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## Village Settlements in Mountainous Tropical Areas, Hotspots of Fecal Contamination as Evidenced by *Escherichia coli* and Stanol Concentrations in Stormwater Pulses

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1 Village settlements in mountainous tropical areas,  
2 hotspots of fecal contamination as evidenced by  
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4 water pulses  
5

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18

## 19 Abstract

20 Fecal bacteria in surface water may indicate threats to human health. Our hypothesis is that village  
21 settlements in tropical rural areas are major hotspots of fecal contamination, because of the number  
22 of domestic animals usually roaming in the alleys, and the lack of fecal matter treatment before  
23 entering river network. By jointly monitoring the dynamics of *Escherichia coli* (*E. coli*) and of seven  
24 stanol compounds during four flood events (July-August 2016) at the outlet of a ditch draining  
25 sewage and surface runoff out of a village of Northern Lao PDR, our objectives were (1) to assess the  
26 range of *E. coli* concentration in the surface runoff washing off from a village settlement, and (2) to  
27 identify the major contributory sources of fecal contamination using stanol compounds during flood  
28 events. *E. coli* pulses ranged from  $4.7 \cdot 10^4$  to  $3.2 \cdot 10^6$  MPN 100 mL<sup>-1</sup>, with particle-attached *E. coli*  
29 ranging from 83 to 100 %. Major contributory feces sources were chicken and human (about 66 and  
30 29 %, respectively), the highest percentage switching from the human pole to the chicken pole  
31 during flood events. Concentrations indicate a severe fecal contamination of surface water during  
32 flood events and suggest that villages may be considered as major hotspots of fecal contamination  
33 pulses into the river network, and thus as point-sources in hydrological models.

34

## 35 Keywords

36 Fecal biomarkers; Fecal Indicator Bacteria; Microbial Source Tracking; EMMA; Freshwater; *E. coli*  
37 partition; Hot moments; Tropical rural areas.

38

## 39 Synopsis

40 Little is known about the contribution of villages to the fecal contamination of surface water in  
41 tropical rural areas. This study documents *E. coli* and stanol concentration levels in surface runoff,  
42 and tracks the origin of the fecal contamination during flood events.

43

## 44 1. Introduction

45 Livestock droppings or human feces, where open defecation is practiced or where sanitation systems  
46 are lacking or deficient, are primary sources of fecal bacteria in the environment <sup>1-3</sup>. Fecal pathogenic  
47 bacteria may threaten human health if present in surface water <sup>4,5</sup>, especially in least developed  
48 countries where about 40 % of the population still use unimproved water sources for domestic needs  
49 <sup>6</sup>.

50 Fecal Indicator Bacteria (FIB), such as *Escherichia coli* (*E. coli*), are transported in water as free cells or  
51 cells attached to soil particles, manure, or sediment <sup>7-10</sup>. In tropical humid rural areas such as the  
52 mountainous areas of Southeast Asia, the ranges of *E. coli* concentrations have already been  
53 investigated during both low flow and high flow periods at the outlet of mixed land use catchments  
54 of various spatial scales <sup>11,12</sup>, highlighting the leading role of surface runoff and soil erosion in *E. coli*  
55 dissemination <sup>13,14</sup>.

56 In rural areas of Southeast Asia, villages concentrate population and domestic animals most often  
57 without wastewater treatment facilities, and the ranges of FIB exported during storms from village  
58 settlements towards river network have not been yet quantified. Similarly, the dynamics of FIB  
59 during storms on impervious surfaces such as compacted soils in village settlements, where surface  
60 runoff is the only vector of diffuse fecal bacteria dissemination, and the attachment of FIB to  
61 suspended sediment in surface runoff, remain to be fully documented. This information is needed to  
62 design and feed catchment-scale hydrological models capable of predicting FIB concentrations during  
63 flood events.

64 The sources of fecal contamination and their relative contributions to the overall fecal contamination  
65 during a flood event are seldom known, although they represent essential information to (1)  
66 understand and model the transfers of fecal bacteria on the catchment scale <sup>15</sup>, and (2) better  
67 manage and mitigate microbial dissemination in the environment <sup>16,17</sup>. Microbial source tracking with  
68 lipid biomarkers such as stanol compounds <sup>18,19</sup> can be used to identify the origin of the fecal  
69 contamination in the environment <sup>20-26</sup>, including stream water <sup>16,21,27-34</sup>. The stanol fingerprint of  
70 feces depends on the animals' diet, their ability to biosynthesize endogenous sterols, and the  
71 composition of the intestinal flora responsible for sterol biohydrogenation into stanols <sup>17</sup>. Unlike  
72 other less persistent, source-specific markers such as caffeine <sup>35</sup>, and similarly to methods using  
73 ratios of fecal source-associated genetic markers <sup>36</sup>, the analysis of a number of stanol compounds  
74 can help identifying different fecal sources and their apportionment. Several ratios of stanol  
75 compounds have been proposed to identify the origin of fecal matter <sup>28,34,37-43</sup>. However, the use of  
76 such ratios has been questioned because they could result in overlaps for a number of animal

77 sources<sup>44</sup>. Multivariate analyses such as principal component analyses (PCA)<sup>45</sup>, possibly associated  
78 to end-member mixing analyses (EMMA), circumvent the problem of ratio specificity to identify the  
79 origin and spatial patterns of fecal matter<sup>17,20,27,34,44,46–49</sup>. However, PCA-based EMMA has not been  
80 tested in a tropical context, although it has the potential to serve as a global method<sup>27</sup>, and it has not  
81 been used to quantify the relative contribution of each fecal contamination source. A number of  
82 studies measured stanol compound concentration in stream suspended sediment<sup>16,21,28,31</sup>, both at  
83 low and high flow<sup>29,30</sup>, but the concentration dynamics and the switch in fecal contamination sources  
84 during flood events, have not been yet investigated.

85 In tropical areas such the mountainous Northern Lao PDR, about 62% of the population lives in rural  
86 areas<sup>50</sup>, in villages with little or no infrastructure for the containment and treatment of human and  
87 animal feces, and of wastewater<sup>6</sup>. Our hypothesis is that village settlements are major hotspots of  
88 fecal contamination because of the number of domestic animals usually roaming in the alleys and the  
89 lack of fecal matter treatment before entering river network. By jointly monitoring the dynamics of *E.*  
90 *coli* and stanol compounds during four flood events (July-August 2016) at the outlet of a ditch  
91 draining sewage and surface runoff out of a village of Northern Lao PDR, our objectives were thus (1)  
92 to assess the range of concentrations of *E. coli* in the surface runoff washing off from a village  
93 settlement, and (2) to identify the major contributory source of fecal contamination using stanol  
94 compounds.

## 95 2. Materials and methods

### 96 2.1. Study design

97 In this study, we quantified the range of *E. coli* concentration in water samples collected at the outlet  
98 of a ditch draining sewage and surface runoff out of a village of Northern Lao PDR during four flood  
99 events. We measured the concentration of *E. coli* in both raw water and filtered water to assess the  
100 percentage of particle-attached *E. coli*. We quantified the concentrations and the relative  
101 abundances of 7 stanol compounds (coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-  
102 ethylepicoprostanol, cholestanol, campestanol, and sitostanol) in three feces sources, namely  
103 human, porcine, and chicken feces, as well as in the suspended sediment of water samples. We  
104 applied PCA-based EMMA on stanol compounds relative abundances in feces to calculate the relative  
105 contribution of the three feces sources to surface runoff fecal contamination during flood events.  
106 Finally, we calculated the amount of *E. coli* originating from the three sources during flood events.

### 107 2.2. Study area

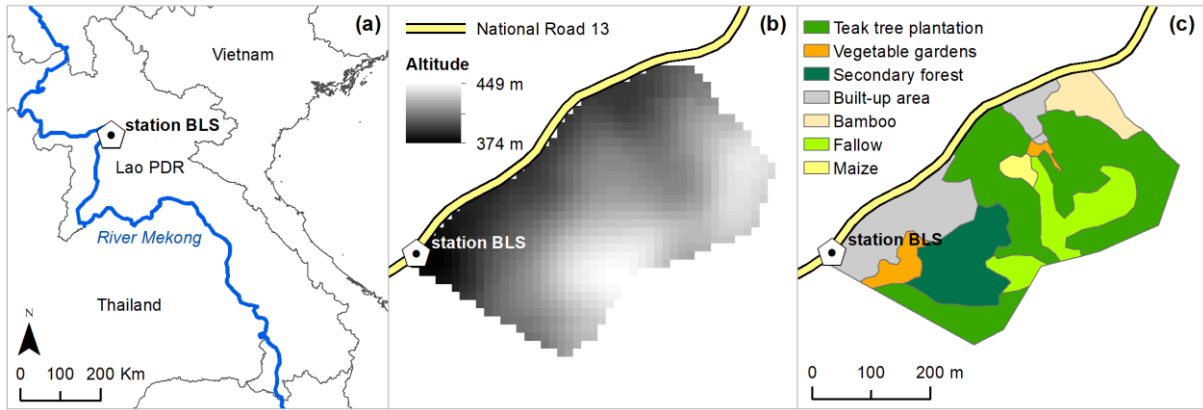
108 The study area is the 13.3-ha south-east part of the Laksip village (Ban Laksip), located 10-km  
109 southeast of Luang Prabang in Northern Lao PDR (Fig. 1a). This study area is part of the 800,000 km<sup>2</sup>

110 Mekong basin. The climate is sub-tropical humid and is characterized by a monsoon regime with a  
111 dry season from November to May, and a rainy season from June to October. The mean annual  
112 temperature is 23.4 °C. The mean annual rainfall is 1,366 mm (CV=0.23), about 71 % (CV=0.09) of  
113 which falls during the rainy season <sup>51</sup>.

114 The sampling and gauging station (latitude: 19.848421; longitude: 102.168295), named BLS after  
115 village name, is located at the outlet of the village drainage area, in a ditch alongside the National  
116 Road 13 (NR13); this ditch collects surface runoff from the drainage area and sewage from several  
117 pipes (piped latrines, bathroom, and kitchen outflows). The drainage area considered in this study is  
118 located between two concrete tubes evacuating water beneath the NR13. Altitude in the considered  
119 drainage area ranges from 374 to 449 m (Fig. 1b). Soil is classified as Acrisol.

120 In the study area, around 5 % of households have no latrines and practice open defecation. Around  
121 80 % of households are equipped with pit latrines that are not connected to the ditch. When these  
122 latrines are full, they are emptied using a pump truck, or they are condemned and new pit latrines  
123 are built. Around 15 % of households are equipped with piped latrines connected to the ditch. These  
124 are the houses built along the ditch, in the downstream part of the study area. Residents take  
125 advantage of certain intense and prolonged rainfall events to open the tap and drain off the  
126 supernatant liquid. However, during the four flood events monitored in our study, we did not  
127 observe flushes from piped latrines. We do not expect sewage piped from bathroom and kitchen to  
128 carry fecal contamination. As for animal droppings, they are sometimes stored as manure or applied  
129 in vegetable gardens, but no manure is imported from outside the village for fertilization. There is  
130 virtually no wildlife due to hunting pressure. Therefore, the fecal contamination in the ditch mostly  
131 comes from surface runoff during rainfall events, through human open defecation and domestic  
132 animal droppings. The ditch is dry during non-storm events, except some intermittent discharge from  
133 sinks and showers.

134 In August 2016, we conducted a detailed land-use survey within the drainage area with a handheld  
135 GPS (Fig. 1c). We classified land use into seven classes, namely: teak tree plantation (20 to 22 years  
136 old monospecific teak tree plantations, predominantly without understory), vegetable gardens,  
137 secondary forest, built-up area, bamboo, fallow (2 to 8 years old spontaneously regrown vegetation  
138 following annual crops), and maize <sup>52</sup>. The built-up area is characterized by impervious surfaces such  
139 as roofs and compacted anthropized soil between dwellings. We also conducted a survey among the  
140 24 households of the built-up area to qualify the possible feces sources (120 humans, 23 pigs, and  
141 111 chickens). Humans, occasionally practicing open defecation <sup>6</sup>, and domestic animals, may traffic  
142 in the surroundings of the built-up area.



143

144 Fig. 1. (a) Location of the BLS gauging and sampling station in Northern Lao PDR, at the outlet of the  
 145 Laksip village (Ban Laksip) drainage area; (b) Elevation within the drainage area (in meters above  
 146 mean sea level; ALOS PALSAR ©JAXA, METI); (c) Land use in August 2016 within the drainage area.

147 **2.3. Sampling of feces sources**

148 To quantify stanol compounds in chicken feces, we collected chicken feces within six randomly  
 149 chosen households of the Laksip village. We mixed equivalent masses of the feces from the six  
 150 households to get a composite sample. The same method was applied to quantify stanol compounds  
 151 in porcine feces. To quantify stanol compounds in human feces, we collected human feces from ten  
 152 randomly chosen inhabitants of the Laksip village. We mixed equivalent masses of the feces from the  
 153 ten inhabitants to get a composite sample. Composite feces samples were dried and kept in the  
 154 refrigerator until the stanol compounds were analyzed. We chose to analyze composite samples of  
 155 feces sources to measure the average concentration of each stanol compound in each feces source,  
 156 and to incorporate the variability of each feces source, with a limited number of analyses of stanol  
 157 compounds.

158 Study procedures for human sample collection and laboratory analysis were reviewed and approved  
 159 by the National Ethics Committee for Health Research, Lao Ministry of Health Council of Medical  
 160 Sciences (reference no. 51/NECHR)<sup>53,54</sup>.

161 **2.4. Flood event monitoring and water sampling**

162 **Rainfall**

163 Rainfall was measured using an automatic weather station (Campbell ARG100, 0.2 mm capacity  
 164 tipping-bucket) located at station BLS (Fig. 1b). Rainfall data was recorded at 1-min time intervals.

165 **Flow rate measurements and water sampling**

166 We measured ditch water level at the BLS gauging station (Fig. 1) with a water level recorder (OTT  
 167 Thalimedes) connected to a data logger, with 1-mm vertical precision at a minimum of 3-min time

168 interval. To relate water level to surface runoff discharge (Q), a control rating curve was determined  
169 using both the velocity area method and the salt dilution method by slug injection.

170 We collected samples of ditch water at BLS sampling station in clean, plastic bottles using an  
171 automatic sampler for the measurement of total suspended sediment concentration ([TSS]), *E. coli*  
172 concentration in raw water ( $[E. coli]$ ), *E. coli* concentration in filtered water ( $[E. coli]_{FREE}$ ), and stanol  
173 compound concentrations. The automatic sampler<sup>55</sup> was triggered by the water level recorder to  
174 collect water every time the water level rose by 2-cm and every time it fell by 4-cm. The bottles were  
175 new empty bottles, supplied by a plastic bottle plant producing bottles for mineral water packaging.  
176 We regularly verified that the bottles were free from *E. coli*<sup>56</sup>.

## 177 2.5. Laboratory analyses of water samples

178 Data of [TSS],  $[E. coli]$ ,  $[E. coli]_{FREE}$ , and of stanol compound concentrations and percentages, are  
179 available on a public repository<sup>57</sup>.

### 180 *E. coli*

181 We removed water samples from the water sampler immediately after the flood event, and stored  
182 them in a cool-box until analysis, which took place within one hour of sampling. We measured  $[E.$   
183  $coli]$  with the standardized microplate method (ISO 9308-3)<sup>56</sup>. A water sub-sample was incubated at  
184 four dilution rates (i.e. 1:2, 1:20, 1:200 and 1:2000) in a 96-well microplate (MUG/EC, BOKAR  
185 DIAGNOSTICS) for 48 h at 44 °C. Ringers' Lactate solution was used for the dilutions and one plate  
186 was used per sample. The number of positive wells for each microplate was noted and the Most  
187 Probable Number (MPN) per 100 mL was determined using the Poisson distribution. The upper limit  
188 of quantification of  $[E. coli]$  using dilution rates 1:2, 1:20, 1:200, and 1:2000, is  $3.2 \cdot 10^6$  MPN 100 mL<sup>-1</sup>.  
189 We measured  $[E. coli]_{FREE}$  by first filtering 5 mL raw water with polycarbonate filters (3 µm porosity;  
190 Whatman) and then applying the standardized microplate method to filtered water.

191 We calculated the percentage of *E. coli* in the particle-attached fraction, i.e. *E. coli* attached to  
192 suspended sediment ( $[E. coli]_{ATT\%}$ ) with equation Eq. 1:

$$[E. coli]_{ATT\%} = \frac{[E. coli] - [E. coli]_{FREE}}{[E. coli]} \times 100 \quad \text{Eq. 1}$$

193 where  $[E. coli]$  is the concentration of *E. coli* in raw water (MPN 100 mL<sup>-1</sup>),  $[E. coli]_{FREE}$  is the  
194 concentration of *E. coli* in the free-living fraction, measured in the filtered water (MPN 100 mL<sup>-1</sup>), and  
195  $[E. coli]_{ATT\%}$  is the percentage of *E. coli* in the particle-attached fraction.



196 Total suspended sediment

197 We measured [TSS] in each sample after filtration of a 200 mL water sub-sample on polycarbonate  
198 filters (0.2  $\mu\text{m}$  porosity; Whatman) followed by evaporation at ambient temperature for 48 h. Dry  
199 filters were kept in the refrigerator until the analysis of dry suspended sediment for stanol  
200 compounds.

201 Stanol compounds

202 We measured the concentrations and the relative abundances of seven stanol molecules in both the  
203 dried feces and the dried suspended sediment: coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-  
204 ethylepicoprostanol, cholestanol, campestanol, and sitostanol.

205 We extracted the total lipid fraction with dichloromethane using an accelerated solvent extractor  
206 (ASE 200, Dionex). Extraction conditions were described previously<sup>44</sup>. The total lipid extract was then  
207 fractionated using solid/liquid chromatography (silica column) to separate the polar fraction. The  
208 polar fraction was derivatized with a mixture of N,O-bis-(trimethylsilyl)trifluoroacetamide and  
209 trimethylchloro-silane (BSTFA + TMCS, 99/1, v/v, Supelco) after addition of 5 $\alpha$ -cholestane (CDN  
210 isotope) as an internal standard. Derivatized samples were analyzed by gas chromatography-mass  
211 spectrometry (GC-MS) using a Shimadzu QP2010plus equipped with a capillary column (Supelco, 60  
212 m x 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness). The temperature of the transfer line was set at 280  $^{\circ}\text{C}$ , and  
213 molecules were ionized by electron impact at an energy of 70 eV. The temperature of the ionization  
214 source was set at 200  $^{\circ}\text{C}$ . Samples were injected in splitless mode at 310  $^{\circ}\text{C}$ . The oven temperature  
215 was programmed from an initial temperature of 200  $^{\circ}\text{C}$  (held for 1 min) then rising to 310  $^{\circ}\text{C}$  at 15  $^{\circ}\text{C}$   
216  $\text{min}^{-1}$  (held for 35 min). Helium was used as the carrier gas, with a flow rate of 1.0  $\text{mL min}^{-1}$ . The  
217 analyses were made in Selective Ion Monitoring (SIM) mode. The compound identification was  
218 performed based on the comparison of the retention times and the mass spectra with available  
219 standards or the literature data (Table SI1)<sup>20</sup>. The quantification was achieved with a 5-point internal  
220 calibration curve (0.1, 0.3, 0.5, 0.7, and 1 ppm) of stanol standards with a constant internal standard  
221 concentration at 0.5 ppm. To ensure the absence of potential contaminants and to verify system  
222 performance during GC-MS measurements<sup>58</sup>, we analyzed two to five blank samples (empty vials)  
223 when beginning the GC-MS sequence, and we ran the 5-point internal calibration curve of stanol  
224 standards at the start, middle and end of each GC-MS sequence (one sequence = 20 samples). A  
225 blank was also analyzed after each real sample during the GC-MS sequence.

226 Limit of quantification (LQ) was 0.01  $\mu\text{g g}^{-1}$ . Limit of detection (LD) ranged from 0.005 to 0.01  $\mu\text{g g}^{-1}$   
227 depending on the stanol compound (Table SI1). For the stanol compounds for which we found  
228 concentrations below the LQ (here epicoprostanol and 24-ethylepicoprostanol), we replaced the

229 concentrations values by the value of the LD. Concentrations of stanol compounds were expressed in  
230  $\mu\text{g g}^{-1}$  of dry matter and converted to  $\mu\text{g L}^{-1}$  by multiplying the concentrations of stanol compounds  
231 by [TSS] in the case of water samples. Hereafter, we refer to [copros]<sub>F</sub>, [epicop]<sub>F</sub>, [24etco]<sub>F</sub>, [24etep]<sub>F</sub>,  
232 [choles]<sub>F</sub>, [campes]<sub>F</sub>, and [sitost]<sub>F</sub>, and to [copros]<sub>WS</sub>, [epicop]<sub>WS</sub>, [24etco]<sub>WS</sub>, [24etep]<sub>WS</sub>, [choles]<sub>WS</sub>,  
233 [campes]<sub>WS</sub>, and [sitost]<sub>WS</sub> as the concentration of coprostanol, epicoprostanol, 24-ethylcoprostanol,  
234 24-ethylepicoprostanol, cholestanol, campestanol, and sitostanol in feces and water samples,  
235 respectively. We calculated the concentration of total stanol compounds in feces ([stanol]<sub>F</sub>) and  
236 water samples ([stanol]<sub>WS</sub>) as the sum of the concentrations of the seven stanol compounds in feces  
237 and water samples, respectively. We analyzed separately coprostanol and the other three 5 $\beta$ -stanol  
238 compounds (epicoprostanol, 24-ethylcoprostanol, and 24-ethylepicoprostanol) because we expected  
239 coprostanol to be the major stanol compound in human feces<sup>17</sup>. We thus calculated the  
240 concentration of 5 $\beta$ -stanol compounds without coprostanol ([5 $\beta$ -w/ocop]<sub>F</sub>) by summing  
241 concentrations of epicoprostanol, 24-ethylcoprostanol, and 24-ethylepicoprostanol in both feces and  
242 water samples ([5 $\beta$ -w/ocop]<sub>WS</sub>). We calculated the concentration of 5 $\alpha$ -stanol compounds in feces  
243 ([5 $\alpha$ -stanol]<sub>F</sub>) and water samples ([5 $\alpha$ -stanol]<sub>WS</sub>) as the sum of the concentrations of 5 $\alpha$ -stanol  
244 compounds, namely cholestanol, campestanol, and sitostanol, in feces and water samples,  
245 respectively.

246 Finally, we calculated the relative abundance, or distribution, in percentage, of the stanol compounds  
247 in the three feces sources and in each water sample by measuring the relative abundance of each  
248 stanol compound compared to the internal standard from the GC-MS characteristic peaks. For each  
249 stanol compound, we integrated the peak area of the selected m/z (Table SI1). We then calculated  
250 the relative abundance of each stanol compound by dividing the area of the compound by the sum of  
251 the peak areas of all the analyzed compounds, and expressed the ratio as a percentage. This way, we  
252 could compare the relative abundances of stanol compounds in both feces sources and water  
253 samples by avoiding stanol concentrations gaps between feces sources (more concentrated) and  
254 water samples (less concentrated), even when concentrations were below the limit of quantification.  
255 Similar to concentrations, we refer to %copros<sub>F</sub>, %epicop<sub>F</sub>, %24etco<sub>F</sub>, %24etep<sub>F</sub>, %choles<sub>F</sub>, %campes<sub>F</sub>,  
256 and %sitost<sub>F</sub>, and to %copros<sub>WS</sub>, %epicop<sub>WS</sub>, %24etco<sub>WS</sub>, %24etep<sub>WS</sub>, %choles<sub>WS</sub>, %campes<sub>WS</sub>, and  
257 %sitost<sub>WS</sub> as the relative abundance of coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-  
258 ethylepicoprostanol, cholestanol, campestanol, and sitostanol in feces and water samples,  
259 respectively. We calculated the sum of the relative abundances of epicoprostanol, 24-  
260 ethylcoprostanol, and 24-ethylepicoprostanol in both feces (%5 $\beta$ -w/ocop<sub>F</sub>) and water samples (%5 $\beta$ -  
261 w/ocop<sub>WS</sub>). We calculated the sum of the relative abundances of cholestanol, campestanol, and  
262 sitostanol in both feces (%5 $\alpha$ -stanol<sub>F</sub>) and water samples (%5 $\alpha$ -stanol<sub>WS</sub>).

## 263 2.6. Correlation analysis

264 We calculated Spearman's rank correlation coefficients between Q, [TSS], [*E. coli*], [copros]<sub>WS</sub>,  
265 [epicop]<sub>WS</sub>, [choles]<sub>WS</sub>, [24etco]<sub>WS</sub>, [24etep]<sub>WS</sub>, [campes]<sub>WS</sub>, [sitost]<sub>WS</sub>, [stanol]<sub>WS</sub>, [5 $\beta$ -w/ocop]<sub>WS</sub>, and  
266 [5 $\alpha$ -stanol]<sub>WS</sub>, with R statistical package version 4.0.3. We considered a correlation to be statistically  
267 significant when its p-value adjusted for multiple comparisons (adjustment method "fdr") was lower  
268 than 0.05.

## 269 2.7. End-member mixing analysis

270 We performed an end-member mixing analysis (EMMA) based on a principal component analysis  
271 (PCA)<sup>45,59</sup> to estimate the relative contributions of the three feces sources (i.e., human, porcine, and  
272 chicken) at the time of each water sampling at the outlet of the Laksip village<sup>44</sup> during the four flood  
273 events. We applied PCA-based EMMA on stanol compounds relative abundances to attenuate the  
274 dilution effect of stanol compounds between feces source samples and water samples, and to  
275 include stanol compound concentrations below LQ.

276 We first built mixing diagrams<sup>60</sup> of stanol compound relative abundances, and checked that the  
277 stanol signatures of the water samples were included in the triangles defined by the three end-  
278 members, i.e., the three feces sources. We discarded stanol compounds for which none of the points  
279 representing a water sample were included in the triangles (here cholestanol): these compounds are  
280 suspected of not having a conservative behavior.

281 We then derived eigenvalues and eigenvectors using PCA (performed with XLSTAT 20.1.1, Addinsoft,  
282 2010) by considering the three end-members as individuals, along with six variables, i.e., the stanol  
283 compound relative abundances in human, porcine, and chicken feces sources, after discarding  
284 cholestanol. We confirmed the *a priori* choice of the three end-members using eigenvalues, and we  
285 used eigenvectors to orthogonally project stanol compound relative abundances in end-members  
286 and in water samples, the latter as supplementary individuals<sup>47</sup>. We used orthogonal projections  
287 along with mixing equations<sup>61</sup> to estimate the relative contributions of the feces sources at each  
288 sampling time.

289 We assessed the uncertainty within the end-members using a Monte-Carlo method<sup>61</sup>: the  
290 mathematical expectation and the standard deviation of the relative abundance of each stanol  
291 compound were estimated for each end-member, based on a literature review of stanol compound  
292 concentration in human<sup>20,27</sup>, chicken<sup>17,46</sup>, and pig<sup>20,27</sup> feces. For each compound, 1000 relative  
293 abundance values were inferred by randomly sampling without replacement a normal distribution  
294 corresponding to both the estimated expectation and the estimated standard deviation. These 1000

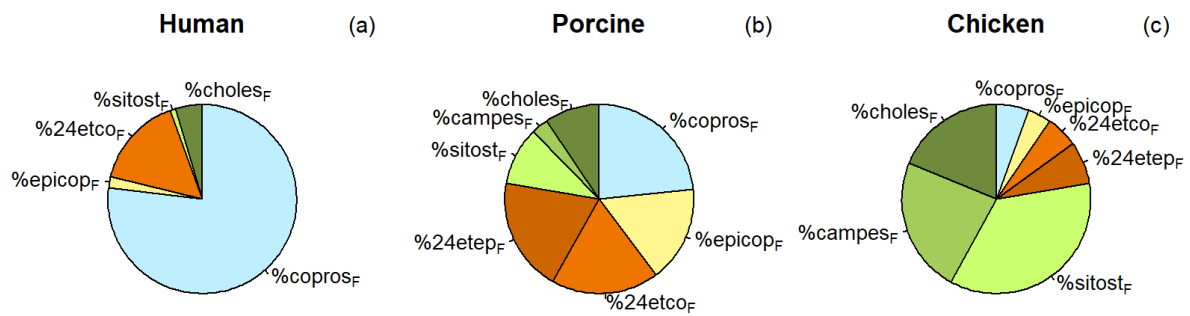
295 new individuals were then included in the PCA to derive the corresponding 1000 eigenvectors and to  
296 calculate the mean and the standard deviation.

### 297 3. Results and discussion

#### 298 3.1. Concentration and relative abundance of stanol compounds in feces

299 As for concentrations, [stanol]<sub>F</sub> in human, porcine, and chicken feces, were 9642.9, 956.2, and 375.4  
300  $\mu\text{g g}^{-1}$ , respectively (Table SI2). As for relative abundances of stanol compounds (Fig. 2), the relative  
301 abundance of 5 $\beta$ -stanol compounds (i.e., %copros<sub>F</sub> and %5 $\beta$ -w/ocop<sub>F</sub>) in human feces was 94 %,  
302 while %5 $\alpha$ -stanol<sub>F</sub> was 6 %. Coprostanol was the major compound (%copros<sub>F</sub>=77 %), followed by 24-  
303 ethylcoprostanol (%24etco<sub>F</sub>=16 %). In porcine feces, the relative abundance of 5 $\beta$ -stanol compounds  
304 was 78 %, while %5 $\alpha$ -stanol<sub>F</sub> was 22 %. Among 5 $\beta$ -stanol compounds, the relative abundance of 5 $\beta$ -  
305 stanol compounds derived from the hydrogenation of cholesterol (coprostanol and epicoprostanol),  
306 was similar to the relative abundance of 5 $\beta$ -stanol compounds derived from the hydrogenation of  
307 sitosterol (24-ethylcoprostanol and 24-ethylepicoprostanol) at 40 and 38 %, respectively. 5 $\beta$ -stanol  
308 compounds without coprostanol (%5 $\beta$ -w/ocop<sub>F</sub>) was 54 %. In chicken feces, the relative abundance  
309 of 5 $\alpha$ -stanol compounds (%5 $\alpha$ -stanol<sub>F</sub>) was 78 %, with sitostanol being the major compound  
310 (%sitost<sub>F</sub>=36 %). 5 $\beta$ -stanol compounds were 22 %, with %copros<sub>F</sub>, %epicop<sub>F</sub>, %24etco<sub>F</sub>, and %24etep<sub>F</sub>,  
311 representing less than 10 % each.

312 Our results are consistent with the relative abundances of stanol compounds documented in the  
313 literature for human, porcine, and chicken feces. Coprostanol is known to be the major stanol  
314 compound in human feces, while other 5 $\beta$ -stanol compounds such as 24-ethylcoprostanol (19 %) and  
315 24-ethylepicoprostanol (20 %) are often major stanol compounds in porcine feces<sup>17,20,62</sup>. 24-  
316 ethylcoprostanol and 24-ethylepicoprostanol are also found in human feces but are less abundant  
317 compared to coprostanol. 5 $\alpha$ -stanol compounds are often major stanol compounds in chicken feces  
318<sup>17,39,46</sup>, although stanol profiles of birds feces are known to greatly vary depending on the animals'  
319 diet<sup>16,17</sup>. These results allow to infer that coprostanol, 5 $\beta$ -stanol compounds without coprostanol,  
320 and 5 $\alpha$ -stanol compounds, mainly originate from human, porcine, and chicken fecal sources,  
321 respectively, and use such inference for further analysis hereafter.



322

323 Fig. 2. Pie charts of the relative abundances of stanol compounds in (a) human feces, (b) porcine  
 324 feces, and (c) chicken feces. %copros<sub>F</sub>: relative abundance of coprostanol (%); %epicop<sub>F</sub>: relative  
 325 abundance of epicoprostanol (%); %24etco<sub>F</sub>: relative abundance of 24-ethylcoprostanol (%);  
 326 %24etep<sub>F</sub>: relative abundance of 24-ethylepicoprostanol (%); %sitost<sub>F</sub>: relative abundance of  
 327 sitostanol (%); %campe<sub>SF</sub>: relative abundance of campestanol (%); %choles<sub>F</sub>: relative abundance of  
 328 cholesterol (%).

### 329 3.2. Concentration of TSS, *E. coli*, and stanol compounds in water samples

330 The four flood events sampled in July and August 2016 covered wide ranges of rainfall duration,  
 331 depth, and intensity (Fig. S11). Rainfall depths on July 20, July 23, August 8, and August 19 were 27,  
 332 51, 25, and 40 mm, respectively, while rainfall durations were 101, 102, 105, and 233 minutes.  
 333 Maximum rainfall intensities were 108, 252, 84, and 132 mm h<sup>-1</sup>, respectively. The rainfall event of  
 334 August 8 actually was a sequence of two rainfall events with maximum intensities of 84 and 72 mm h<sup>-1</sup>,  
 335 respectively. According to rainfall time-series analysis for the synoptic station of Luang Prabang<sup>63</sup>,  
 336 the flood events of July 20 and of August 8 correspond to a cumulative probability lower than 0.01  
 337 (return period of 1 year) and can be classified as “small” events. The flood event of August 19  
 338 corresponds to a cumulative probability ranging between 0.01 and 0.1 and can be classified as a  
 339 “medium” event. The flood event of July 23 corresponds to a cumulative probability ranging between  
 340 0.1 and 0.5 (return period of 2 years) and can be classified as a “large” event. The four flood events  
 341 were thus representative of the common rainfall conditions prevailing during the rainy season in  
 342 northern Lao PDR. Q ranged from 10 to 3491 L s<sup>-1</sup>, with maxima of 410, 3491, 561, and 3085 L s<sup>-1</sup> on  
 343 July 20, July 23, August 8, and August 19, respectively. A total of 29 water samples were collected  
 344 during the four flood events, including five, five, nine, and ten water samples on July 20, July 23,  
 345 August 8, and August 19, respectively (Fig. S11).

346 [TSS] ranged from 1.0 to 20.2 g L<sup>-1</sup> (Fig. 3a), with maximum [TSS] of 9.1, 6.3, 11.7, and 20.2 g L<sup>-1</sup> on  
 347 July 20, July 23, August 8, and August 19, respectively. These concentrations are in the range of [TSS]  
 348 measured during twelve flood events sampled from 2011 to 2015 at the outlet of the adjacent 60-ha

349 mixed land use Houay Pano catchment ( $0.1\text{-}25.7\text{ g L}^{-1}$ )<sup>13</sup>. As in the case of these previous [TSS]  
350 measurements, eroded suspended particles were detached from bare soil surfaces found in the  
351 alleys of the village and in the teak tree plantations grown without understory (Fig. 1). Bare soils  
352 most often display a high rate of crusting, which induces high surface runoff<sup>64,65</sup> and, in turn,  
353 generates high soil erosion in areas of lower surface shear-stress resistance<sup>66</sup>.

354 [*E. coli*] ranged from  $4.7\cdot 10^4$  to  $3.2\cdot 10^6$  MPN 100 mL<sup>-1</sup> (Fig. 3b). Maximum [*E. coli*] were  $1.8\cdot 10^6$ ,  
355  $3.2\cdot 10^6$ ,  $1.6\cdot 10^6$ , and  $2.9\cdot 10^5$  MPN 100 mL<sup>-1</sup> on July 20, July 23, August 8, and August 19,  
356 respectively. The two first water samples collected on July 23 resulted in [*E. coli*] above the limit of  
357 quantification, to be related to the *a priori* choice of the four dilution rates with the standardized  
358 microplate method. This implies that [*E. coli*] are under-estimated for these two time points. Overall,  
359 the concentrations are higher than the range of [*E. coli*] measured at the outlet of the adjacent  
360 Houay Pano catchment ( $1.6\cdot 10^2\text{-}7.4\cdot 10^4$  MPN 100 mL<sup>-1</sup>)<sup>13</sup>, or during high flow in a range of Mekong  
361 tributaries in Lao PDR ( $8.0\cdot 10^4$  MPN 100 mL<sup>-1</sup>)<sup>11</sup>, but they are in the range of the maximum [*E. coli*]  
362 measured in the Mekong Delta at the end of the rainy season ( $1.1\cdot 10^6$  MPN 100 mL<sup>-1</sup>)<sup>21</sup>. [*E. coli*] are  
363 also within the range of [*E. coli*] measured in the influent of wastewater treatment plants (WWTP)  
364 ranging from  $10^4$  to  $10^7$  CFU or MPN 100 mL<sup>-1</sup><sup>67-71</sup>. All these values exceed the 500 cells per 100 mL<sup>-1</sup>  
365 FIB threshold provided by the World Health Organization for freshwater<sup>72</sup>, associated to a 10 %  
366 chance of gastroenteritis per single exposure<sup>73</sup>. Bathing in such running water, as children often do  
367 to refresh themselves from the heat during the hot and rainy season<sup>4</sup>, may thus pose a serious  
368 health risk.

369 [*E. coli*]<sub>ATT%</sub> ranged from 83 to 100 %, with a median of 97 % (Fig. 3c). These percentages are similar  
370 to the ranges measured in *in situ* mesocosms installed within the Houay Pano catchment (91-99 %),  
371 for [TSS] up to  $0.5\text{ g L}^{-1}$ <sup>74</sup>. The range of [*E. coli*]<sub>ATT%</sub> is narrower than the range measured in grab  
372 samples along the Red River during one year (9.7-100 %), for [TSS] up to  $0.6\text{ g L}^{-1}$ <sup>9</sup>. Hence, [*E. coli*] at  
373 the outlet of the ditch may be mostly driven by the detachment of soil-associated human and animal  
374 feces with soil erosion during flood events, and most of the fecal contamination is attached to  
375 particles, as are stanol compounds<sup>21,23</sup>.

376 None of the stanol compound concentrations measured in the particulate phase during the four  
377 flood events was below the detection limit. Two stanol compounds were often below the  
378 quantification limit: epicoprostanol and 24-ethylepicoprostanol. [stanol]<sub>WS</sub> ranged from 1.3 to 591.0  
379  $\mu\text{g L}^{-1}$ , [copros]<sub>WS</sub> ranged from 0.3 to 293.0  $\mu\text{g L}^{-1}$ , [5 $\beta$ -w/ocop]<sub>WS</sub> ranged from 0.4 to 222.3  $\mu\text{g L}^{-1}$ ,  
380 while [5 $\alpha$ -stanol]<sub>WS</sub> ranged from 0.4 to 75.7  $\mu\text{g L}^{-1}$  (Fig. 3d,e,f,g).

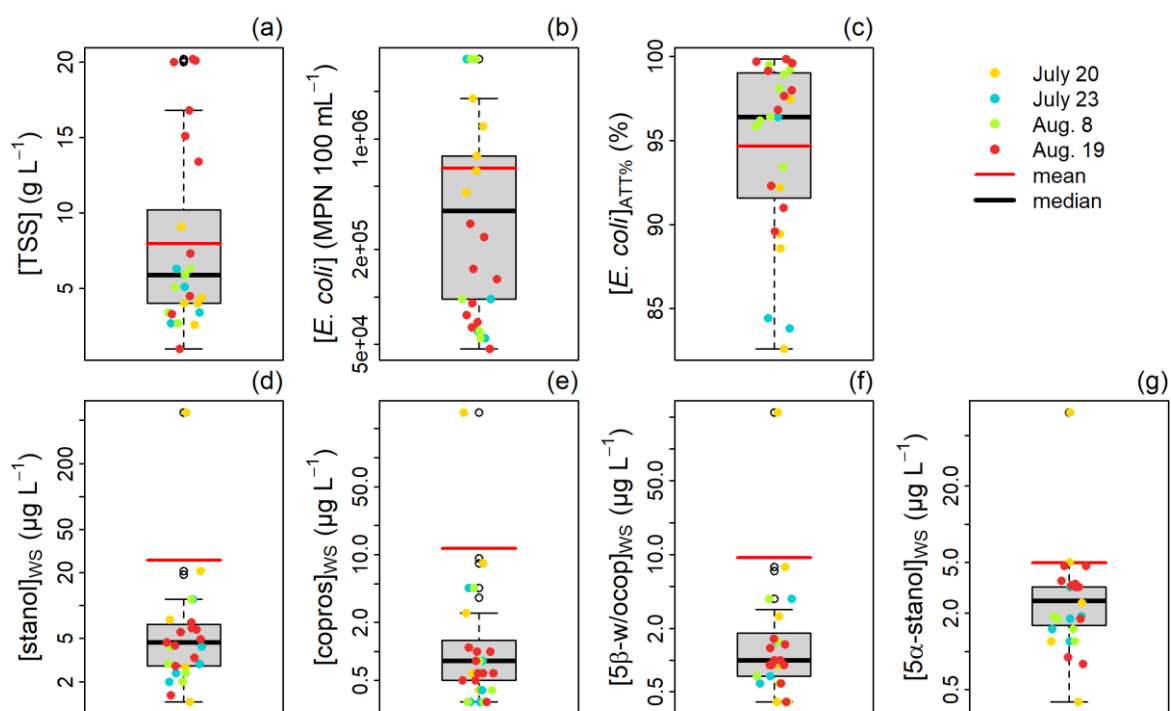
381 The range of [copros]<sub>WS</sub> is higher than the ranges measured in the Mekong Delta (0.001-97.1 µg L<sup>-1</sup>)  
382 <sup>29</sup>, in Western Malaysia rivers and in the Mekong Delta (2\*10<sup>-5</sup>-13.5 µg L<sup>-1</sup>) <sup>21</sup>, in streams of Manaus  
383 area in Brazil (0.03-46.92 µg L<sup>-1</sup>) <sup>34</sup>, and in Solo and Serayu rivers in Indonesia (<LD-0.68 µg L<sup>-1</sup>) <sup>31</sup>, but  
384 in general lower than the range of [copros]<sub>WS</sub> reported for WWTP influent in Japan (290.03-327.8 µg  
385 L<sup>-1</sup>) <sup>21</sup> and in the USA (385 µg L<sup>-1</sup>) <sup>75</sup>.

386 The range of [epicop]<sub>WS</sub> (<LQ-12.8 µg L<sup>-1</sup>) is higher than the ranges measured in Western Malaysia  
387 rivers and in the Mekong Delta (5\*10<sup>-6</sup>-1.579 µg L<sup>-1</sup>) <sup>21</sup>, and in streams of Manaus area in Brazil (<LD-  
388 2.28 µg L<sup>-1</sup>) <sup>34</sup>, but in general lower than the range of [epicop]<sub>WS</sub> reported for WWTP influents in  
389 Japan (5.08-12.18 µg L<sup>-1</sup>) <sup>21</sup>. [24etep]<sub>WS</sub> ranged from <LQ to 3.9 µg L<sup>-1</sup> while [24etco]<sub>WS</sub> ranged from  
390 0.4 to 205.6 µg L<sup>-1</sup> (Fig. SI2). [24etco]<sub>WS</sub> reported for the streams of Manaus area in Brazil ranged  
391 from <LQ to 9.03 µg L<sup>-1</sup> <sup>34</sup>. [24etco]<sub>WS</sub> reported for the WWTP influent in the USA was 140 µg L<sup>-1</sup> while  
392 [epicop]<sub>WS</sub> and [24etep]<sub>WS</sub> were below the limit of detection <sup>75</sup>. In our study, given the number of  
393 values below the quantification limit for both [epicop]<sub>WS</sub> and [24etep]<sub>WS</sub>, [5β-w/ocop]<sub>WS</sub> mostly  
394 reflects [24etco]<sub>WS</sub>.

395 The range of [choles]<sub>WS</sub> (0.2-53.0 µg L<sup>-1</sup>; Fig. SI2) is higher than the ranges measured in Western  
396 Malaysia rivers and in the Mekong Delta (6\*10<sup>-4</sup>-3.485 µg L<sup>-1</sup>) <sup>21</sup>, in streams of Manaus area in Brazil  
397 (0.02-2.67 µg L<sup>-1</sup>) <sup>34</sup>, and in Solo and Serayu rivers in Indonesia (0.052-0.468 µg L<sup>-1</sup>) <sup>31</sup>. [choles]<sub>WS</sub> was  
398 in the range reported for WWTP influent in Japan (23.95-31.27 µg L<sup>-1</sup>) <sup>21</sup>. The ranges of [campes]<sub>WS</sub>  
399 and of [sitost]<sub>WS</sub> (0.1-7.7 µg L<sup>-1</sup> and 0.1-15.0 µg L<sup>-1</sup>, respectively; Fig. SI2) are higher than the  
400 [campes]<sub>WS</sub> and [sitost]<sub>WS</sub> measured in Solo and Serayu rivers in Indonesia (<LD-0.261 µg L<sup>-1</sup> and <LD-  
401 0.476 µg L<sup>-1</sup>, respectively) <sup>31</sup>. [sitost]<sub>WS</sub> measured in streams of Manaus area in Brazil ranged from <LQ  
402 to 1.54 µg L<sup>-1</sup> <sup>34</sup>. [choles]<sub>WS</sub>, [campes]<sub>WS</sub>, and [sitost]<sub>WS</sub> reported for the WWTP influent in the USA  
403 were 40, 55, and 23 µg L<sup>-1</sup>, respectively <sup>75</sup>. Hence, the range of [5α-stanol]<sub>WS</sub> is higher than the [5α-  
404 stanol]<sub>WS</sub> measured in Solo and Serayu rivers in Indonesia (0.072-1.206 µg L<sup>-1</sup>) <sup>31</sup>, but lower than the  
405 [5α-stanol]<sub>WS</sub> measured in the USA WWTP influent (118 µg L<sup>-1</sup>) <sup>75</sup>.

406 The concentrations of *E. coli* and of stanol compounds measured at the outlet of the Laksip village  
407 during the four flood events were generally higher than the concentrations measured in the other  
408 tropical rivers, whatever the hydrological conditions, while they were lower or in the same range  
409 than the concentrations measured in the WWTP influent. Since concentrations are measured in  
410 surface runoff and are thus diluted by rainfall, the range of both [*E. coli*] and [stanol]<sub>WS</sub> can be  
411 considered high. In the environment, the highest stanol compound concentrations are often found at  
412 sampling sites located near domestic outfalls, and concentrations tend to decrease with distance  
413 from the sources, involving mixing and dilutions processes <sup>23,34,76-78</sup>. Hence, lower concentrations of

414 stanol compounds found in e.g. the Mekong Delta <sup>21,29</sup> are consistent with higher concentrations at  
 415 the outlet of a village located within a headwater catchment such as the Laksip village. A common  
 416 feature in rural areas of developing countries is that although some families have individual  
 417 treatment systems (septic tanks), surface runoff water and most of wastewater are not stored nor  
 418 treated before entering the river network. Besides, [*E. coli*] in surface runoff and at the soil surface  
 419 are positively associated <sup>79</sup>, and urbanized areas are known to increase FIB concentration due to  
 420 impervious surfaces and failing septic systems <sup>80</sup>, two features common to our study area. Hence,  
 421 both high [stanol]<sub>ws</sub> and high [*E. coli*] suggest that ditch water is highly contaminated by fecal matter  
 422 and that villages in rural areas can be considered as hotspots of fecal contamination during flood  
 423 events. Villages may thus be described as point-sources of fecal contamination in distributed or semi-  
 424 distributed hydrological models.



425  
 426 Fig. 3. Water quality during four flood events sampled in 2016 at the BLS sampling station, Laksip  
 427 village, Northern Lao PDR. (a) [TSS]: concentration of total suspended sediment (g L<sup>-1</sup>); (b) [*E. coli*]:  
 428 concentration of *Escherichia coli* (MPN 100 mL<sup>-1</sup>); (c) [*E. coli*]<sub>ATT%</sub>: percentage of *Escherichia coli*  
 429 attached to suspended sediment (%); (d) [stanol]<sub>ws</sub>: sum of the concentrations of seven stanol  
 430 compounds (coprostanol, epicoprostanol, cholestanol, 24-ethylcoprostanol, 24-ethylepicoprostanol,  
 431 campestanol, and sitostanol) (μg L<sup>-1</sup>); (e) [copros]<sub>ws</sub>: concentration of coprostanol (μg L<sup>-1</sup>); (f) [5β-  
 432 w/ocop]<sub>ws</sub>: sum of the concentrations of epicoprostanol, 24-ethylcoprostanol, and 24-  
 433 ethylepicoprostanol (μg L<sup>-1</sup>); (g) [5α-stanol]<sub>ws</sub>: sum of the concentrations of cholestanol,  
 434 campestanol, and sitostanol (μg L<sup>-1</sup>). Y-axes for [*E. coli*] and stanol compounds concentration are



435 shown on log scales. Each boxplot contains the extreme of the lower whisker (dashed line), the lower  
436 hinge (thin line), the median (bold line), the upper hinge (thin line), and the extreme of the upper  
437 whisker (dashed line). The whiskers extend to the most extreme data point which is no more  
438 than 1.5 times the interquartile range from the box.

### 439 3.3. Spatial origin of TSS, *E. coli*, and stanol compounds measured in water samples

440 [TSS] was positively correlated to [campes]<sub>WS</sub> ( $r=0.46$ ,  $p\text{-value}=0.026$ ) and [sitost]<sub>WS</sub> ( $r=0.54$ ,  $p\text{-}$   
441  $\text{value}=0.007$ ) while [*E. coli*] was positively correlated to [copros]<sub>WS</sub> ( $r=0.81$ ,  $p\text{-value}<0.001$ ), [epicop]<sub>WS</sub>  
442 ( $r=0.55$ ,  $p\text{-value}=0.007$ ), [choles]<sub>WS</sub> ( $r=0.53$ ,  $p\text{-value}=0.009$ ), [24etco]<sub>WS</sub> ( $r=0.83$ ,  $p\text{-value}<0.001$ ),  
443 [24etep]<sub>WS</sub> ( $r=0.64$ ,  $p\text{-value}=0.001$ ), and to [5 $\beta$ -w/ocop]<sub>WS</sub> ( $r=0.81$ ,  $p\text{-value}<0.001$ ) (Fig. S13).  
444 Concentrations of the stanol compounds were in general positively correlated among each other. Q  
445 was positively correlated to [TSS] ( $r=0.76$ ,  $p\text{-value}<0.001$ ) and negatively correlated to [*E. coli*] ( $r=-$   
446  $0.55$ ,  $p\text{-value}=0.006$ ). No significant correlation was found between [*E. coli*] and [TSS].

447 Soil erosion is expected to drive both [*E. coli*], *E. coli* being mostly transported attached to suspended  
448 particles, and [stanol]<sub>WS</sub>, stanol compounds being mostly bound to particulate matter<sup>21,23</sup>. The  
449 discrepancy in correlations suggests that the sources of suspended sediment, *E. coli*, and stanol  
450 compounds, may not always coincide: suspended sediment may come either closely from the alleys  
451 of the village or more remotely from the teak tree plantations grown without understory<sup>65</sup>, while *E.*  
452 *coli* may preferentially come from the built-up area<sup>81</sup> (Fig. 1). *E. coli*-free suspended sediment may  
453 get enriched with attached *E. coli* while transferred from remote areas towards the built-up area and  
454 eventually to the ditch, or conversely, dilute the concentration of suspended sediment loaded with  
455 attached *E. coli* originating from soil eroded in the built-up area. The absence of significant  
456 correlation between [*E. coli*] and [5 $\alpha$ -stanol]<sub>WS</sub> suggests that the origin of [5 $\alpha$ -stanol]<sub>WS</sub> may be not  
457 only the chicken contamination source in the built-up area<sup>17,31</sup>, but also teak tree plantations where  
458 soil erosion is known to be high<sup>64</sup>. Indeed, campesterol and sitosterol have been commonly used as  
459 plant biomarkers or as markers of terrigenous organic matter<sup>17,20,25,46</sup>, and if chicken were roaming in  
460 the teak tree plantations, the correlation between [*E. coli*] and both [TSS] and Q would have been  
461 stronger and positive. In addition, [*E. coli*] and [stanol]<sub>WS</sub> generally decreased when reaching the  
462 flood event peak (see detail provided in Supporting Information, including Fig. S11 and Fig. S12),  
463 indicating a dilution of the concentration by surface runoff. Hence, the correlation between [*E. coli*]  
464 and both [copros]<sub>WS</sub> and [5 $\beta$ -w/ocop]<sub>WS</sub> suggests that fecal contamination is mostly associated to  
465 human and porcine feces sources<sup>79</sup> and seems to imply that fecal matter production is concentrated  
466 in the area close to the ditch outlet, i.e., in the built-up area (Fig. 1).

467 The complementary information given by both the microbial indicator and the biomarkers provides a  
468 multi-metrics evaluation of water quality<sup>19,82,83</sup>. In our study, [*E. coli*] was correlated to [copros]<sub>WS</sub>  
469 ( $r=0.81$ ;  $r^2=0.66$ ) and to [24etco]<sub>WS</sub> ( $r=0.83$ ;  $r^2=0.69$ ) (Fig. SI3). Strong positive associations between  
470 [*E. coli*] and [copros]<sub>WS</sub> were already highlighted in the Mekong Delta ( $r^2=0.86$  and  $0.91$  during rainy  
471 and dry seasons, respectively)<sup>21,29</sup>. Besides, reported half-lives of dissolved-phase coprostanol, 24-  
472 ethylcoprostanol, and sitostanol were 3.8, 4.4, and 5.4 days in aerobic and dark conditions at 18 °C<sup>35</sup>,  
473 while reported half-lives of *E. coli* near the study area were 0.5-5 days depending on the suspended  
474 sediment settling and sunlight exposure conditions<sup>74</sup>. The interval between each monitored flood  
475 event and the previous one of similar return period was 1.8-5.7 days. Since the production of fecal  
476 matter is virtually constant in the Laksip village, and considering the half-lives of both stanol  
477 compounds and *E. coli*, no decay of neither *E. coli* nor stanol compounds is expected between fecal  
478 matter production and flood events, and during flood events (typically 1-2 hours for the four flood  
479 events). However, positive correlation between [*E. coli*] and [stanol]<sub>WS</sub> ( $r=0.67$ ,  $p\text{-value}<0.001$ ) (Fig.  
480 SI3) imply that the source of *E. coli* likely coincides with most of the sources of stanol compounds,  
481 and that fecal input in ditch water is fresh<sup>17</sup>. Hence, the dynamics of [copros]<sub>WS</sub>, [5 $\beta$ -w/ocop]<sub>WS</sub>, and  
482 to a lesser extent [stanol]<sub>WS</sub> are consistent with the dynamics of [*E. coli*], and confirm that *E. coli* is a  
483 relevant FIB in the study area.

#### 484 3.4. Relative abundance of stanol compounds in water samples

485 The relative abundance of stanol compounds in water samples varied during flood events (Fig. SI4):  
486 %copros<sub>WS</sub> ranged from 7 to 56 %, while %5 $\beta$ -w/ocop<sub>WS</sub> ranged from 13 to 28 %, and %5 $\alpha$ -stanol<sub>WS</sub>  
487 ranged from 19 to 79 %. %copros<sub>WS</sub> decreased from the onset of the flood event until a minimum  
488 occurring after the discharge peak, i.e., when %5 $\alpha$ -stanol<sub>WS</sub> reached its maximum, while %5 $\beta$ -  
489 w/ocop<sub>WS</sub> remained relatively stable. Maximum %copros<sub>WS</sub> and %5 $\alpha$ -stanol<sub>WS</sub> indicate that the  
490 contribution of both human and chicken feces sources, along with possible terrigenous stanol  
491 compounds, are high in the surface runoff washing off the alleys of the Laksip village<sup>17,20</sup>.  
492 Epicoprostanol is usually used as an indicator of the level of treatment or age of the fecal matter.  
493 High %copros<sub>WS</sub> (7-56 %) and low %epicop<sub>WS</sub> (3-8 %) suggest that the fecal matter is little or not  
494 treated before entering the river water network<sup>37,38</sup>, confirming that villages in rural areas contribute  
495 to decrease surface water quality.

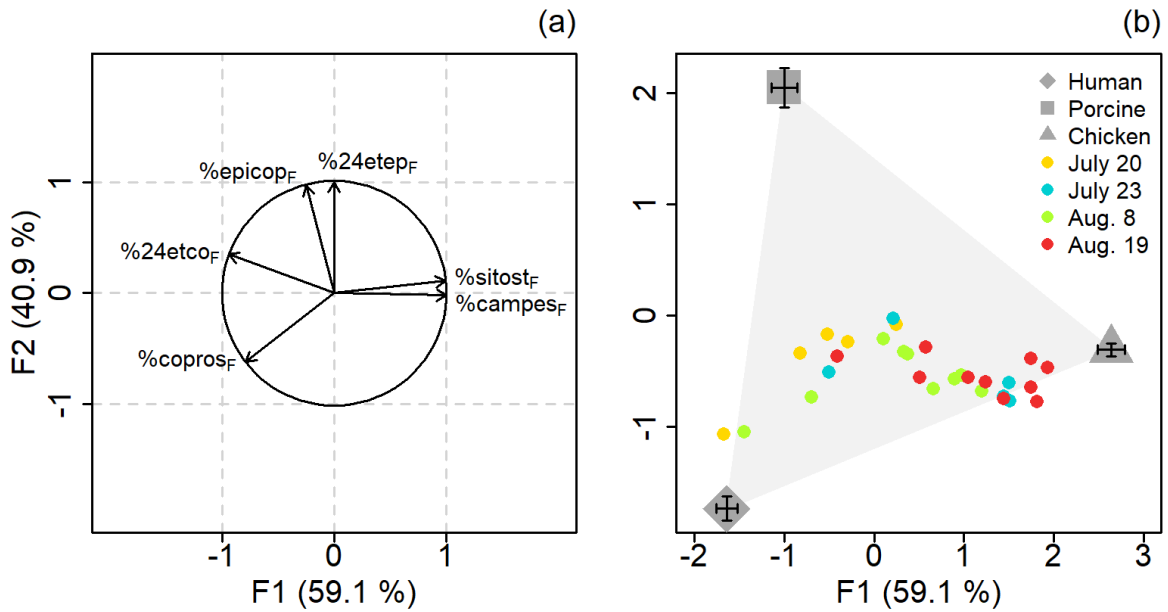
#### 496 3.5. Separation of feces sources with PCA in a tropical context

497 Mixing diagrams show that the relative abundances of the stanol compounds in water samples were  
498 mostly included in the triangles defined by the relative abundances of the stanol compounds in feces  
499 sources, except for %choles<sub>WS</sub>, for which none of the relative abundances were included in the  
500 triangles (Fig. SI5). Hence, we discarded cholesterol before computing PCA.

501 Stanol compounds in water samples not included in the triangles defined by the relative abundance  
502 of the stanol compounds in feces sources may mean that a source, represented by a pole of the  
503 triangle, is inaccurately defined, highly variable, or even missing. In this study, a source other than  
504 the chicken could contribute to the concentration of cholestanol, campesterol, and sitosterol, in  
505 surface runoff: these three compounds have been commonly used as plant biomarkers or as markers  
506 of terrigenous organic matter<sup>17,20,25,46</sup>, but they may also originate from domestic vegetable oils  
507 discharged in sewage<sup>76,84</sup>. As for %24etco<sub>WS</sub> and %24etep<sub>WS</sub>, a source other than the porcine could  
508 contribute, such as caprine<sup>20</sup>. This source could have been missed during the survey because  
509 possibly present in a small number, or not owned by the 24 households located in the built-up area.  
510 Similarly, uncounted dogs could contribute to %copros<sub>WS</sub><sup>20</sup>.

511 The first (F1) and second (F2) components of the PCA explained 59 % and 41 %, respectively, of the  
512 total variance (Fig. 4a). The main contributive variables on the axis F1, in decreasing order of  
513 importance, were %campes<sub>F</sub> (28 %), %sitost<sub>F</sub> (28 %), %24etco<sub>F</sub> (25 %), and %copros<sub>F</sub> (18 %), while the  
514 main contributive variables on the axis F2 were %24etep<sub>F</sub> (41 %), %epicop<sub>F</sub> (38 %), and %copros<sub>F</sub> (16  
515 %) (Table SI3). The PCA analysis clearly separated the 5 $\alpha$ -stanol compounds and the 5 $\beta$ -stanol  
516 compounds without coprostanol along axis F1, and revealed that %copros<sub>F</sub> discriminated the human  
517 feces source, while %epicop<sub>F</sub> and %24etep<sub>F</sub> discriminated the porcine feces source, and %sitost<sub>F</sub> and  
518 %campes<sub>F</sub> discriminated the chicken feces source (Fig. 4b). The case of %24etco<sub>F</sub> is ambiguous, since  
519 it is located between the poles defined by the porcine and the human feces sources: %24etco<sub>F</sub> in  
520 both human and porcine feces sources represents 16 and 19 % of the stanol compounds,  
521 respectively, thus a similar percentage that prevents the discrimination of the two feces sources by  
522 using %24etco<sub>F</sub> only<sup>20</sup>. It confirms that PCA based on six conservative stanol compounds measured in  
523 human, porcine, and chicken feces, was more powerful to separate the three feces sources than  
524 using a single compound, and that PCA was successful to separate feces sources in a tropical context  
525<sup>27</sup>.

526 When plotted within the stanol signature of the feces sources (Fig. 4b), the stanol signatures of the  
527 29 water samples collected during the four flood events were mostly discriminated along axis F1,  
528 with most of the samples located near the pole defined by the chicken feces source, and to a lesser  
529 extent towards the pole defined by the human feces source.



530

531 Fig. 4. Principal component analysis (PCA) applied to three individuals (human, porcine, and chicken  
 532 feces sources) and to six variables (%copros<sub>F</sub>, %epicop<sub>F</sub>, %24etco<sub>F</sub>, %24etep<sub>F</sub>, %campes<sub>F</sub>, %sitost<sub>F</sub>): (a)  
 533 PCA correlation circle; (b) PCA score plot of stanol compounds relative abundance in feces sources,  
 534 with the stanol signature in dry suspended sediment of 29 water samples collected during four flood  
 535 events in 2016 at the BLS sampling station, Laksip village, Northern Lao PDR. F1: principal component  
 536 1; F2: principal component 2. The numbers between brackets are the variance explained by each  
 537 component. Standard deviation is shown as error bars.

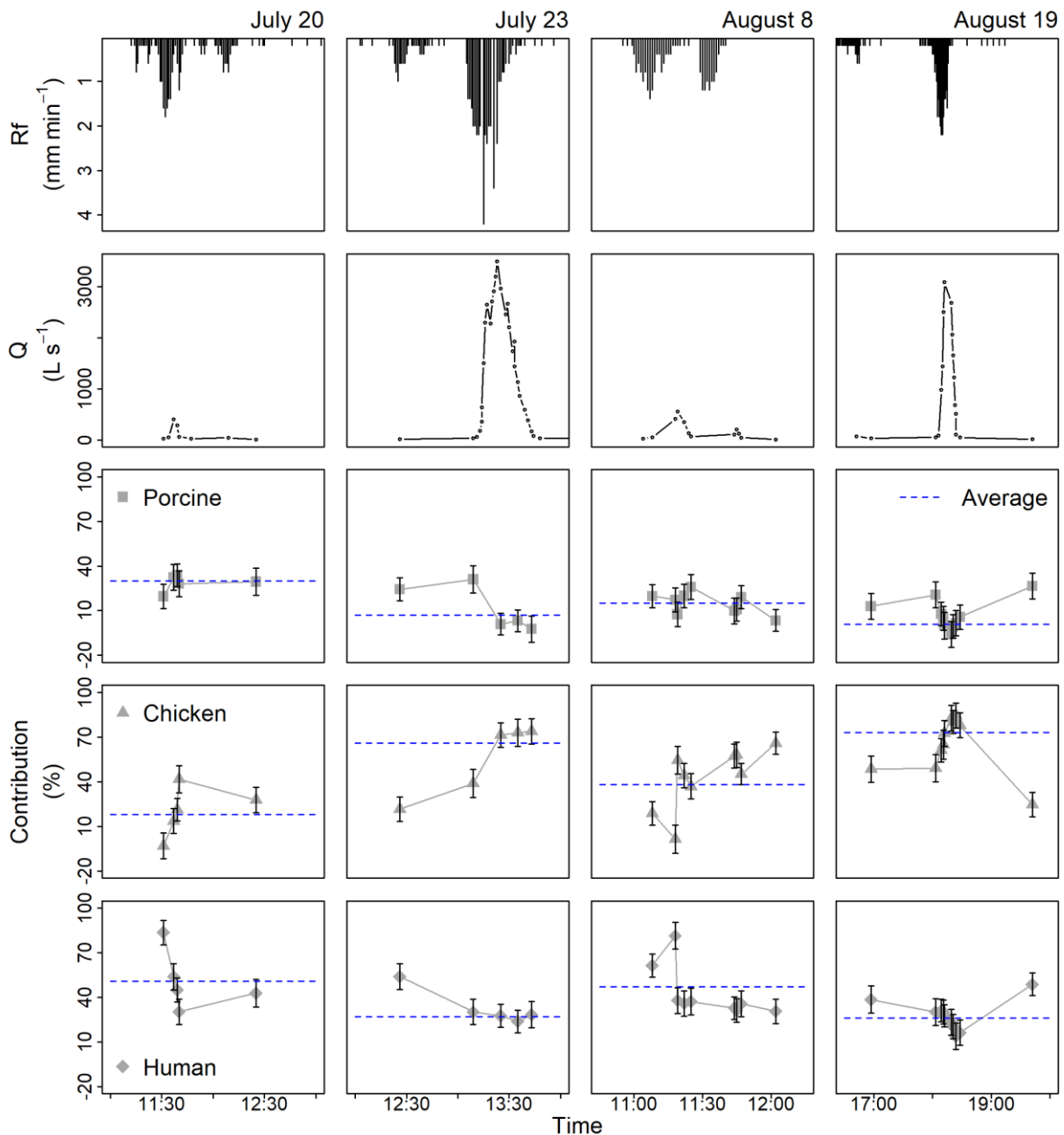
### 538 3.6. Dynamics of the contributions of the fecal contamination sources during flood events

539 The relative contributions of human, porcine, and chicken feces sources at each sampling time during  
 540 flood events ranged from 14±9 to 84±8 %, -6±9 to 34±8 %, and -3±9 to 84±8 %, respectively (Fig. 5).  
 541 Negative values reflect an inaccurately defined pole, the variability of the pole, or the absence of a  
 542 pole in the analysis, or stanol compounds that are not fully conservative. Here however, these  
 543 negative values are within the confidence interval, the average standard deviation of the relative  
 544 contributions of the three feces sources being ±8 %. The contribution of the human feces source  
 545 generally decreased from flood event onset to shortly after flood event peak, while the contribution  
 546 of the chicken feces source generally increased during flood event. The increasing contribution of the  
 547 chicken source may be explained by the fact that (1) human sources are generally closer to the ditch  
 548 outlet than the chicken source; with a shorter travel time to the outlet, human sources are thus first  
 549 detected and exhausted, and (2) 5α-stanol compounds may not only originate from the chicken  
 550 source, but also from terrigenous organic matter originating from agricultural and forest areas  
 551 located more remotely than the built-up area.

552 Discharge-weighted average contributions per flood event of human, porcine, and chicken feces  
553 sources ranged from 26 to 51 %, 1 to 30 %, and 18 to 73 %, respectively (Fig. 5). The average  
554 contribution per flood event of porcine feces sources was in general the smallest. The highest  
555 average contributions of the chicken feces source were found for the highest values of rainfall depth,  
556 rainfall maximal intensity, and maximal discharge, while the highest average contributions of both  
557 human and porcine feces sources were found for the lowest values of rainfall depth, rainfall maximal  
558 intensity, and maximal discharge. Discharge-weighted average contributions over the four flood  
559 events of human, porcine, and chicken feces sources were 29, 5, and 66 %, respectively.

560 The three feces sources produce different concentrations of *E. coli*: humans produce about  $1.6 \cdot 10^{11}$   
561 *E. coli* cap<sup>-1</sup> day<sup>-1</sup>, while pigs and chickens produce about  $1.3 \cdot 10^{10}$  and  $6.8 \cdot 10^8$  *E. coli* cap<sup>-1</sup> day<sup>-1</sup>,  
562 respectively<sup>79</sup>. Multiplied by the units of each feces source in the village (see Section 2.2), the  
563 amount of *E. coli* produced is  $1.92 \cdot 10^{13}$ ,  $2.99 \cdot 10^{11}$ , and  $7.55 \cdot 10^{10}$  *E. coli* day<sup>-1</sup> for human, pigs, and  
564 chickens, respectively. The proportions of *E. coli* load from each feces source calculated from the  
565 latter, i.e. the *E. coli* input into the system from literature review, is therefore 98.1, 1.5, and 0.4 % for  
566 human, pigs, and chickens, respectively. The discrepancies between the proportions calculated from  
567 *E. coli* input and from stanol-based discharge-weighted average contributions can be explained by (1)  
568 preferential surface runoff paths (e.g., is the area convex or concave), and (2) human habits and  
569 animal management (e.g., surface runoff flow into areas favored for open defecation and/or animal  
570 droppings; animals free-ranging or restricted to a fenced area; surface runoff flow into fenced areas).  
571 As an example, for the human source, the difference between the *E. coli* load calculated from human  
572 density and the *E. coli* load calculated from stanol-based average contributions depends in part on  
573 human access to sanitation, i.e., latrines that could store fecal matter which would therefore not be  
574 measured at the village outlet during the four flood events; however, having latrines at home does  
575 not imply that open defecation is not practiced from time to time.

576 *E. coli* exports at the ditch outlet, calculated over the flood events defined as the period lasting from  
577 the first sampling to the last sampling, ranged from  $2.6 \cdot 10^7$  to  $6.4 \cdot 10^7$  MPN. Using the above-  
578 mentioned stanol-based contributions for each sampling time, *E. coli* originating from human,  
579 porcine, and chicken feces sources ranged from  $1.2 \cdot 10^7$  to  $2.8 \cdot 10^7$  MPN,  $0.1 \cdot 10^7$  to  $1.0 \cdot 10^7$  MPN,  
580 and  $0.6 \cdot 10^7$  to  $3.3 \cdot 10^7$  MPN, respectively, during flood events. Surface water fecal contamination of  
581 human origin is especially a concern in terms of risk assessment, since the risk posed to human  
582 health appears substantially higher when ingested water is contaminated by human sources  
583 compared to other animal sources such as birds, poultry, or pigs<sup>85</sup>. One explanation for this is the  
584 host-specific nature of viruses such as noroviruses<sup>86,87</sup>.



585

586 Fig. 5. Rainfall, discharge, and contribution in percentage (%) of porcine, chicken, and human feces  
 587 sources in dry suspended sediment during four flood events sampled in 2016 at the BLS sampling  
 588 station, Laksip village, Northern Lao PDR. Rf: rainfall ( $\text{mm min}^{-1}$ ); Q: discharge ( $\text{L s}^{-1}$ ). Standard  
 589 deviation is shown as error bars. Dotted lines are the average contributions of the feces source  
 590 calculated over the flood event defined as the period lasting from the first sampling to the last  
 591 sampling.

### 592 3.7. Summary

593 To our knowledge, this study is the first one to document the concentrations of fecal bacteria  
 594 exported out of a village settlement in a tropical mountainous area of Southeast Asia. It is also the

595 first one to describe the dynamics of in-stream fecal contamination using biomarkers such as stanol  
596 compounds during flood events.

597 We showed that *E. coli* pulses in surface runoff ranged from  $4.7 \cdot 10^4$  to  $3.2 \cdot 10^6$  MPN 100 mL<sup>-1</sup>: such  
598 concentrations indicate a severe fecal contamination of surface water, and suggest that village  
599 settlements in tropical rural areas may be considered as major hotspots of fecal contamination  
600 pulses into the river network. Major contributory sources of fecal contamination were human and  
601 chicken (up to 51 and 73 %, respectively), the highest percentage switching from the human pole to  
602 the chicken pole during flood events. The chicken source may be overestimated since chicken-  
603 associated stanol compounds may also be terrigenous or plant-related.

604 In addition, we showed that the range of *E. coli* attached to suspended sediment in surface runoff  
605 was 83-100 %. Concentration of total stanol compounds ranged from 1.3 to 591.0 µg L<sup>-1</sup> during flood  
606 events. Concentrations of 5β-stanol, including coprostanol and 24-ethylcoprostanol, were consistent  
607 with the concentrations of *E. coli*, confirming that *E. coli* is a relevant FIB in the context of tropical  
608 village settlements.

609 The transferability of these results to other tropical humid catchments or village areas is ensured  
610 provided (1) the production of fecal matter is virtually constant, (2) there is no contributions from pit  
611 latrines with aged sewage, and (3) the travel time of fecal contaminants during flood events is short.

612 Our results concerning the export of *E. coli* and its attachment to suspended sediment will make it  
613 possible to better parameterize village settlements as point-sources in distributed or semi-  
614 distributed hydrological models. Such models can be used to test scenarios (improved hygiene  
615 practices and sanitation <sup>6</sup>, improved land use management to increase rain water infiltration and  
616 decrease surface runoff and soil erosion <sup>64</sup>, improved management of livestock and manure <sup>88</sup>), and  
617 to suggest better ways of managing and mitigating microbial dissemination in the environment.

618

## 619 Supporting Information

620 Details regarding hysteresis loops analysis from the concentration of TSS, *E. coli*, and stanol  
621 compounds measured in water samples (Pages S2-S3); Rainfall, discharge, and water quality (Fig. S11,  
622 page S4); [TSS]-Q, [E. coli]-Q, [copros]<sub>WS</sub>-Q, [choles]<sub>WS</sub>-Q, [24etco]<sub>WS</sub>-Q, [24etep]<sub>WS</sub>-Q, [campes]<sub>WS</sub>-Q,  
623 and [sitost]<sub>WS</sub>-Q hysteresis loops (Fig. S12, page S5); Spearman's rank correlation coefficient matrix of  
624 log-transformed discharge and concentrations of water quality variables in water samples (Fig. S13,  
625 page S6); Rainfall, discharge, and relative abundance of stanol compounds in water samples (Fig. S14,  
626 page S7); Mixing diagrams of the relative abundance of seven stanol compounds in feces sources and

627 in water samples (Fig. SI5, page S8); Names of the stanol compounds analyzed in this study, m/z  
628 values used for the identification and quantification, and information on quantification compounds  
629 (Table SI1, page S9); Concentration and relative abundance of the seven stanol compounds measured  
630 in porcine, chicken, and human feces (Table SI2; page S10); Factorial coordinates and contribution of  
631 variables on the F1 and F2 axes calculated from the Principal Component Analysis (PCA) based on the  
632 relative abundance of six stanol compounds measured in human, porcine, and chicken feces sources  
633 (Table SI3, page S11); [TSS]-Q, [*E. coli*]-Q, [copros]<sub>WS-Q</sub>, [choles]<sub>WS-Q</sub>, [24etco]<sub>WS-Q</sub>, [24etep]<sub>WS-Q</sub>,  
634 [campes]<sub>WS-Q</sub>, and [sitost]<sub>WS-Q</sub> hysteresis loops (Table SI4, page S12).

635

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644



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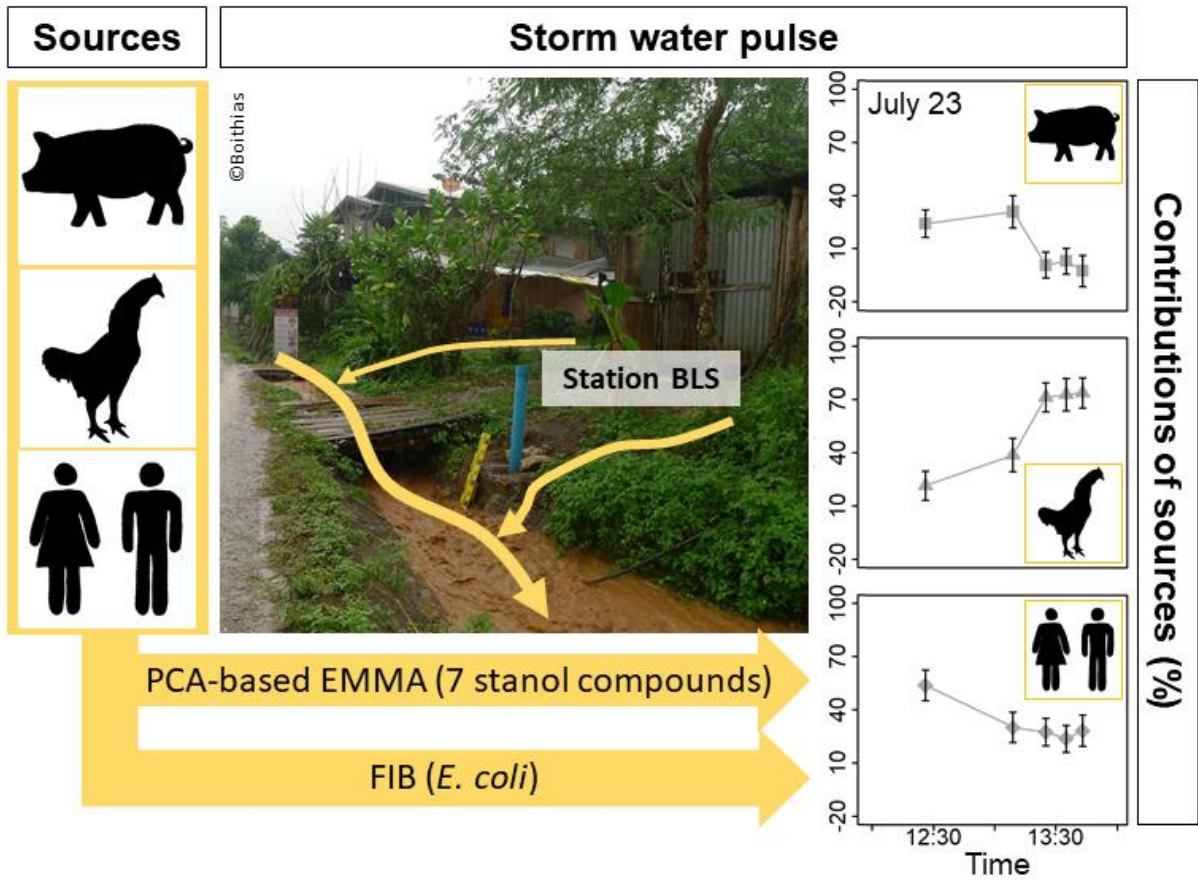
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