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1 **Soil ingestion, a key determinant of exposure to environmental contaminants. The case**  
2 **study of chlordecone exposure in free-range pigs in the French West Indies.**

3

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15

16

17 <sup>1</sup>Non-standard abbreviations sorted in alphabetical order

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<sup>1</sup> BW: body weight, CLD: chlordecone, CR: Creole breed of pigs, DM: dry matter, FM: fresh matter, HP: high pasture paddock, LP: low pasture paddock, LW: Large White breed of pigs, MRL: maximum residue limit, MW: metabolic weight, SP: sweet potato paddock

19 **ABSTRACT**

20

21 Ingested soil may expose free-range animals to environmental pollutants. In pigs, soil  
22 ingestion is few described whereas their burrowing behaviour suggests that it could be high.  
23 Although highly productive pigs are generally reared indoor, free-range farming is increasing  
24 in view of ethical considerations for animal welfare and is a common practice for subsistence  
25 agriculture systems. The experiment lasted 8 weeks (2 for adaptation, 6 for measurements)  
26 with 24 growing pigs of Guadeloupean Creole (CR) or Large White (LW) breeds. Pigs were  
27 assigned to 3 outdoor treatments: high pasture HP (>60 days of regrowth), low pasture LP  
28 (35 days of regrowth), and sweet potato SP (sweet potato field). Titanium (soil marker) and  
29 chromium (faecal output marker) contents of faeces, vegetation and soil samples were used to  
30 estimate individual daily soil ingestions. The average, 10<sup>th</sup> and 90<sup>th</sup> percentiles were 440, 200  
31 and 726 g of dry soil per 100 kg body weight, respectively, without significant differences  
32 between the 3 outdoor treatments or the 2 breeds but with a significant period (i.e. week of  
33 measurements) × treatment interaction ( $P<0.001$ ). In the French West Indies, animals may be  
34 exposed to chlordecone (CLD), a very persistent organochlorine insecticide. Simulations of  
35 CLD tissue contamination due to ingestion of contaminated soil were carried out and  
36 compared to the maximum residue limit. These results show that grazing management needs  
37 to be adapted to effectively limit soil ingestion by pigs and the impact of a contaminated  
38 environment on the sustainability of pig systems.

39

40 **Keywords:** soil intake; feeding behaviour; plant cover; exposure risk; soil-bound pollutants;  
41 chlordecone

42

## 1. Introduction

Literature developed methodologies to show that free-range animals might ingest soil during exploration of outside runs or paddocks (Jurjanz et al., 2014; Mayland et al., 1975; Roberts and Longhurst, 2002) and to quantify it. Even if the ingestion of some invertebrates of the pedofauna can take place (Rose and Williams, 1983), the ingestion of soil is generally of little nutritional value, as the presence of soil in the digestive tract would decrease the digestibility of ingested diet (Jurjanz et al., 2014). In addition, the ingestion of soil has been shown to be a significant exposure pathway of free-range animals to environmental pollutants (Ayrault et al., 2016; Cooke et al., 1996; Johnsen and Aaneby, 2019; Rychen et al., 2013). Indeed, soil can keep over very long-time concentrations of heavy metals, organic pollutants or radionuclides (Comte et al., 2022). Therefore, the studies of soil ingestion are less motivated by nutritional or zootechnical questions but much more by environmental and food safety approaches. To the best of our knowledge, this question has been little studied in pigs where only one study gives quantifications for free-range lactating sows (Jurjanz and Roinsard, 2014).

Although some breeds of pigs are more often reared indoor, societal expectations in favour of animal welfare are encouraging farmers to provide outdoor areas for the animals, and this is a criterion in the specifications for organic farming if the control of African swine fever is met (Martínez Avilés et al., 2019). Moreover, pigs are reputed to their digging activity and to explore easily soil whenever they have an access to (Høøk Presto et al., 2008). Furthermore, pigs are frequently an element of self-catering agricultural systems as they are able to valorize different byproducts of the garden or the household and by consequence would efficiently participate in the food delivery for the family. These agricultural systems are of especial interest as they are more frequent in informal systems not covered by the

68 governmental monitoring programs to control exposure to environmental pollutions (Kagira  
69 et al., 2010; Thutwa et al., 2020). Moreover, informal animal rearing would concern more  
70 often the less well-off parts of the population, which have generally a more vulnerable health  
71 status. The fact that soil may carry environmental pollutants in the food chain especially to  
72 these populations strengthen the need to evaluate the degree of soil ingestion of pigs in such  
73 informal rearing systems and how it can be limited.

74 In this frame, the Caribbean context has a higher importance as home reared pigs are part of  
75 the traditional food habits and were frequently raised outside in a tropical climate  
76 characterized by episodes of strong rainfall (Sousa Junior et al., 2014). Therefore, these free-  
77 range systems can cumulate all risk factors: species with an elevated digging activity raised  
78 on easily soiled vegetation due to humid climate. The presence of a sanitary crisis due to the  
79 pollution of the environment by the organochlorine pesticide chlordane (CLD) would  
80 reinforce the need to quantify the soil ingestion by free-range pigs and to find out  
81 management tools to limit their soil ingestion. In Guadeloupe, as in many tropical and  
82 subtropical regions (Robinson et al., 2011), swine production is based on a variety of farming  
83 systems, including specialized industrial and landless farms with high pig density, or small  
84 family farms with Creole and/or crossbreeds reared in low input conditions (including free-  
85 range pigs or tethered to a tree) (Gourdine et al., 2021).

86 Therefore, the present work studies the ingestion of soil in free-range pigs raised on different  
87 types of tropical vegetation covers in Caribbean conditions. Besides this main objective, the  
88 study target to investigate potential differences in soil ingestion between the local Creole  
89 breed in comparison to the European Large White breed.

90

## 91 **2. Materials and methods**

92

93        *2.1.Ethics statement*

94

95 All animal care handling techniques and procedures as well as the procedures for sampling  
96 were approved by the Ethics Committee of French Guyana and West Indies n° 069 (Comité  
97 d’Ethique en Matière d’Expérimentation Animale des Antilles et de la Guyane, CEMEAAG)  
98 authorized by the French Ministry of Higher Education, Research and Innovation, under the  
99 project number APAFIS#6070-2016070721289156v3. The experiment was performed at the  
100 INRAE Experimental indoors facilities of PTEA (Plateforme Tropicale d’Expérimentation  
101 sur l’Animal) and outdoor facilities of PEYI (Plateforme Expérimentale sur le végétal et les  
102 agrosYstèmes innovants en milieu tropical) according to the certificate number A 971-18-02  
103 of authorization to experiment on living animals issued by the French Ministry of  
104 Agriculture.

105

106        *2.2.Experimental design and animal management*

107

108 A total of 24 growing pigs of two different breeds (12 Creole CR and 12 Large White LW)  
109 were used on the experimental facilities of INRAE in Guadeloupe (GPS 16°12’13” N,  
110 61°39’24”W). The experimental design consisted of three outdoors treatments: high pasture  
111 (HP, pasture with grass more than 60 days of regrowth age); low pasture (LP; pasture with  
112 grass at 35 days of regrowth age) and sweet potato (SP; sweet-potato field). The 24 pigs were  
113 randomly selected at 13 weeks of age ( $20.6 \pm 3.6$  kg and  $28.9 \pm 5.2$  kg BW, for CR and LW  
114 pigs, respectively), from the same litters and were affected in each treatment to have half-  
115 siblings represented in each treatment (4 CR and 4 LW pigs). All the pigs fed the same before  
116 joining the different experimental treatments. The experiment began by 2 weeks of adaptation

117 of the pigs to experimental conditions (wk1-wk2), followed by 6 weeks of measurements on  
118 animals, vegetation and soil (wk3-wk8) (Fig. SM1, supplementary material).

119 The total area for each treatment was 880 m<sup>2</sup> (10 x 88 m) of ferralitic alluvial soils, divided in  
120 one plot of 100 m<sup>2</sup> (10 x 10 m; 12.5 m<sup>2</sup> per pig) for adaptation (electric fences, pastures for  
121 HP and LP, and sweet-potato field for SP) and 6 plots of 130 m<sup>2</sup> (10 x 13 m<sup>2</sup>) for the  
122 measurement periods. The 8 pigs per treatment grazed a plot for one week, from Monday  
123 (day1) to Sunday (day7) (16.25 m<sup>2</sup> per pig per week). The plot was delimited by a mobile  
124 electric fence powered by a solar battery. Every Monday morning (at about 07:00), the pigs  
125 were moved to another plot. For each treatment, the pigs had access to two shades (1 m<sup>2</sup> per  
126 pig) and to 2 barrels near the shades, containing 100 L of water each, which were filled twice  
127 per day so that the pigs never run out of water, for drinking and for mud wallow. The HP and  
128 LP pastures did not contain any cultivated crops, but only natural grasses. The SP field was  
129 the results of a planting of sweet-potato cuttings of more than three nodes every 20 cm on a  
130 row and 1.60 m between rows (corresponding to 33 000 cuttings per ha). The plots were  
131 clean weeded before planting and one month after planting. In LP treatment, the plots were  
132 previously mown to 3 cm 35 days before the introduction of the pigs, to ensure the same age  
133 of regrowth and the same vegetation stage. The LP and HP diets were based on grass  
134 available on the plot, cracked corn (containing <25 µg Ti·g<sup>-1</sup> DM and <20 µg Cr·g<sup>-1</sup> DM; 750  
135 g FM·d<sup>-1</sup> from wk1 to wk4, and 1000 g FM·d<sup>-1</sup> from wk5 to wk8; DM = 87.8 % FM), and a  
136 supplementation of 250 g FM·d<sup>-1</sup> of a protein feed (composed by 96% of soya bean meal and  
137 0.6 % of chromium oxide; containing 100 µg Ti·g<sup>-1</sup> DM and 4215 µg Cr·g<sup>-1</sup> DM). The SP  
138 diets were based on sweet-potato leaves and tubers available on the plot (the pigs had to dig  
139 the soil to catch tubers), a limited amount of non-cultivated grasses available in the sweet-  
140 potato field, cracking corn (400 g FM·d<sup>-1</sup>) and the same supplementation (250 g FM·d<sup>-1</sup>) of  
141 protein feed than LP and HP treatments. The protein feed was given individually in feeding

142 boxes for all treatments in two daily meals: 150 g fresh matter (FM) in the morning and 100 g  
143 FM in the afternoon.

144

### 145 *2.3. Sampling, measurements and analysis*

146

147 Pregrazing surface height of herbage sward of both HP and LP treatments were measured on  
148 Monday morning *via* a rising plate herbometer (Fig. SM1, supplementary material). Grass  
149 was sampled on the HP treatment plot (outside the experimental paddocks). Potato tubers and  
150 aerial parts (leaves and stems) of potatoes were harvested on the SP treatment plot (outside  
151 the experimental paddocks) and weighted to estimate the available biomass per surface unit.  
152 The potato allowance was estimated in kg dry matter (DM) per period, as well as in g DM per  
153 kg body weight (BW). Based on BW of each pig in each period, an average supply per CR  
154 pig and per LW pig was estimated (CR pigs being lighter than LW ones, the calculation was  
155 done separately for the two breeds). Samples of corn and protein feed were taken weekly. All  
156 feedstuffs samples were dried (65°C up to constant weight) and crushed in a ball mill.

157 The behaviour of the pigs was studied twice during the experimental period, at the beginning  
158 (the third day of wk3) and in the middle (the fourth day of wk7) of the experiment. During 24  
159 continuous hours (from 06:00 to 06:00 of the next day), the physical (rest, feeding, rooting,  
160 ...) and feeding (water, leaves, sweet potatoes, concentrate) activities were recorded every  
161 five minutes. Animals were weighed each Monday morning during the transfer to the new  
162 experimental barn on a scale (PM110, Maréchalle, Chauny). During the distribution of the  
163 protein feed, an individual sample of faeces was taken from each animal from Tuesday  
164 (day2) to Friday (day5), dried (65°C up to constant weight) and crushed in a ball mill.



165 Each morning (from Monday to Friday, at about 08:00), a standardized picture (from the  
166 south-east corner) of each plot (HP, LP and SP) was taken with a digital camera.  
167 Consequently, each plot within each treatment had a total of 6 pictures (from Monday to  
168 Friday, day1 to day5, and the Monday after pigs had changed plot, called day8) (Fig. SM1,  
169 supplementary material). These images were used to quantify the changes in monitor the  
170 disappearance of the vegetation cover (see section 2.5).

171 Soil samples were taken once the animals have been moved to the next barn (15 cm, 3  
172 elementary samples grouped to one sample of each experimental barn, i.e. each week). After  
173 manual separation of gravel (>2 mm) and roots, soil samples were dried and crushed in a ball  
174 mill. Titanium (in the form of titanium dioxide  $\text{TiO}_2$ ) and chromium (Cr) were analysed in all  
175 samples (vegetation, feedstuffs, faeces and soil) by SARM service of CNRS-CRPG  
176 laboratory (Vandœuvre-lès-Nancy, France). The samples were dissolved by melting with  
177 lithium metaborate and recovering the melt with diluted HCl. Melting was carried out in a  
178 muffle furnace progressively raised to 1000°C (200°C/h).  $\text{TiO}_2$  was measured by UV-visible  
179 spectrophotometry (measurement at 470 nm of the titanium-chromotropic acid complex at pH  
180 3.5) on an AGILENT CARY60 instrument. Cr was measured by flame atomic absorption  
181 spectrometry on an AGILENT SPECTRAA 240FS instrument. Regarding Quality Control,  
182 all measurements were validated by measuring certified reference materials included in the  
183 analysis series. The reference materials analysed are BE-N, UB-N, SO-1, SO-2, BCR-  
184 CRM060, GSS-7, CRM-055, BCR-CRM414, GXR-5 and GXR-2 (Jochum et al., 2005). For  
185 Quality Assurance, the limit of detection (LOD) was 25 ppm for  $\text{TiO}_2$  and 20 ppm for Cr. It  
186 was calculated on the measurements of minimum 10 x experimental blank. Uncertainty  
187 values vary depending on the content determined in each sample and are based on statistical  
188 calculations extrapolated from measurements of reference materials and duplicates of the  
189 experimental samples. For Cr the relative uncertainty is <20% for contents below 100 ppm,

190 and <5% for contents above 100 ppm. For TiO<sub>2</sub> the relative uncertainty varies from 10 to  
191 20% for contents below 200 ppm, to <5% for contents above 1000 ppm.

192

#### 193 *2.4. Evaluation of soil ingestion*

194

195 The ingestion of soil was estimated at each experimental week based on faecal samples taken  
196 between Tuesday (day2) and Friday (day5). The method consisted of estimating the amount  
197 of the internal soil marker TiO<sub>2</sub> excreted in faeces, by considering that all faecal TiO<sub>2</sub> was  
198 originated from soil according to the very low TiO<sub>2</sub> levels found in plants and crops, 200  
199 times lower than those found in soil (Table 1). Therefore, the daily faecal output was  
200 estimated *via* the external marker Cr<sub>2</sub>O<sub>3</sub>. Indeed, the daily applied of chromium, obtained by  
201 multiplying the amount of protein feed daily ingested (refusals were considered) by the  
202 chromium content of the protein feed, was divided by the chromium concentration in the  
203 faeces:

$$204 \text{ Daily faecal output [kg DM}\cdot\text{d}^{-1}] = \text{protein feed ingestion [g DM]} \times \text{Cr in protein feed } [\mu\text{g} \\ 205 \text{ Cr}\cdot\text{g}^{-1} \text{ DM}] / \text{Cr in faeces } [\mu\text{g Cr}\cdot\text{g}^{-1} \text{ DM}] \times 1000$$

206 Then, the daily soil ingestion was estimated *via* the concentration of the soil-specific internal  
207 marker TiO<sub>2</sub> in faecal output and in soil:

$$208 \text{ Daily soil ingestion [g}\cdot\text{d}^{-1}] = \text{TiO}_2 \text{ in faeces } [\mu\text{g TiO}_2\cdot\text{g}^{-1} \text{ DM}] \times \text{faecal output [g DM}\cdot\text{d}^{-1}] / \\ 209 \text{TiO}_2 \text{ in soil } [\mu\text{g TiO}_2\cdot\text{g}^{-1} \text{ dry soil}].$$

210

#### 211 *2.5. Statistical analyses*

212

213 Statistical analyses were performed using R software (version 4.1.1) (R Development Core  
214 Team, 2020). Pregrazing sward surface heights were analysed in a linear model to compare  
215 HP and LP treatments, period and treatment  $\times$  period interaction. The latter was not  
216 significant and remove from final model. Tukey's *post-hoc* test was used for multiple  
217 comparisons between the 6 experimental periods.

218 For the behaviour data, the data collected every five minutes were converted in time spent in  
219 physical of feeding activities. Each behaviour trait (eating, rooting, ...) was analyzed  
220 separately by robust analysis of variance (ANOVA) with the WRS2 package, using firstly a  
221 three-way robust ANOVA: the effects of treatment (HP, LP vs. SP), breed (CR vs. LW) and  
222 period (wk3 vs. wk7) and within-animal effects, due to repeated measurements (wk3 and  
223 wk7). Pairwise group comparisons were performed within each breed  $\times$  treatment interactions  
224 and when interactions were found not significant ( $P>0.05$ ), two-way ANOVA was performed  
225 and pairwise group comparisons were performed within each treatment (HP, LP and SP).  
226 Pearson correlations between estimated ingestion levels and the time spent for rooting  
227 activities were calculated to check the linear relationship between soil ingestion and rooting  
228 activities.

229 The daily pictures of plots were analyzed using the method proposed by Mahieu et al. (2019).  
230 The aim of this method is to discriminate defoliation patterns based on a pixel index, the  
231 visible atmospherically resistant index for green matter (VARIgreen). As reported by Mahieu  
232 et al. (2019), the pixel index values were distributed in three categories: i) category A  
233 contained pixels associated with bare soil, mud and/or soil litter; ii) category B contained  
234 pixels associated with stems and other senescent plant parts with low chlorophyll levels; and  
235 iii) category C contained pixels associated with green matter. The percentage of pixels were  
236 calculated for each category and were normalized with the arcsine of the square root of the  
237 proportions. The transformed data were analyzed using ANOVA of the R package agricolae,

238 with the effects of treatment (HP, LP vs. SP), day (1 to 5 and 8) and the interaction between  
239 day and category (A, B and C).

240 Animal variables (BW, faecal production, soil ingestion) were tested using linear mixed  
241 models with type of paddock (HP, LP, SP), period, breed (CR, LW) and their two-by-two  
242 interactions as fixed effects; and individual as a random effect (R package nlme). Individual  
243 animals were used as the experimental unit. Type of paddock  $\times$  breed interaction was never  
244 significant and remove from final models. Only the interactions of period with the type of  
245 paddock, or with the breed, were retained in the final models when they were significant  
246 ( $P < 0.05$ ) or tended to be ( $0.05 < P < 0.1$ ). The 'lsmeans' function (R package lsmeans) was  
247 used for multiple comparisons (Tukey method for adjusting p.values).

248

### 249 **3. Results**

250

#### 251 *3.1. Paddock characteristics*

252

253 Herbage and potato quality, as well as stocking rate, expressed in kg BW per m<sup>2</sup> per period,  
254 are detailed in Table 1. Pregrazing sward surface heights were significantly higher on HP  
255 than LP paddocks with an average gap of 12.9 cm ( $P < 0.001$ ; Table 1). For both LP and HP  
256 treatments, the sward heights in wk8 (27.0 cm) were significantly higher than the sward  
257 heights in weeks 3 to 6 (19.3 to 21.9 cm;  $P < 0.001$ ). Sward heights ranged from 14.2 to 21.2  
258 cm and from 23.9 to 32.9 cm for LP and HP paddocks respectively. Treatment  $\times$  period  
259 interaction was not significant.

260 The potato biomass on SP treatment was estimated to 38.4 and 18.7 kg DM per period for  
261 tubers and leaves/stems respectively. This corresponded to a daily potato allowance of  
262 approximately 20.1 and 10.1 g DM $\cdot$ kg<sup>-1</sup> BW for tubers and leaves/stems respectively. In

263 relation to the BW of the pigs this would be equivalent to a daily allowance of 615 and 756 g  
264 DM tubers per pig, and 300 and 368 g DM leaves/stems per pig, for CR and LW breeds  
265 respectively.

266

### 267 *3.2. Pig behaviour and impacts on paddocks*

268

#### 269 *3.2.1. Pig behaviour*

270

271 The interaction effects of breed with treatment and period on rooting, drinking and resting  
272 activities were not significant ( $P>0.20$ ). Rooting activities significantly increased from wk3  
273 to wk7 in HP and LP treatment but remained the same in SP treatment (Fig. 1). Eating  
274 activities were found to be different between breeds ( $P <0.01$ ). Irrespective of the treatment,  
275 CR pigs spent much more time eating than LW pigs. Eating (Fig. 2) and drinking (Fig. SM2,  
276 supplementary material) activities in outdoor conditions significantly increased or trended to  
277 increase from wk3 to wk7 (except for CR pigs in HP conditions and for pigs in SP conditions,  
278 for eating and drinking activities, respectively). Consequently, time dedicated for resting  
279 decreased from wk3 to wk7 (Fig. SM3, supplementary material).

280

#### 281 *3.2.2. Soiling and depletion of the vegetation*

282

283 The ANOVA analysis of the percentage of pixels showed that the latest was not affected by  
284 either treatment ( $P>0.8$ ) or week ( $P>0.6$ ). At the opposite, the percentage of pixels was  
285 affected by the interaction between days of pasture and VARIgreen category ( $P<0.001$ ).  
286 Figure 3 illustrates that the proportion of pixels corresponding to green matter (category C)  
287 but also to stems and senescent plant parts (category B) decreased with the day of pasture and

288 it is closed to zero after pasture (day 8). At the opposite, the percentage of pixels  
289 corresponded to bare soil, mud and/or soil litter (category A) increased and the value was  
290 closed to 100 % after moving pigs to the next plot (day 8). The percentage of pixels of C  
291 category (green matter) dropped dramatically from the first to the second day of pasture and  
292 inversely the percentage of pixels of A category increased. These results of the picture  
293 analysis were in line with our observations (Fig. SM4, supplementary material).

294

### 295 *3.3. Soil ingestion*

296

#### 297 *3.3.1. Influence of the type of paddock and the period*

298

299 There was a significant type of paddock  $\times$  period interaction for the four variables: BW  
300 ( $P < 0.05$ ), daily faecal output ( $P < 0.05$ ), faecal  $\text{TiO}_2$  content ( $P < 0.001$ ) and daily soil  
301 ingestions ( $P < 0.001$ ; Fig. 4; Table 2). Expressed in g DM per pig per day, the means per  
302 treatment and period ranged from 91.9 (wk4) to 130.6 (wk5), from 131.2 (wk7) to 262.1  
303 (wk4), and from 80.3 (wk5) to 178.4 (wk8), for HP, LP and SP treatments respectively.  
304 Regarding the individual values, daily soil ingestions expressed in g DM per pig ranged from  
305 38.5 to 258.6, from 58.4 to 514.5, and from 35.2 to 310.8, for HP, LP and SP treatments  
306 respectively. In LP treatment, two values were particularly high for one pig due to its high  
307 faecal output and high  $\text{TiO}_2$  contents in faeces. The latest were kept in the dataset as they did  
308 not appear abnormal, but they pull the average of the LP treatment upwards (Fig. 4).  
309 Irrespective of the period (wk3 or wk7), no significant correlations were found between  
310 rooting behaviour and the level of soil ingestion (first behaviour data set during wk3:  $r =$   
311 0.18,  $P = 0.40$ ; second behaviour data set during wk7:  $r = -0.34$ ,  $P = 0.10$ ).

312

313        3.3.2. *Influence of BW and breed*

314

315 Pig's BW was not different between the three treatments (i.e. the three types of paddock: HP,  
316 LP and SP) ( $33.0 \pm 1.4$  kg, mean  $\pm$  SE) but was significantly higher for LW than CR pigs  
317 ( $P < .001$ ; Table 1). Pigs' BW increased during the six periods from 26.9 to 39.3 kg (on  
318 average + 12.4 kg in 5 weeks). The effect of breed was only significant on daily soil ingestion  
319 when the latter was expressed per animal and not per unit BW. The breed effect was therefore  
320 confounded with the BW effect since CR pigs were lighter than LW ones. Indeed, BW was  
321 on average 29.3 kg for pigs of CR breed and 36.7 kg for pigs of LW breed. During the  
322 experiment, CR pigs have grown from 23.8 to 34.9 kg and LW pigs have grown from 30.0 to  
323 43.6 kg. The BW difference between CR and LW pigs was on average 6.2 kg at the start and  
324 8.6 kg at the end of the experiment. Daily soil ingestions were on average 116.2 and 162.9 g  
325 DM per pig, and 410.5 and 469.2 g DM per 100 kg BW, for CR and LW breeds respectively  
326 (Fig. 5). The 10<sup>th</sup> and 90<sup>th</sup> percentiles were 181.8 and 662.6 g DM per 100 kg BW for CR,  
327 and 218.1 and 789.8 g DM per 100 kg BW for LW. Period  $\times$  breed and treatment  $\times$  breed  
328 interactions were not significant for all variables tested ( $P > 0.05$ ; Fig. 5; Table 3).

329

330        **4. Discussion**

331

332        *4.1. Soil ingestion in pigs and possible drivers*

333

334 We hypothesized that soil ingestion would increase from HP, LP to SP due to i) the  
335 difference in pasture allowance between HP and LP; ii) the higher rooting activities in SP  
336 than LP and HP since pigs had to root to find sweet-potato tubers. In contrast to our  
337 expectations, soil ingestion levels did not differ between treatments, with a daily average of

338 440 g DM per 100 kg BW, while we observed higher rooting activities in SP than LP and HP  
339 and higher pregrazing sward heights in HP than LP. A high variability in soil ingestions  
340 between pigs and between periods was observed with average, 10<sup>th</sup> and 90<sup>th</sup> percentiles at  
341 439.9, 200.2 and 726.3 g DM per 100 kg BW, respectively.

342 The BW of the animals remained similar between the three types of paddocks and the pigs  
343 grew by about 353 g per day (i.e. +12.4 kg between the 1<sup>st</sup> and 6<sup>th</sup> week of measurements).  
344 This growth is consistent with the references for these breeds fed with these types of diets in  
345 our conditions (Gourdine et al., 2018), so it appears that the three types of paddocks allowed  
346 the pigs to meet their nutritional requirements for maintenance and growth. Therefore, we  
347 consider that the animals on the LP treatment were not nutritionally limited.

348 Although the 24-hour behavioural observations in weeks 3 and 7 showed that pigs in the SP  
349 paddock spent more time for rooting activities, this did not result into higher soil ingestion.  
350 The higher rooting activities on the SP treatment may be explained by the expression of  
351 natural foraging behaviour reinforced here by the search for underground food in contrast to  
352 the aerial grass available on the LP and HP treatments. Several hypotheses can be put forward  
353 to try to explain the rooting and/or soil ingestion behaviour, such as the natural exploratory  
354 behaviour of pigs (Studnitz et al., 2007), the search for nutritional elements as pedofauna,  
355 minerals, trace elements or fibres. However, even if the nutrient intakes from the different  
356 diets of the three treatments have not been quantified, the possibility of a restriction in  
357 minerals or trace elements is unlikely.

358 According to the picture analysis of the dirty grass or bare soil evolution over a period, the  
359 condition of the paddocks remained fairly similar between the three treatments but with a  
360 clear degradation from one day to the next. From day 5 (i.e. the last day of faces collection),  
361 the paddocks had a fairly high proportion of bare soil (85.1 %). On the morning of the 8<sup>th</sup> day



362 (i.e. the day the pigs were moved to a new paddock for a next period), the bare soil, mud  
363 and/or soil litter represented almost the entire surface. Faeces were collected from day 2 to  
364 day 5 of each period, so soil ingestion was assessed in the first half of each period when  
365 sufficient vegetation was still available to observe differences between treatments if there had  
366 been any.

367 The period effect on soil ingestion was not related to the increase in stocking rate over time,  
368 although the increase in grazing pressure from one period to another may increase the impact  
369 of trampling and soiling of the grass, but the highest soil ingestions not being concentrated in  
370 the last periods. The first experimental period was the rainiest with 49 mm the day the  
371 animals entered the first experimental paddock, and 45 mm the day before. These conditions  
372 could explain at least in part the higher soil uptake observed in wk3, particularly in the LP  
373 treatment. Such an effect of humidity on soil ingestion has been shown previously in sheep  
374 (Abrahams and Steigmajer, 2003) and cattle (Collas et al., 2019). The consequences of the  
375 heavy rainfall may be more impactful on the short grass of LP than on the HP and SP  
376 treatments where the tall grass, or the aerial parts of the potatoes, can more easily attenuate  
377 the "splash" effect linked to the soil projections on the grass (Dreicer et al., 1983; Hinton et  
378 al., 1995). The results of this study suggest that the amplitude of soil ingestion is more related  
379 to the natural rooting behaviour of pigs in comparison with other species, the variability in  
380 climatic conditions and the time spent in each plot.

381

#### 382 *4.2. Soil ingestion of pigs in comparison to other species*

383

384 This very first quantification in growing pigs showed average daily ingestion of soil between  
385 116 and 171 g per animal. That is quite similar to these reported for cattle, also in Caribbean

386 systems (Collas et al., 2019), but the large different BW change their significations. Indeed,  
387 the comparison of ingestions are generally carried out for growing animals by putting the  
388 ingested amount in ratio to 100 kg of BW or for comparison between species relative to the  
389 metabolic weight (MW, correspond to  $BW^{0.75}$ ). In such a frame, the measured soil ingestion  
390 of these free-range pigs of 368 to 548 g per 100 kg of BW are much higher than ingestions  
391 reported in growing cattle in Caribbean systems of less than 100 g per 100 kg BW (Collas et  
392 al., 2020, 2019; Jurjanz et al., 2017), or less than 200 g per 100 kg BW in growing broilers  
393 (Jurjanz et al., 2015). By expressing per kg MW, cattle and broilers ingested generally not  
394 more than 4 g DM per kg MW (Collas et al., 2019; Jurjanz et al., 2017, 2015), grazing horses  
395 and lactating sows around 5 g  $DM \cdot kg^{-1}$  MW (Jurjanz et al., 2021; Jurjanz and Roinsard,  
396 2014), whereas these free-range pigs in tropical conditions ingest much more with 9 to 13 g  
397  $DM \cdot kg^{-1}$  MW (Table 2). These comparisons confirm clearly that the natural digging  
398 behaviour of pigs would result in a significantly higher soil ingestion than in other species  
399 what would, by consequence, expose such rearing systems much more to pollutants in a  
400 context of contaminated areas.

401

402 *4.3. Implications for animal exposure to soil-bound pollutants and human health – the*  
403 *case of chlordecone*

404

405 During grazing, pigs ingest soil and this is enhanced by rooting activity. Although this natural  
406 behaviour may allow ingestion of pedofauna and roots or mineral supplementation (Edwards,  
407 2003), it also presents risks as pigs are exposed to the full range of contaminants that may be  
408 present in the soil, which may have serious repercussions on their health and/or the safety of  
409 the products. In the French West Indies, nearly one fifth of the agricultural soil in  
410 Guadeloupe and two fifths of the agricultural soil in Martinique are at risk of CLD

411 contamination (Comte et al., 2022; Ministère de la Transition Ecologique, 2017). Due to its  
412 strong persistence, this insecticide, used against the banana weevil (*Cosmopolites sordidus*)  
413 until its ban in the 1990s, caused soil pollution and the contamination of water and  
414 ecosystems. A major challenge for this territory, as for any territory affected by such a health  
415 crisis, is to maintain ecosystem services, in particular the food produced, and the economic  
416 activities that depend on them (Perrette et al., 2020).

417 The concentration of CLD in perirenal adipose tissue (reference at the slaughterhouse for  
418 comparison with the maximum residue limit MRL) was simulated as a function of the daily  
419 soil ingestion and the concentration of CLD in the soil ( $CLD_{soil}$ ). The CLD concentration in  
420 serum ( $\mu\text{g/mL}$ ) was first estimated by dividing the maintenance dose by the clearance. The  
421 maintenance dose is the amount of CLD daily ingested ( $\mu\text{g CLD}\cdot\text{kg}^{-1}\text{ BW}$ ) obtained by  
422 multiplying daily soil ingestion by  $CLD_{soil}$ . Daily soil ingestions (CR: 164.2, 72.7 and 265.0 g  
423 DM per pig; LW: 246.3, 114.5 and 414.6 g DM per pig; average, 10th and 90th percentiles,  
424 respectively) were calculated for each breed using values expressed per 100 kg BW, obtained  
425 for CR and LW pigs in this study, and the average BW of free-range growing pigs (CR: 40  
426 kg; LW: 52,5 kg; average between post-weaning and slaughtering BW considering linear  
427 relation, with 20 and 60 kg for CR pigs, 25 and 80 kg for LW pigs, respectively). Different  
428  $CLD_{soil}$  from 0.01 to 0.1  $\text{mg}\cdot\text{kg}^{-1}$  were considered in these simulations. The clearance ( $\text{mg}\cdot\text{kg}^{-1}$   
429  $^1$  per day) was obtained from Fourcot et al. (2020) for both breeds (average of the two breeds  
430 and separately). Finally, the CLD concentrations in perirenal adipose tissue ( $\mu\text{g per g of fat}$ )  
431 were obtained by dividing the serum concentration by the tissue partition coefficient ( $K_p$ ).  
432 The  $K_p$  in tissue is obtained by the ratio of concentrations between serum and tissue (Fourcot,  
433 2020). Simulated results show that when  $CLD_{soil}$  is 0.1  $\text{mg}\cdot\text{kg}^{-1}$ , CLD concentrations in  
434 perirenal adipose tissue exceed the MRL set at 0.02 mg CLD per kg of fat regardless of breed  
435 and soil ingestion. When  $CLD_{soil}$  is 0.05  $\text{mg}\cdot\text{kg}^{-1}$ , only the 10% of animals ingesting the least

436 amount of soil complied for both breeds (with a concentration 2 times lower than the MRL).  
437 When  $CLD_{soil}$  is  $0.03 \text{ mg}\cdot\text{kg}^{-1}$ , only the 10% of animals that ingest the most soil do not  
438 comply for both breeds. For  $CLD_{soil}$  of  $0.01$  and  $0.02 \text{ mg}\cdot\text{kg}^{-1}$ , all animals comply (Table  
439 SM5, supplementary material). These simulations on the compliance of animals at slaughter,  
440 in relation to their ingestion of soil and the level of soil contamination, may contribute to  
441 establishing thresholds for regulating soil use in agriculture (Li, 2020). Our results therefore  
442 raise the question of the values to be considered in the risk assessment. Should we consider  
443 the mean or median ingestion levels, the 90 or 95 percentiles or outliers? Since self-  
444 consumption is a widespread practice in the French West Indies, this point is of crucial  
445 importance for self-consumers, who can therefore be markedly overexposed. Free-range  
446 livestock farming is a relatively common practice in the tropics in self-sufficient animal  
447 husbandry and has many advantages in terms of economy, environment, animal welfare and  
448 legislation (European organic regulation), so it is important to be able to secure these farming  
449 systems against health risks. A precise determination of daily soil ingestion is therefore  
450 necessary for the assessment of the health risk.

451 Our study shows that a significant amount of soil can be ingested by pigs on a daily basis. In  
452 order to limit the exposure of pigs to environmental contaminants, especially persistent  
453 organic pollutants, it is necessary to adapt grazing practices (i.e. stocking rate, optimal time  
454 step for changing plots, to limit soil ingestion. Thus, soil quality (texture, structure and  
455 composition), particularly the amount of soil pollutants, and climatic parameters has to be  
456 considered in grazing management. Even if grass is initially available in the paddock, the  
457 foraging behaviour of the pig can quickly remove the grass, leaving bare or even turned over  
458 ground for the remainder of the time spent in the paddock (Edwards, 2003). Thus,  
459 maintaining sufficient grass availability and/or increasing the supply of concentrate feed may  
460 help to limit soil ingestion associated with foraging and rooting on pasture (Jakobsen et al.,

461 2015; Stern and Andresen, 2003). Rivera Ferre et al. (2000) observed a high frequency of  
462 rooting in unrunng sows compared to nose-rung sows, but animal welfare implications limit  
463 this practice as a recommendation to reduce soil ingestion (Horrell et al., 2001).

464 The present study focused on soil ingestion, which is an important route of exposure of  
465 animals to environmental contaminants. However, other matrices can also contribute to  
466 animal exposure such as food and water consumption. The biomass of sweet potato tubers on  
467 the SP treatment plot was assessed which provides information on the amount offered to the  
468 animals (i.e. 38.4 kg DM per paddock of 130 m<sup>2</sup> offered per week, which corresponds to 20.1  
469 g DM/kg BW<sup>-1</sup> per day). From the pictures of the paddocks (Fig SM4, supplementary  
470 material), it can be considered that the pigs consumed almost all the available potatoes, which  
471 allows to roughly estimate the daily intake of potato tubers (i.e. 615 and 756 g DM per pig for  
472 CR and LW breeds respectively) based on the average BW of the animals of both breeds. The  
473 CLD concentration in potato tubers was estimated according to Achard et al. (2007) using a  
474 proportional relationship with the CLD content of the soil and a coefficient adapted for  
475 ferralitic soils such as the study plots. These data make it possible to evaluate the intake of  
476 CLD via the ingestion of sweet potato tubers. Using the average soil ingestions obtained in  
477 this study for the CR and LW pigs in the SP treatment, together with the CLD contamination  
478 of the soil to be simulated, it is possible to estimate the intake of CLD via soil ingestion. For  
479 soil at 0.05 mg CLD.kg<sup>-1</sup> for example, this would correspond to a CLD intake of 9.95 (CR)  
480 and 12.17 (LW) µg, with 5.92 (CR) and 7.21 (LW) µg via soil, and 4.03 (CR) and 4.96 (LW)  
481 µg via sweet potato tubers. The soil contribution to exposure would be 60% with this kind of  
482 diet.

483 Drinking water can also expose animals to pollutants as CLD, especially for unmonitored  
484 spring water or ponds. However, this route of exposure is easier to control if farmers can use  
485 potable water drinking supply as a limit has been set at 0.1 µg CLD.L<sup>-1</sup>. Considering a water

486 consumption of 10% BW (i.e. on average 0.29 and 0.37 L per day for CR and LW pigs  
487 respectively under the conditions of this study) (Chambre d'Agriculture de Bretagne, 2021),  
488 this would represent an intake of CLD up to 0.29 and 0.37  $\mu\text{g}$  for CR and LW pigs  
489 respectively in the case of water reaching the potability threshold of 0.1  $\mu\text{g CLD.L}^{-1}$ .

490

## 491 **5. Conclusions**

492

493 This study provided references on soil ingestion by free-range pigs by comparing two breeds  
494 (Creole and Large White) and three types of outdoor paddocks (pasture with two different  
495 grass availabilities and sweet potato field). The results show that daily soil ingestions are not  
496 influenced by breed or paddock type in this experiment, but this type of management exposes  
497 pigs to substantial soil ingestions about half a kg of dry soil per animal per day. When free-  
498 range farming is carried out in an area where the soil is contaminated, soil ingestion levels  
499 such as those observed in this study can result in contamination of pigs, and their tissues, at  
500 values sometimes exceeding the MRL. In this context, adapting farming practices to limit pig  
501 exposure to soil-bound pollutants, in particular persistent organic pollutants as some  
502 phytosanitary molecules, appears inevitable to ensure the safety of animal products.

503

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505

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514

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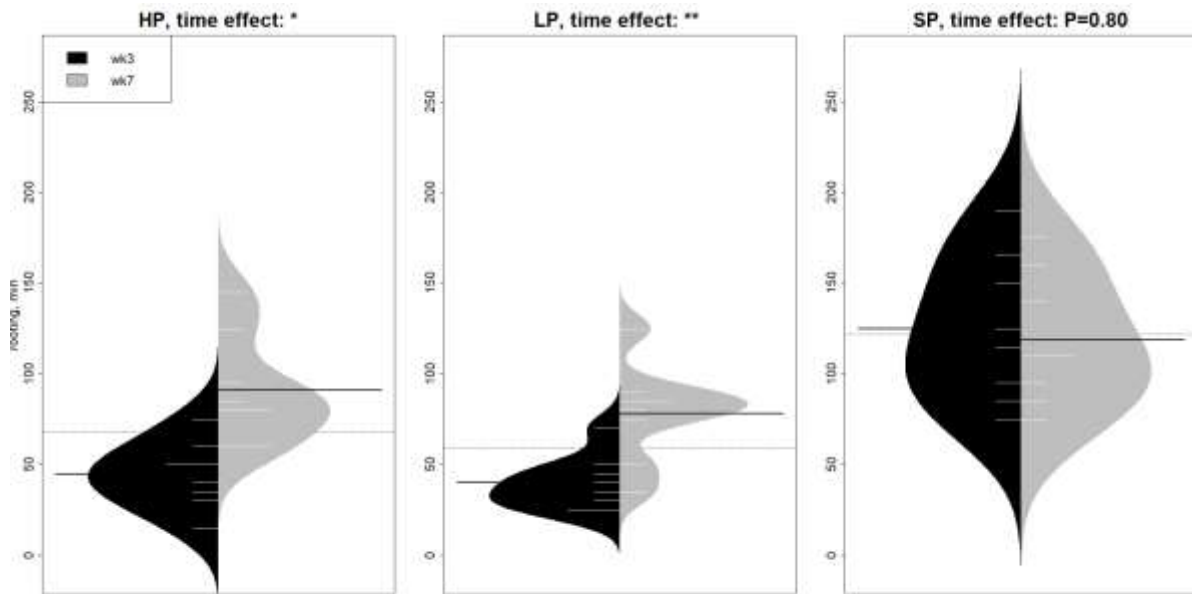
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668

669 **Figures**

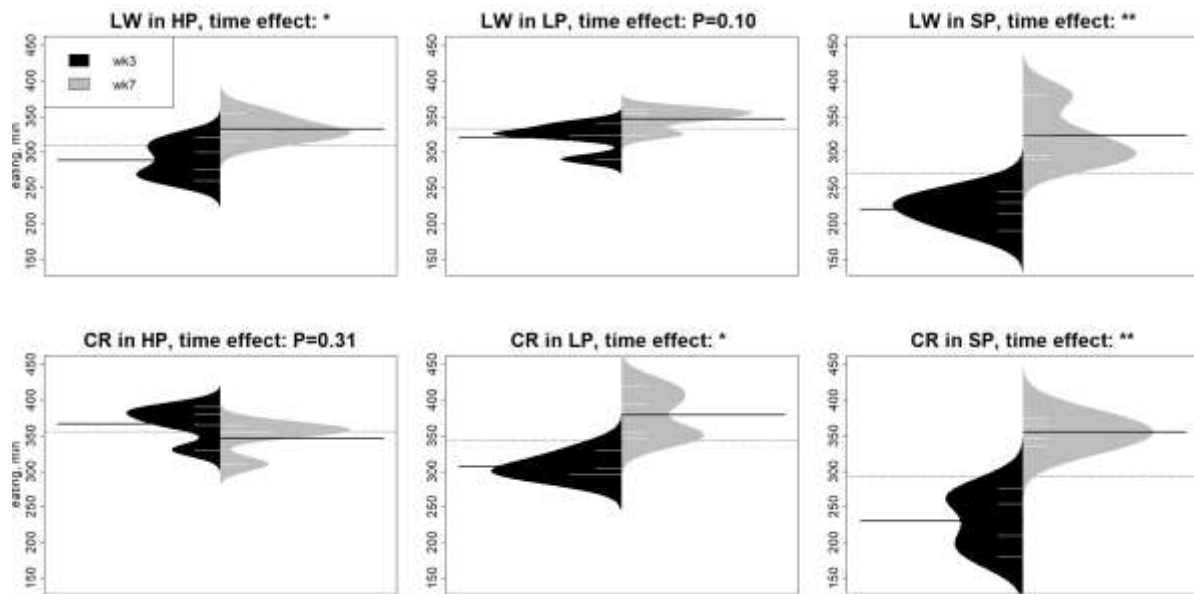
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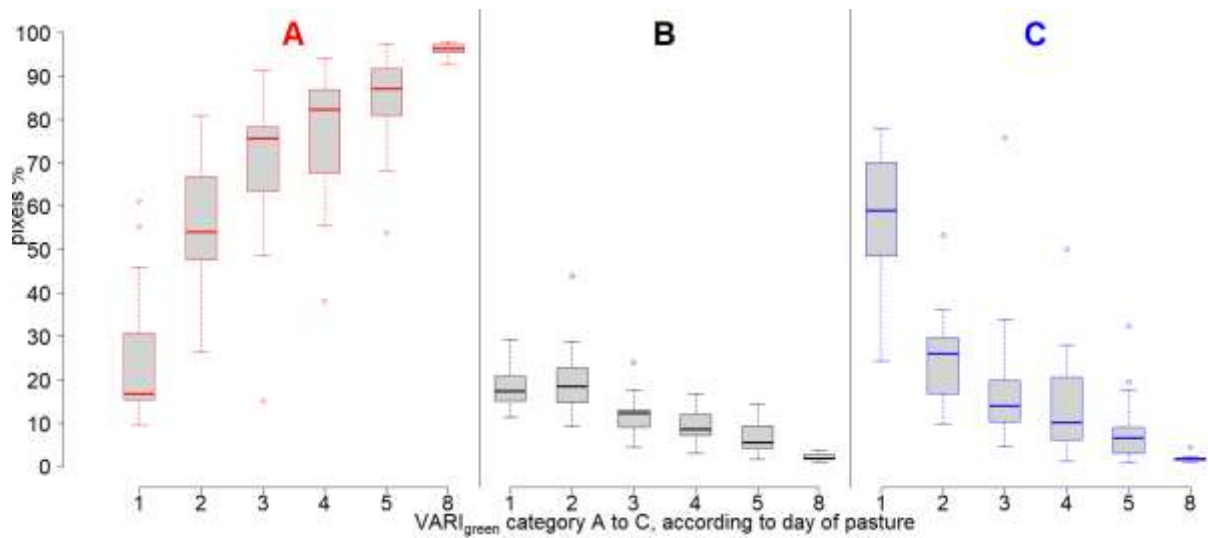
672 **Fig. 1.** Beanplot of rooting activities (time spent within 24-h, in min) of outdoor pigs reared  
673 in HP (pasture with grass more than 60 days of regrowth age), LP (pasture with grass at 35  
674 days of regrowth age) or SP (sweet-potato field) conditions. The horizontal bars correspond  
675 to the means of the distributions (if they were normal): one mean for the black distribution,  
676 one mean for the grey and one mean for the joint distribution. Asterisks indicate significant  
677 difference (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ) between wk3 and wk7 within treatment.

678



679  
 680 **Fig. 2.** Beanplot of eating activities (time spent within 24-h, in min) of outdoor pigs reared in  
 681 HP (pasture with grass more than 60 days of regrowth age), LP (pasture with grass at 35 days  
 682 of regrowth age) or SP (sweet-potato field) conditions. Asteriks indicate significant  
 683 difference (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ) between wk3 (in black) and wk7 (in grey)  
 684 within breed  $\times$  treatment interactions (LW: Large White, CR: Creole).

685

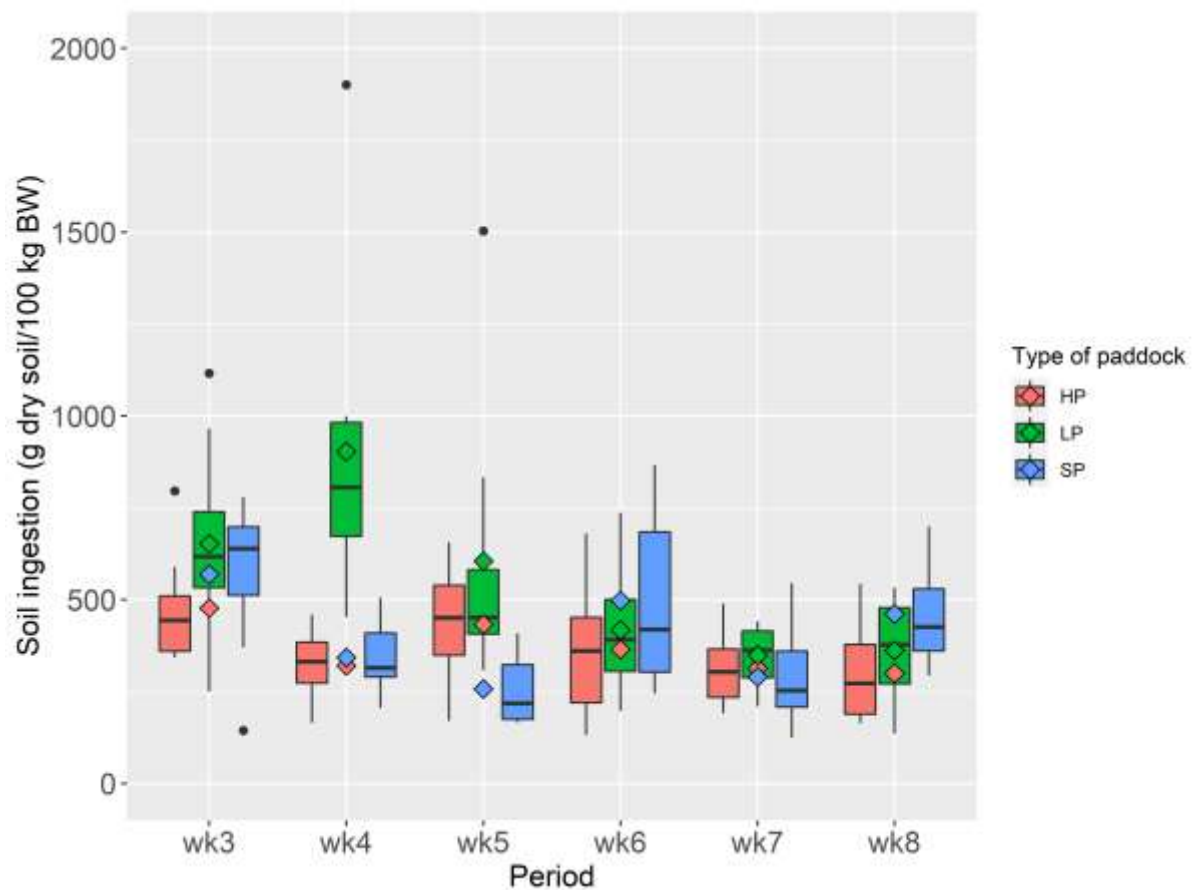


686

687 **Fig. 3.** Boxplot of the percentage of pixels in each VARIgreen category (A: bare soil, mud,  
 688 and/or soil litter; B: stems and senescent plant parts; and C: green matter: leaves and sheaths)  
 689 according to the day of pasture (1 to 5: first to fifth day of pasture; 8: the first day after  
 690 pasture).

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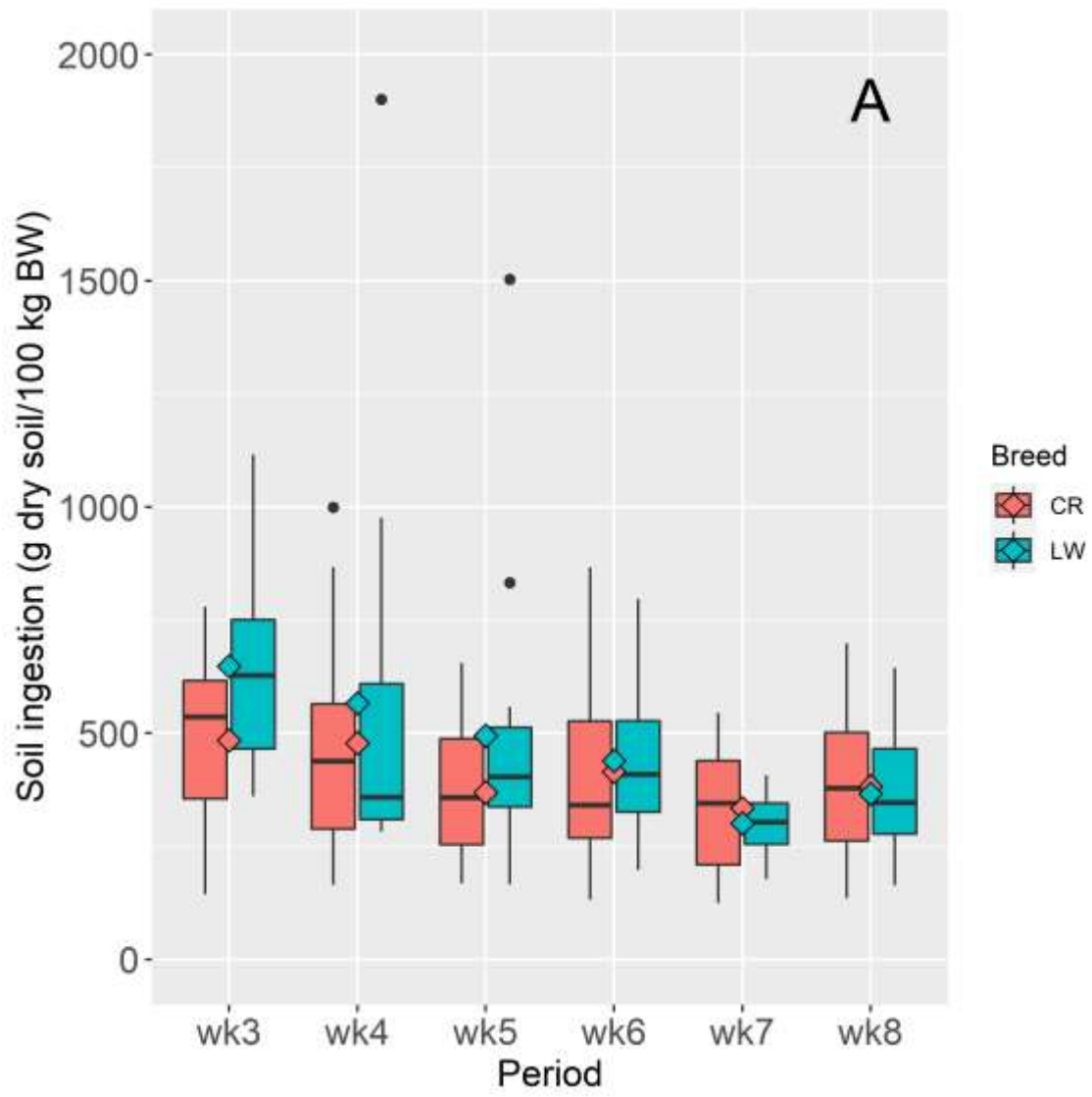




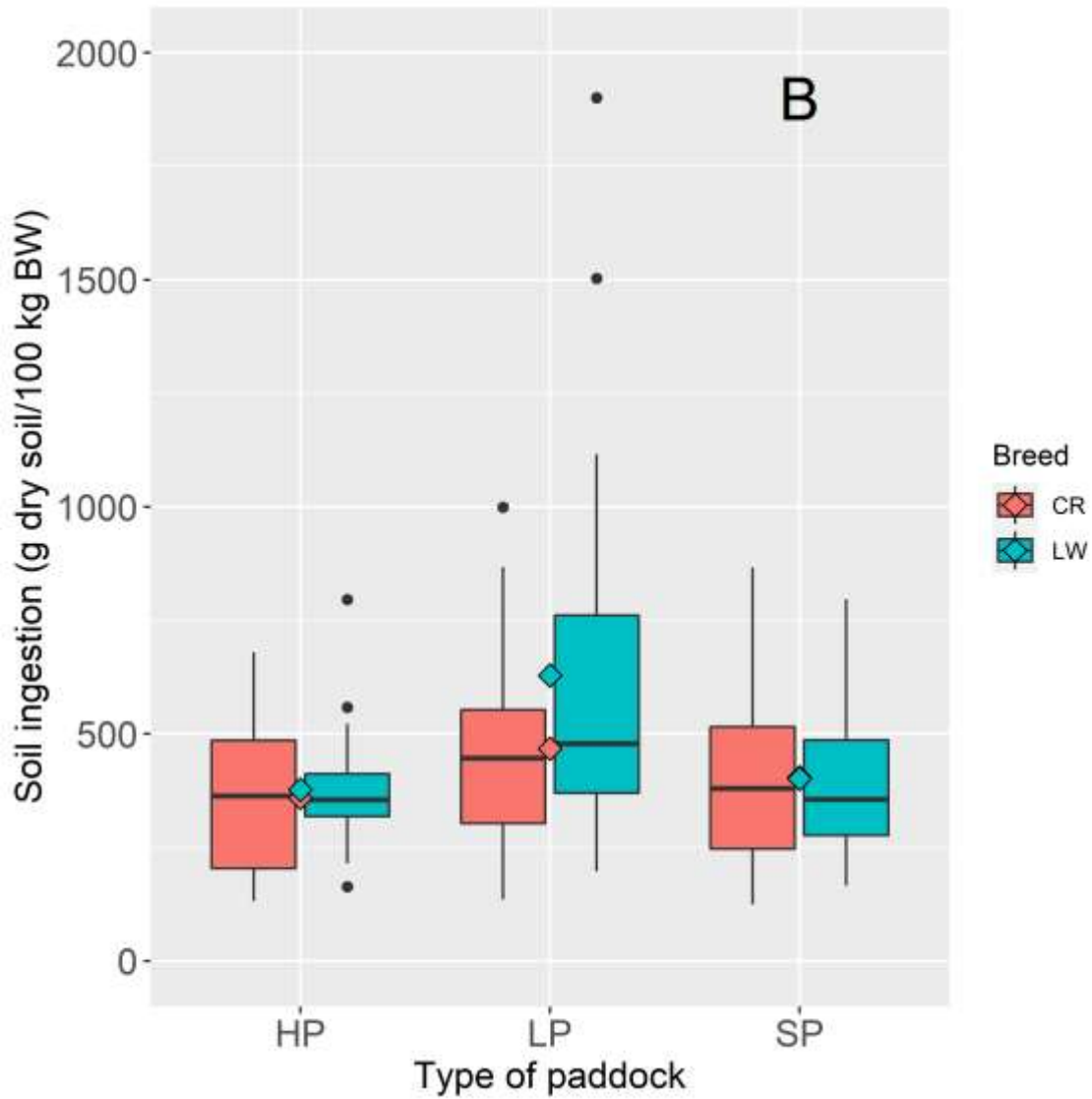
692

693 **Fig 4.** Effect of type of paddock (HP: pasture with grass more than 60 days of regrowth age;  
 694 LP: pasture with grass at 35 days of regrowth age; SP: sweet-potato field) and experimental  
 695 week (wk3 to wk8) on the ingestion of soil (the diamonds represent the averages of each  
 696 treatment for each period).

697



698



699

700 **Fig. 5.** Effect of breed (CR: Creole; LW: Large White) and experimental period (A) (wk3 to  
 701 wk8) or type of paddock (B) (HP: pasture with grass more than 60 days of regrowth age; LP:  
 702 pasture with grass at 35 days of regrowth age; SP: sweet-potato field) on the relative  
 703 ingestion of dry soil, i.e. per 100 kg of BW.

704

705 **Tables**

706

707 **Table 1.** Grazing management, vegetation and soil characteristics according to the paddock  
 708 type (HP: pasture with grass more than 60 days of regrowth age; LP: pasture with grass at 35  
 709 days of regrowth age; SP: sweet-potato field) (average value of the 6 weeks for each type of  
 710 paddock).

Item	Type of paddock			
	HP	LP	SP	
			Tubers	Leaves/stems
<b>Grazing management</b>				
Daily surface area, m <sup>2</sup> per animal per period	16.25	16.25	16.25	
Stocking rate, kg BW per m <sup>2</sup>	2.00	2.05	2.04	
Stocking rate (week 3), kg BW per m <sup>2</sup>	1.64	1.66	1.67	
Stocking rate (week 8), kg BW per m <sup>2</sup>	2.39	2.44	2.42	
Sward height, cm	28.6	15.7	-	
<b>Vegetation characteristics</b>				
Dry matter content, g·kg <sup>-1</sup>	24.8	23.1	28.2	16.2
Organic matter content, g·kg <sup>-1</sup> DM	779	-	881	801
Crude protein content, g·kg <sup>-1</sup> DM	62	-	56	122
Neutral detergent fiber content, g·kg <sup>-1</sup> DM	669	-	60	302
Cr <sup>1</sup> content, µg·g <sup>-1</sup> DM	< 20	< 20	< 20	< 20
Ti <sup>2</sup> content, µg·g <sup>-1</sup> DM	36	59	< 25	69
<b>Ti and Cr levels in soils</b>				
Cr content, µg·g <sup>-1</sup> DM	<20	<20	<20	
Ti content, µg·g <sup>-1</sup> DM	14223	14082	13282	

711  ${}^1\text{Cr}$  = chromium.

712  ${}^2\text{Ti}$  = titanium.

713

714 **Table 2.** Soil ingestion by pigs according to the type of paddock (HP: pasture with grass  
 715 more than 60 days of regrowth age; LP: pasture with grass at 35 days of regrowth age; SP:  
 716 sweet-potato field), period and their interaction.

Item	Type of paddock				<i>P</i> -value		
	HP	LP	SP	RSD <sup>1</sup>	Type of paddock	Period	Type of paddock × Period
Body weight (BW), kg	32.5	33.3	33.2	0.84	NS <sup>2</sup>	<.0001	0.043
Faecal production							
Faecal Cr <sup>3</sup> content, µg·g <sup>-1</sup>	3118	3050	3109	498.3	NS	0.004	0.068
Daily faecal output, g	357	387	343	66.5	NS	0.058	0.015
DM per pig							
Daily faecal output, g							
DM per 100 kg BW							
Faecal Ti <sup>4</sup> content, µg·g <sup>-1</sup>	4585 <sub>b</sub>	6052 <sub>a</sub>	4924 <sub>ab</sub>	800.6	0.036	<.0001	<.0001
Soil ingestion							
Soil ingestion, g DM per pig	116 <sub>b</sub>	171 <sub>a</sub>	131 <sub>ab</sub>	41.1	0.050	0.024	<.0001
Soil ingestion, g DM per 100 kg BW	368	548	403	145.0	0.081	<.0001	<.0001
Soil ingestion, g DM per kg MW <sup>5</sup>	8.7	12.9	9.6	3.26	0.069	NS	<.0001

717 <sup>a-b</sup>Means within a row with different superscripts differ (*P* <0.05).

718 <sup>1</sup>RSD = residual standard deviation.

719 <sup>2</sup>NS = non-significant ( $P > 0.1$ ).

720 <sup>3</sup>Cr = chromium.

721 <sup>4</sup>Ti = titanium.

722 <sup>5</sup>MW = metabolic weight.

723

724 **Table 3.** Soil ingestion by pigs according to the breed (CR: Creole; LW: Large White) and its  
 725 interaction with the period (same model and continuation of the results presented in Table 2).

Item	Breed			P-value	
	CR	LW	RSD <sup>1</sup>	Breed	Breed × Period
Body weight (BW), kg	29.3	36.7	0.84	<.001	<.0001
Faecal production					
Faecal Cr <sup>2</sup> content, µg·g <sup>-1</sup>	3676	2509	498.3	<.0001	0.009
Daily faecal output, g DM per pig	284	440	66.5	<.0001	0.019
Daily faecal output, g DM per 100 kg					
BW					
Faecal Ti <sup>3</sup> content, µg·g <sup>-1</sup>	5442	4932	800.6	NS <sup>4</sup>	NS
Soil ingestion					
Soil ingestion, g DM per pig	116	163	41.1	0.015	0.089
Soil ingestion, g DM per 100 kg BW	411	469	145.0	NS	NS
Soil ingestion, g DM per kg MW <sup>5</sup>	9.4	11.4	3.26	NS	0.077

726 <sup>1</sup>RSD = residual standard deviation.

727 <sup>2</sup>Cr = chromium.

728 <sup>3</sup>Ti = titanium.

729 <sup>4</sup>NS = non-significant ( $P>0.1$ ).

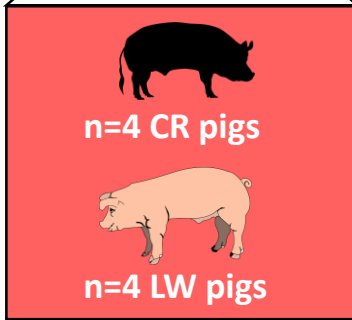
730 <sup>5</sup>MW = metabolic weight.

731

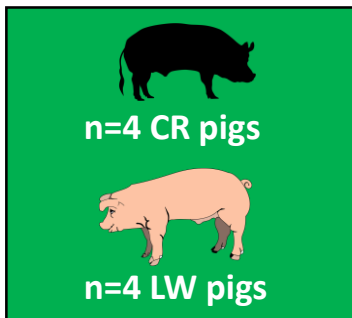


wk3 → wk4 → wk5 → wk6 → wk7 → wk8

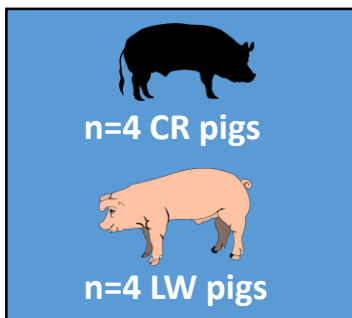
**6 experimental weeks wk3 to wk8**  
(preceded by 2 adaptation weeks wk1 and wk2)  
Pigs moved to a new paddock each week



**High Pasture HP**

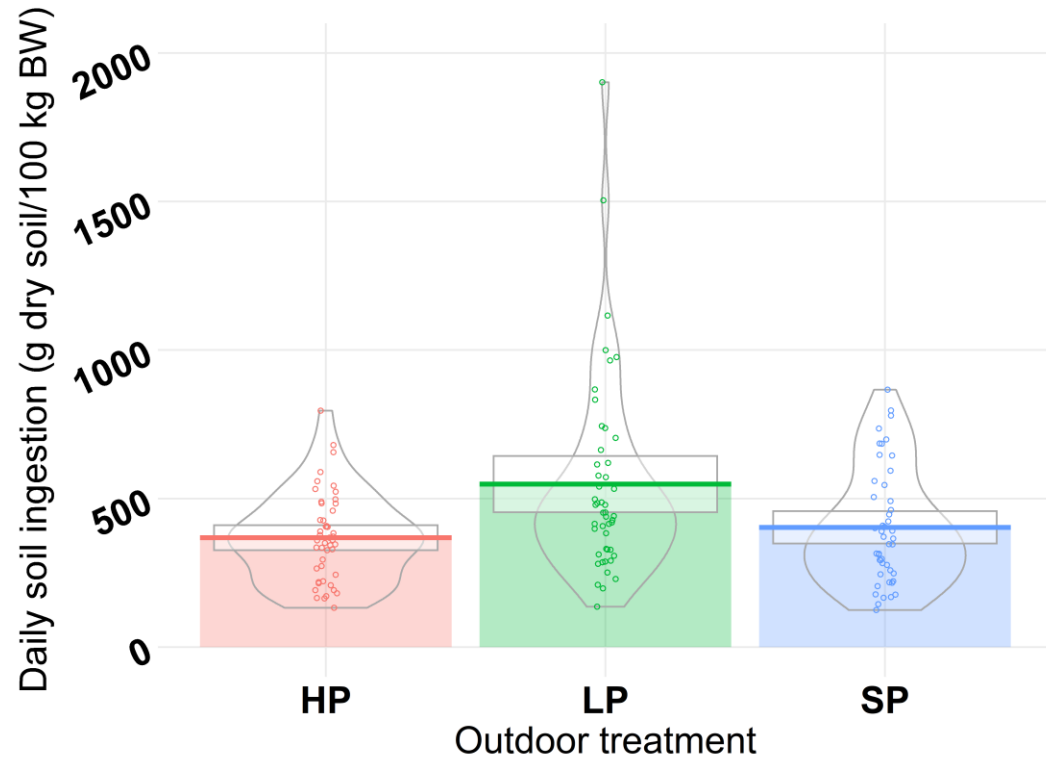


**Low Pasture LP**



**Sweet Potato SP**

**3 outdoor treatments (HP, LP, SP), 24 free-range pigs, 2 breeds**  
(CR: Creole of Guadeloupe, LW: Large White)



**Individual daily soil ingestion of pigs**

No difference between the 3 treatments and the 2 breeds

Reference data for predictive models of  
**pig exposure to soil-bound pollutants**