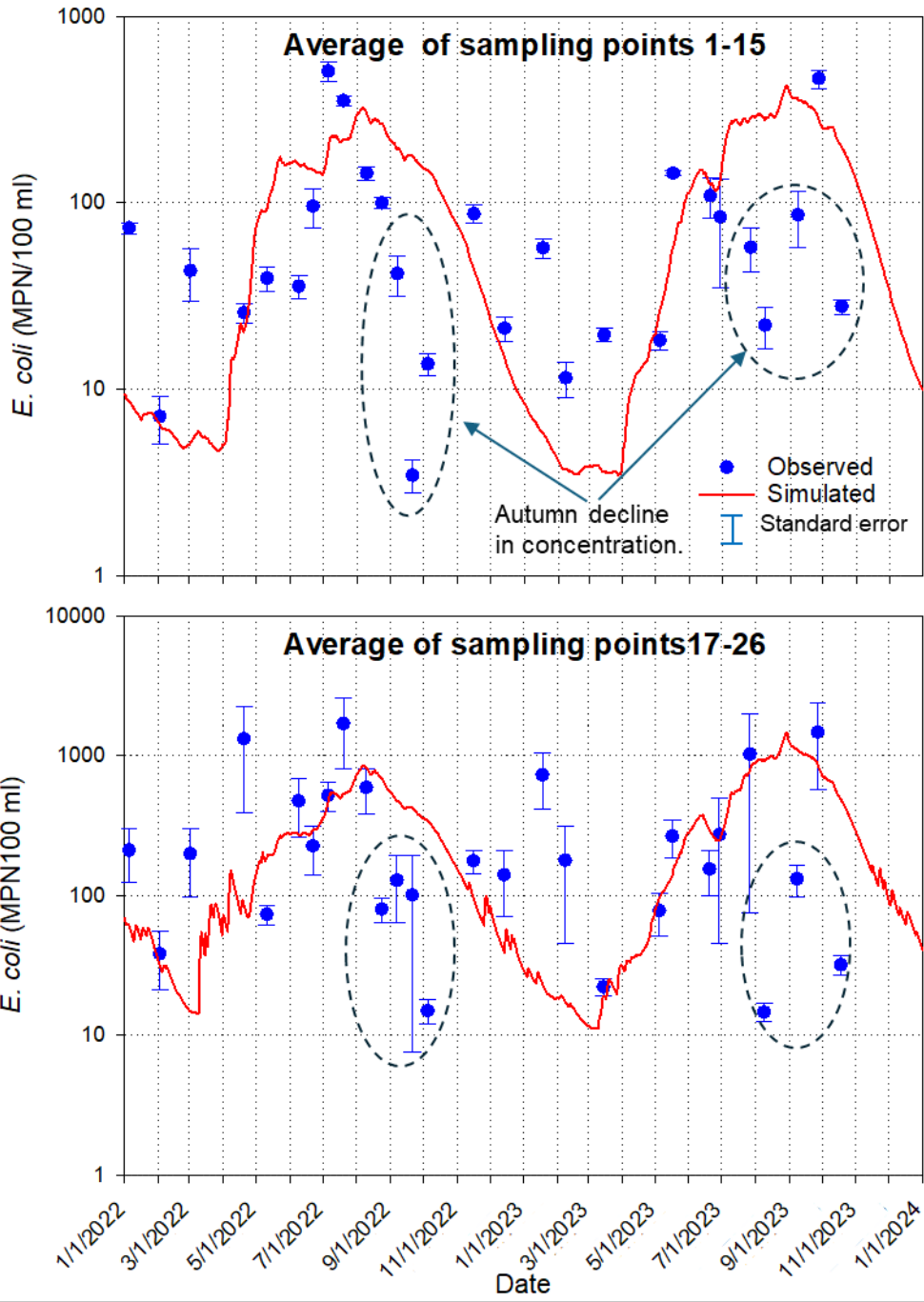
390

391 Simulated and observed concentrations of *E. coli* were each averaged over the interior and nearshore sampling
392 locations (Figure 13). Averaged values of concentrations at the nearshore locations (17-26) were approximately three times
393 higher than the average of concentrations sampled at interior locations (1-15). Elevated nearshore concentrations may be
394 explained by flushing bacteria to the pond during runoff and direct excretion by bathing cattle, which, according to monitoring,
395 spent most of their time grouped close to the shore.

396 The summary data in Figure 13 show that the model describes seasonal average concentration patterns relatively well.
397 However, observed concentrations significantly declined during autumn 2022 and 2023 compared to the model simulation.
398 Clear, shallow water can cause deeper penetration of solar radiation. De Brauwere et al. (2014) indicate that the inactivation
399 of bacteria is caused by UV irradiation. To account for the latter, some models add a term to the overall decay parameter due
400 to sunlight (McCorquodale et al., 2004) or use a decay term depending on the intensity of the solar radiation (Kashefipour et
401 al., 2006). The decrease in the nutrients in water in the fall can also be the reason for the reduction of *E. coli* concentration.

402 The descriptive statistics of measured and simulated average concentrations were close when computed over the
403 observation period. The minimum and maximum averages across the pond concentrations were 3.56 and 525.1 for observed
404 and 4.08 and 479.4 MPN (100 mL) for simulated data, respectively. The mean logarithms of average concentrations were
405 1.70 ± 0.47 and 1.99 ± 0.67 for the measured and simulated data, respectively. The correlation coefficient between measured
406 and simulated logarithms of average concentrations was 0.483. This value was significant at a 0.01 significance level and
407 indicated a moderate correlation.

408



409

410 Figure 13. Average *E. coli* concentrations in the pond's interior (1-15) and nearshore (17-26) sampling locations. Dashed circles
 411 indicate an autumn decline in concentrations.

412

413 4. Discussion

414 Applying mechanistic fate and transport models relies on model parameter values typically obtained via model calibration.
415 Several factors make the site-specific calibration of microbial fate and transport parameters difficult and sometimes unfeasible.
416 The spatiotemporal variability of microbial concentrations in ponds is very high (Havelaar et al., 2017; Stocker et al., 2022),
417 indicating the need to collect large numbers of samples. The collection and analysis of those samples appear to be unfeasible.
418 That precludes establishing a proper monitoring program for efficient model calibration. The high spatiotemporal variability
419 emphasized the scale mismatch between the water sample size and the water volume, and this sample is used to characterize a
420 much larger water volume used for mass balance computation in the hydrological models.

421 Flow sub-model parameters have satisfactorily estimated more easily obtainable properties, such as clay or sand content
422 and bulk density (Schaap et al., 2001). Analogous predictive relationships have not been developed, partially because the
423 potential predictors of microorganism survival or release rate have been shown to be dependent on environmental variables
424 that were themselves variable in space and time.

425 Compendia of microorganism release and survival parameters (Park et al., 2016) show that the parameter values
426 encompass wide numerical ranges and that it is challenging to attribute fate and transport parameter values to specific
427 environmental conditions or management practices. These factors so far substantially limit the applicability of microbial fate
428 and transport modeling. On the other hand, such modeling is in demand due to the need for projections of microbial water
429 quality changes due to environmental changes, adaptation practices, site-specific trade-offs between different water quality
430 aspects, multiple ownership and management along or around irrigation water sources, etc.

431 In this situation, a relatively important question is: What accuracy can be expected in fate and transport predictions made
432 with average or typical fate and transport parameter values? In other words, given the high spatial variability of microbial
433 concentrations, how significant are the differences between observed and simulated concentrations with average parameter
434 concentrations? The answers to those questions are surprisingly scarce, as the published modeling reports focus on calibration
435 results. These questions have not been researched for agricultural ponds. Results of this work show that if the animal behavior
436 patterns are known, the seasonal trends and magnitudes of pond water microbial pollution can be estimated. Quantifying such
437 patterns for cattle ponds has not been done so far. However, it presents a promising avenue for research.

438 We realize that the system parameters we have studied are subject to multiple sources of uncertainty. Quantifying this
439 uncertainty and running ensemble simulations with parameters treated as random values presents an interesting avenue for
440 future research. Results of this work, obtained with mean parameter values from various sources, indicate that determining the
441 statistical properties of the bacteria sources can be a first feasible step in that direction.

442 The present model does not incorporate explicit simulation of soil and manure erosion, suspended sediment transport,
443 settling, or resuspension processes. This omission may underestimate bacterial concentrations in receiving waters during high-
444 flow events, when sediment resuspension, potentially exacerbated by cattle trampling or other disturbances can act as an
445 important secondary source of fecal indicator bacteria. Numerous studies have highlighted the significance of sediment-

446 associated bacterial transport and resuspension in agricultural watersheds (e.g., Jamieson et al., 2005; Pandey et al., 2012;
447 Bradshaw et al., 2021). Future model extensions could benefit from coupling a sediment transport sub-module to more
448 comprehensively capture these interactions.

449 The current implementation does not include site-specific calibration or formal sensitivity analysis of key parameters (e.g.,
450 die-off rates, sorption coefficients as listed in Tables 1 and 2). While these steps would provide valuable insights into parameter
451 influence and model behavior, and are recommended for future applications, the present work prioritizes proof-of-concept
452 integration and qualitative assessment over quantitative optimization. Similarly, the model currently incorporates a single
453 dominant removal process (first-order die-off/inactivation) to maintain simplicity during initial coupling; additional
454 mechanisms (e.g., settling, resuspension, or biotic interactions) were not included but represent promising extensions. Such
455 enhancements could better elucidate ecosystem functioning and inform management strategies to enhance bacterial removal
456 in pond systems, as supported by prior modeling efforts in watershed-scale microbial fate and transport (e.g., Ferguson et al.,
457 2010; Bradford et al., 2013; Cho et al., 2016).

458 This study contributes to the current state-of-the-art in several ways. First, it applies a fully mechanistic three-dimensional
459 surface–subsurface hydrological model to simulate microbial fate and transport in and around a cattle pond. Unlike many
460 previous studies that rely on simplified or lumped representations of microbial transport, the present approach explicitly
461 resolves hydrological processes controlling bacterial transport through surface runoff and subsurface flow pathways at the
462 watershed scale. Second, the model explicitly accounts for direct microbial loading caused by cattle entering the pond and
463 depositing manure in the water. The magnitude of this source term was estimated from the observed presence of cattle in the
464 pond using time-lapse imagery obtained from multiple trail cameras. To our knowledge, such direct observational
465 quantification of livestock behavior has rarely been incorporated into mechanistic watershed-scale microbial transport models.
466 Third, the study evaluates the ability of a mechanistic model to reproduce observed *E. coli* dynamics with minimal calibration
467 using primarily literature-based parameter values. This approach addresses a common limitation in agricultural water quality
468 studies, where extensive datasets required for model calibration are rarely available. Finally, the modeling framework provides
469 quantitative insight into the relative importance of different contamination pathways, including surface runoff from manure
470 deposited on pasture and direct microbial loading by cattle entering the pond. The results demonstrate that direct excretion by
471 cattle in the pond can dominate microbial inputs under certain management conditions. Overall, this work demonstrates how
472 mechanistic watershed-scale modeling combined with observational data on livestock behavior can be used to improve
473 understanding and prediction of microbial contamination in agricultural ponds.

474

475

476

477 5. Conclusions

478 This study developed the first mechanistic numerical model for bacteria transport from grazing land around a cattle pond. The
479 model was based on the HGS software, simulating fully coupled surface-subsurface flow and transport. The model was tested
480 to simulate *E. coli* fate and transport in a small pastureland watershed in Georgia, USA, using observations from 2021-2023.
481 The primary goal was to simulate the temporal and spatial distribution of *E. coli* concentration in the pond. All parameters for
482 this simulation were taken from the literature or estimated from published data. The exception was the rate of *E. coli* input to
483 the pond from the direct excretion of feces from animals, since such data were not found in the literature. The non-calibrated
484 model could mimic *E. coli* temporal concentration patterns and peak times reasonably well in most of the pond's sampling
485 locations. There were seasonal differences in correspondence between simulated and measured *E. coli* time series, and the
486 magnitude of concentration peaks was poorly predicted in some sampling locations. Predictions of the average across-pond
487 concentrations were expected to be moderately accurate. Quantification of microbial inputs for cattle ponds has not been done
488 so far. Still, it presents a promising avenue to estimate the microbial water quality in cow ponds using the data accumulated in
489 past research.

490

491

492 Author contributions

493 AY: methodology, investigation, formal analysis, software, writing - original draft; AC: supervision, methodology,
494 investigation, data acquisition, resources, formal analysis, writing – review and editing; JW: data acquisition, resources; OP:
495 resources, writing – review and editing, RH: supervision, software; YP: conceptualization, project administration, funding
496 acquisition.

497 Declaration of competing interest

498 The authors declare that they have no known competing financial interests or personal relationships that could have appeared
499 to influence the work reported in this paper.

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503 assistance cooperative agreements 58-8042-3-030 "Modeling Fate and Transport of Indicator and Pathogenic Organisms to
504 Assess Microbial Water Quality of Irrigation Water Sources, "and 58-8042-0-064 "Monitoring and Modeling Microbial
505 Quality of Irrigation Water"

506 **Data availability**

507 Data will be made available on request.

508

509

510 **Appendix A: Equations to calculate *E. coli* concentration released from cowpats at the soil surface (3rd-kind boundary**
511 **condition concentration).**

512 For day t , the content of microorganism in the applied manure is calculated by (Martinez et al., 2013):

513

$$514 \quad m_i(t) = m_i(t - 1)e^{-k_{s,m}} \quad (A1)$$

516

515 Where $k_{s,m}$ is the rate coefficient which is the function of temperature T at time t , (d^{-1})

517

$$518 \quad k_{s,m} = \begin{cases} k_{s,m,1}, & t \leq t_{s,m,1} \\ k_{s,m,2}(T), & t > t_{s,m,1} \end{cases} \quad (A2)$$

519

520 $t_{s,m,1}$ is the duration of the first stage, d , T is the average daily temperature, $^{\circ}C$, and $k_{s,m,1}$ and $k_{s,m,2}(T)$ the survival rates at the
521 first and second survival stages are respectively.

522 The bacterial population may grow, remain stable, or die off during the first survival stage, and decrease during the second
523 stage of survival. On the second stage, the values of $k_{s,m,2}(T)$ can be described with the Q10 model (Martinez et al., 2013).

524

$$525 \quad k_{s,m,2}(T) = k_{s,m,2}(20)Q_{10,m}^{\frac{T-20}{10}} \quad (A3)$$

526

527 where $k_{s,m,2}(20)$ is the survival rate at $20^{\circ}C$, $Q_{10,m}$ reflects the sensitivity of $k_{s,m,2}$ to a temperature that is equal to the change
528 in survival rate occurring as temperature changes by $10^{\circ}C$.

529 The concentration of released microorganism C_m is calculated according to Bradford and Schijven (2002) as

$$530 \quad C_{man}(t) = \frac{dM_{man}}{Rdt} = \frac{M_0\alpha_m}{R} (1 + \alpha_m\beta_m t)^{-(1+1/\beta_m)} \quad (A4)$$

531

$$532 \quad C_m(t) = m_i E_r C_{man}(t) \quad (A5)$$

533

534 Where M_{man} is the cumulative cowpat mass released into the aqueous phase (g), R is rain intensity, cm/h , α_m (h^{-1}) and β_m are
535 fitting parameters defining the shape of the release curve, and M_0 is the initial mass of cowpats (g/cm^2), C_{man} is the aqueous

536 manure concentration (g cm^{-3}), m_i is content of microorganism in the cowpats (CFU g^{-1}), E_r is microorganism release
537 efficiency.
538

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