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1 **Experimental and modelling evidence of splash effects on manure borne**

2 ***Escherichia coli* washoff**

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23

24 **Summary:** In tropical montane South-East Asia, recent changes in land use have induced  
25 increased runoff, soil erosion and in-stream suspended sediment loads. Land use change is  
26 also contributing to increased microbial pathogen dissemination and contamination of stream  
27 waters. *Escherichia coli* (*E. coli*) is frequently used as an indicator of faecal contamination.  
28 Field rain simulations were conducted to examine how *E. coli* is exported from the surface of  
29 upland, agricultural soils during runoff events. The objectives were to characterize the loss  
30 dynamics of this indicator from agricultural soils contaminated with livestock waste, and to  
31 identify the effect of splash on washoff. Experiments were performed on nine 1 m<sup>2</sup> plots,  
32 amended or not with pig or poultry manure. Each plot was divided into two 0.5 m<sup>2</sup> sub-plots.  
33 One of the two sub-plots was protected with a mosquito net for limiting the raindrop impact  
34 effects. Runoff, soil detachment by raindrop impact and its entrainment by runoff, and *E. coli*  
35 loads and discharge were measured for each sub-plot. The results show that raindrop impact  
36 strongly enhances runoff generation, soil detachment and entrainment and *E. coli* export.  
37 When the impact of raindrops was reduced with a mosquito net, total runoff was reduced by  
38 more than 50%, soil erosion was on average reduced by 90% and *E. coli* export from the  
39 amended soil surface was on average 3 to 8 times lower. A coupled physics-based approach  
40 was performed using the Cast3M platform for modelling the time evolutions of runoff, solid  
41 particles detachment and transfer, and bacteria transport that were measured for one of the  
42 nine plots. After estimation of the saturated hydraulic conductivity, soil erodibility, and  
43 attachment rate of bacteria, model outputs were consistent with measured runoff coefficients,  
44 suspended sediment and *E. coli* loads. This work therefore underlines the need to maintain  
45 adequate vegetation at the soil surface to avoid the erosion and export of soil borne potential  
46 pathogens towards downstream aquatic systems.

47 **Keywords:** Faecal Indicator Bacteria (FIB); Raindrop impact; Runoff; Physics-based  
48 modelling; Tropical agro-ecosystems; Lao PDR

## 49 **1. Introduction**

50 Globally, there are nearly 1.7 billion cases of diarrhoeal disease every year making it the  
51 second leading cause of death in children under five years old (World Health Organisation  
52 2014). A large proportion of this disease load is directly related to the ingestion of water  
53 contaminated with microbial pathogens of faecal origin. In urban areas, the majority of faecal  
54 contamination is of human origin. However, in rural areas, livestock can also be a significant  
55 source of microbial pollution (Gagliardi and Karns 2000; Unc and Goss 2004).

56 Several bacteria are used as indicators of faecal contamination. However, *Escherichia coli* (*E.*  
57 *coli*) has emerged as one of the most appropriate indicators of microbial contamination of  
58 natural waters (Ishii and Sadowsky 2008; Rochelle-Newall et al. 2015). The presence or  
59 absence of faecal indicator bacteria (FIB) indicates whether or not faecal contamination is  
60 potentially present.

61 Runoff from manured fields and pastures, as well as direct deposition of animal waste into  
62 water are traditionally viewed as important mechanisms of *E. coli* contamination in rural  
63 watersheds (Jamieson et al. 2004). The importance of surface runoff during storm events as a  
64 mechanism of transport of soil bound *E. coli* was also highlighted by Causse et al. (2015).  
65 Ribolzi et al. (2016) showed that the erosion of particles from the soil surface during storm  
66 events is a strong source of *E.coli* in rural, montane streams. This is of importance as the  
67 majority of enteric bacteria in aquatic systems are associated with sediments and these  
68 associations influence their survival and transport characteristics (Jamieson et al. 2005;  
69 Muirhead et al. 2006; Wilkinson et al. 1995).

70 Several authors have demonstrated that overland flow and splash effect (i.e. impact of  
71 raindrops on soil surface) are major determinants for soil surface particle detachment and  
72 displacement at the plot scale in inter-rill zones (Lacombe et al. 2018; Luk 1979; Quansah  
73 1981; Ziegler et al. 2000). Detached soil particles are then transported downstream by the

74 splash related droplets and overland flow. Splash and overland flow are interacting processes:  
75 kinetic energy dissipation of raindrop impact induces the soil surface sealing, which  
76 drastically reduces infiltration (Assouline and Mualem 1997) and favours ponding and  
77 overland flow (Mügler et al. 2019); splash results from the compression of the water at the  
78 soil surface and thus requires an optimal film thickness for the greatest efficiency which is in  
79 the order of 10 mm (Kinnell 1993). Other factors influence the intensity of splash  
80 detachment: (i) kinetic energy of drops, which can be increased or reduced depending on the  
81 ground cover (Lacombe et al. 2018) or slope angle (Ribolzi et al. 2011); (ii) soil texture, with  
82 fine sands being the most sensitive; (iii) soil organic matter content that increases the  
83 cohesion of soil particles (Armenise et al. 2018); (iv) soil moisture with low splash  
84 detachment when soils are dry and increasingly higher with increasing soil moisture. These  
85 processes are often measured on plots of 1 m<sup>2</sup> (Patin et al. 2018).

86 During the rainy season in tropical humid regions, rainfall intensities are high and the splash  
87 effect is known to be a determining factor for the detachment of soil particles (Ziegler et al.  
88 2000). Therefore, given the anticipated links between rain intensity, erosion and the export of  
89 soil surface *E. coli* during rain events, it can be hypothesised that the splash effect will have a  
90 strong impact on the export of *E. coli* from the soil surface.

91 Modelling experiments with a physics-based approach is a useful way to understand the  
92 physical processes involved. Numerical simulations allow to test different hypotheses on  
93 runoff production and the transport of sediments and bacteria. For example, Guber et al.  
94 (2009, 2011) coupled the kinematic wave overland flow model KINEROS2 with a  
95 convective-dispersive overland transport equation for modelling overland transport of  
96 bacteria. The model successfully simulated the release and transport of faecal coliforms on  
97 vegetated and bare plots. In a recent paper, we used the physics-based Cast3M code for  
98 modelling the impact of raindrops on hydraulic conductivity and overland flow intensity on

99 steep slopes under high-intensity rainfall (Mügler et al. 2019). The present paper follows on  
100 from that work and characterizes the transport of FIB using complementary experimental and  
101 modelling approaches.

102 Rain simulation experiments have been used to examine soil erosion, organic matter export  
103 and the impact of various land use practices on soil erosion (Janeau et al. 2014; Le et al. 2020;  
104 Tataro et al. 2008). In the present paper, we applied this technique to understand how an  
105 indicator of faecal contamination is exported from upland soils used for agricultural activities  
106 during a rain event. The objectives of this work were to characterize the loss dynamics of *E.*  
107 *coli* from agricultural soils contaminated with livestock waste and to partition total  
108 detachment into the splash and hydraulic components.

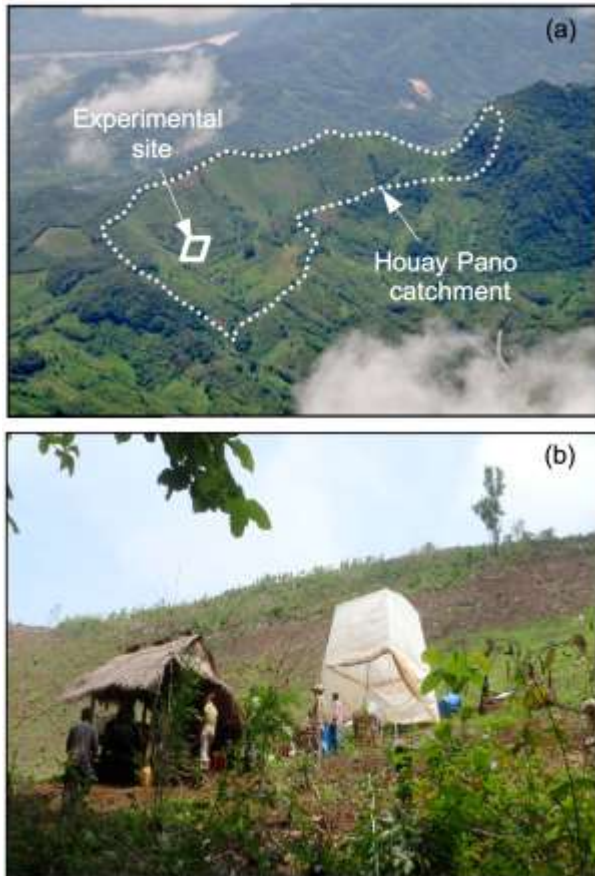
## 109 **2. Materials and methods**

### 110 *2.1. Study area*

111 The rain simulation experiments were conducted in a small mountain Laotian catchment, the  
112 Houay Pano catchment (Fig. 1a). This catchment belongs to the Critical Zone Observatory M-  
113 Tropics (Multiscale TROPICAL Catchments, <https://mtropics.obs-mip.fr>). It can be considered  
114 as being representative of the montane rural agro-ecosystems of South-East Asia (steep  
115 slopes, high rainfall intensities during the rainy season, « slash and burn » agricultural system,  
116 contaminated water with microbial pathogens of faecal origin). Shale and schist soil are the  
117 most widespread in the catchment and the soil is slightly acidic (Ribolzi et al. 2011). The  
118 study area is subject to a tropical climate with heavy rains up to 280 mm h<sup>-1</sup> (Valentin et al.  
119 2008). Experiments were carried out during the dry season. The agricultural production  
120 system is based on a slash and burn technique. Main land use has recently switched from  
121 crops and fallow toward teak plantations (Ribolzi et al. 2017). Animal husbandry is now  
122 developing. However, animal density remains low with two or three pigs in the lower sections

123 of the catchment and some chickens (~20) in both the lower and upper sections (Rochelle-  
124 Newall et al. 2016).

125



126

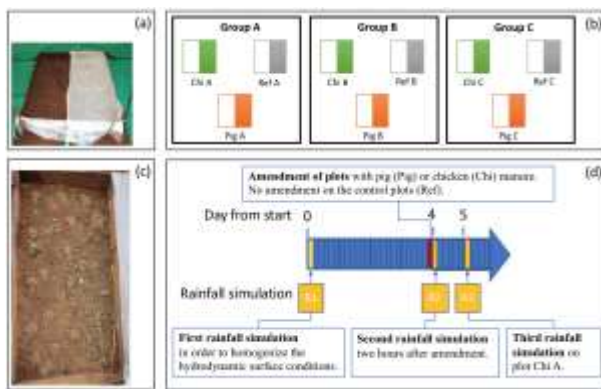
127 **Fig. 1** Study site: **(a)** Aerial view of the Houay Pano catchment (white dotted polygon) and  
128 location of the experimental site (white rhombus); **(b)** Picture showing an overview of the  
129 hillslope and the experimental site with the rainfall simulator tent on the right

130

## 131 2.2. Experimental design

132 Nine 1 m<sup>2</sup> plots were implanted following the method of Janeau et al. (2003) on a steep slope  
133 of the upper part of the catchment (Fig. 1b). All the plots had roughly the same steep slope  
134 (~48%). The purpose of the study was not to investigate the influence of the slope on the  
135 intensity of splash detachment. Indeed, this point has already been studied at Houay Pano by

136 Ribolzi et al. (2011). The soil characteristics (slope, bulk density and texture) are given in  
 137 Table SI-1 (Online Resource 1). The experimental design was similar to that used by Mügler  
 138 et al. (2019). Each plot was divided into two sub-plots (1 m downslope  $\times$  0.5 m perpendicular  
 139 to the slope) and one of the sub-plot was covered with a 2-mm grid size wire screen (a  
 140 mosquito net) (Fig. 2a). The role of the net was to reduce the raindrop impact effects on the  
 141 soil surface. The nine plots (Fig. 2b) were divided into triplicated treatment groups, denoted  
 142 A, B, and C: controls with no amendments (Ref A, Ref B and Ref C) or amended with pig  
 143 manure (Pig A, Pig B, and Pig C) or poultry manure (Chi A, Chi B, and Chi C).  
 144



145  
 146 **Fig. 2** Experimental design: **(a)** image of one 1-m<sup>2</sup> metallic frame divided into two sub-plots  
 147 without mosquito net (left) and with mosquito net (right); **(b)** Diagram showing the nine plots  
 148 (pairs of 9 sub-plots without mosquito net shown in white, associated with 9 sub-plots covered  
 149 with mosquito net shown in colour) divided in triplicated treatment groups (A, B, C): controls  
 150 with no amendments (Ref A, Ref B, and Ref C) or amended with pig manure (Pig A, Pig B, and  
 151 Pig C) or poultry manure (Chi A, Chi B, and Chi C); **(c)** Image of one sub-plot partially covered  
 152 with disconnected small patches of chicken manure ; **(d)** Chronological sequence for the three  
 153 rainfall simulations (R1, R2 and R3) and time of amendment with pig or chicken manure



### 154 2.3. Rainfall simulation

155 A portable rainfall simulator was used for the three successive rainfall simulations (Fig. 1b).  
156 The characteristics of the rainfall simulations were similar to those used and described in  
157 Mügler et al. (2019): a constant rainfall intensity  $\sim 90 \text{ mm h}^{-1}$  during 60 min. The plots were  
158 protected with a plastic cover between simulations to prevent the possible modification of the  
159 soil surface characteristics and moisture content from natural precipitation. The rain water  
160 used for the simulations was pumped from an upland stream adjacent to the plots and samples  
161 were collected to determine the background levels of contamination for the rain simulations  
162 (Table SI-2, Online Resource 1).

163 The hydrodynamic surface conditions of the nine plots were homogenized by the first rainfall  
164 simulation, denoted R1, which was performed without amendment (Fig. 2d). The second  
165 rainfall simulation, denoted R2, was carried out on the nine plots 4 days after R1. It was  
166 conducted two hours after the input of livestock waste to the Chi and Pig plots (Fig. 2d). No  
167 manure was added to the control Ref plots. For the Chi plot of group A, denoted Chi A plot, a  
168 third rainfall simulation, denoted R3, was conducted 24 hours after R2 (Fig. 2d).

169 Overland flow from each  $0.5 \text{ m}^2$  sub-plot was collected in a large and clean bucket during  
170 each rain simulation. Samples of water were collected from each bucket at the end of each  
171 rainfall simulation for the determination of total suspended sediment concentration (TSS,  $\text{g l}^{-1}$ ),  
172 and *E. coli* numbers (MPN  $100 \text{ ml}^{-1}$ ). These samples represent the average concentration  
173 in the runoff water from the  $0.5 \text{ m}^2$  sub-plot. During the rainfall simulations R2 and R3 on the  
174 Chi A plot, in addition to the average sample, five additional samples were collected over the  
175 duration of the simulation to provide an estimation of the time course of *E. coli* export in  
176 runoff during two successive rain events. The time course measurements during R3 were only  
177 conducted on the Chi A plot. The other plots were destroyed by a very strong natural rainfall  
178 event before third series of rain simulations could be completed. As a consequence of the

179 complexity of running this type of experiment in a tropical, upland rural terrain, the rainfall  
180 simulations were performed on consecutive days (Fig. 2d). However, care was taken to ensure  
181 that the interval of time between simulations was the same throughout the experiment.

#### 182 2.4. Manure treatments

183 Pig and chicken manure was collected from six properties in the village of Ban Lak Sip. The  
184 manure from each animal type was pooled and mixed. For each 0.5 m<sup>2</sup> sub-plot, 84 g of pig  
185 manure or 62 g of chicken waste were mixed with 250 ml of water used for rainfall  
186 simulation. These amounts were selected in order to compensate for the higher anticipated  
187 *E. coli* loading in chicken manure (Rosebury 1962). Table 1 gives the *E. coli* loads of the  
188 manure applied to the plots before the second rainfall simulation, R2. Amended plots were  
189 only partially covered by disconnected small patches of manure (Fig. 2c).

190

191 **Table 1:** Most probable number (MPN) of *E. coli* with the associated uncertainty for the soil of  
192 the study area and the collected pig (Pig) and poultry (Chi) faeces (wet-weight).

	Measured <i>E. coli</i> (MPN g <sup>-1</sup> )	Error (-)	Error (+)
Soil	5133	1027	12133
Chicken	1.80 × 10 <sup>9</sup>	1.02 × 10 <sup>9</sup>	2.80 × 10 <sup>9</sup>
Pig	0.27 × 10 <sup>9</sup>	0.17 × 10 <sup>9</sup>	0.43 × 10 <sup>9</sup>

193

194

#### 195 2.5. Determination of TSS and *E. coli*

196 The concentration of Total Suspended Sediments (TSS) was measured in each 330 ml sample  
197 after filtration on 0.45 µm porosity cellulose acetate pre-weighted filters (Sartorius) and  
198 evaporation at 105 °C for 48 h.

199 *E. coli* was quantified by standardised microplate method (ISO 8308-3, MUGEC; BioKar®).  
200 This method, which has been used with success in this watershed (Boithias et al. 2016;  
201 Causse et al. 2015; Ribolzi et al. 2016), is briefly described below. 200 µl of sample were  
202 inoculated into each well of the 96-well plate. Six serial dilutions from 1/20 to 1/200000 were  
203 used and one plate was used per sample, giving 16 wells per dilution. The limit of detection is  
204 low: 15 MPN 100 ml<sup>-1</sup>. Samples were treated within four hours of collection and were  
205 incubated at 44°C for 48 hours. *E. coli* was determined from the number of positive wells  
206 using a statistical Poisson distribution (MPN calculator, Build 22 by Mike Curiale).

## 207 *2.6. Flow, erosion and bacteria transport modelling*

208 The coupling of surface and subsurface flows was performed within a Darcy multidomain  
209 approach (Weill et al. 2009). In this approach, flow in the runoff layer, in the unsaturated zone,  
210 and in the saturated zone are modelled with a diffusive wave approximation of the Saint-Venant  
211 equations, the Darcy equation, and the Richards equation, respectively. The characteristic of the  
212 Darcy multidomain approach is to write all these equations in the same way in the whole  
213 domain as a single Richards diffusion type equation that describes both surface and subsurface  
214 flows. A fuller description of the method is provided in the Supplementary Methods section  
215 (Online Resource 1). In the present paper, as validated in Mügler et al. (2019), the saturated  
216 hydraulic conductivity  $K_s$  was assumed constant in space at the 1-m<sup>2</sup> plot scale but was assumed  
217 to decrease with time during the rainfall event due to seal formation according to the following  
218 exponential function:

$$219 \quad K_s(t) = K_\infty + (K_0 - K_\infty)e^{-(Rt/\tau_1)} \quad (1)$$

220 where  $R$  is the rainfall rate [LT<sup>-1</sup>],  $\tau_1$ , a dimensioning parameter [L], and  $K_0$  and  $K_\infty$ , the initial  
221 and final hydraulic conductivities [LT<sup>-1</sup>], respectively (Assouline 2004; Mügler et al. 2019;  
222 Ribolzi et al. 2011; Silburn and Connolly 1995).

223 Erosion by soil detachment and entrainment in the runoff layer was modelled with the following  
 224 sediment mass balance equation

$$225 \quad \frac{\partial hc}{\partial t} + \frac{\partial qc}{\partial x} = e(x,t) \quad (2)$$

226 where  $c$  is the depth-averaged sediment concentration [ $L^3$  of sediment /  $L^3$  of water],  $h$  is the  
 227 water depth in the runoff layer [ $L$ ], and  $q = h \times u$  is the water flux [ $L^2T^{-1}$ ], with  $u$ , the runoff  
 228 velocity. The source term  $e$  [ $LT^{-1}$ ] is usually divided into two contributions: the rate of particle  
 229 detachment by raindrop impact (hereinafter called splash erosion  $e_s$ ), and the net rate of particle  
 230 detachment by flow (hereinafter called hydraulic erosion  $e_h$ ). At small scale, the dominant  
 231 erosion mechanism is splash (Kinnell 2005). As a consequence, at the 1-m<sup>2</sup> plot scale, we  
 232 neglected the hydraulic erosion  $e_h$ , and only modelled the splash erosion  $e_s$ . Splash erosion is  
 233 modelled in the same way as in the KINEROS code (Woolhiser et al. 1990)

$$234 \quad e_s(x,t) = c_f(t) \times k(h) \times (R - I)^2 \quad (3)$$

235 where  $R$  and  $I$  are the rainfall rate and the infiltration rate in the soil [ $LT^{-1}$ ], respectively. For  
 236 modelling surface crusting during the rainfall event, we made the choice to model the soil  
 237 erodibility  $c_f(t)$  [ $L^{-1}T$ ] in the same way as we modelled the saturated hydraulic conductivity  
 238  $K_s(t)$  (Eq. (1))

$$239 \quad c_f(t) = c_\infty + (c_0 - c_\infty)e^{-(Rt/\tau_2)} \quad (4)$$

240 where  $\tau_2$  is a dimensioning parameter [ $L$ ], and  $c_0$  and  $c_\infty$  are the initial and final soil  
 241 erodibilities [ $L^{-1}T$ ], respectively.

242 The function  $k(h)$  in Eq. (3) models the decrease of splash erosion rate as surface water depth  
 243 increases according to the following formulation

$$244 \quad k(h) = e^{-c_h \times h} \quad (5)$$

245 where  $c_h$  is the damping coefficient [ $L^{-1}$ ], which is estimated according to Woolhiser et al.  
 246 (1990) as follows

247  $c_h = 2/d_r$  (6)

248 where  $d_r$  is the raindrop diameter.

249 Transport of bacteria in the runoff layer was modelled with the following mass balance equation  
 250 (Guber et al. 2011)

251 
$$\frac{\partial h C_b}{\partial t} + \frac{\partial q C_b}{\partial x} = \frac{\partial}{\partial x} \left( a_L q \frac{\partial C_b}{\partial x} \right) - \frac{\partial S_m}{\partial t} - d(k_a C_b - k_d \rho S_s) - (1 - k_s) I C_b + R C_{ir}$$
 (7)

252 where  $C_b$ ,  $S_m$ ,  $S_s$ , and  $C_{ir}$  are the cell concentrations in the runoff layer, in the manure applied to  
 253 the soil surface, in the solid phase of the soil mixing zone, and in the rainfall water, respectively.

254 The mixing zone is the soil surface layer that actively interacts with runoff. Its depth and the soil  
 255 bulk density in this zone are denoted  $d$  and  $\rho$ , respectively. The parameters  $a_L$ ,  $k_a$ ,  $k_d$ , and  $k_s$  are  
 256 the dispersivity [L], the attachment and detachment rates of bacteria at the solid phase [ $T^{-1}$ ], and  
 257 the straining coefficient that models the filter due to vegetation, respectively.

258 The mass conservation of bacteria in the soil mixing zone was given by

259 
$$d\rho \frac{\partial S_s}{\partial t} = d(k_a C_b - k_d \rho S_s) + k_f (1 - k_s) I C_b$$
 (8)

260 where  $k_f$  denotes the fraction of infiltrated cells that are filtered out within the soil-mixing zone.  
 261 However, Martinez et al. (2014) concluded after a global sensitivity analysis that this parameter  
 262 was not relevant. Furthermore, the best calibrations of simulations of transport of bacteria  
 263 performed by Guber et al. (2009, 2011) were obtained with  $k_s \sim 1$ , showing that the main bacteria  
 264 exchange between runoff and soil is attachment of bacteria at the solid phase. As a consequence,  
 265 we took both  $k_f$  and  $k_s$  equal to one.

266 The irreversible release of bacteria from the surface applied manure was modelled according to  
 267 Guber et al. (2011) as follows

268 
$$\frac{\partial S_m}{\partial t} = -C_0 h_m \alpha_m (1 + \alpha_m \beta_m t)^{-(1+1/\beta_m)}$$
 (9)

269 where  $C_0$  is the initial cell concentration in the applied manure [ $\text{cell L}^{-3}$ ],  $h_m$  is the thickness of  
270 the applied manure, and  $\alpha_m$  and  $\beta_m$  are two parameters that characterize the shape of the release  
271 curve.

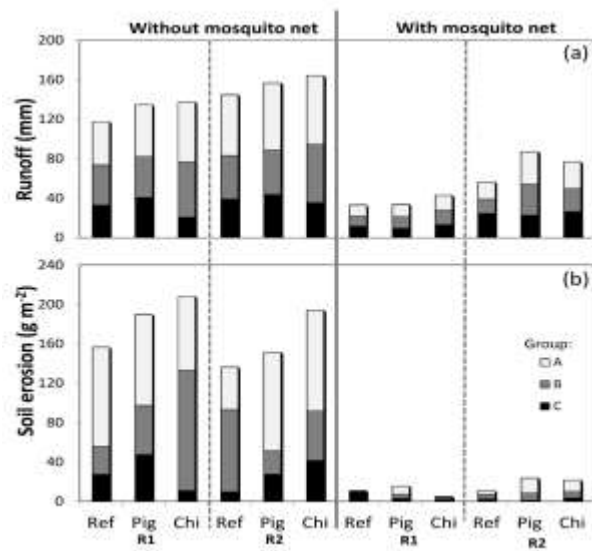
272 All equations were solved with the numerical code Cast3M. More information about the  
273 numerical method and its validation can be found in (Kollet et al. 2017; Mügler et al. 2011;  
274 Mügler et al. 2019; Weill et al. 2009). The bacteria transport module was validated with  
275 experimental data already published (Guber et al. 2009). Results are described in the  
276 Supplementary Methods section (Online Resource 1).

## 277 **3. Results**

### 278 *3.1. Runoff and erosion*

279 In general, runoff increased from the first simulation (R1) to the second simulation (R2) in all  
280 of the plots, regardless of amendment or whether or not netting was present. The average and  
281 the standard deviation of the runoff volume from the nine sub-plots where the splash effect  
282 was present (those without the netting) was equal to 43 ( $\pm 12$ ) mm during R1 and increased to  
283 52 ( $\pm 13$ ) mm during R2 (Fig.3a, left side). In the sub-plots where the splash effect was  
284 reduced (those with netting), this average runoff volume increased from 12 ( $\pm 2$ ) mm to 25  
285 ( $\pm 6$ ) mm (Fig.3a, right side). The pattern was less clear for soil detachment, although there  
286 was for most cases a slight tendency towards decreased soil detachment in R2. Average soil  
287 detachment and entrainment in the nine sub-plots where the splash effect was present was  
288 equal to 62 ( $\pm 38$ )  $\text{g m}^{-2}$  during R1 and decreased to 54 ( $\pm 34.0$ )  $\text{g m}^{-2}$  during R2 (Fig.3b, left  
289 side). In the sub-plots where the splash effect was reduced, this average soil erosion was very  
290 low both during R1 (4 ( $\pm 4$ )  $\text{g m}^{-2}$ ) and R2 (6 ( $\pm 5$ )  $\text{g m}^{-2}$ ) (Fig.3b, right side).

291



292

293

**Fig. 3** Bar plots showing cumulated **(a)** runoff depth and **(b)** soil erosion from the eighteen

294

0.5m<sup>2</sup> sub-plots (i.e. nine without plus nine with mosquito net) divided into triplicated

295

treatment groups (A, B, C). Controls with no amendments (Ref) or amended with pig manure

296

(Pig) or poultry manure (Chi). Measurements were conducted during the first rainfall

297

simulation (R1, before manure application) and the second rainfall simulation (R2, after

298

manure application)

299

300

Large differences in runoff volume and soil erosion were observed between the sub-plots with

301

and without mosquito netting (Fig. 3). This pattern of higher runoff in the splash sub-plots

302

was common to all treatments and all rainfall simulations, regardless of whether manure was

303

added or not. The average runoff volume was ~4 and ~2 times higher in the nine sub-plots

304

where the splash effect was present than in the nine sub-plots where the splash effect was

305

reduced during the first (R1) and second rainfall simulation (R2), respectively. Similarly,

306

average soil erosion on the nine sub-plots where the splash effect was present was nearly one

307

order of magnitude higher than in the sub-plots where the splash effect was reduced after both

308

R1 and R2.

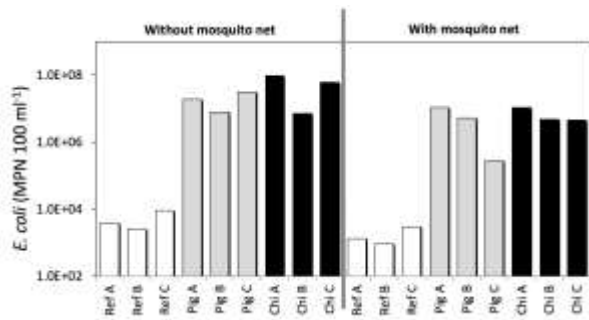
309 3.2. *Escherichia coli* load and dynamics

310 The chicken faeces used contained more than six times more *E. coli* than pig faeces (Table 1).

311 The average concentrations of *E. coli* in runoff collected in the sample bucket at the end of R2

312 were four orders of magnitude higher in the plots that were amended with manure (Fig. 4).

313



314

315 **Fig. 4** *E. coli* concentration in runoff water from both sub-plots without mosquito net (left  
316 side, with splash effect) and with mosquito net (right side, with limited splash effect) for the  
317 triplicated plots Ref, Pig, and Chi for rainfall simulation R2

318

319 The average concentrations of *E. coli* in runoff from the Ref plots were  $5.2 \times 10^3 (\pm 3.5 \times 10^3)$   
320 and  $1.7 \times 10^3 (\pm 1.1 \times 10^3)$  MPN 100 ml<sup>-1</sup> for the splash and non-splash plots, respectively.

321 These values are of the same order of magnitude as the *E. coli* concentrations measured in the  
322 stream water used for the rain during these Ref rainfall simulations (Table SI-2, Online

323 Resource 1). *E. coli* concentrations increased to  $1.9 \times 10^7 (\pm 1.1 \times 10^7)$  and  $5.5 \times 10^6 (\pm 5.4 \times$

324  $10^6)$  MPN 100 ml<sup>-1</sup> for the Pig splash and non-splash plots and  $5.5 \times 10^7 (\pm 4.6 \times 10^7)$  and  $6.7$

325  $\times 10^6 (\pm 3.7 \times 10^6)$  MPN 100 ml<sup>-1</sup> for the Chi splash and non-splash, respectively. Comparing

326 the splash and non-splash plots, the concentrations of *E. coli* were 3, 3.5 and 8 times lower for  
327 Ref, Pig and Chi, respectively.

328 The concentration of *E. coli* in runoff was significantly correlated with suspended sediment

329 concentrations in the splash sub-plots ( $r^2 = 0.68$ ;  $p < 0.05$ ) for R2 and R3 on the Chi A sub-

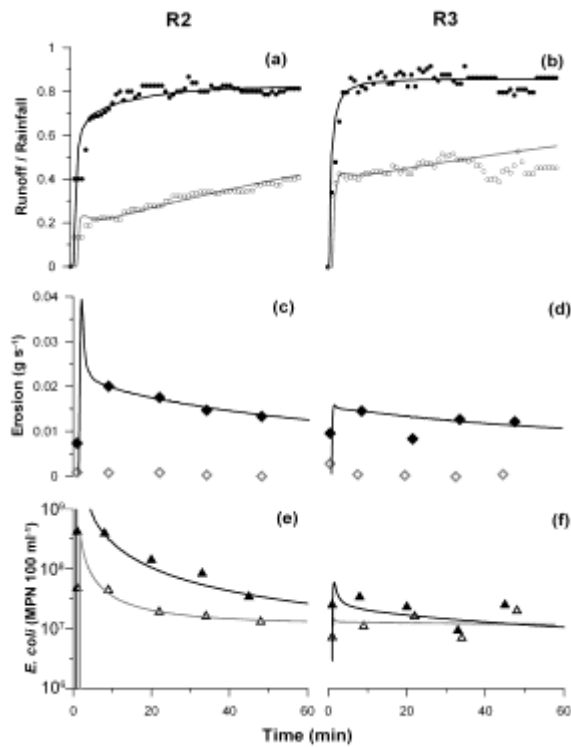


330 plots. No significant correlation was observed for the non-splash sub-plots (Fig. SI-2, Online  
331 Resource 1).

### 332 3.3. *Surface runoff modelling*

333 The modelling approach was used to simulate the time course experiment performed on the two  
334 Chi A sub-plots during two successive rainfall simulations R2 and R3. Figures 5a-b display both  
335 experiment and model overland flow coefficients. This coefficient is equal to the ratio between  
336 the overland flow rate and the rainfall rate. Model outputs were obtained with the parameter  
337 values listed in Table SI-3 in the Supplementary Methods section (Online Resource 1). These  
338 values are the same as those already used and discussed in Mügler et al. (2019). Only the  
339 saturated hydraulic conductivity was estimated. The characteristics of the time evolutions  $K_s(t)$   
340 used for modelling the runoff (cf. Eq. (1)) are given in Table 2. Runoff in the sub-plot without a  
341 mosquito net (splash plots) was correctly modelled with a fast decreasing  $K_s(t)$ , with the same  
342  $\tau_1$  value for the two successive rainfalls ( $\tau_1 = 21.4$  mm), and with values of  $K_0$  and  $K_\infty$  that  
343 were higher in the modelling of R2 than in R3. On the contrary, runoff in the sub-plot with a  
344 mosquito net was correctly modelled with a slow decreasing  $K_s(t)$ , with a nearly one order of  
345 magnitude higher value for  $\tau_1$  ( $\tau_1 = 187.5$  mm). Here again, the value of  $K_0$  decreased from R2  
346 to R3. However, the value for  $K_\infty$  was the same for both rainfalls. The smaller values of  $K_0$  in  
347 the modelling of runoff on the sub-plot without a mosquito net are probably due to the raindrop  
348 impact during the preliminary rainfall (before the manure application).

349



350

351 **Fig. 5** Evolution over time on plot Chi A of the runoff coefficient (Runoff/Rainfall) obtained

352 during R2 (a) and R3 (b); the sediment outputs obtained during R2 (c) and R3 (d); *E. coli*

353 concentrations obtained during R2 (e) and R3 (f). In each figure, empty and filled symbols

354 correspond to the experimental results for the sub-plots with and without a mosquito net,

355 respectively. Solid lines correspond to the modelled evolutions with mosquito net (grey) and

356 without mosquito net (black)

357 **Table 2:** Estimated parameter values for the time evolution of saturated hydraulic conductivity

358  $K_s(t)$  given by Eq. (1) and obtained for the two Chi A sub-plot experiments, with and without

359 raindrop impact, and for two successive rainfall simulations R2 and R3.

Sub-plot	Rainfall simulation	$\tau_1$ (mm)	$K_0$ (mm h <sup>-1</sup> )	$K_\infty$ (mm h <sup>-1</sup> )
without a mosquito net	R2	21.4	40	18
without a mosquito net	R3	21.4	20	14
with a mosquito net	R2	187.5	84	16
with a mosquito net	R3	187.5	62	16

360

361 *3.4. Erosion modelling*

362 Figures 5c-d show the evolutions over time of both measured and modelled sediment outputs  
363 during R2 and R3 on the Chi A sub-plots. The comparison of sediment outputs with or without a  
364 mosquito net clearly shows the large effect of raindrop impact on erosion. Sediment outputs  
365 from the sub-plot for which the surface soil is protected from raindrop impact by a mosquito net  
366 are negligible indicating that splash erosion is negligible when the raindrop impact is reduced. It  
367 also supports the assumption that hydraulic erosion is negligible at the 1-m<sup>2</sup> plot scale. As a  
368 consequence, only the experiment with the raindrop impact was modelled with Eqs (2)-(6).

369 For a  $\sim 90 \text{ mm h}^{-1}$  rainfall rate, the raindrop diameter  $d_r$  is approximately equal to 1.34 mm  
370 (Mügler et al. 2019). Thus, according to Eq. (6), the damping coefficient  $c_h \sim 1493 \text{ m}^{-1}$ . Table 3  
371 lists the values of the other erosion parameters which were estimated from the experimental  
372 time evolutions of the sediment outputs plotted in Figs. 5c-d.

373

374 **Table 3:** Estimated parameter values for the time evolution of soil erodibility  $c_f(t)$  given by  
375 Eq. (4) and obtained for the sub-plot experiment without a mosquito net, and for two successive  
376 rainfall simulations R2 and R3.

Sub-plot	Rainfall simulation	$\tau_2$ (mm)	$c_0$ (s m <sup>-1</sup> )	$c_\infty$ (s m <sup>-1</sup> )
without a mosquito net	R2	75	21	8
without a mosquito net	R3	75	15	8

377

378 After rainfall, crusts covered nearly 98% of the surface of the unprotected sub-plot and 77% of  
379 the surface of the protected sub-plot, which was already crusted prior to the experiments. The

380 most impervious crusts, namely gravel and erosion crusts, developed only on the unprotected  
381 sub-plot, covering 1.5% and 6% of the surface, respectively (Mügler et al. 2019).

### 382 3.5. *E. coli* transport modelling

383 Figures 5e-f show *E. coli* concentrations in runoff water at the outlet of the Chi A sub-plots  
384 during R2 and R3. The numbers of exported *E. coli* are clearly higher on the sub-plots whose  
385 soil surface is not protected from raindrop impact. Table 4 lists the values of two parameter sets,  
386 denoted as Model 1 and Model 2 that were used for the bacteria transport modelling. Most of  
387 the parameter values in the two sets are the same because they were either measured or  
388 determined from the literature. As mentioned in Table 1, the MPN of *E. coli* in poultry faeces  
389 was equal to  $1.8 \times 10^9$  MPN g<sup>-1</sup>, and 62 g of chicken waste were applied to each sub-plot. As a  
390 consequence, we took  $C_{ohm} = 2.23 \times 10^{11}$  MPN m<sup>-2</sup>. Furthermore, as the *E. coli* concentration in  
391 the stream water used for the rain during each rainfall simulation was several orders of  
392 magnitude lower than  $C_{ohm}$  (Table SI-2 in Online Resource 1), the last term in Eq. (7) was  
393 removed ( $C_{ir} = 0$ ).

394 Parameters that characterize the shape of the release curve according to Eq. (9) were estimated  
395 from the fit of experimental release of faecal coliform from manure on plots (Guber et al. 2006).  
396 Guber et al. (2006) showed that the value of  $\alpha_m$  in Eq. (9) was linearly related to the rainfall rate  
397  $R$  (cm h<sup>-1</sup>) according to the following relationship:  $\alpha_m = 0.036 + 0.86 \times R$ . For bare clay loam  
398 plots and irrigation rates  $\sim 6$  cm h<sup>-1</sup>, they found that experimental evolutions were correctly  
399 fitted with  $\beta_m = 8$ . As a consequence, we took these values (see Table 4).

400

401

402

403 **Table 4:** Parameter values for the bacteria transport modelling.

Parameters	Symbol	Unit	Model 1	Model 2
Initial cell concentration	$C_{0h_m}$	MPN m <sup>-2</sup>	2.23×10 <sup>11</sup>	same value
Cell concentration in the rainfall	$C_{ir}$	MPN m <sup>-2</sup>	0	same value
Shape of the release curve	$\alpha_m$	h <sup>-1</sup>	0.036 + 0.86× $R$ ( $R$ in cm h <sup>-1</sup> )	same value
	$\beta_m$	-	8	same value
Thickness of the active soil layer	$D$	M	0.01	same value
Dispersivity	$a_L$	M	0	same value
Partitioning coefficient	$K_d$	ml g <sup>-1</sup>	54	same value
Attachment rate	$k_a$	s <sup>-1</sup>	0	0.015 (estimated)
Detachment rate	$k_d = k_a / ((\rho/\theta_s) \times K_d)$	s <sup>-1</sup>	0	1.4×10 <sup>-4</sup>

404

405 The thickness of the soil mixing zone,  $d$ , was set at 1 cm (Cho et al. 2016; Guber et al. 2011).

406 Dispersivity was neglected at the 1-m<sup>2</sup> plot scale ( $a_L = 0$ ). The partitioning coefficient  $K_d$ , which  
 407 is defined as  $k_d / ((\rho/\theta_{sat}) \times k_d)$ , was assumed to be related to the clay content in the soil as follows  
 408 (Pachepsky et al. 2006)

$$409 \quad K_d = A \times (CLAY)^B \quad (10)$$

410 where CLAY is the clay percentage in soil, and parameters  $A = 10^{-1.6 \pm 0.9}$ , and  $B = 1.98 \pm 0.7$ . This  
 411 relation gave us  $K_d = 54$  ml g<sup>-1</sup>. Finally, the only difference between Model 1 and Model 2 was  
 412 that the interaction between the mixing zone and the runoff was neglected in Model 1 ( $k_a = k_d =$   
 413  $0$ ), although the attachment and detachment of bacteria to the solid phase, and the infiltration of  
 414 bacteria in the soil were taken into account in Model 2 ( $k_a \neq 0$  and  $k_d \neq 0$ ). Hence, in Model 1,

415 all parameters were estimated *a priori*. None were calibrated. On the contrary, in Model 2, the  
416 attachment rate of bacteria at the solid phase,  $k_a$ , had to be estimated from experimental results.  
417 Thus, the detachment rate of bacteria,  $k_d$ , was calculated as  $k_d = k_a / ((\rho / \theta_{sat}) \times K_d)$ .  
418 In Figs. 5e-f, the black solid lines correspond to the evolutions over time of *E. coli* export  
419 obtained during the two successive simulated rainfalls with Model 1. Except at the beginning of  
420 runoff when the simulated concentration is very high because the runoff layer is extremely thin,  
421 the numerical results are in good agreement with the experimental results on the sub-plot with  
422 raindrop impact, both during the first and the second rainfall (filled triangles in Figs. 5e-f). This  
423 result was obtained without calibration. In contrast, the exported bacteria concentrations  
424 measured at the outlet of the sub-plot whose surface soil was protected from raindrop impact  
425 (empty triangles in Figs. 5e-f) were correctly simulated with Model 2 (grey solid lines in Figs.  
426 5e-f) after estimation of the attachment rate  $k_a$  to the value  $0.015 \text{ s}^{-1}$ .

## 427 **4. Discussion**

428 Runoff from manured fields and pastures is known to be an important mechanism by which  
429 organisms of faecal origin are transferred to streams highlighting the dominance of diffuse  
430 sources of contamination over point sources in rural watersheds (Collins et al. 2005). This is  
431 particularly the case in rural areas of developing countries where solid and liquid waste from  
432 humans and other animals is released into the environment without treatment. We used field  
433 rain simulations and modelling to examine how *E. coli* are exported from the surface of  
434 upland, agricultural soils during runoff events.

### 435 *4.1. Effect of raindrop impact on overland flow and soil detachment and entrainment*

436 When the impact of raindrops was reduced, total runoff was on average reduced by 70%  
437 during the first rainfall simulation and by 50% during the second one (Fig. 3a). The purpose  
438 of the modelling approach was not to perform a precise calibration of all parameters but rather

439 to investigate the behaviour of some of them. Runoff was correctly modelled with a saturated  
440 hydraulic conductivity decreasing with time during the rainfall event (Figs. 5a-b). This  
441 decreasing conductivity of the soil was faster for the sub-plot without a mosquito net than for  
442 the protected sub-plot. This behaviour can be attributed to the formation of more structural  
443 crusts on the unprotected soil surface subject to the high kinetic energy raindrop impacts  
444 (Mügler et al. 2019).

445 As shown by many previous studies ( Wei et al. 2007; Lacombe et al. 2018), the intensity of  
446 rain and soil detachability are directly related. As Ziegler et al. (2000) observed, splash effects  
447 dominate over hydrologic processes in soil detachment. Figures 3 and 5 show that rain splash  
448 not only enhances runoff generation but also greatly contributes to soil detachment and  
449 entrainment. Such a high value of soil detachment rate has already been noticed on bare soils.  
450 For example, Patin et al. (2018) obtained in the same catchment a mean soil loss per rainfall  
451 event of  $154 \pm 53 \text{ g m}^{-2}$  on bare soils. This rate dropped to  $2 \text{ g m}^{-2}$  when the soil was left  
452 fallow. In our experiments, the mosquito net, which reduces the splash erosion by 90%, plays  
453 the same role as a low vegetation cover.

#### 454 *4.2. Effect of raindrop impact on bacteria export*

455 Figures 4 and 5e-f show that rain splash also has a strong impact on the export of *E. coli* from  
456 the soil surface. The modelling approach used in the work presented here allows the  
457 determination of the main processes involved in the transport of bacteria. In this approach  
458 (Eqs. 7-9), bacteria are assumed to be released from the surface applied manure into the  
459 runoff water. Then, they can interact or not with the soil surface layer. In this zone, called the  
460 “soil mixing zone”, bacteria can attach or detach from the solid phase. In the sub-plot where  
461 the raindrop impact was not reduced (without mosquito net), the high experimental bacteria  
462 export was correctly modelled without any exchange of bacteria between runoff and mixing  
463 zone. This behaviour is in agreement with experimental and numerical results of Guber et al.

464 (2009) that we used to validate our modelling approach. Guber et al. (2009) calibrated the  
465 mass exchange rate between the runoff and the mixing zone to zero for modelling the export  
466 of bacteria from bare plots subject to high rainfall intensities ( $58 < R < 73 \text{ mm h}^{-1}$ ).  
467 In contrast, less bacteria were exported from the sub-plot where raindrop impact was reduced  
468 (with mosquito net). In this case, a part of the bacteria that were released from the manure into  
469 the runoff water interacted with the soil surface layer. This lower bacteria export from a  
470 protected sub-plot was correctly modelled by taking into account the exchange of bacteria  
471 between the overland flow and the soil by attachment and detachment. This behaviour is in  
472 agreement with model results of Guber et al. (2011). Indeed, from the calibration of field-  
473 scale experiments subjected to rainfall rates lower than  $14 \text{ mm h}^{-1}$ , Guber et al. (2011)  
474 concluded that adsorption and desorption of bacteria released from dairy bovine manure were  
475 not negligible. The  $k_a/k_d$  ratio that they obtained from calibration ( $k_a/k_d \sim 122$ ) is comparable to  
476 the value that we obtained ( $k_a/k_d \sim 107$ ).

#### 477 *4.3. Effect of successive rainfalls*

478 As can be seen in Fig. 3a, for each plot, runoff depth increases during successive rainfalls.  
479 This increase was correctly modelled by a decrease of the initial hydraulic conductivity  $K_0$   
480 from one rainfall to the next (Table 2 and Figs 5a-b). Effect of successive rainfalls on soil  
481 detachment is more contrasted. On one hand, there was a tendency towards decreased soil  
482 detachment and entrainment from the sub-plots without mosquito net during successive  
483 rainfalls (Fig. 3b). On the other hand, when the impact of raindrops was reduced, soil  
484 detachment and entrainment was negligible on the non-amended subplots during both the R1  
485 and R2 rainfalls. It only slightly increased during R2 on the amended Pig A and Chi A sub-  
486 plots perhaps because of the entrainment of some manure by runoff. It is also potentially due  
487 to an indirect effect linked to the evolution of the deposit between the two rainfall simulations  
488 (e.g. solar radiation, biological activity) that would have made the deposit easier to mobilize.



489 When the impact of raindrop was reduced by the mosquito net, the concentration of *E. coli* in  
490 runoff decreased mainly during the first 20 min of rainfall R2 (Fig. 5e). Thereafter, the  
491 concentration decreased only very slightly during the following rainfall R3 (Fig. 5f). When  
492 the sub-plots were not protected with a mosquito net, concentrations of *E. coli* in runoff were  
493 ten times higher and decreased during the successive rainfalls R2 and R3 (Figs 5e-f).  
494 Although the data from the rainfall R3 should be viewed with caution given that only one  
495 experiment was possible, the data point towards a decreasing dynamic over successive  
496 rainfalls. This decline of the peak bacteria release between the once-wet faecal deposit and  
497 subsequent rainfall events has long been observed (e.g. Kress and Gifford 1984). It has been  
498 attributed to the leaching effect of the first rainfall on bacteria in the faecal deposit.

## 499 **5. Conclusion**

500 Rainfall simulation experiments at the plot scale carried out with or without raindrop impact on  
501 bare or amended soils led to the following conclusions:

- 502 • Runoff production, soil detachment and entrainment and *E. coli* export are strongly  
503 enhanced by raindrop impact. When the impact of raindrops was reduced with a  
504 mosquito net, soil erosion was on average reduced by 90% and *E. coli* export from the  
505 amended soil surface was on average 3 to 8 times lower. These results underline the  
506 strong impact of the splash effect on the export of *E. coli* from the soil surface.
- 507 • The temporal evolution of runoff, soil and *E. coli* exports during two successive rainfall  
508 simulations were correctly modelled with a physics-based approach with a soil hydraulic  
509 conductivity and a soil erodibility that exponentially decreased over time of exposure to  
510 rainfall. These decreasing parameters model the splash effect on the soil surface  
511 properties.
- 512 • Both experimental and modelling approaches showed the leaching effect of the first  
513 rainfall on bacteria in the faecal deposit.

- 514       • When the impact of raindrops was reduced, the lower bacteria export from the protected  
515           soil was correctly modelled by taking into account the exchange of bacteria between the  
516           overland flow and the soil by attachment and detachment.

517

518   These results highlight the strong mobilization and transport of faecal indicator bacteria from  
519   steep slopes under high intensity rainfall such as usually occur in rural Southeast Asia. The  
520   links between land cover, or vegetation and soil erosion have been previously identified (i.e.  
521   Lacombe et al. 2018) and recent work demonstrated the importance of vegetation cover in  
522   reducing the export of soil microbial communities from soils (Le et al. 2020). The work  
523   presented here builds on this work and shows that by reducing the splash effect, through low  
524   cover, such as surface vegetation, can significantly reduce microbial pathogen dissemination  
525   and, by extension, the contamination of stream waters. This is particularly important in areas  
526   where access the adequate sanitation is limited or non-existent and where untreated surface  
527   water is used for domestic requirements.

528

529

530 **Declarations**

531

532 **Ethics approval and consent to participate**

533 Not applicable

534 **Consent for publication**

535 Not applicable

536 **Availability of data and materials**

537 The datasets used and analysed during the current study are available from the corresponding  
538 author on reasonable request.

539 **Competing interests**

540 The authors declare that they have no competing interests.

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545 **Authors' contributions**

546 All authors contributed to the work presented in the manuscript. Olivier Ribolzi and Emma  
547 Rochelle-Newall and Oloth Sengtaheuanghoung contributed to the study conception and  
548 design. Material preparation and field data collection were performed by Jean-Louis Janeau,  
549 Thierry Henri-des-Tureaux, Keooudone Latsachack, Marion Viguier, Christian Valentin and  
550 Olivier Ribolzi. Laboratory analysis were conducted by Chanthamousone Thammahacksa,  
551 Emilie Jardé, Marion Viguier and Emma Rochelle-Newall. Modelling was performed by  
552 Claude Mügler. The first draft of the manuscript was written by Claude Mügler, Olivier  
553 Ribolzi and Emma Rochelle-Newall, all authors commented on previous versions of the  
554 manuscript. All authors read and approved the final manuscript.

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696 **Figure captions:**

697

698 **Fig. 1**

699 Study site: **(a)** Aerial view of the Houay Pano catchment (white dotted polygon) and location of  
700 the experimental site (white rhombus); **(b)** Picture showing an overview of the hillslope and the  
701 experimental site with the rainfall simulator tent on the right

702

703 **Fig. 2**

704 Experimental design: **(a)** image of one 1-m<sup>2</sup> metallic frame divided into two sub-plots without  
705 mosquito net (left) and with mosquito net (right); **(b)** Diagram showing the nine plots (pairs of 9  
706 sub-plots without mosquito net shown in white, associated with 9 sub-plots covered with  
707 mosquito net shown in colour) divided in triplicated treatment groups (A, B, C): controls with  
708 no amendments (Ref A, Ref B, and Ref C) or amended with pig manure (Pig A, Pig B, and  
709 Pig C) or poultry manure (Chi A, Chi B, and Chi C); **(c)** Image of one sub-plot partially covered  
710 with disconnected small patches of chicken manure ; **(d)** Chronological sequence for the three  
711 rainfall simulations (R1, R2 and R3) and time of amendment with pig or chicken manure

712

713 **Fig. 3**

714 Bar plots showing cumulated **(a)** runoff depth and **(b)** soil erosion from the eighteen 0.5m<sup>2</sup>  
715 sub-plots (i.e. nine without plus nine with mosquito net) divided into triplicated treatment  
716 groups (A, B, C). Controls with no amendments (Ref) or amended with pig manure (Pig) or  
717 poultry manure (Chi). Measurements were conducted during the first rainfall simulation (R1,  
718 before manure application) and the second rainfall simulation (R2, after manure application)

719

720

721 **Fig. 4**

722 *E. coli* concentration in runoff water from both sub-plots without mosquito net (left side, with  
723 splash effect) and with mosquito net (right side, with limited splash effect) for the triplicated  
724 plots Ref, Pig, and Chi for rainfall simulation R2

725

726 **Fig. 5**

727 Evolution over time on plot Chi A of the runoff coefficient (Runoff/Rainfall) obtained during  
728 R2 (**a**) and R3 (**b**); the sediment outputs obtained during R2 (**c**) and R3 (**d**); *E. coli*  
729 concentrations obtained during R2 (**e**) and R3 (**f**). In each figure, empty and filled symbols  
730 correspond to the experimental results for the sub-plots with and without a mosquito net,  
731 respectively. Solid lines correspond to the modelled evolutions with mosquito net (grey) and  
732 without mosquito net (black)