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## Quantifying the effect of overland flow on *Escherichia coli* pulses during floods: use of a tracer-based approach in an erosion-prone tropical catchment

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1 Quantifying the effect of overland flow on  
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26

27 **Abstract**

28 Bacterial pathogens in surface waters threaten human health. The health risk is especially high in  
29 developing countries where sanitation systems are often lacking or deficient. Considering twelve flash-  
30 flood events sampled from 2011 to 2015 at the outlet of a 60-ha tropical montane headwater catchment  
31 in Northern Lao PDR, and using *Escherichia coli* as a fecal indicator bacteria, our objective was to quantify  
32 the contributions of both surface runoff and sub-surface flow to the in-stream concentration of *E. coli*  
33 during flood events, by (1) investigating *E. coli* dynamics during flood events and among flood events and  
34 (2) designing and comparing simple statistical and mixing models to predict *E. coli* concentration in  
35 stream flow during flood events. We found that in-stream *E. coli* concentration is high regardless of the  
36 contributions of both surface runoff and sub-surface flow to the flood event. However, we measured the  
37 highest concentration of *E. coli* during the flood events that are predominantly driven by surface runoff.  
38 This indicates that surface runoff, and causatively soil surface erosion, are the primary drivers of in-  
39 stream *E. coli* contamination. This was further confirmed by the step-wise regression applied to  
40 instantaneous *E. coli* concentration measured in individual water samples collected during the flood  
41 events, and by the three models applied to each flood event (linear model, partial least square model,  
42 and mixing model). The three models showed that the percentage of surface runoff in stream flow was  
43 the best predictor of the flood event mean *E. coli* concentration. The mixing model yielded a Nash-  
44 Sutcliffe efficiency of 0.65 and showed that on average, 89% of the in-stream concentration of *E. coli*  
45 resulted from surface runoff, while the overall contribution of surface runoff to the stream flow was  
46 41%. We also showed that stream flow turbidity and *E. coli* concentration were positively correlated, but  
47 that turbidity was not a strong predictor of *E. coli* concentration during flood events. These findings will  
48 help building adequate catchment-scale models to predict *E. coli* fate and transport, and mapping the  
49 related risk of fecal contamination in a global changing context.

50

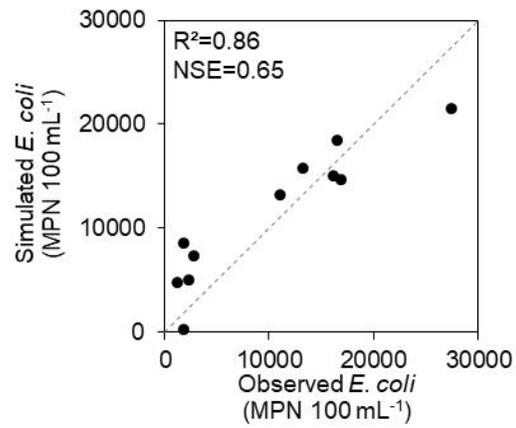
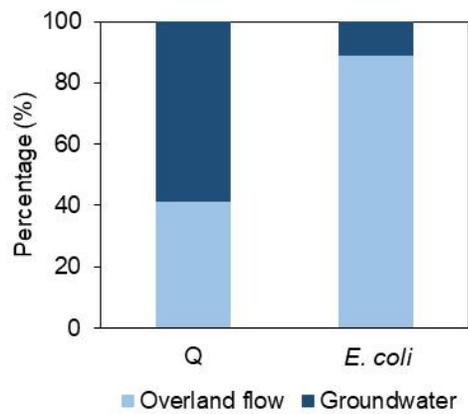
51 **Keywords**

52 Surface runoff; Fecal Indicator Bacteria; Storm flow; Land-use change; Surface-sub surface flow

53 separation; northern uplands of Lao PDR

54

55 **Graphical abstract**



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57

## 58 1. Introduction

59 The presence of bacterial pathogens in surface waters threatens human health. Pathogenic bacteria are  
60 etiological agents of several waterborne diseases such as diarrhea, which is globally a leading cause of  
61 death among all ages (1.31 million deaths in 2015) (Troeger et al., 2017). These bacterial pathogens can  
62 be of fecal origin, e.g. from cattle or from human feces where open defecation is practiced or where  
63 sanitation systems are lacking or deficient (Exley et al., 2015; Tong et al., 2016). In developing countries,  
64 untreated surface water is often used for drinking, cooking, bathing and washing, thus exposing the  
65 population to a high health risk (Boithias et al., 2016).

66 Catchment microbial response depends on complex interactions between spatial patterns of land use,  
67 soil types, antecedent conditions of soil, and rainfall characteristics (Kim et al., 2018; McKergow and  
68 Davies-Colley, 2010; Pachepsky et al., 2018; Strauch et al., 2014). In the tropics, the presence in stream  
69 water of Fecal Indicator Bacteria (FIB), such as *Escherichia coli* (*E. coli*), is known to be partly driven by  
70 surface runoff (Causse et al., 2015; Ribolzi et al., 2016a) and groundwater flow (Chuah and Ziegler, 2018).  
71 However, the underlying mechanisms of FIB dynamics during storms remain to be fully documented. For  
72 example, there is a need to better quantify the respective contributions of surface and sub-surface flows  
73 to stream flow (e.g. using geochemical tracers) to understand and model the transfers of *E. coli* at the  
74 catchment scale during a flood event (Cho et al., 2016; Kim et al., 2017) and to predict the relative  
75 contributions of surface and sub-surface flows to the overall in-stream *E. coli* contamination (Chin, 2011).

76 *E. coli* is known to be transported in water as free cells or cells attached to particles of soil, manure or  
77 sediment (Garcia-Armisen and Servais, 2009; Krometis et al., 2007; Soupir et al., 2010). While stream  
78 flow turbidity is often higher in tropical areas, as compared to temperate areas, suspended sediments  
79 are known to carry *E. coli* (Nguyen et al., 2016). However, the relationships between stream flow  
80 turbidity, suspended sediments concentration, and *E. coli* concentration, and their possible hysteretic  
81 patterns during flood events, have not been yet fully explored.

82 Several studies in the tropics have investigated the relationships between suspended sediments  
83 concentration and turbidity in rivers and lakes (Martinez et al., 2009; Robert et al., 2017, 2016; Ziegler et  
84 al., 2014), and few studies investigated the relationships between suspended sediments concentration  
85 and *E. coli* concentration (Boithias et al., 2016; Nguyen et al., 2016). However, to our knowledge, no  
86 study has yet investigated the relationships between turbidity and *E. coli* concentration. Given that in-  
87 stream *E. coli* concentration can vary rapidly during a flood event, a high-frequency sampling is required  
88 to characterize this variability. The latter is both time consuming and expensive. Therefore, a proxy-  
89 based approach is required and there is a need to verify if stream flow turbidity can be used as a proxy of  
90 *E. coli* concentration during flood events.

91 In rural Southeast Asia, such as the uplands of Northern Lao PDR, livestock grazing and the lack of  
92 adequate sanitation systems result in high levels of fecal contamination in surface waters during both  
93 high and low flow seasons (Boithias et al., 2016; Nguyen et al., 2016; Rochelle-Newall et al., 2016). In  
94 addition, Northern Lao PDR, like other mountainous regions in South-East Asia, is experiencing rapid  
95 changes in land use (Riboldi et al., 2017; Turkelboom et al., 2008). Annual crops are replaced by tree  
96 plantations (e.g. teak) with limited understorey and litter coverage. This land-use change decreases soil  
97 water infiltration and increases surface runoff and soil erosion (Lacombe et al., 2018; Riboldi et al., 2017;  
98 Song et al., 2020; Ziegler et al., 2004), but its impact on bacteria export has not yet been investigated.

99 Considering twelve flash-flood events sampled from 2011 to 2015 at the outlet of a 60-ha montane  
100 tropical headwater catchment in Northern Lao PDR, our objective was thus to quantify the contributions  
101 of both surface runoff and sub-surface flow to the in-stream concentration of *E. coli* during flood events,  
102 by (1) investigating *E. coli* dynamics during flood events and among flood events and (2) designing and  
103 comparing simple statistical and mixing models to predict *E. coli* concentration in stream flow during  
104 flood events.

## 105 2. Materials and methods

### 106 2.1. Study design

107 In this paper, we quantify the relative roles of both surface runoff and sub-surface flow in the  
108 contamination of stream water by *E. coli* in a tropical montane catchment. To do so, we measured fine  
109 temporal-scale discharge and stream flow electrical conductivity to calculate the relative contributions of  
110 surface runoff and sub-surface flow to total stream flow. For twelve flash-flood events from 2011 to  
111 2015 (named with the letters A to L), we then investigated statistical relationships between surface  
112 runoff, sub-surface flow, *E. coli* concentration, together with turbidity, total suspended sediments  
113 concentration, land-use features (areal percentage of land cover and *E. coli* input), and flood event-  
114 integrated variables, i.e. total rainfall, maximum rainfall intensity, rainfall duration, soil antecedent  
115 conditions, peak discharge, total volume of stream water exported during the flood event, and flow  
116 coefficient. For each flood event, we applied a mixing model to calculate the relative contributions of  
117 surface runoff and of sub-surface flow to the overall in-stream contamination by *E. coli*, by assigning to  
118 each one of these two flow components a characteristic bacteria concentration based on *E. coli*  
119 measurements in overland flow (overland flow *E. coli* end-member) and in groundwater (groundwater *E.*  
120 *coli* end-member).

### 121 2.2. Study area: the Houay Pano catchment

122 The 0.6 km<sup>2</sup> Houay Pano headwater catchment is located 10 km south of Luang Prabang city in Northern  
123 Lao PDR (Fig. 1a), within the 800 000 km<sup>2</sup> Mekong basin. This experimental site (Boithias et al., 2020) is  
124 part of the critical zone observatories' network named Multiscale TROPICAL Catchments (M-TROPICS),  
125 which belongs to the French Research Infrastructure OZCAR (Gaillardet et al., 2018). This catchment can  
126 be considered as being representative of the montane agro-ecosystems of South-East Asia. The climate is  
127 sub-tropical humid and is characterized by a monsoon regime with a dry season from November to May,  
128 and a wet season from June to October. The mean annual (2001-2019) temperature is 23.4 °C while the

129 mean annual rainfall is 1 366 mm (CV=0.23), about 71 % (CV=0.09) of which falls during the wet season.  
130 Altitude within the catchment is 435-716 m (Fig. 1b) and the slope gradient is 1-135 % (mean=52 %). The  
131 Laksip village, located downstream the S4 station (Fig. 1b), includes 484 inhabitants (Census of 2015).

### 132 2.3. Land use and land-use change

133 Detailed land-cover surveys and mapping were conducted each year from 2011 to 2015 within the  
134 catchment (Boithias et al., 2020). The annual areal percentages of fallow (Fallow), teak trees (Teak),  
135 annual crops (Annual crop), and annual crops grown under young teak trees (Teak+Crop) were calculated  
136 using QGIS 2.6. We assessed the monthly *E. coli* input from 2011 to 2015 within the catchment with the  
137 monthly counting of human and domestic animal (swine and poultry) traffic within the catchment, with  
138 the daily feces production of humans and domestic animals, and with the *E. coli* content in their feces  
139 (Causse et al., 2015).

### 140 2.4. Rainfall measurements

141 Rainfall was measured by an automatic weather station (Campbell BWS200 equipped with ARG100, 0.2  
142 mm capacity tipping-bucket) located within the catchment (Fig. 1b). Data was recorded at 6-min time  
143 interval from 2011 to 2012, and at 1-min time interval from 2013 to 2015. Data from 2013 to 2015 was  
144 then cumulated into a 6-min time series for consistency with the 2011-2012 sub-period.

### 145 2.5. Flow rate measurements and water quality monitoring

146 We measured stream water level at the S4 gauging station of the catchment outlet (Fig. 1b) within a V-  
147 notch weir equipped with a water level recorder (OTT Thalimedes) connected to a data logger, with 1-  
148 mm vertical precision at a minimum of 3-min time interval. To relate water level to discharge, a control  
149 rating curve was determined using both the velocity area method and the salt dilution method by slug  
150 injection. Samples of stream water were collected at S4 gauging station in clean, plastic bottles using an

151 automatic sampler (Automatic Pumping Type Sediment Sampler, ICRISAT) for the measurement of Total  
152 Suspended Sediments concentration ([TSS]), turbidity (Turbidity), Electrical Conductivity at 25°C (EC), and  
153 *E. coli* concentration (*[E. coli]*). The automatic sampler was triggered by the water level recorder to  
154 collect water after every 2-cm water level change during flood rising and every 5-cm water level change  
155 during flood recession.

156 [TSS] was measured in each sample after filtration on 0.2 µm porosity cellulose acetate filters (Sartorius)  
157 and evaporation at 105 °C for 48 h. Turbidity was determined with a turbidity meter (EUTECH  
158 Instruments TN-100) and EC with a conductivity meter (WTW340).

159 For nine flood events (events A, B, F-L), we measured *[E. coli]* with the standardized microplate method  
160 (ISO 9308-3). A water sub-sample was incubated at four dilution rates (i.e. 1:2, 1:20, 1:200 and 1:2000) in  
161 a 96-well microplate (MUG/EC, BIOKAR DIAGNOSTICS) for 48 h at 44 °C. Ringers' Lactate solution was  
162 used for the dilutions and one plate was used per sample. The number of positive wells for each  
163 microplate was noted and the Most Probable Number (MPN) per 100 mL was determined using the  
164 Poisson distribution. For three flood events (events C-E), we measured *[E. coli]* following the membrane-  
165 filter method (EPA Method 1603). A small quantity of each water sample was filtered through the filter  
166 membrane, which retained the bacterial cells. After filtration, this membrane was placed on a selective  
167 medium (Sartorius NKS Endo nutrient pads), and incubated at 44 °C for 24 h. Shiny green *E. coli* colonies  
168 were directly counted and expressed as Colony Forming Units (CFU) per 100 mL. Although "colony  
169 forming unit" techniques and "most probable number" techniques are known to give highly correlated  
170 results (e.g. Cho et al., 2010; Lušić et al., 2016; Wohlsen et al., 2006), we previously ensured that the two  
171 methods were consistent in the Houay Pano catchment conditions (unpublished work).

172 In addition, we monitored surface runoff in the different land uses of the catchment from 2011 to 2015  
173 using 1-m<sup>2</sup> microplots (Patin et al., 2018). We annually installed between 6 and 29 microplots to collect

174 surface runoff water samples during each rainfall event and to measure EC with WTW340. In 2012 and  
175 2014, we used sub-samples of surface runoff water to measure *E. coli* concentration in surface runoff as  
176 described previously. We calculated the mean concentration of *E. coli* in surface runoff by weighing the  
177 *E. coli* concentration exported from each land use by the annual areal percentage of each land use ( $[E.$   
178  $coli]_{OF-TOT}$ ). We also measured the *E. coli* concentration in water samples taken from three piezometers  
179 (Ribolzi et al., 2018) and calculated the mean concentration of *E. coli* in groundwater ( $[E. coli]_{GW-TOT}$ ).

## 180 2.6. Electrical conductivity-based hydrograph separation

181 We used a tracer-based approach to separate storm hydrographs into ‘event water’ and ‘pre-event  
182 water’. This approach relies on a simple mixing model with two end-members and EC as a tracer. It is of  
183 relatively low cost compared to e.g. isotopic tracers and was successfully tested in the study catchment  
184 (Ribolzi et al., 2018). Based on previous field observations and measurements performed in the same  
185 study catchment (Patin et al., 2012; Ribolzi et al., 2011; Vigiak et al., 2008), the two end-members of the  
186 model (i.e. overland flow EC end-member in event water, and groundwater EC end-member in pre-event  
187 water) can be interpreted in terms of hydrological processes. Event water mainly includes infiltration  
188 excess that produces overland flow along hillslopes. Pre-event water relates to groundwater that feeds  
189 the stream during the storm event, plus the water in the stream channel prior to the storm event, which  
190 is also related to groundwater outflows. As suggested by Collins and Neal (1998), we verified the linearity  
191 between EC and the concentration of a conservative tracer to control the relevance of the EC-based  
192 approach in our context (Ribolzi et al., 2018). The mixing model applied to individual samples is  
193 described by the following equations:

$$Q = Q_{OF} + Q_{GW} \quad \text{Eq. 1}$$

$$Q \times EC = Q_{OF} \times EC_{OF} + Q_{GW} \times EC_{GW} \quad \text{Eq. 2}$$

194 where  $Q$  is the instantaneous stream water discharge at the catchment outlet ( $L s^{-1}$ ),  $Q_{OF}$  is the  
195 instantaneous discharge of overland flow, i.e., event water or surface runoff ( $L s^{-1}$ ),  $Q_{GW}$  is the

196 instantaneous discharge of groundwater, i.e., pre-event water or sub-surface flow ( $L s^{-1}$ ), EC is the  
197 instantaneous electrical conductivity measured in the stream ( $\mu S cm^{-1}$ ),  $EC_{OF}$  is the electrical conductivity  
198 in overland flow (overland flow EC end-member;  $\mu S cm^{-1}$ ), approximated from electrical conductivity  
199 measurements in samples of overland flow collected at the soil surface on hillslopes draining to the  
200 stream, and  $EC_{GW}$  is the electrical conductivity in groundwater (groundwater EC end-member;  $\mu S cm^{-1}$ ),  
201 approximated from the stream electrical conductivity at the beginning of the flood event, since  
202 groundwater is the only supply of water to the stream during inter-storm flow periods (Ribolzi et al.,  
203 2005). For each individual sample, we calculated the relative contributions of  $Q_{OF}$  and of  $Q_{GW}$  to  $Q$  based  
204 on Eq. 2, namely  $Q_{OF}\%$  and of  $Q_{GW}\%$  (in %).

205 We assessed the uncertainty in estimating the contributions of overland flow and groundwater flow to  
206 the total stream flow using the formulation of Genereux (1998), which assigns an uncertainty specific to  
207 each term in Eqs. 1 and 2. The accuracy of  $Q$  is considered to be within  $\pm 10\%$  of the measured value,  
208 while the uncertainty of EC is approximately  $\pm 5\%$  (Ribolzi et al., 2018). The uncertainties of both  $EC_{OF}$  and  
209  $EC_{GW}$  are estimated from the coefficients of variation (CV) of overland flow and groundwater samples,  
210 respectively.

## 211 2.7. Flood-event variables

212 For each flood event, the mean concentration of *E. coli* ( $[E. coli]_{mean}$ ) is equivalent to the average of the  
213 instantaneous *E. coli* concentrations ( $[E. coli]$ ) measured during the event, weighted by the  
214 corresponding measurements of instantaneous discharge. We considered 13 candidate explanatory  
215 variables to predict  $[E. coli]_{mean}$ :

- 216 - Rainfall duration ( $R_D$ , in min), total rainfall ( $R_{TOT}$ , in mm), maximum rainfall intensity ( $RI_{MAX}$ , in mm  
217  $h^{-1}$ );

- 218 - Antecedent Precipitation Index (API, in mm), as a proxy of soil moisture conditions, calculated as  
 219  $API_n = (API_{n-1} + P_{n-1}) \times e^{-\alpha \times t}$  where  $API_{n-1}$  is the antecedent precipitation index prior to  
 220 rain event n-1 (mm),  $P_{n-1}$  is the rainfall precipitated during rain event n-1 (mm),  $\alpha$  is the  
 221 calibration coefficient usually set at 0.5 ( $\text{day}^{-1}$ ), and  $t$  is the duration between  $P_n$  and  $P_{n-1}$  (day)  
 222 (Descroix et al., 2002);
- 223 - Peak discharge during flood event ( $Q_{MAX}$ , in  $\text{L s}^{-1}$ ), total volume of stream water during flood  
 224 event ( $Q_{TOT}$ , in  $\text{m}^3$ ), total volume of surface runoff, i.e. overland flow, during flood event ( $Q_{OF-TOT}$ ,  
 225 in  $\text{m}^3$ ) and its contribution to  $Q_{TOT}$  ( $Q_{OF-TOT}\%$ , in %), and volume of sub-surface flow during flood  
 226 event ( $Q_{GW-TOT}$ , in  $\text{m}^3$ ) and its contribution to  $Q_{TOT}$  ( $Q_{GW-TOT}\%$ , in %);
- 227 - Flow coefficient of the flood event ( $K_E$ , no dimension), calculated as the ratio between  $Q_{TOT}$  and  
 228  $R_{TOT}$ ;
- 229 - Flood-event mean concentration of total suspended sediments concentration ( $[TSS]_{mean}$ , in  $\text{g L}^{-1}$ ),  
 230 calculated by weighting [TSS] by the discharge, and flood-event mean turbidity ( $Turbidity_{mean}$ , in  
 231 NTU), calculated by weighting Turbidity by time.

## 232 2.8. Hysteresis

233 Combined with EC-based hydrograph separation, the analysis of rotational direction, curvature, and  
 234 trend of hysteresis relationships can be used to interpret the relative contributions from surface water  
 235 and groundwater, and analyte sources (Holz, 2010; Long et al., 2017). Here, we analyzed hysteresis  
 236 patterns of EC, Turbidity, [TSS] and [*E. coli*] against discharge; [TSS], Turbidity and [*E. coli*] against  $Q_{OF}$ ;  
 237 Turbidity and [*E. coli*] against [TSS]; and [*E. coli*] against Turbidity. For each flood, we considered straight  
 238 relationships when the p-value of the Pearson correlation was below 0.001. The threshold was  
 239 voluntarily chosen very demanding to distinguish straight relationships from other hysteretic patterns.

## 240 2.9. Statistical analysis

241 We calculated correlations and regressions to identify explanatory variables predicting [*E. coli*] and [*E.*  
242 *coli*]<sub>mean</sub>. Statistics were calculated with R statistical package version 3.4.3 (correlations), Minitab 18.1  
243 (stepwise regression), and XLSTAT 20.1.1 (partial least square regression). An explanatory variable was  
244 considered to be statistically significantly different from zero when its p-value, derived from Student's t-  
245 test, was lower than 0.05.

### 246 2.9.1. Modelling instantaneous *E. coli* concentration [*E. coli*]

247 We first calculated Pearson correlations between [*E. coli*] and the eight hydrological variables measured  
248 or calculated for each sample (EC, Q, Q<sub>OF</sub>, Q<sub>GW</sub>, Q<sub>OF</sub>%, Q<sub>GW</sub>%, [TSS], and Turbidity). In addition, to select  
249 the best set of explanatory variables predicting [*E. coli*], we derived linear regressions from the 'step-  
250 wise regression' selection algorithm. This selection intended to maximize the prediction R<sup>2</sup> (R<sup>2</sup><sub>pred</sub>)  
251 calculated by leave-one-out cross-validations. This performance criterion reflects the ability of the model  
252 to predict observations that were not used in the model calibration. Its maximization leads to greater  
253 parsimony in the number of explanatory variables (Helsel and Hirsch, 2002). We verified the required  
254 homoscedasticity of the model's residuals by visual inspection. Multi-collinearity among the selected  
255 explanatory variables was avoided by ensuring that the Variance Inflation Factor (VIF) never exceeded  
256 the value of 8 (Helsel and Hirsch, 2002). We didn't mix instantaneous measurements with flood-event  
257 variables or with variables assessed annually (Annual crop, Teak+Crop, Teak, and Fallow) or monthly (*E.*  
258 *coli* input).

### 259 2.9.2. Modelling mean concentration of *E. coli* per flood event [*E. coli*]<sub>mean</sub>

260 We first calculated Pearson correlations between [*E. coli*]<sub>mean</sub> and the thirteen hydro-meteorological  
261 variables measured or calculated for each flood event (Table 1) and the five land-use related variables  
262 (Annual crop, Teak+Crop, Teak, Fallow, and *E. coli* input). In addition, we used Partial Least Square (PLS)

263 regression to detect dependencies between variables. PLS is able to handle datasets with a number of  
264 variables higher than the number of observations. It is also poorly sensitive to multi-collinearity, and  
265 handles missing data by imputation. The importance of each projected variable is estimated by the  
266 Variable Importance in the Projection (VIP). We discarded the variables for which VIP values were below  
267 1 (Ribolzi et al., 2016b; Wold, 1995).

## 268 2.10. Mixing model of flood-event mean concentration of *E. coli*

269 We applied a simple mixing model with two end-members to predict  $[E. coli]_{mean}$  and to separate the  
270 contributions of both 'event water' and 'pre-event water' to the in-stream *E. coli* concentrations. Here  
271 the two end-members of the model are *E. coli* concentration in event water (overland flow *E. coli* end-  
272 member or  $[E. coli]_{OF-TOT}$ ) and *E. coli* concentration in pre-event water (groundwater *E. coli* end-member  
273 or  $[E. coli]_{GW-TOT}$ ). The mixing model applied to flood-event mean variables is described by the following  
274 equations:

$$Q_{TOT} = Q_{OF-TOT} + Q_{GW-TOT} \quad \text{Eq. 3}$$

$$Q_{TOT} \times [E. coli]_{mean} = Q_{OF-TOT} \times [E. coli]_{OF-TOT} + Q_{GW-TOT} \times [E. coli]_{GW-TOT} \quad \text{Eq. 4}$$

275 where  $Q_{TOT}$  is the total volume of stream water during flood event ( $m^3$ ),  $Q_{OF-TOT}$  is the total volume of  
276 surface runoff, or overland flow, during flood event ( $m^3$ ),  $Q_{GW-TOT}$  is the total volume of sub-surface flow,  
277 or groundwater flow, during flood event ( $m^3$ ),  $[E. coli]_{mean}$  is the flood-event mean concentration of *E. coli*  
278 (MPN 100 mL<sup>-1</sup>),  $[E. coli]_{OF-TOT}$  is the mean concentration of *E. coli* exported in overland flow (MPN 100  
279 mL<sup>-1</sup>), calculated from *E. coli* concentration measured in samples of overland flow collected at the soil  
280 surface on hillslopes draining to the stream, and  $[E. coli]_{GW-TOT}$  is the mean concentration of *E. coli* in  
281 groundwater (MPN 100 mL<sup>-1</sup>), calculated from *E. coli* concentration measured in groundwater. This  
282 approach was shown to yield a comparable or even higher performance when predicting in-stream  
283 pathogen concentrations compared with more complex fate and transport models (Chin, 2011). We  
284 applied the mixing model to the twelve flood events and compared its performance with the

285 performance of the PLS model. For each flood event, we calculated the relative contributions of [*E.*  
286 *coli*]<sub>OF-TOT</sub> and of [*E. coli*]<sub>GW-TOT</sub> to [*E. coli*]<sub>mean</sub> based on Eq. 4, namely [*E. coli*]<sub>OF-TOT</sub>% and of [*E. coli*]<sub>GW-TOT</sub>%  
287 (in %).

## 288 3. Results

### 289 3.1. *E. coli* dynamics during flood events and among flood events

290 The areal percentage of land use evolved from 2011 to 2015 (Fig. SI1): annual crop decreased from 28%  
291 to 4% of the catchment area, and teak and annual crop decreased from 12% to 0%, while teak increased  
292 from 18% to 36%, and fallow increased from 29% to 46%. Areal percentage of forest was constant at 8%.  
293 The *E. coli* input into the catchment decreased from a monthly average of  $2 \times 10^{15}$  in 2011 to a monthly  
294 average of  $3 \times 10^{14}$  in 2015 (Fig. SI2). Over the 2011-2015 period, the cumulated *E. coli* input for swine and  
295 poultry accounted for 0.01-0.38 % of the total *E. coli* input.

296 In the meantime, a total of 294 discharge peaks were recorded between January 1, 2011, and December  
297 31, 2015 (Fig. 2), in response to a range of rainfall events (Fig. SI3, see interquartile ranges): 50 % of  $R_{TOT}$   
298 ranged between 7 and 22 mm, 50 % of  $R_{MAX}$  ranged between 24 and 60  $\text{mm h}^{-1}$ , and 50 % of  $R_D$  ranged  
299 between 49 and 235 min.  $Q$  ranged from 0 to 1 807.6  $\text{L s}^{-1}$  ( $30 \text{ L s}^{-1} \text{ ha}^{-1}$ ), with a mean  $Q$  of  $33.3 \text{ L s}^{-1}$ .

300 We monitored twelve flood events (A to L) for stream water quality. Datasets for each of the twelve  
301 flood events are complete except EC measurements lacking for event A and Turbidity measurements  
302 lacking for event G, due to measurement devices' breakdown. A total of 99 stream water samples was  
303 collected: [TSS] ranged from 0.02 to 25.7  $\text{g L}^{-1}$  while Turbidity ranged from 191.8 to 13 480 NTU (Fig. 3).  
304 *E. coli* were detected in all samples and [*E. coli*] ranged between 160 and 74 000 MPN 100  $\text{mL}^{-1}$  (Fig. 3).  
305 For the twelve flood events, rainfall was followed by an increase of  $Q$ , Turbidity, [TSS] and [*E. coli*] (Fig.  
306 3). However, the peaks of  $Q$ , Turbidity, [TSS] and [*E. coli*] were often asynchronous (full description is  
307 given in the supplementary information). Hysteresis loops showed complex patterns mixing straight  
308 lines, simple clockwise or anti-clockwise loops, figures-of-eight or multiple hysteresis loops (summary is  
309 presented in Table SI1, full description is given in the supplementary information, including Figs. SI4-SI7).

310 The uncertainty bands enclosing  $Q_{OF}$  and  $Q_{GW}$  in Fig. 3 show that the hydrograph could be clearly  
311 separated for each flood event. Flood event C was the only event where the stream flow was virtually  
312 100% groundwater flow. Excluding flood event C,  $Q_{OF-TOT}\%$  ranged between 17 and 80% (Fig. 4a). The  
313 average  $Q_{OF-TOT}\%$  was 41% (CV=0.59).

314 The twelve flood events corresponded to a range of storm characteristics in terms of  $R_{TOT}$ ,  $R_{I_{MAX}}$  and  $R_D$   
315 (Fig. SI3, Fig. 2).  $Q_{MAX}$  ranged from 31.7 to 967.9  $L s^{-1}$  (Table 1, Fig. 3). Three flood events occurred after  
316 dry periods (events B, G, and I:  $API < 10$ ), whereas others occurred shortly after a previous flood event  
317 (events D, E, and J:  $API > 30$ ) or later (events A, C, F, H, K, and L:  $10 < API < 20$ ) (Table 1).  $[E. coli]_{mean}$  ranged  
318 from 1 125 and 27 375 MPN 100  $mL^{-1}$ .

319 Mean  $EC_{OF}$  was 55  $\mu S cm^{-1}$  (CV=0.7) over the 2011-2015 period (65 surface runoff water samples). Mean  
320  $EC_{GW}$  was 256  $\mu S cm^{-1}$  (CV=0.42) among the twelve flood events.  $[E. coli]_{OF-TOT}$  was 24 880, 27 292, 27 441,  
321 26 987, and 26 935 MPN 100  $mL^{-1}$  in 2011, 2012, 2013, 2014, and 2015, respectively (26 surface runoff  
322 water samples).  $[E. coli]_{GW-TOT}$  was 277 MPN 100  $mL^{-1}$  (6 groundwater samples).

## 323 3.2. Statistical analysis

### 324 3.2.1. Modelling instantaneous *E. coli* concentration [*E. coli*]

325 Considering all water samples collected during the flood events (Fig. SI8), [*E. coli*] was positively  
326 correlated to  $Q$  ( $r=0.54$ ,  $p < 0.001$ ),  $Q_{OF}$  ( $r=0.56$ ,  $p < 0.001$ ),  $Q_{OF}\%$  ( $r=0.51$ ,  $p < 0.001$ ), [TSS] ( $r=0.43$ ,  $p < 0.001$ ),  
327 and Turbidity ( $r=0.43$ ,  $p < 0.001$ ), and was negatively correlated to EC ( $r=-0.31$ ,  $p=0.003$ ), and to  $Q_{GW}\%$  ( $r=-$   
328  $0.51$ ,  $p < 0.001$ ). Turbidity was positively correlated to [TSS] ( $r=0.82$ ,  $p < 0.001$ ).

329 From the stepwise regression, [*E. coli*] was best predicted by [TSS] and  $Q_{OF}$  (Eq. 5):

$$[E. coli] = 4995 + 689 \cdot [TSS] + 41.49 \cdot Q \quad \text{Eq. 5}$$

330 T-values and p-values of the coefficients are given in Table SI2. The  $R^2$  of the model is 35.3 % and the  
331  $R^2_{\text{pred}}$  is 24.42 %.

### 332 3.2.2. Modelling mean concentration of *E. coli* per flood event $[E. coli]_{\text{mean}}$

333  $[E. coli]_{\text{mean}}$  was positively correlated to  $Q_{\text{MAX}}$  ( $r=0.71$ ,  $p=0.009$ ),  $Q_{\text{TOT}}$  ( $r=0.59$ ,  $p=0.042$ ),  $Q_{\text{OF-TOT}}$  ( $r=0.72$ ,  
334  $p=0.012$ ), and  $Q_{\text{OF-TOT}}\%$  ( $r=0.93$ ,  $p<0.001$ ), and was negatively correlated to  $Q_{\text{GW-TOT}}\%$  ( $r=-0.93$ ,  $p<0.001$ )  
335 (Fig. SI9).

336 From the above, we found that  $Q_{\text{OF-TOT}}\%$  was the variable the most positively correlated to  $[E. coli]_{\text{mean}}$ .

337 The linear model is:

$$[E. coli]_{\text{mean}} = 332 \cdot Q_{\text{OF-TOT}}\% - 3749 \quad \text{Eq. 6}$$

338 The  $R^2$  between predicted and observed  $[E. coli]_{\text{mean}}$  was 0.87 while the Nash-Sutcliffe efficiency (NSE)  
339 was 0.84 (Fig. 5a). Excluding event C that was driven by groundwater flow only (and for which the linear  
340 model predicted a negative value),  $[E. coli]_{\text{mean}}$  predicted by the linear model ranged from 1981 to 22  
341 825 MPN 100 mL<sup>-1</sup>.

342 These latter trends are confirmed by the PLS regression (Fig. 6a), which shows that Axis 1 mostly explains  
343 the variables  $Q_{\text{OF-TOT}}\%$  ( $r=0.863$ ),  $Q_{\text{GW-TOT}}\%$  ( $r=-0.863$ ),  $Q_{\text{MAX}}$  ( $r=0.859$ ), and  $Q_{\text{OF-TOT}}$  ( $r=0.815$ ) whereas Axis 2  
344 mostly explains the variable  $RI_{\text{MAX}}$  ( $r=0.563$ ). Hence, Axis 1 corresponds to variables that are strongly  
345 related to stream water whereas Axis 2 corresponds to variables describing rainfall and soil moisture  
346 conditions. Accordingly, the twelve flood events were scattered along the two axes, with the flood  
347 events of highest  $[E. coli]_{\text{mean}}$  in the right panel (Fig. 6b). The variables with VIP values above 1 were  $Q_{\text{OF-}}$   
348  $\text{TOT}\%$ ,  $Q_{\text{GW-TOT}}\%$ ,  $Q_{\text{MAX}}$ ,  $Q_{\text{OF-TOT}}$ , and  $Q_{\text{TOT}}$  when considering one component and  $Q_{\text{OF-TOT}}\%$ ,  $Q_{\text{GW-TOT}}\%$ ,  
349  $[\text{TSS}]_{\text{mean}}$ ,  $Q_{\text{MAX}}$ , and  $Q_{\text{OF-TOT}}$  when considering two components (Fig. 6c; Table SI3). The statistical model  
350 given by the PLS is given in Table SI3. The  $R^2$  between predicted and observed  $[E. coli]_{\text{mean}}$  was 0.83 while  
351 the NSE was 0.78 (Fig. 5b). Excluding event C that was driven by groundwater flow only (and for which

352 the PLS model predicted a negative value),  $[E. coli]_{\text{mean}}$  predicted by the PLS model ranged from 1 067 to  
353 23 237 MPN 100 mL<sup>-1</sup>.

354  $[E. coli]_{\text{mean}}$  predicted by the mixing model ranged from 277 to 21 572 MPN 100 mL<sup>-1</sup>. The R<sup>2</sup> between  
355 predicted and observed  $[E. coli]_{\text{mean}}$  was 0.86 while the NSE was 0.65 (Fig. 5c). Flood event C was the only  
356 event where the percentage of *E. coli* was 100% from groundwater flow. Excluding flood event C,  $[E.$   
357  $coli]_{\text{OF-TOT}}\%$  ranged from 95 to virtually 100% (Fig. 4b). The average  $[E. coli]_{\text{OF-TOT}}\%$  was 89% (CV=0.33).

358

359

## 360 4. Discussion

### 361 4.1. High *E. coli* concentration pulses match high overland flow pulses

362 The analysis of the twelve monitored flood events (Table 1) shows that  $[E. coli]_{\text{mean}}$  was high regardless of  
363 the flood event characteristics:  $[E. coli]_{\text{mean}}$  ranged between 1 125 and 27 375 MPN 100 mL<sup>-1</sup>. The order of  
364 magnitude of the maximum  $[E. coli]$  is about 10<sup>5</sup> MPN 100 mL<sup>-1</sup>. All these values exceed the 1 000  
365 MPN 100 mL<sup>-1</sup> threshold provided by e.g. the European Directive 2006/7/EC for bathing water quality.

366 Although rainfall is known to reactivate hydrological connectivity (Bracken et al., 2013), rainfall  
367 characteristics such as  $R_{\text{TOT}}$ ,  $RI_{\text{MAX}}$ , and  $R_{\text{D}}$ , did not appear as strong explanatory variables of  $[E. coli]_{\text{mean}}$   
368 (Fig. 6 and Fig. SI9). Variations in  $[E. coli]$  and  $[E. coli]_{\text{mean}}$  between flood events may be explained by  
369 rainfall spatial distribution and the timing between rainfall events: the rainfall distribution will determine  
370 which areas draining animal and human manure are activated, whereas the timing between events may  
371 indicate which catchment microbial stocks have accumulated to high levels (McKergow and Davies-  
372 Colley, 2010). In Houay Pano catchment, variations in concentration between flood events may also be  
373 explained by *E. coli* input distribution at the soil surface, as the land use is mixed (Fig. SI1). Indeed, based  
374 on this twelve-event dataset and hysteresis analysis, we could not find any clear, consistent trend at the  
375 outlet of the Houay Pano catchment (Table SI1) and we thus could not identify any primary bacteria  
376 source.

377 The land use change in Houay Pano catchment was rapid and led to the simultaneous increase of teak  
378 tree plantations and of fallow areas (Riboldi et al., 2017). Previous plot- and catchment-scale results  
379 showed higher surface runoff and suspended matter export when teak tree plantations increased  
380 (Lacombe et al., 2018; Mügler et al., 2019; Riboldi et al., 2017; Song et al., 2020), suggesting that higher  
381 numbers of *E. coli* could be transferred to the river. However, land use characteristics such as land use  
382 variables (Annual crop, Teak+Crop, Teak, Fallow, and *E. coli* input) did not appear as strong explanatory

383 variables of  $[E. coli]_{\text{mean}}$  (Fig. 6 and Fig. SI10). Growing teak trees and fallow requires less people in the  
384 field compared to annual crops. Since field workers practice open defecation, less workers imply lower  
385 input of *E. coli* into the catchment. The contribution of domestic animals to the overall *E. coli* input being  
386 only 0.01-0.38 % of the total *E. coli* input, the consequence of the extension of teak tree plantation and  
387 of fallow in Houay Pano from 2011 to 2015 (Fig. SI1) is the overall decrease of the *E. coli* input into the  
388 catchment over the same period (Fig. SI2).

389 The highest  $[E. coli]_{\text{mean}}$  was often associated to flood events with highest  $Q_{\text{MAX}}$  (events J and L) and  
390 dominant surface runoff ( $Q_{\text{OF-TOT}}\%>50$ : events D, and I-L), confirming the role of surface runoff, and  
391 subsequent soil surface erosion, in *E. coli* transfers to the stream (Causse et al., 2015) (Table 1, Fig. 6).  
392 For flood events where sub-surface flow was dominating ( $Q_{\text{OF-TOT}}\%<50$ : events B, C, and E-H),  $[E. coli]_{\text{mean}}$   
393 was in general lower, although exceeding 1 000 MPN 100 mL<sup>-1</sup>. High *E. coli* concentrations even when  
394 sub-surface flow was dominating suggest that streambanks and the streambed may release stored *E. coli*  
395 (Chu et al., 2011; Park et al., 2017; Stocker et al., 2018) since sediment deposited at the bottom of the  
396 stream may act as an *E. coli* reservoir (Pachepsky et al., 2017; Rochelle-Newall et al., 2015; Smith et al.,  
397 2008). In fact, the concentration of *E. coli* in Houay Pano streambed sediment is about 40 000 MPN g<sup>-1</sup>  
398 (Ribolzi et al., 2016a), but we cannot exclude that *E. coli* is simultaneously transferred from the hillslope  
399 with surface runoff, if hillslope soil surface is highly contaminated. We also found both clockwise and  
400 anti-clockwise  $[E. coli]$ -[TSS] and  $[E. coli]$ -Turbidity hysteresis loops, and in some cases figure-of-eight  
401 patterns (Table SI1, Fig. SI6 and Fig. SI7). This further suggests that the erosion of bacteria stores is not  
402 strictly driven by soil erosion, but also by in-stream sediment resuspension (Evrard et al., 2016; Gourdin  
403 et al., 2015; Huon et al., 2017). In parallel, possibly less *E. coli*-contaminated sub-surface flow, or return  
404 flow, may dilute in the stream the *E. coli* concentration originating from surface runoff and thus mitigate  
405 the in-stream microbial contamination.

## 406 4.2. Predictive models of *E. coli* concentration: performances and usefulness

407 To our knowledge, few studies have reported predictive models using independent variables to explain  
408 *E. coli* concentration and, to the best of our knowledge, they were all developed in temperate areas  
409 (Chen and Chang, 2014; Hathaway et al., 2010). The models suggested by Chen and Chang (2014)  
410 included antecedent precipitation, stream temperature, and TSS concentration, and gave an  $R^2$  of 0.27-  
411 0.61 depending on the season and the catchment. The model suggested by Hathaway et al. (2010)  
412 included temperature, rainfall, and humidity, and had an  $R^2$  of 0.7462. The variety of explanatory  
413 variables among models reflects the variety of driving processes driving *E. coli* fate and transport among  
414 catchments.

### 415 4.2.1. Modelling instantaneous *E. coli* concentration [*E. coli*]

416 [*E. coli*] was equally correlated to both [TSS] and Turbidity ( $r=0.43$ ,  $p<0.001$ ; Fig. SI8) while Turbidity was  
417 correlated with [TSS] ( $r=0.82$ ,  $p<0.001$ , Fig. SI8). The strong correlation between Turbidity and [TSS] is in  
418 line with the results obtained in other studies in tropical areas, such as Ziegler et al. (2014) for rivers in  
419 Thailand and Robert et al. (2017) in West African lakes and ponds. The relationships between [TSS] and  
420 [*E. coli*] and between Turbidity and [*E. coli*] are qualified by hysteretic trends (Figs. SI6 and SI7,  
421 respectively) during flood events. Notably, little hysteresis is observed between [TSS] and Turbidity (Fig.  
422 SI6). Hysteretic trends may illustrate the uncertainty when predicting *E. coli* by a proxy such as turbidity:  
423 sources of sediments may not exactly coincide with sources of *E. coli*. For example, these relationships  
424 may not apply in large mixed-land use catchments, because of the multiple, geographically separated, *E.*  
425 *coli* and turbidity sources. This may limit the usefulness of using turbidity as a proxy of *E. coli* near the  
426 catchment outlet (McKergow and Davies-Colley, 2010). Another explanation of the hysteresis is the  
427 nature and the properties of the suspended sediments, that may change during the flood event: bacteria  
428 may be less prone to attach to sand-rich suspended particles (Oliver et al., 2007). Similarly, hysteresis in  
429 Turbidity-[TSS] relationships may be explained by changing reflectance properties of the suspended

430 sediments depending on particle size, shape, mineralogy, aggregation/flocculation, dissolved light-  
431 absorbing matter and bubbles, because of the spatial heterogeneity of rainfall and suspended sediment  
432 sources at the catchment scale (Navratil et al., 2011; Ziegler et al., 2014). A last uncertainty source in the  
433 relationships between [*E. coli*], [TSS], and Turbidity, is the possible exhaustion of the *E. coli* stock within  
434 the catchment after a succession of flood events.

435 From the step-wise regression analysis, the predictive model for [*E. coli*] includes [TSS] and  $Q_{OF}$  as  
436 explanatory variables (Eq. 5). The two explanatory variables reflect the two processes driving *E. coli*  
437 transport within the catchment:  $Q_{OF}$  reflects the mobilization of *E. coli* with relative contributions of  
438 surface and sub-surface flows along the flood event, whereas [TSS] reflects the attachment of *E. coli* to  
439 soil particles and/or streambed resuspended sediments (Garcia-Armisen and Servais, 2009; Nguyen et  
440 al., 2016). Overall, higher *E. coli* concentration is related to higher surface runoff and higher suspended  
441 matter in the stream flow. The value of the intercept term in Eq. 5 corresponds to *E. coli* concentration  
442 during base flow (Boithias et al., 2016; Kim et al., 2018), i.e. when [TSS] and  $Q_{OF}$  tend to zero because  
443 there is no surface runoff during inter-storm periods, in other words when groundwater is the only  
444 supply to stream flow and EC tends to  $EC_{GW}$ .

445 The two variables best predicting [*E. coli*], namely [TSS] and  $Q_{OF}$ , can be assessed with proxies. [TSS]  
446 could be interchanged with Turbidity, since [TSS] and Turbidity are correlated, as discussed above.  
447 Similarly, EC appears strongly related to  $Q_{OF}$  (and Q) by a hyperbolic function (Fig. SI8). Using EC as a  
448 proxy of Q has already been proposed in Alpine headwaters (Cano-Paoli et al., 2019). Both of these  
449 proxies can be monitored *in situ* at high frequencies.

#### 450 4.2.2. Modelling mean concentration of *E. coli* per flood event [*E. coli*]<sub>mean</sub>

451 The PLS regression analysis (Table SI3) confirmed the analysis of the correlation matrix (Fig. SI9):  $Q_{OF-TOT}\%$   
452 and  $Q_{GW-TOT}\%$  were the best predictors of [*E. coli*]<sub>mean</sub> ( $r= 0.93$  and  $-0.93$ , respectively). Land uses that

453 favor higher  $Q_{OF-TOT}\%$  or lower  $Q_{GW-TOT}\%$ , such as erosion-prone teak trees plantations (Lacombe et al.,  
454 2016; Ribolzi et al., 2017), or more intense rainfall events, will lead to higher in-stream concentrations of  
455 *E. coli* (Fig. 6a and Fig. SI10) and thus higher fecal contamination risk. Hence, a model based on  $Q_{OF-TOT}\%$   
456 or  $Q_{GW-TOT}\%$  appears relevant to predict  $[E. coli]_{mean}$ . Such a basic tool may help local and national  
457 stakeholders to assess the fecal contamination risk by testing global change scenarios in target  
458 catchments.

459 In this study, the mixing model of  $[E. coli]_{mean}$  based on  $Q_{OF-TOT}\%$  and  $Q_{GW-TOT}\%$  had a  $R^2$  of 0.86 and a NSE  
460 of 0.65. The NSE value is lower than the NSE values' range reported by Chin (2011) in six nested  
461 catchments in Georgia, United-States. However, in our study the values of both overland flow and  
462 groundwater end-members were measured in the field, conversely to Chin (2011) who numerically  
463 optimized the values of the two end-members. The performance of the mixing model is lower in terms of  
464 NSE than those of the PLS and the linear models (NSE = 0.78 and 0.84, respectively). However, the PLS  
465 model is difficult to implement because of the large number of variables it implies, and both the linear  
466 and the PLS models are constrained by their validity domains.

467 Within a catchment, the sources of *E. coli* are the *E. coli* transferred from hillslopes with surface runoff,  
468 as discussed in section 4.1, and the resuspension from the streambed, whereas the sinks of *E. coli* are the  
469 deposition of the bacterium on the streambed and its decay in the water column. Since rainfall events in  
470 the Houay Pano catchment last 0.5-5 hours, considering a decay rate of  $0.3-0.6\text{ d}^{-1}$  (Nguyen et al., 2016),  
471 the removal of *E. coli* in the water column from bacteria population decay is negligible during a flood  
472 event. Since the mixing model is able to accurately predict the flood-event mean concentration of *E. coli*  
473 at the catchment outlet, the deposition of *E. coli* on the streambed is either negligible or compensated  
474 by the resuspension. The percentage of *E. coli* resuspended from streambed during a flood is about 11%  
475 (Ribolzi et al., 2016a), which implies that the compensated *E. coli* deposition, e.g. in small wetlands along  
476 the main stream, is about 11% as well. Finally, since the possible sources and sinks of *E. coli* are negligible

477 compared to the bacterium transport processes during flood events, the approximation of the simple  
478 model that only considers two end-members, namely the *E. coli* concentration in both surface runoff and  
479 groundwater, is acceptable.

480 Hence, we could calculate the relative contributions of surface runoff and of sub-surface flow to the in-  
481 stream *E. coli* contamination. Excluding flood event C that was driven by groundwater flow, more than  
482 95% of  $[E. coli]_{\text{mean}}$  resulted from overland flow, even for flood events where the percentage of overland  
483 flow was small (<20%, events E and G). The average contribution of overland flow to the flood-event  
484 mean concentration of *E. coli* was 89%, while the average contribution of overland flow to the flood-  
485 event mean stream flow was 41% (Fig. 4). In other words, the contribution of groundwater to the in-  
486 stream *E. coli* contamination was low during flood events, even though the contribution of groundwater  
487 to stream water flow was greater than that of overland flow, i.e. when base flow index (BFI) was over  
488 0.5. In comparison, with similar BFI values, Chin (2011) reported bacteria loads from surface runoff in the  
489 range of 80–90%, with the remainder mostly originating from base flow, and negligible background loads  
490 from the catchment.

491

492 5. Conclusion

493 To our knowledge, few papers have reported the statistical relationships between *E. coli* and its  
494 environmental drivers (meteorological, hydrological, and land use variables) and the present study is the  
495 first one to investigate *E. coli* dynamics during flood events in a tropical humid catchment. We conclude  
496 that:

- 497 - Regardless of the contribution of sub-surface flow to the flood, in-stream *E. coli* concentration is  
498 high, suggesting that the streambed *E. coli* store is high. However, highest concentrations of *E.*  
499 *coli* are measured for the flood events that are driven by surface runoff, suggesting that surface  
500 runoff, and consequently soil erosion, are the primary drivers of in-stream *E. coli* contamination;
- 501 - The three predictive models (linear model, partial least square model, and mixing model) show  
502 that the percentage of surface runoff in stream flow is the best predictor of the flood event  
503 mean concentration of *E. coli*;
- 504 - A simple mixing model based on the relative contributions of both overland flow and  
505 groundwater, and on the *E. coli* concentration in both overland flow and groundwater, is reliable  
506 to predict in-stream flood-event mean *E. coli* concentration (NSE=0.65). On average, 89% of the  
507 in-stream concentration of *E. coli* is supplied by surface runoff, while the overall contribution of  
508 surface runoff to the stream flow is 41%.
- 509 - Stream flow turbidity and *E. coli* concentration are positively correlated, but turbidity is not a  
510 strong predictor of *E. coli* concentration during flood events;

511 Predictive models based on turbidity and electrical conductivity may be used to provide real-time  
512 estimates of in-stream *E. coli* concentration, whereas a mixing model may provide flood-event mean  
513 information. Such simple models may help to assess the impact of global change on in-stream *E. coli*  
514 contamination. They could thus be used to assess the risk of water borne diseases such as diarrhea, in  
515 rural areas where mammals, including humans, practice open defecation, and to design early warning

516 systems. Applied over long periods of time, the models might be used to calculate *E. coli* input-output  
517 load balances. Such basic modelling studies would thus help to assess the long-term impact of land-use  
518 change on the microbial quality of surface water.

519 Future work is required to better understand the pathways of FIB at catchment scale. For example, there  
520 is a need to characterize the interactions between ground and surface waters, and the role of the  
521 hyporheic zone, as sources of contaminant. Furthermore, the partition between free and particle-  
522 attached *E. coli* should be quantified to better understand and predict the probability that bacteria is  
523 deposited on the streambed during the flood recession stage and re-suspended in the water column  
524 during the next flood rising stage. These findings are of primary importance to build adequate  
525 catchment-scale models to accurately simulate *E. coli* fate and transport, and thus better assess fecal  
526 contamination risk in a global changing context.

527

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536

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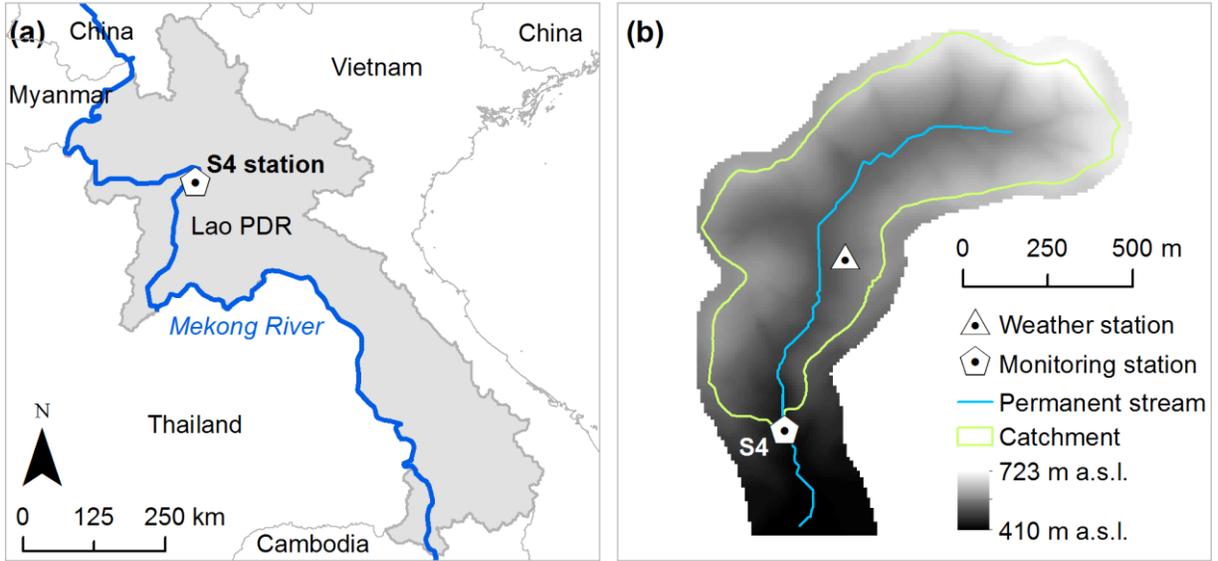
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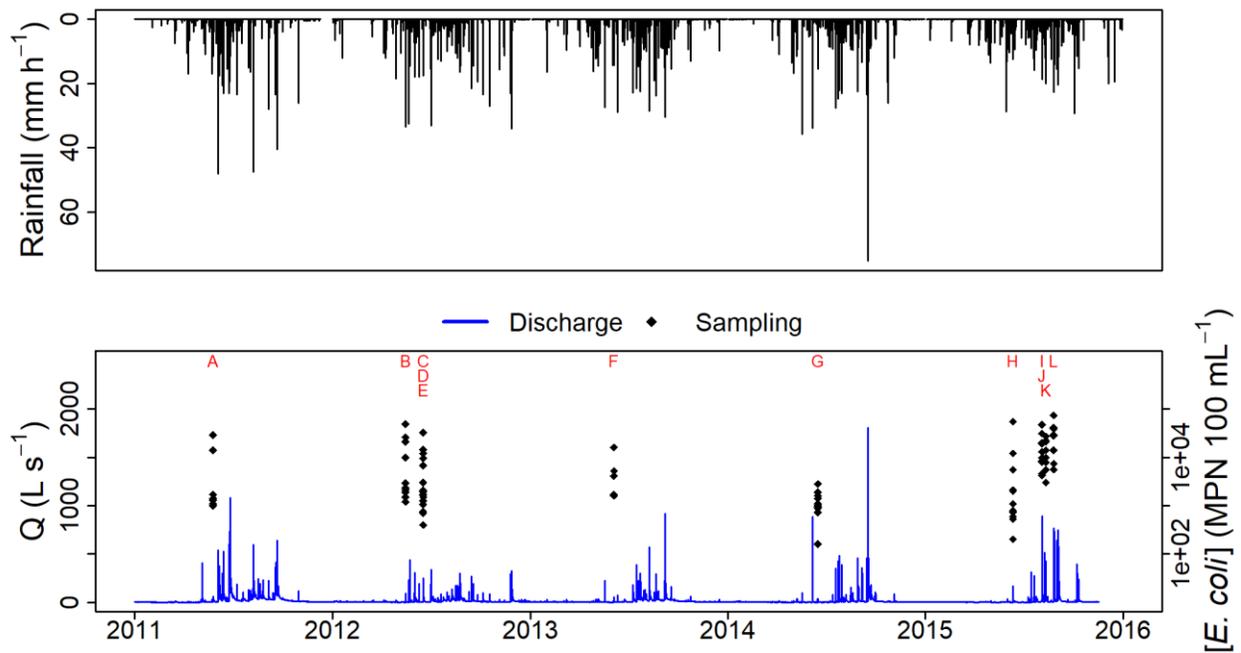
777 Fig. 1. (a) Location of the S4 outlet of the Houay Pano catchment in northern Lao PDR; (b) River gauging  
778 and sampling station S4, weather station, and altitudes. Altitudes are given in meters above sea level (m  
779 a.s.l.).

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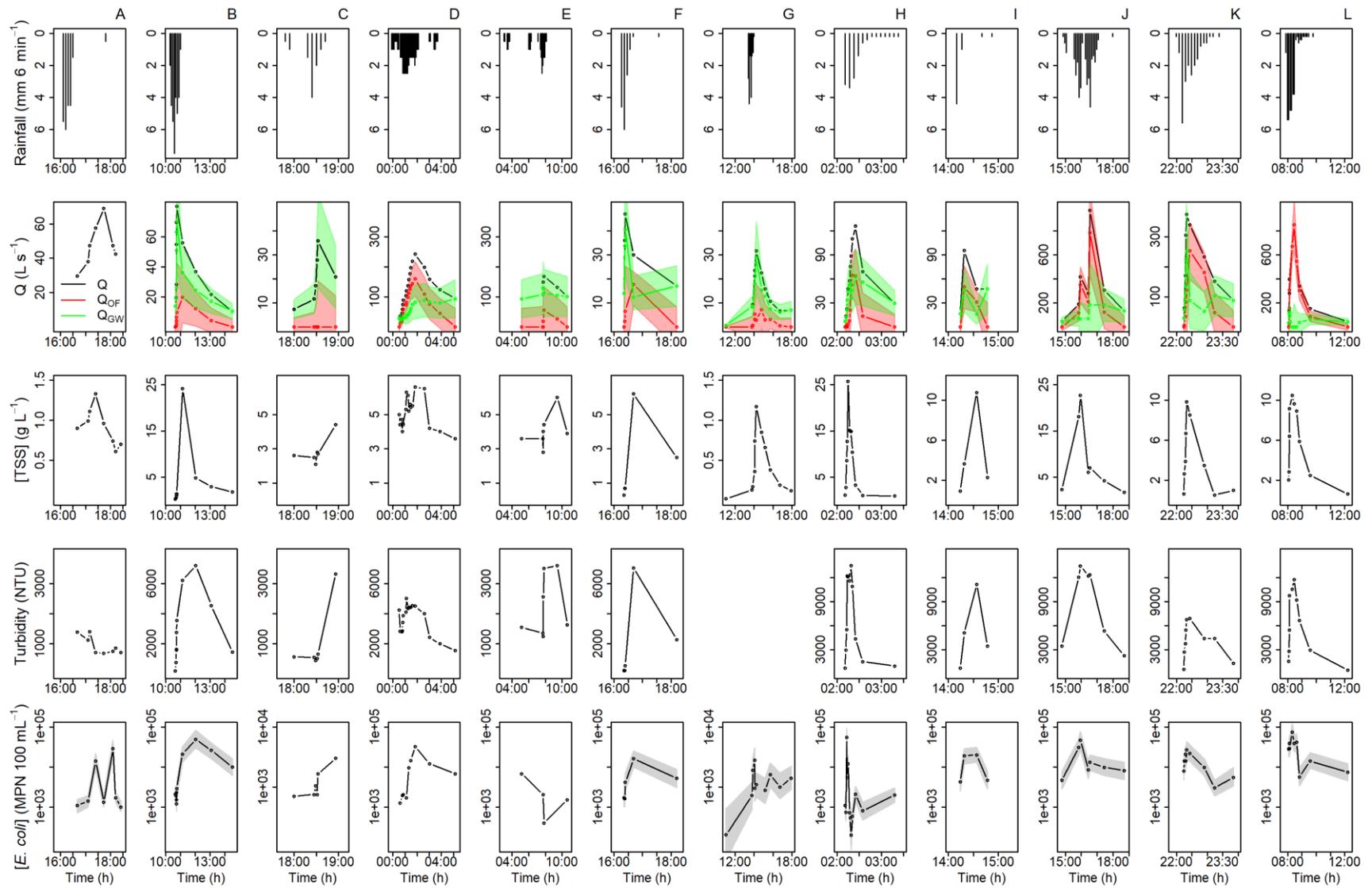
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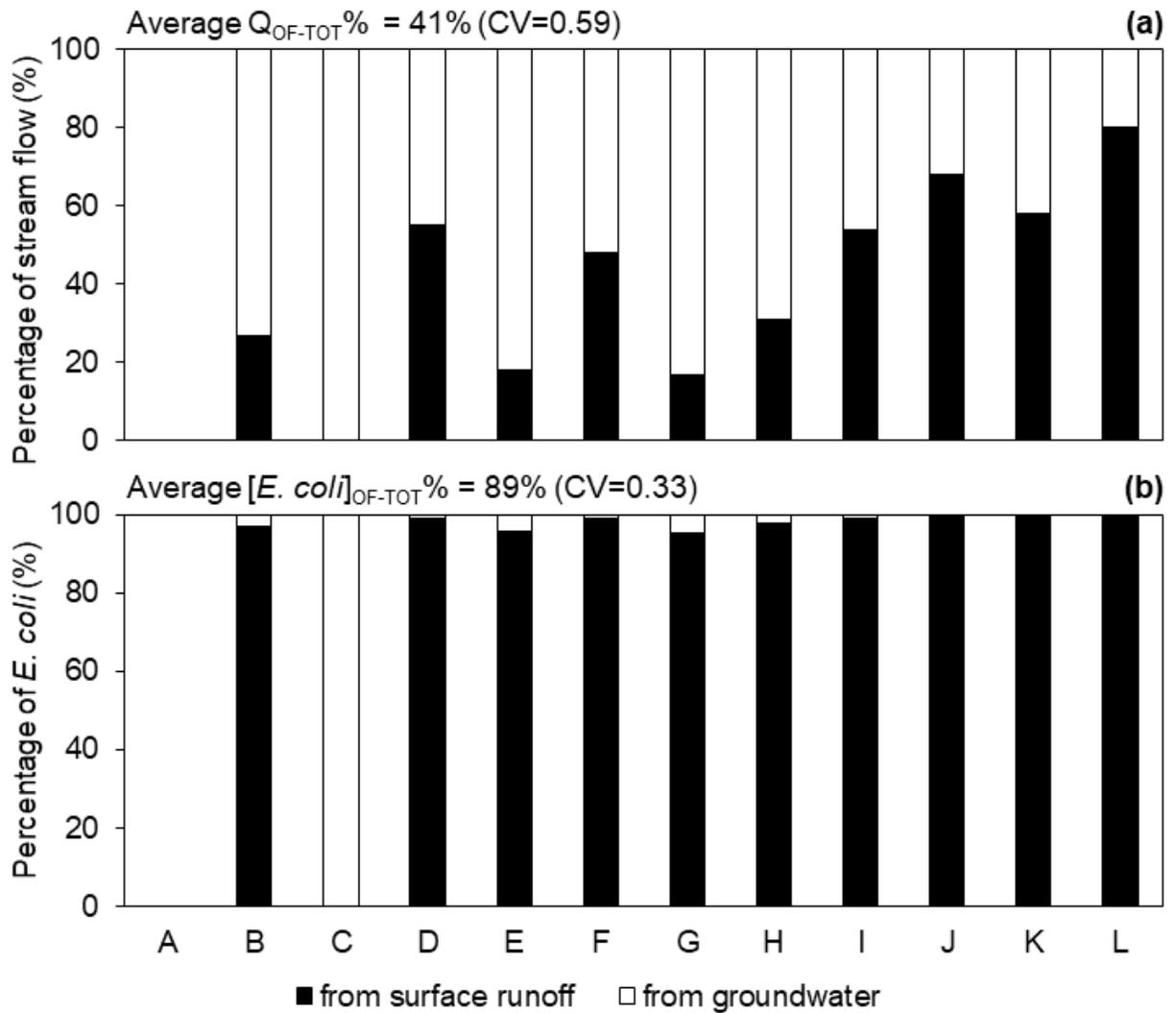
785 Fig. 2. Rainfall ( $\text{mm h}^{-1}$ ) and discharge ( $Q$ , in  $\text{L s}^{-1}$ ) for the 2011-2015 period, and *E. coli* concentration ( $[E.$   
 786 *coli], in  $\text{MPN } 100 \text{ mL}^{-1}$ ) for twelve flood events from 2011 to 2015 at the outlet of the Houay Pano  
 787 catchment, northern Lao PDR. The flood events of 16-17 June 2012 (C, D, and E) and of 4 August 2015 (I  
 788 and J) have been decomposed into 3 and 2 separate flood events. Details of these two flood events is  
 789 shown in Fig. 3. Although  $[E. coli]$  measures for events C-E were expressed in  $\text{CFU } 100 \text{ mL}^{-1}$ , they are  
 790 reported as  $\text{MPN } 100 \text{ mL}^{-1}$  for the sake of simplicity.*

791



793 Fig. 3. Monitored flood events from 2011 to 2015 at the outlet of the Houay Pano catchment, northern Lao PDR. Rainfall: rainfall (mm); Q:  
794 discharge ( $L s^{-1}$ );  $Q_{OF}$ : surface runoff ( $L s^{-1}$ );  $Q_{GW}$ : sub-surface flow ( $L s^{-1}$ ); [TSS]: total suspended sediments concentration ( $g L^{-1}$ ); Turbidity: turbidity  
795 (NTU); [*E. coli*]: *E. coli* concentration (MPN 100 mL<sup>-1</sup>). Measurements of turbidity were lacking for event G. Although [*E. coli*] measures for events  
796 C-E were expressed in CFU 100 mL<sup>-1</sup>, they are reported as MPN 100 mL<sup>-1</sup> for the sake of simplicity. Red and green bands for  $Q_{OF}$  and  $Q_{GW}$ ,  
797 respectively, are uncertainty bands calculated with the Genereux (1998) method. Grey bands for [*E. coli*] are uncertainty intervals given by  
798 Poisson distribution when using the standardized microplate method.

799



800

801 Fig. 4. Contributions (in %) of overland flow and of groundwater flow in (a) flood-event total volume of

802 stream flow, and in (b) flood-event total *E. coli* number. The twelve flood events were sampled from

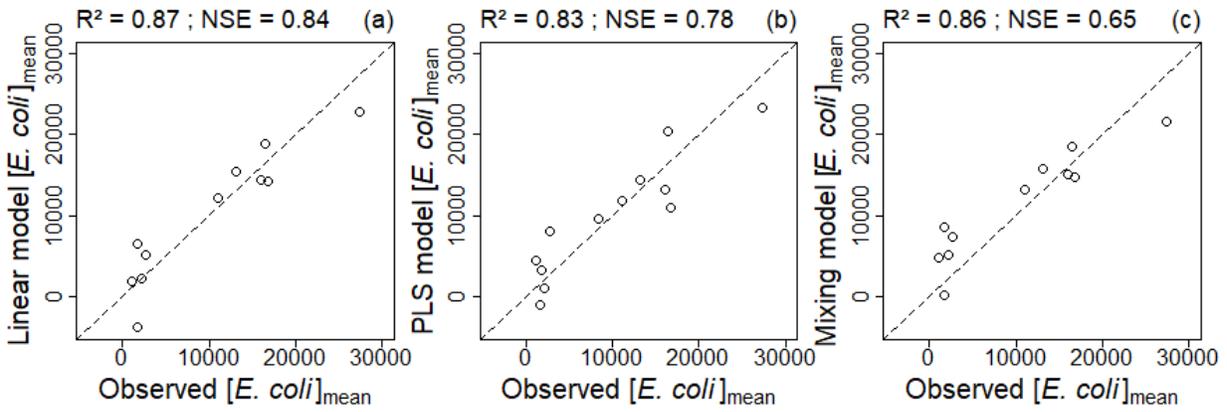
803 2011 to 2015 at the outlet of the Houay Pano catchment, northern Lao PDR. Measurements of electrical

804 conductivity were missing for event A. Average  $Q_{OF-TOT}\%$  and average  $[E. coli]_{OF-TOT}\%$  are the average

805 contribution of overland flow to the in-stream flow and the average percentage of *E. coli* resulting from

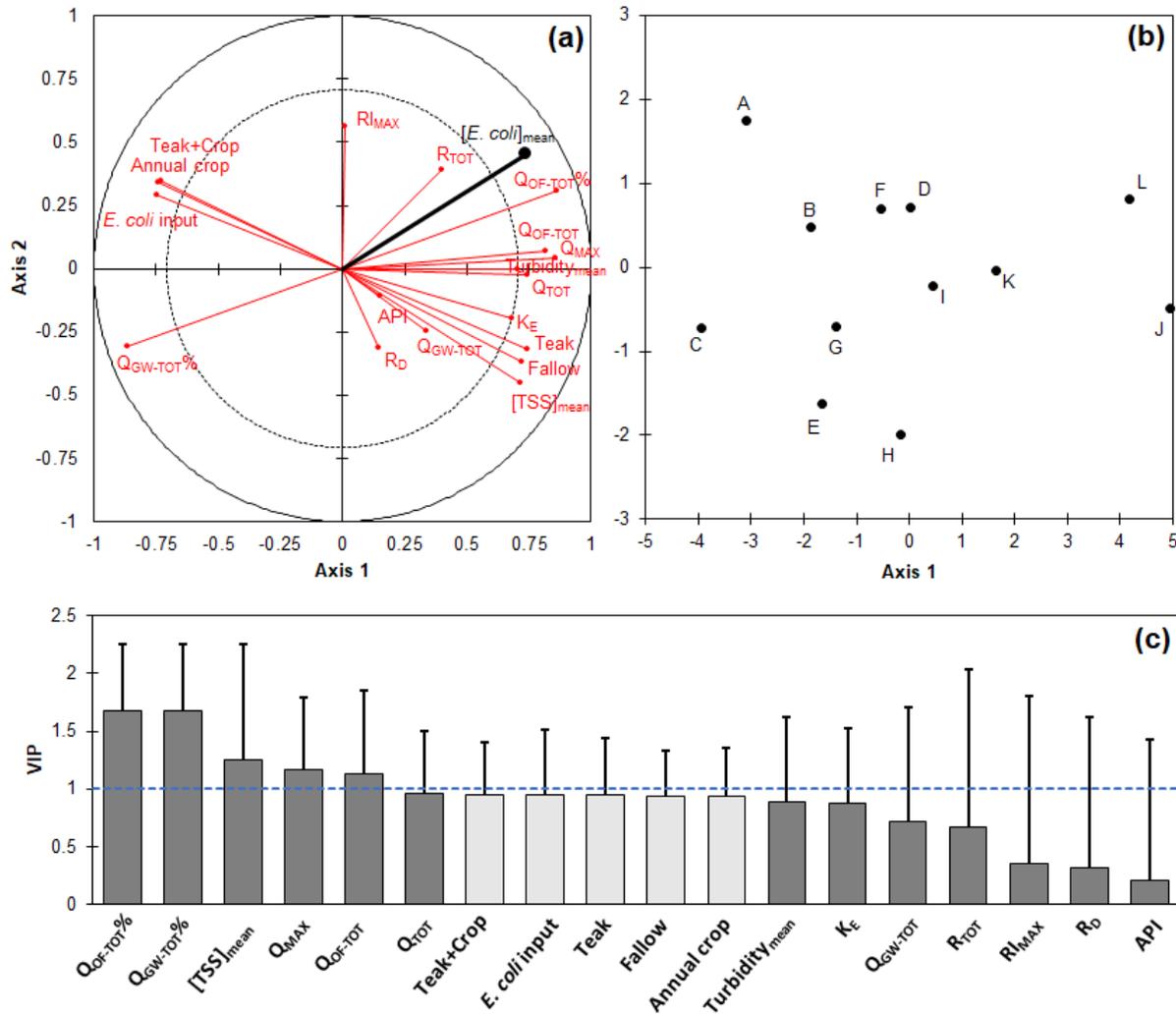
806 overland flow, respectively. CV: coefficient of variation.

807



808

809 Fig. 5. Mean *E. coli* concentration per flood event ( $[E. coli]_{\text{mean}}$ ): comparison of models' predictions with  
 810 observed values (MPN 100 mL<sup>-1</sup>). (a)  $Q_{\text{OF-TOT}}\%$ -based linear model against observed  $[E. coli]_{\text{mean}}$ ; (b) PLS  
 811 model against observed  $[E. coli]_{\text{mean}}$ ; (c) Mixing model against observed  $[E. coli]_{\text{mean}}$ .  $R^2$ : coefficient of  
 812 determination; NSE: Nash-Sutcliffe Efficiency. The twelve flood events were sampled from 2011 to 2015  
 813 at the outlet of the Houay Pano catchment, northern Lao PDR.



814

815 Fig. 6. Partial Least Square regression analysis where the flood-event mean concentration of *E. coli* ( $[E.$

816 *coli]\_{mean}) is explained by hydro-meteorological and land-use variables: (a) *E. coli* located in the*

817 correlations circle with 13 meteorological and hydrological variables and 5 land-use variables; (b) Map of

818 observations (twelve flood events from 2011 to 2015 at the outlet of the Houay Pano catchment,

819 northern Lao PDR); (c) Variable Importance in Projection (VIP) of the 18 variables for two components.

820  $R_D$ : event rainfall duration;  $R_{TOT}$ : cumulated rainfall over the event;  $R_{I_{MAX}}$ : maximum rainfall intensity; API:

821 antecedent precipitation index;  $Q_{MAX}$ : peak discharge during the event;  $Q_{TOT}$ : total volume of stream

822 water exported during the event;  $Q_{OF-TOT}$ : total volume of surface runoff exported during the event;  $Q_{OF-$

823  $TOT\%$ : percentage of surface runoff during the event calculated as  $Q_{OF-TOT}/Q_{TOT} * 100$ ;  $Q_{GW-TOT}$ : total volume

824 of sub-surface flow exported during the event;  $Q_{GW-TOT}\%$ : percentage of sub-surface flow during the  
825 event calculated as  $Q_{GW-TOT}/Q_{TOT} * 100$ ;  $K_E$ : flow coefficient calculated as  $Q_{TOT}/R_{TOT}$ ;  $[TSS]_{mean}$ : flood-event  
826 mean total suspended sediments concentration;  $Turbidity_{mean}$ : flood-event mean turbidity; Fallow:  
827 annual percentage of the catchment area covered by fallow; Teak: annual percentage of the catchment  
828 area covered by teak trees; Annual crop: annual percentage of the catchment area covered by annual  
829 crops; Teak+Crop: annual percentage of the catchment area covered by annual crops grown under young  
830 teak trees; *E. coli* input: monthly *E. coli* input within the catchment.

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832 Table 1. Hydro-meteorological characteristics of the selected flood events from 2011 to 2015 at the outlet of the Houay Pano catchment,  
833 northern Lao PDR.

Event	Rainfall			Discharge						Water quality				
	Start date Time	R <sub>D</sub> (min)	R <sub>TOT</sub> (mm)	RI <sub>MAX</sub> (mm h <sup>-1</sup> )	API (mm)	Q <sub>MAX</sub> (L s <sup>-1</sup> )	Q <sub>TOT</sub> (m <sup>3</sup> )	Q <sub>OF-TOT</sub> (m <sup>3</sup> )	Q <sub>OF-TOT</sub> % (%)	K <sub>E</sub> (%)	n (-)	[TSS] <sub>mean</sub> (g L <sup>-1</sup> )	Turbidity <sub>mean</sub> (NTU)	[ <i>E. coli</i> ] <sub>mean</sub> (MPN 100 mL <sup>-1</sup> )
A	05/25/2011 16:06	24	22.5	60	15.5	69.1	305.5			2.3	8	1.0	993.6	8388
B	05/15/2012 10:18	42	33.5	75	2.8	80.3	553.4	146.7	27	2.7	10	4.9	5466.0	2672
C	06/16/2012 17:48	54	10.5	40	15.0	35.8	69.7	0.0	0	1.1	6	2.9	594.2	1682
D	06/17/2012 00:00	228	39	25	48.6	242.0	2620.8	1432.4	55	11.2	18	5.3	3252.9	16053
E	06/17/2012 03:00	306	19	25	40.6	168.4	2471.3	440.9	18	21.6	7	4.1	2578.0	2181
F	06/04/2013 16:11	82	14.6	60	12.3	47.0	215.6	103.3	48	2.5	5	2.0	5860.3	11020
G	06/16/2014 13:19	36	15.4	44	9.3	31.7	222.6	38.4	17	2.4	11	0.6		1125
H	06/12/2015 02:06	74	13.2	34	11.1	125.8	308.9	96.4	31	4.0	12	10.1	3696.0	1748
I	08/04/2015 13:22	50	6	44	4.3	95.2	127.4	69.1	54	3.8	4	4.8	6809.8	16776
J	08/04/2015 14:49	131	34.6	46	30.9	967.9	5554.2	3796.0	68	26.7	7	9.8	7790.9	16403
K	08/11/2015 21:59	81	20.8	56	17.4	374.7	1378.9	804.9	58	11.0	9	6.0	5205.8	13112
L	08/26/2015 07:48	116	32.4	54	16.2	849.6	3903.6	3118.3	80	20.0	10	8.2	4822.8	27375

834 R<sub>D</sub>: duration of rainfall event; R<sub>TOT</sub>: cumulated rainfall of the event; RI<sub>MAX</sub>: maximum rainfall intensity; API: antecedent precipitation index; Q<sub>MAX</sub>: peak discharge  
835 during the flood event; Q<sub>TOT</sub>: total volume of stream water exported during the event; Q<sub>OF-TOT</sub>: total volume of surface runoff exported during the event; Q<sub>OF-</sub>  
836 <sub>TOT</sub>%: percentage of surface runoff during the event calculated as Q<sub>OF-TOT</sub>/Q<sub>TOT</sub>\*100; K<sub>E</sub>: flow coefficient calculated as Q<sub>TOT</sub>/R<sub>TOT</sub>; n: sample size; [TSS]<sub>mean</sub>: flood-  
837 event mean total suspended sediments concentration; Turbidity<sub>mean</sub>: flood-event mean turbidity; [*E. coli*]<sub>mean</sub>: flood-event mean *E. coli* concentration.  
838 Measurements of EC and of Turbidity were lacking for events A and G, respectively. Although *E. coli* measurements for events C-E were expressed in CFU 100  
839 mL<sup>-1</sup>, they are reported as MPN 100 mL<sup>-1</sup> for the sake of simplicity.