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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 m² plots,
32 amended or not with pig or poultry manure. Each plot was divided into two 0.5 m² 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 m² (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.

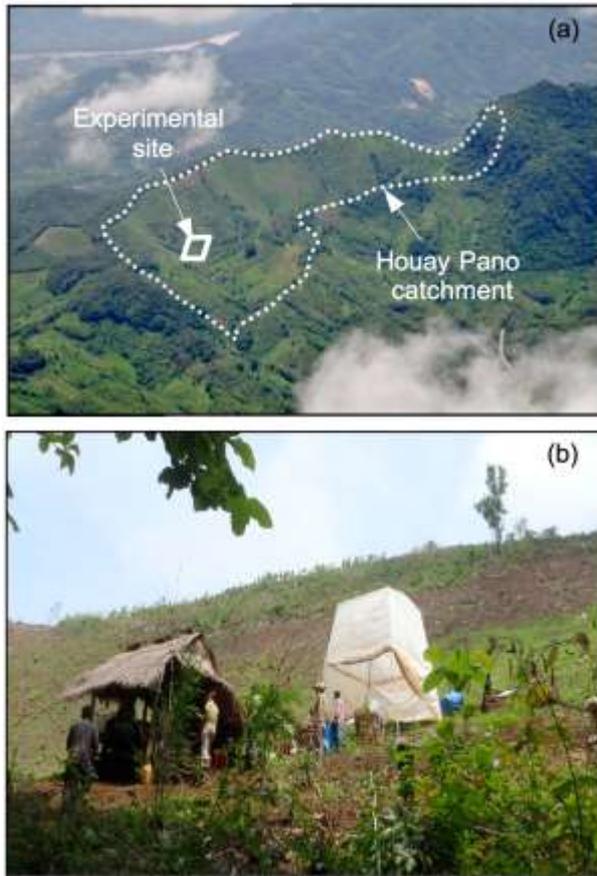
109 **2. Materials and methods**

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⁻¹ (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

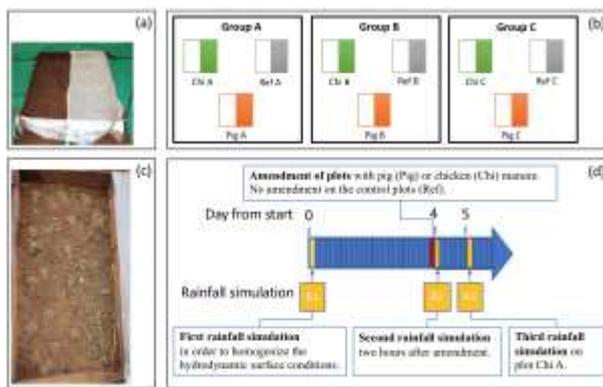
127 **Fig. 1** Study site: **(a)** Aerial view of the Houay Pano catchment (white dotted polygon) and
128 location of the experimental site (white rhombus); **(b)** Picture showing an overview of the
129 hillslope and the experimental site with the rainfall simulator tent on the right

130

131 2.2. Experimental design

132 Nine 1 m² 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-m² 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

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 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, g l^{-1}),
172 and *E. coli* numbers (MPN 100 ml^{-1}). These samples represent the average concentration
173 in the runoff water from the 0.5 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 m² 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 ⁻¹)	Error (-)	Error (+)
Soil	5133	1027	12133
Chicken	1.80 × 10 ⁹	1.02 × 10 ⁹	2.80 × 10 ⁹
Pig	0.27 × 10 ⁹	0.17 × 10 ⁹	0.43 × 10 ⁹

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⁻¹. 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-m² 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⁻¹], τ_1 , a dimensioning parameter [L], and K_0 and K_∞ , the initial
221 and final hydraulic conductivities [LT⁻¹], 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-m² 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 τ_2 is a dimensioning parameter [L], and c_0 and c_∞ 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 ρ , 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 [cell L^{-3}], h_m is the thickness of
270 the applied manure, and α_m and β_m are two parameters that characterize the shape of the release
271 curve.

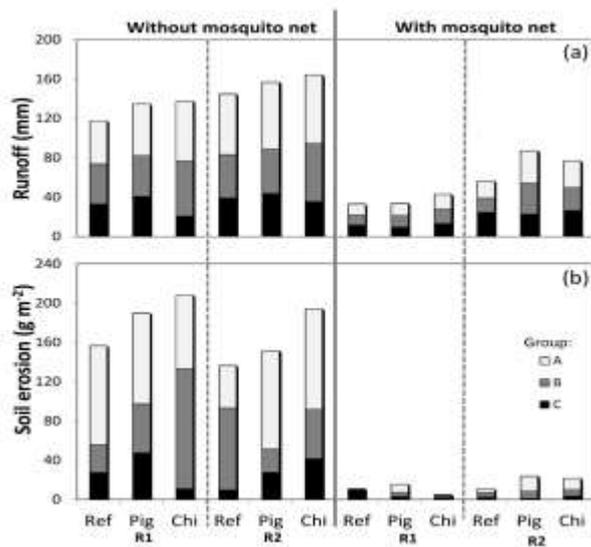
272 All equations were solved with the numerical code Cast3M. More information about the
273 numerical method and its validation can be found in (Kollet et al. 2017; Mügler et al. 2011;
274 Mügler et al. 2019; Weill et al. 2009). The bacteria transport module was validated with
275 experimental data 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 (± 12) mm during R1 and increased to
283 52 (± 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 (± 2) mm to 25
285 (± 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 (± 38) g m^{-2} during R1 and decreased to 54 (± 34.0) 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 (± 4) g m^{-2}) and R2 (6 (± 5) 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 0.5m² 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.

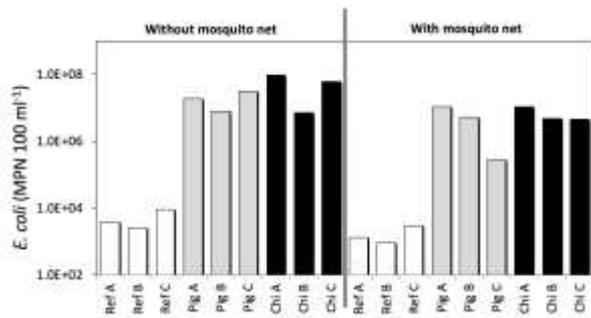
309 3.2. *Escherichia coli* load and dynamics

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⁻¹ 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⁻¹ 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⁻¹ 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

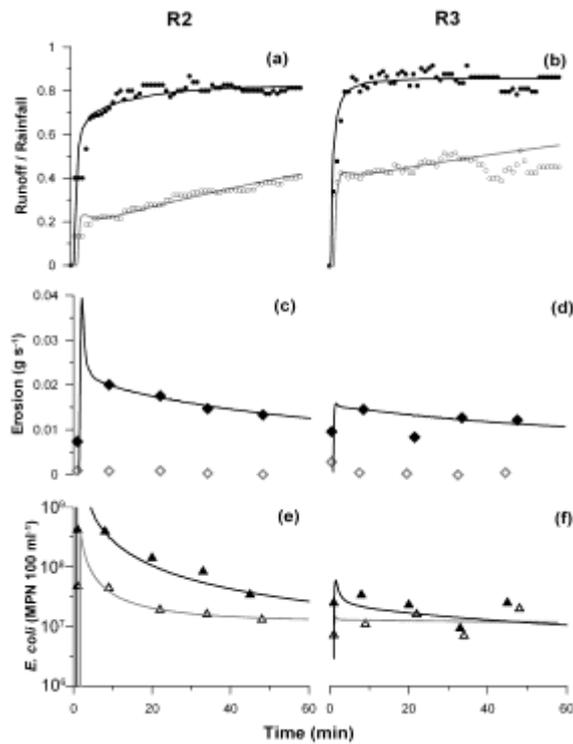
329 concentrations in the splash sub-plots ($r^2 = 0.68$; $p < 0.05$) for R2 and R3 on the Chi A sub-

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 τ_1 value for the two successive rainfalls ($\tau_1 = 21.4$ mm), and with values of K_0 and K_∞ 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 τ_1 ($\tau_1 = 187.5$ mm). Here again, the value of K_0 decreased from R2
346 to R3. However, the value for K_∞ 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	τ_1 (mm)	K_0 (mm h ⁻¹)	K_∞ (mm h ⁻¹)
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-m² 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	τ_2 (mm)	c_0 (s m ⁻¹)	c_∞ (s m ⁻¹)
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 1.8×10^9 MPN g⁻¹, 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⁻². 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 α_m in Eq. (9) was linearly related to the rainfall rate
397 R (cm h⁻¹) according to the following relationship: $\alpha_m = 0.036 + 0.86 \times R$. For bare clay loam
398 plots and irrigation rates ~ 6 cm h⁻¹, 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 ⁻²	2.23×10^{11}	same value
Cell concentration in the rainfall	C_{ir}	MPN m ⁻²	0	same value
Shape of the release curve	α_m	h ⁻¹	$0.036 + 0.86 \times R$ (R in cm h ⁻¹)	same value
	β_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 ⁻¹	54	same value
Attachment rate	k_a	s ⁻¹	0	0.015 (estimated)
Detachment rate	$k_d = k_a / ((\rho / \theta_s) \times K_d)$	s ⁻¹	0	1.4×10^{-4}

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-m² 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⁻¹. 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 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 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 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
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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-m² 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 0.5m²
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)