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## Accepted Manuscript

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Cattle exposure to chlordecone through soil intake. The case-study of tropical grazing practices in the French West Indies.

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Non-standard abbreviations sorted in alphabetical order:

ADLIB: *ad libitum* daily herbage allowance; CLD: chlordecone; DHA: daily herbage allowance; DM: dry matter; DMD: dry matter digestibility; DRY: dried soil surface; DSA: daily surface area allowance; DUG: during grazing; HDL: high-density lipoprotein; HIGH: high daily herbage allowance; HUM: water-saturated soil surface; LOW: low daily herbage allowance; MRL: maximum residue limit; OM: organic matter; POG: post-grazing; PRG:

pre-grazing; SADUG: soil amount on during-grazing herbage; SAPOG: soil amount on post-grazing herbage; SAPRG: soil amount on pre-grazing herbage; SAUH: soil amount on unwashed herbage; SIA: soil intake amount; SIR: soil intake rate as a percentage of dry matter intake; SSH: sward surface height; SSM: soil surface moisture; TiDUG: titanium content in unwashed herbage sampled during grazing; TiH: titanium content in washed herbage; TiPOG: titanium content in unwashed herbage sampled post-grazing; TiPRG: titanium content in unwashed herbage sampled pre-grazing; TiS: titanium content in soil

## ABSTRACT

Ingested soil is a major vector of organic contaminants from environment to free-ranged animals, particularly for grazing herbivores. Therefore, a better understanding of processes driving soil intake may provide new insights to limit animal exposure to contaminants and ensure safety of animal products. To maintain the supply service of livestock farming activities in contaminated areas, it is necessary to design adapted farming practices aiming at controlling the risk for human health. This study was conducted in the French West Indies, where chlordecone, an organochlorine insecticide previously used to protect banana plantation against the black weevil and banned since 1993, has polluted nearly twenty percent of agricultural surfaces since the 1970s. A crossover study design was performed to estimate soil intake by twelve tethered Creole young bulls according to different grazing practices. The objectives were to characterize the influence of (i) daily herbage allowance (LOW, HIGH, ADLIB: 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively); (ii) and soil surface moisture (SSM) testing grazing on a water-saturated (HUM) vs dried (DRY) ground. The herbage offer was managed via the allocated surfaces varying the chain length as animal holders commonly do in informal Caribbean systems. The results evidenced an increase in soil intake with DHA reduction (2.1 to 3.8% of DM intake;  $P < 0.05$ ) and with SSM increase (2.4 to 3.6% of DM intake;  $P < 0.05$ ). Herbage offer reduction involved a closer-to-the-ground grazing with shorter post-grazing sward surface height (82.2 to 63.3 mm;  $P < 0.001$ ), and both herbage offer reduction and SSM increase amplified sward soiling (measured from titanium content in unwashed herbage and image analysis). This work showed that soil intake is unavoidable even when herbage offer is very generous. The animals will significantly increase soil intake when herbage offer would be at 150 g DM/kg BW<sup>0.75</sup> or less, especially when the grazed surface is humid.

*Keywords:* soil ingestion; pasture allowance; soil surface moisture; beef cattle; contaminant; exposure risk

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## 1. Introduction

Soil is one of the main reservoirs of environmental contaminants worldwide and several studies pointed out soil as being one of the main matrices for contaminant transfer to outside-reared animals (Duarte-Davidson and Jones, 1996; Fries, 1996). Sorption properties of many persistent organic pollutants enable soil to retain and accumulate them over long periods. Therefore, the exposure of free-range animals to contaminants is linked to the amount of ingested soil which needs to be precisely estimated for risk evaluation purposes. Soil intake by free-ranged cattle has been described in free or strip grazing systems in different temperate zones, mostly evaluating the impact of season (Healy, 1968; Thornton and Abrahams, 1983; Abrahams and Thornton, 1994) or management conditions of animals as supplementation or herbage offer (Fries et al., 1982; Jurjanz et al., 2012). An extrapolation from data obtained in grazing systems in temperate zones would not allow an accurate quantification of soil intake by animals in humid tropical zones, due to large differences in the nature of the soil and the structure of the herbaceous stratum.

In the French West Indies, chlordane (CLD), an organochlorine insecticide, was used to control the banana weevil *Cosmopolites sordidus* from 1972 until its ban in 1993. This persistent organic pollutant is widely spread in soils from Guadeloupe and Martinique, with CLD contamination of 1/5 and 2/5 of the agricultural area respectively (Cabidoche and Lesueur Jannoyer, 2011). Cabidoche et al. (2009) reported differences in CLD contamination of soils according to soil type, with concentrations in the 0-30 cm layer reaching 1, 4 and 37 mg CLD/kg for nitisols, ferralsols and andosols respectively. CLD is mainly persistent in the first 0-10 cm layer with concentrations in untilled andosol reaching 50 mg CLD/kg. The chemical and physical properties of CLD explain its remanence in soils. This molecule is very adsorbable, due to its high partitioning coefficient ( $K_{oc}$ ) between the sorbed part on soil organic matter (OM) (estimated by the soil organic carbon ranging from 35 to 85 g/kg for

andosols) and the dissolved part in water, and very few volatile in aqueous solution (vapor tension =  $3.10^{-2}$  Pa at 25°C; INCHEM, 1984). Jurjanz et al. (2014) studied the relative bioavailability of soil-bound CLD in ruminants and did not reported any CLD sequestration by soil binding, meaning that CLD ingested by animals from contaminated soil is bioavailable during digestive process. This emphasizes the need to integrate exposure to contaminated soil in risk assessments.

Food products of animal origin are major contributors for population exposure to chlordecone, particularly for informal supply routes, as self-production, gifts and roadside sellings (Kannari study; ANSES, 2017). Fishing products and eggs were identified as the main contributors which conducted to spatialized fishing bans and, for poultry, to several studies on egg transfer (Jondreville et al., 2013, 2014) and estimations of plant and soil intake (Jurjanz et al., 2014, 2015). Knowledge on factors affecting exposure to persistent organic pollutants on free-ranged cattle is more restricted, especially in tropical zone, whereas there are regularly carcass of beef cattle which exceed the maximal residue limit (MRL). This situation will be intensify with the decrease of MRL in perirenal fat from 100 to 27 µg/kg in 2019 (Triplet study; ANSES, 2018) with serious socio-economic consequences for farmers.

Significant proportions of Caribbean livestock farmers raise attached animals (*i.e.* tethered animals with a chain from their neck to a post and allowed to graze in a limited circular area) and manage a limited number of animals in non-standard grazing areas (e.g. roadside, sugar cane, banana or orchard fields). A previous study (Jurjanz et al., 2017) showed in post-tethered Creole young bulls a steep increase of soil intake from 4.4 to 9.3 % of ingested DM when herbage offer decreased from 128 to 71 g DM/kg BW<sup>0.75</sup> at a very short sward height after grazing (<40 mm). When post-tethering grazing was carried out with a relatively short chain (*i.e.* limited area offered to animals) and a low herbage offer (linked to sward height and biomass), Jurjanz et al. (2014) observed that animals grazed closer to the ground which



could favor soil intake. This previous results suggest that an increased herbage offer may result in a significant reduction of soil intakes. It would be very valuable to estimate soil intake by cattle receiving more generous herbage allowances to know the minimal soil intake which cannot be avoided, even when herbage offer is not a limiting factor, and therefore should be integrated as baseline in risk assessments. It would allow to determine to what extent free-ranged cattle may be exposed to chlordecone independently to herbage offer, and to search for other variation factors to adjust grazing practices to limit animal exposure.

In Caribbean conditions, heavy rainfalls result regularly in very humid soil surfaces what may be a worsening factor through a reinforced trampling effect which may favor sward soiling and indirect intake of soil particles adhered on herbage (Smith and Jones, 2000).

To limit animal exposure to chlordecone through soil intake, the aim of this study was to investigate in cattle at what degree soil intake can be reduced by increasing herbage offer, either on dry or water-saturated soil surface.

## **2. Materials and methods**

### *2.1. Animals, treatments and experimental design*

The experiment was performed at the Tropical Platform for Experimentation on Animal of the French National Institute for Agricultural Research (INRA-PTEA, Petit-Bourg, Guadeloupe, France, 16°12'05''N, 61°39'53''W; altitude: 111 m above sea level) from 04 February to 28 April 2017. It was conducted in farm-like conditions in accordance to the European Union and French laws on animal care and welfare (APAFIS#5527-2016050608133139). Twelve young bulls of the breed Creole of Guadeloupe (*Bos taurus*) were allocated to two groups of six animals, each group being tested in a crossover design

during six successive periods (Fig. 1). A crossover study design means that all animals of the experiment undergo the same treatments at different periods. Each animal is its own control since the same animal is exposed to different treatments during several successive periods. A crossover design contains as many periods as treatments. Animals were constrained by a chain from their neck to a post and allowed to graze in a limited circular area (Fig. 2). This post-tethered grazing method was chosen because it allows precise individual measurements without the need for handling that would disturb the animals and above all because it allows precise control of the quantities of grass offered to each individual. Moreover, this traditional post-tethered grazing system reflects well the local practice of small holders. The twelve young bulls were born between 30 April and 4 July 2016 and were weaned at seven months old. Three to eight weeks after weaning, the animals were accustomed to post-tethered grazing during two weeks on a plot neighboring experimental ones and covered by the same herbage type. The twelve animals were allocated to two groups regarding their age and their speed of adaptation to post-tethered grazing. At the start of the experiment, animals were on average 267 days old and weighed 158 kg BW (estimated with barymetric ribbon). Within each group, the six young bulls alternatively received a low (LOW), high (HIGH), or *ad libitum* (ADLIB) daily herbage allowance (DHA), corresponding to 100, 150 and 300 g DM/kg BW<sup>0.75</sup> respectively, with two animals receiving the same DHA at each period. Each animal-DHA combination was run during two periods to test two conditions of soil surface moisture (SSM): a period with sprinkler irrigation to obtained water-saturated soil surface (HUM: soil water potential < 10 kPa), and a period without irrigation to maintain the dried soil surface (DRY: soil water potential > 30 kPa) (Fig. 1; Fig. 2). The experiment was carried out during the 'Carême' season, which has relatively few rainfalls, to ensure the dried soil surface conditions. Mean daily rainfall was 1.8±0.5 mm for DRY treatment periods, mean daily Penmann evapotranspiration was 4.0±0.1 mm, and minimum and maximum

temperatures along the experiment were on average  $20.4\pm 0.1$  and  $27.8\pm 0.1^{\circ}\text{C}$  respectively (mean  $\pm$  SE) without any difference between both groups. Each period was composed from three to six days of adaptation, and eight days of measurements (days 1 to 8; Fig. 3). The duration of adaptation was extended until six days when animals received a different DHA than the one of the previous period, or if SSM conditions were not reached yet. The experiment lasted 77 days for the first group (04 February to 21 April), with six 11 to 12-day periods (04-14 February, 14-24 February, 27 February-10 March, 13-24 March, 01-08 April, 10-21 April), and 67 days for the second group (21 February to 28 April), with six 8 to 12-day periods (21 February-03 March, 06-17 March, 20-31 March, 01-08 April, 08-15 April, 17-28 April). Three periods lasted 8 days (*i.e.* 3 days of adaptation and 5 days of measurements) due to meteorological constraints. At each period, herbage sampling, required for nutritional quality and sward soiling analyses, began two days before fecal collection (realized for intake estimation) to take into account transit time in digestive tract and obtain herbage samples representative of ingested herbage.

## *2.2. Plot preparation, grazing management and vegetation characteristics*

Three neighboring grass fields, mainly composed of Para grass (*Brachiaria mutica*), were grazed during the experiment, being a total of 2.65 ha with two plots of 1 ha each and one of 0.65 ha. In order to ensure identical vegetation stage for measurements, fields were mown beforehand, and animals could always graze on a fresh plot. Each plot was grazed between two and four times with two to four weeks of pasture growth between two passages. During HUM conditions, night irrigation was set up, to limit evaporation losses, from day 3 to day 7 for each period (Fig. 3). The flow was controlled and evaluated to 5 mm/hour resulting to an experimental irrigation of 20 mm per night before grazing. When rainfall attained this value,

irrigation was stopped. Every morning at 0730 h, each animal was offered fresh herbage by moving its post on a new area to control DHA and limit daily intake fluctuations. Grazing circles were spaced of 2 m minimum to avoid any overlap between individual grazing and defecating areas. The three contrasted DHA levels (*i.e.* 100, 150 and 300 g DM/kg BW<sup>0.75</sup> corresponding to 2.8, 4.1 and 8.4 kg DM/100 kg BW for LOW, HIGH and ADLIB respectively; Table 1) were obtained as follows: each day the available biomass per unit area was estimated by measuring the sward surface height (SSH, 15 measurements with a rising plate herbometer for each individual plot to be grazed) and applying a biomass-SSH regression established before the experimental period following the method previously described by Jurjanz et al. (2017). The grazing area corresponding to each animal was then calculated according to its BW and DHA, and the chain length adjusted. Post-grazing SSH was also measured every day from 15 random points per circle after removing the animals. Herbage was sampled during the days 2 to 5 of each period, near each grazing circle to not interfere with the DHA, to obtain four daily herbage samples using the methodology described by Jurjanz et al. (2017). These four daily samples were pooled to analyze the contents of ash (incineration at 550°C during 6 h), to calculate then OM, as well as titanium (Ti) (see '2.7. Soil intake' section for analytical details). Pre-grazing SSH and chemical composition of herbage were similar along the experiment whatever the treatment (Table 1).

### 2.3. Moisture of soil surface

During the pre-experimental period, sensors (Watermark<sup>®</sup>, Irrrometer Company, Riverside, USA) were installed on experimental plots to record soil water potential which informs on the water availability in the upper layer of soil. Lower or equal to 10 kPa, soil was considered water-saturated (objective for HUM conditions), whereas higher or equal to 30 kPa, soil

surface was considered dried (objective for DRY conditions). Soil water potential was read every morning before to move the animals on fresh herbage (Fig. 3).

The SSM scoring, adapted from the Food and Agriculture Organization soil adhesion scoring protocol, was performed on three elementary samples per grazing circle on the morning of days 2 to 7 of each period, just before the animals were moved in. Scores range from 0 to 3 and it was considered that DRY conditions corresponded to scores between 0 and 1 (friable to faintly sticky soil), whereas in HUM conditions, scores were between 2 and 3 (sticky to pasty soil). The retained score per circle was the average of three scores, each one evaluated by a different observer. Soil water potential, recorded by sensors, and SSM scoring were two complementary parameters to control SSM factor. The two SSM levels were consistent with the objectives and significantly contrasted with soil water potential power of 8.9 and 33.8 kPa and SSM scores of 2.24 and 0.79 for HUM and DRY conditions respectively (Table 1).

#### *2.4. Body weight and dry matter intake*

Every month BW was individually estimated with a barymetric ribbon to adapt chain length to DHA (expressed per kg of metabolic BW) taking into account individual BW variations along the experiment. These BW measurements were linearly modellized to retain one monthly value used to estimate relative DM and soil intake.

Daily DM intake was estimated for each animal for the last five days of each period (day 7 to day 11; Fig. 3) following the methodology previously described by Jurjanz et al. (2017) which first calculate daily OM intake from fecal output over 24 h and OM digestibility estimated from fecal CP content (expressed in percentage of OM) according to Boval et al. (1996). Chemical analyses were realized to determine OM, ash (incineration at 550°C during

6 h), crude protein (Dumas method, AOAC Official Method 990.03, AOAC International 2005), and Ti (see '2.5. Soil intake' section for analytical details) contents.

### 2.5. Soil intake

Thirty soil samples were carried out with an auger (top 5 cm depth) in the three experimental plots. Each sample was constituted from a pool of ten subsamples carried out in the same plot. In order to avoid herbage damaging, soil sampling was carried out at the end of the experiment. Gravel (*i.e.* stones and coarse sand > 2 mm) and pieces of vegetation were removed by wet sieving. Pooled samples were then dried at 60°C until constant weight before analyzing their DM and Ti contents.

The extraction of Ti from soil, herbage and feces was performed on an aliquot of each sample via mineralisation with HNO<sub>3</sub>/HCl (soil and feces) and HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (herbage) in closed tubes placed in a digestion system under a gradual heating mode, exposed to microwave energy (feces only). Concentrations were determined in triplicate, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, X Series II Model, Thermo Fischer Scientific, Courtaboeuf, France). Validity of analytical method was checked by means of standard reference materials. Limit of quantitation was 0.35 µg Ti/g DM.

Soil intake rate was calculated for each animal as a percentage of its DM intake according to Beyer et al. (1994). The method was described in a previously experiment (Jurjanz et al. 2017). Soil intake amount was then calculated for each animal using its DM intake and soil intake rate.

### 2.6. Sward soiling

Contribution of grazing to sward soiling was evaluated by two complementary methods: Ti content in unwashed herbage sampled at different times, and picture analyses.

For Ti analyses, unwashed herbage was cut with a grass edging shear (2 cm above the ground level) at three times: before grazing (on the morning when animals are moved on their new surfaces), during grazing (on the afternoon after 8 h of grazing) and after grazing (on the morning after animals left surfaces they were offered the day before). Sampling was performed during the days 2 to 5 of each period by cutting six 1 m-strips, each one out of one different grazing circle, to form the daily pre-grazing sample. The four daily samples were dried 48 h at 60°C then pooled to form one sample per period representing pre-grazing herbage offered to the six animals of the group. For during and post-grazing herbage, one sample per animal per day was done inside each grazing circle, along a radius from peripheral to center (where the chain was attached). The four daily samples collected at each time were dried and pooled to obtain one 'during-grazing' sample and one 'post-grazing' sample per animal per period. Ti contents of pooled pre-, during- and post-grazing herbage samples were measured using the previously described methodology. These analyses allowed to estimate the soil amount adhered on unwashed herbage (SAUH) at the three different times by the following equations:

$$(4a) \quad \text{SAPRG} = (\text{TiPRG} - \text{TiH}) \times 10^3 / \text{TiS}$$

$$(4b) \quad \text{SADUG} = (\text{TiDUG} - \text{TiH}) \times 10^3 / \text{TiS}$$

$$(4c) \quad \text{SAPOG} = (\text{TiPOG} - \text{TiH}) \times 10^3 / \text{TiS}$$

where SAPRG, SADUG and SAPOG are the soil amounts on herbage sampled pre-, during-, and post-grazing respectively (mg soil/g DM); TiPRG, TiDUG and TiPOG are Ti contents in unwashed herbage sampled pre-, during-, and post-grazing respectively ( $\mu\text{g Ti/g DM}$ ); and TiH and TiS are Ti contents in washed herbage and soil respectively ( $\mu\text{g Ti/g DM}$ ).

Photographs of the grazed circles were taken each morning after the animals had left (corresponding to post-grazing sampling), as well as on areas just before grazing (pre-grazing samples). The  $VARI_{green}$  index was calculated for each pixel from the zones of interest as the ratio  $(G-R)/(G+R-B)$  (Ballesteros et al., 2014), where R, G and B are the values of the red, green and blue channels. The pixels were then classified as soil, mud or dead material (CI-A,  $VARI_{green} < -0.00673$ ), green material (CI-C,  $VARI_{green} > 0.0616$ ) and senescent leaves or stems (CI-B) for  $VARI_{green}$  ranging from -0.00673 to 0.0616. Then, the percentage of pixels corresponding to each of the three classes was calculated to compare the images before and after grazing in order to evaluate the effects of the studied treatments.

### *2.7. Statistical analyses*

Statistical analyses were performed using R software (version 3.3.2) (R Development Core Team, 2016). Grazing management (including SSH), moisture of soil surface and animal (BW, OM, DM and soil intake) variables were tested using linear mixed models with DHA and SMM as fixed effects, and individual nested within groups (since each animal belongs either to the group 1 or to the group 2 along the experiment) as a random effect ('nlme' package). Individual animals were used as the experimental unit. DHA x SMM interaction was never significant so it was removed from final models. The 'glht' function of the 'multcomp' package (Tukey's test) ( $P < 0.05$ ) was used for multiple comparisons between DHA levels. Sward soiling variable (SAUH) was tested using the same model adding sampling time to DHA and SSM as fixed effect. The DHA x SSM and triple interactions were never significant so they were removed from final model. For picture analyses, the pixel percentages of each image were  $\arcsin(\sqrt{\cdot})$  transformed to normalize the residuals. The



treatment effects were tested using an ANOVA followed by multiple comparison of means (Tukey's test). Means and standard errors were displayed as back-transformed values.

### 3. Results

#### 3.1. Body weight, dry matter intake and sward surface height

Animal body weight was on average 171 kg along the experiment (Table 2) and increased during the experiment from  $158 \pm 6$  kg to  $184 \pm 7$  kg (mean  $\pm$  SE).

The animals ingested more at the highest herbage allowance (ADLIB) than at both more restrictive ones (HIGH and LOW) (2.53 vs 2.10 kg DM/100 kg BW;  $P < 0.001$ , Table 2).

DHA reduction resulted in an increase of the DM ingested herbage/DM offered ratio with significant differences between each DHA level (from 0.30 for ADLIB to 0.69 for LOW;  $P < 0.001$ ).

Whereas sward heights were similar before grazing whatever the DHA treatment (108.5 mm;  $P > 0.05$ ), it differed after grazing with the lower values reported for both more restrictive herbage allowances (66.6 mm for LOW and HIGH vs 82.2 mm for ADLIB;  $P < 0.001$ ; Table 2).

#### 3.2. Soil intake

Fecal Ti content was significantly higher for LOW than for ADLIB DHA ( $P < 0.05$ ), and for HUM than for DRY SSM conditions ( $P < 0.05$ ; Table 3). When animals were grazing on the LOW DHA, they ingested 3.75% of soil in their total daily DM intake. This level is nearly two times higher than the soil intake estimated for animals receiving an *ad libitum* herbage

allowance (2.12%;  $P < 0.05$ ; Table 3). Soil intake rate estimated for HIGH DHA was intermediate without a significant difference to both other offers ( $P > 0.05$ ). Regarding soil moisture effect, soil intake rate was 1.5 times higher during HUM than during DRY conditions (3.58 vs 2.43%;  $P < 0.05$ ). The higher soil intake rate was obtained with the most restrictive herbage offer on a water-saturated soil surface (LOW-HUM: 4.66%), whereas animals receiving an *ad libitum* herbage offer on a dried soil surface realized the lower soil intake rate (ADLIB-DRY: 2.08%). Soil intake rates corresponding to the four other herbage offer-soil surface moisture combinations were to an intermediary level (HIGH-HUM: 3.92%; ADLIB-HUM: 2.16%; LOW-DRY: 2.85%; HIGH-DRY: 2.36%).

When the ingested amount of soil was considered, the estimations ranged from 52.4 (ADLIB) to 71.8 (LOW) g DM/100 kg BW according to DHA level, and from 55.9 (DRY) to 71.0 (HUM) g DM/100 kg BW according to SSM condition, but differences were not significant whatever the factor considered ( $P > 0.05$ ; Table 3).

### 3.3. Sward soiling

Sward soiling, estimated by the soil amount on unwashed herbage sampled before, during and after grazing, increased during the 24 h in each grazing circle with soil amounts significantly higher on post- than pre-grazing herbage (38.0 vs 1.8 mg/g DM whatever DHA or SSM effects;  $P < 0.001$ ). This impact of grazing on sward soiling essentially occurred during the eight first hours of grazing in each area since no statistical difference was observed for during- vs post-grazing sampling date (Fig. 4).

Sward soiling was particularly pronounced as herbage allowance was reduced (LOW: 34.3 vs HIGH and ADLIB: 18.0 mg/g DM;  $P < 0.001$ ), and soil surface was water-saturated (HUM: 34.0 vs DRY: 12.9 mg/g DM;  $P < 0.001$ ). The sampling date x SSM interaction was significant

( $P < 0.001$ ) and resulted in an increasing sward soiling during grazing in HUM conditions, whereas differences between sampling dates were less contrasted in DRY conditions (Fig. 4B). The higher soiling levels, reported for post-grazing-LOW or post-grazing-HUM combinations, correspond to approximately 10% of herbage DM (Fig. 4).

Results of image analysis are consistent with soil contents in after grazing herbage. The green material removal (after vs before grazing) was most pronounced and the percentage of Cl-A pixels increased as the forage offer decreased (Fig. 5). The Cl-A pixel percentage increase was also higher for HUM than for DRY (Fig. 5).

#### 4. Discussion

##### *4.1. Daily herbage allowance and soil surface moisture effects on dry matter and soil intake*

The three contrasted levels of DHA involved a gradient of DM intake and significantly different DM of ingested herbage/DM offered ratios (0.69, 0.52 and 0.30 for LOW, HIGH and ADLIB respectively; Table 2). This result confirms the restriction constraint for animals grazing the LOW DHA which might ingest parts of plants less digestible than animals grazing HIGH and ADLIB DHA.

Both DHA and SSM influenced soil intake rate, which increased with reduction of DHA and increase of SSM. Previous studies focusing on soil intake by cattle already evidenced a DHA effect as in Jurjanz et al. (2012, 2017) in French temperate or tropical pastures respectively. According to pasture allowance, Jurjanz et al. (2012) reported average soil intake rate from 1 to 6 % DM intake (*i.e.* 0.17 to 0.83 kg DM/day) in Holstein dairy cows daily receiving around 290 and 170 g DM/kg BW<sup>0.75</sup>. Soil intake rate of 4.4 and 9.3 % DM intake (*i.e.* 0.13

and 0.16 kg DM/day) were reported by Jurjanz et al. (2017) in Creole of Guadeloupe young bulls daily receiving around 130 and 70 g DM/kg BW<sup>0.75</sup>/day. Most of the soil intake studies by ruminants were conducted in temperate countries mainly located in North America (Mayland et al., 1977, cattle; Kirby and Stuth, 1980, steers), Western Europe (Green et al., 1995, cattle; Abrahams and Blackwell, 2013, sheep) and New-Zealand (Healy, 1968, dairy cows; Roberts and Longhurst, 2002, sheep). Soil intake by domestic herbivores in tropical conditions is poorly documented whereas several factors such as tropical tether-grazing practices, humid meteorological conditions or type of soils or vegetation, could differently impact this parameter when compared to temperate grazing conditions.

Several authors analyzed the seasonal pattern of soil ingestion by diverse herbivore species in different climate zones. In mule deer in Colorado, Arthur and Alldredge (1979) reported greatest soil intake during the spring (29.6 g/day) and winter (18.3 g/day) compared to summer and autumn (less to 10 g/day). In the same way, sheep grazing in an arid area realized highest soil intake during winter and lowest during summer with a range from 71 to 163 g/day according to seasonal variations (Vaithiyathan and Singh, 1994). In wild herbivores in Namibia, Turner et al. (2013) observed higher soil intake rates in wet than in dry seasons. Seasonal pattern of soil intake can be linked to several variation factors such as growing vegetation stage (sward height and palatability), vegetation community structure and composition, animal feeding preferences and digestive physiology, or meteorological conditions (wind velocity, rainfall, SSM; Turner et al., 2013). To our knowledge, the present experiment is the first study analyzing the impact of SSM on soil intake by beef cattle. Soil intake rate increased 1.5 times when animals grazed on a water-saturated ground (HUM SSM) in comparison to dry conditions. The SSM condition was the same for the 6 animals of each group at each period and changed from a period to another; by this way, SSM effect was confused with period effect but the six experimental periods of each group were alternatively

realized in HUM or DRY SSM conditions to avoid the influence of sward evolution along the experiment. Sward soiling by soil particles adherence on leave or stem surface of herbaceous plants due to raindrop splash, resuspension or animal trampling was described in literature (Hinton, 1992; Rafferty et al., 1994). Influence on sward soiling of both variation factors tested in this experiment (DHA and SSM) was discussed in the following section.

*4.2. Daily herbage allowance and soil surface moisture impacts for sward surface height and sward soiling, and consequences for soil intake*

Animals adapted their feeding behavior to DHA variations by eating a larger proportion of the pre-grazing SSH (PRG SSH, 108.5 mm on average) which resulted in contrasted post-grazing SSH (POG SSH) between ADLIB (82.2 mm) and HIGH and LOW DHA (66.6 mm). Jurjanz et al. (2017) reported lower pre- (in average 84.4 mm) and post-grazing SSH (32.7 and 52.0 mm for res- and non-restrictive DHA). Precautions are requested when comparing the results of this study to those from Jurjanz et al. (2017) because of higher pre-grazing SSH in the present study.

Sward soiling was evaluated by picture analyses and Ti content in unwashed herbage used to estimate the amount of soil on herbage. Results indicate a non-negligible sward soiling for LOW DHA and HUM SSM but this method does not allow to distinguish the part of the daily amount of ingested soil coming from an indirect intake of soiled sward (soil particles adhered on vegetation), to the one coming from soil directly ingested from the ground. The effect of grazing activity was apprehended with the three times of measurement (before, during and after grazing) which evidenced sward soiling amplification along the 24 h on the same area to graze with significant differences for pre-grazing vs during and post-grazing herbage. Sward soiling was not significantly different between during- and post-grazing samples which

means that impact of grazing is more important during the first hours in a new area (during grazing herbage was sampled after 8h of grazing). Between during- and post-grazing sampling, grazing activity had a lower impact on sward soiling, perhaps due since animals were resting during the night.

DHA had an impact on post-grazing SSH, which could influence herbage accessibility and prehension and the risk to ingest soil by grazing close-to-the-ground soiled herbage. Ingestion of soiled sward could also be important when SSM is high since it amplifies the consequences of animal trampling and chain moving and the amount of soil particles which can be adhered on herbage. In addition, soil ingestion during grooming cannot be ruled out, as the coat is often soiled by mud in wet conditions (Herlin and Andersson, 1996).

#### *4.3. Implication for animal exposure to chlordecone and human health*

Significant differences in soil intake rates were observed between the three DHA and the two SSM levels but not for the amount of ingested soil. This is due to the higher DM intake realized by the animals receiving HIGH or ADLIB DHA or grazing in DRY SSM, compared to LOW DHA and HUM SSM respectively. The DHA influences the daily intake of herbage, consequently the average daily gain. If the conditions of this experiment were applied for the entire duration of the stock raising, animals receiving LOW DHA would need a longer grazing period to reach the same final level of slaughter BW as those receiving HIGH or ADLIB DHA. For CLD, a pollutant with a half-life averaging 44 days in cattle (Mahieu et al., 2012), and animals equally balanced between inputs and outputs of pollutant, we can consider the following equation:

$$(6) \quad k \times BW \times CLD_{\text{body}} = B \times SIR \times DMI \times CLD_{\text{soil}}.$$

In this equation,  $k$  is the daily elimination rate in cattle, being 0.016 for CLD (Mahieu et al., 2018);  $BW$  is the average body weight of fattening Creole young bulls at pasture, being 250 kg (quasi-linear growing with average  $BW$  of 153 kg at weaning and 341 kg at slaughtered; INRA Antilles-Guyane, 2014);  $B$  is the relative bioavailability of the pollutant, being 1 (Jurjanz et al., 2014);  $SIR$  and  $DMI$  are the daily soil intake rate (as a percentage of  $DMI$ ) and dry matter intake respectively;  $CLD_{body}$  and  $CLD_{soil}$  are the CLD contents in the animal resulting from soil intake exposure, and in the soil respectively.

Based on the results of the present study, with soil intake rates of 2.08 and 4.66%, and  $DMI$  of 2.70 and 1.84 kg DM/100 kg  $BW$  for young bulls grazing in ADLIB-DRY and LOW-HUM conditions respectively, and considering  $CLD_{soil}$  of 1 mg/kg, the estimations of  $CLD_{body}$  were 35 and 54  $\mu\text{g/kg BW}$  for animals in ADLIB-DRY and LOW-HUM grazing conditions respectively, being a value more than 1.5 times higher for the most restrictive offer on a water-saturated soil surface.

Pasture practices therefore influence the soil intake by grazing cattle and their exposure to soil-bound CLD. Thus, accurate measurements of soil intake constitute an important requirement in risk evaluation regarding CLD contamination. If CLD intake rate is higher than CLD elimination rate, which depends on exposure dose and duration and CLD half-life, it results in a CLD bioaccumulation into the organism, especially in edible tissues as muscle and liver, this latter being reported as the most bio-accumulative organ. Lastel et al. (2016, 2018) studied the CLD concentrations in tissues (liver, empty carcass, shaft muscle and perirenal adipose tissue) of growing goats exposed to CLD by intravenous injections (1 mg CLD/kg  $BW$ ; Lastel et al., 2016) or oral daily exposure (0.05 mg CLD/kg  $BW$  during 21 days; Lastel et al., 2018). They observed a higher CLD concentration in the liver and in the muscle than in the perirenal adipose tissue (values reaching 97.7, 11.5 and 0.73 mg CLD/g fresh matter respectively; Lastel et al., 2018). These results could be explained by the high

affinity of CLD to plasmatic proteins as albumin and high-density lipoprotein (HDL) (Soine et al., 1982). There is the need to understand what are the factors determining cattle exposure to chlordecone in order to help farmers to adapt their farming systems and methods to address the effects of pollution. In this way, they will be able to produce animals and animal products that comply with the regulations. According to the geographical zone considered, soil may contribute in a significant extent to animal exposure to a large range of contaminants as pathogens (Turner et al., 2013), radionuclides (Andersson et al., 2001), or metallic (Smith et al., 2009) and organic pollutants (Mamontova et al., 2007; Jurjanz et al., 2014).

## **5. Conclusions**

This work confirms the influence of herbage offer on soil intake in tether-grazing cattle and brings references for a larger range of situations. Comparing water-saturated vs dried ground, this study demonstrated for the first time, to our knowledge, that soil surface moisture is an important factor modulating soil intake by animals. This study allowed to obtain references on the daily soil intake realized by cattle in different situations of tether-grazing, and enable to propose to farmers grazing practices recommendations to limit soil intake by cattle in chlordecone contaminated areas. This will help to maintain farming activities while ensuring food safety of animal products and preserving human health. These results could be extrapolated to the whole tropical zone where tether-grazing practices are commons and meteorological conditions impacting vegetation and soil surface moisture are similar. Further investigation is needed for a better understanding of soil intake determinants, as soil class (clay content and composition, aggregate stability, etc.), sward height or seasonality factors.

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**Figure captions**

**Fig. 1.** Experimental design of the experiment representing the three daily herbage allowance (DHA) levels (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively) and the two soil surface moisture (SSM) levels (HUM, DRY; *i.e.* water-saturated or dried soil surface) tested during periods 1 to 6, and 7 to 12, for both groups of 6 animals respectively.

**Fig. 2.** Photographs of the experimental design for the first group of six young bulls grazing on a water-saturated soil surface (HUM soil surface moisture) after 8 h of grazing (in the afternoon) (left) and 24 h of grazing (in the morning before to move their post on a new area of fresh herbage) (right). The irrigation equipment is visible on the right of the photograph on the left.

**Fig. 3.** Measurements distribution during each period with three to six days of adaptation and eight days of measurement (SSH: sward surface height; DHA: daily herbage allowance; DSA: daily surface area allowance). This experimental schedule was repeated during periods 1 to 6, and 7 to 12, for both groups of 6 animals respectively.

**Fig. 4.** Soil amounts adhered on herbage (mg/g DM), estimated from Ti contents of herbage sampled pre-grazing (PRG), during grazing (DUG, after 8h of grazing) and post-grazing (POG), according to three daily herbage allowance levels (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively; A) and soil surface moisture (HUM, DRY; *i.e.* water-saturated or dried soil surface; B) (a,b = statistical difference at  $P < 0.05$ ).

**Fig. 5.** Boxplots of the percentage of pixels classified into three categories according to VARIgreen: "Cl-A" corresponds to bare soil, mud-soiled and dead plant organs; "Cl-B" to

stems and senescent organs; "Cl-C" to green leaf sheaths and blades. Solid lines join class back-transformed means for each treatment with standard errors as vertical segments. Within each class, boxplots topped with different letters or symbols differ significantly ( $P < 0.05$ ). The three-boxplot sets are displayed for the images taken before grazing and after grazing according to the three daily herbage allowance levels (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively) and soil surface moisture (HUM, DRY; *i.e.* water-saturated or dried soil surface).



**Table 1. Grazing management, sward characteristics and moisture of soil surface according to daily herbage allowance (DHA) (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively) and soil surface moisture (SSM) (HUM, DRY; *i.e.* water-saturated or dried soil surface).**

Item	DHA			SSM			P-value	
	LOW	HIGH	ADLIB	HUM	DRY	RSD <sup>1</sup>	DHA	SSM
Grazing management								
DHA, g DM/kg BW <sup>0.75</sup>	99.8 <sup>c</sup>	148.8 <sup>b</sup>	301.4 <sup>a</sup>	185.8	180.9	11.8	<0.001	0.080
DHA, kg DM <sup>2</sup> /100 kg BW <sup>3</sup>	2.77 <sup>c</sup>	4.12 <sup>b</sup>	8.35 <sup>a</sup>	5.14	5.02	0.31	<0.001	0.108
DSA <sup>4</sup> , m <sup>2</sup> /animal	23.64 <sup>c</sup>	34.81 <sup>b</sup>	69.63 <sup>a</sup>	41.84	43.55	5.23	<0.001	0.169
Radius of grazing circle, m	2.73 <sup>c</sup>	3.32 <sup>b</sup>	4.69 <sup>a</sup>	3.54	3.62	0.19	<0.001	0.078
Chain length, m	2.06 <sup>c</sup>	2.64 <sup>b</sup>	4.01 <sup>a</sup>	2.86	2.94	0.19	<0.001	0.081
Estimated biomass, g DM/m <sup>2</sup>	203	207	209	213	199	32	0.820	0.079
Herbage quality								
DM content, g/kg	148	148	148	142	142	2.5e-14	-	0.991
OM content, g/kg	908	908	908	908	908	5	-	0.935
DM								
NDF <sup>5</sup> content, g/kg	688	688	688	692	681	6.9e-14	-	0.488

DM

Moisture of soil

surface

Soil water	21.38	21.38	21.38	8.94 <sup>b</sup>	33.8	13.42	-	<0.001
potential, kPa					2 <sup>a</sup>			

Pre-grazing soil	1.49	1.54	1.52	2.24 <sup>a</sup>	0.79 <sup>b</sup>	0.36	0.880	<0.001
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moisture score

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<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

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

<sup>2</sup>DM = dry matter.

<sup>3</sup>BW = body weight.

<sup>4</sup>DSA = daily surface area allowance.

<sup>5</sup>NDF = neutral detergent fiber (analyses realized by UpScience Labs laboratory, Château-Thierry, France).

**Table 2. Body weight (BW), daily organic matter (OM) and dry matter (DM) intake, intake/offer ratio and sward surface height (SSH) according to daily herbage allowance (DHA) (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively) and soil surface moisture (SSM) (HUM, DRY; *i.e.* water-saturated or dried soil surface).**

Item	DHA			SSM			P-value	
	LOW	HIGH	ADLIB	HUM	DRY	RSD <sup>1</sup>	DHA	SSM
BW, kg	171.2 <sup>a</sup>	171.7 <sup>a</sup>	170.8 <sup>a</sup>	172.6 <sup>a</sup>	169.9 <sup>a</sup>	10.1	0.953	0.273
OM intake								
Daily fecal output, kg OM/100 kg BW	0.48 <sup>b</sup>	0.51 <sup>b</sup>	0.57 <sup>a</sup>	0.52	0.52	0.08	<0.001	0.900
OM digestibility	0.73 <sup>b</sup>	0.74 <sup>ab</sup>	0.75 <sup>a</sup>	0.72 <sup>b</sup>	0.76 <sup>a</sup>	0.02	0.034	<0.001
Daily OM intake, kg OM/100 kg BW	1.79 <sup>b</sup>	2.01 <sup>b</sup>	2.30 <sup>a</sup>	1.91 <sup>b</sup>	2.16 <sup>a</sup>	0.360	<0.001	0.006
DM intake								
Daily DM intake, g DM/kg BW <sup>0.75</sup>	71.2 <sup>b</sup>	80.0 <sup>b</sup>	91.2 <sup>a</sup>	76.1 <sup>b</sup>	85.5 <sup>a</sup>	14.3	<0.001	0.007
Daily DM intake, kg DM/100 kg BW	1.98 <sup>b</sup>	2.22 <sup>b</sup>	2.53 <sup>a</sup>	2.11 <sup>b</sup>	2.38 <sup>a</sup>	3.92	<0.001	0.005
DM of ingested herbage/DM offered ratio	0.69 <sup>a</sup>	0.52 <sup>b</sup>	0.30 <sup>c</sup>	0.47 <sup>b</sup>	0.54 <sup>a</sup>	0.11	<0.001	0.007
SSH <sup>2</sup>								
Pre-grazing SSH,	105.4	106.9	113.1	113.0	103.9	23.8	0.499	0.113

mm

Post-grazing	63.3 <sup>b</sup>	69.9 <sup>b</sup>	82.2 <sup>a</sup>	74.6	69.0	14.1	<0.001	0.095
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SSH, mm

Pre - post-	42.1 <sup>a</sup>	37.0 <sup>ab</sup>	30.9 <sup>b</sup>	38.4	34.9	15.0	0.042	0.339
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grazing SSH

difference, mm

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<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

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

<sup>2</sup>SSH = sward surface height.

**Table 3. Soil intake rate (SIR) and daily soil intake amount (SIA) according to daily herbage allowance (DHA) (LOW, HIGH, ADLIB; *i.e.* 100, 150, 300 g DM/kg BW<sup>0.75</sup> respectively) and soil surface moisture (SSM) (HUM, DRY; *i.e.* water-saturated or dried soil surface).**

Item	DHA			SSM			P-value	
	LOW	HIGH	ADLIB	HUM	DRY	RSD <sup>1</sup>	DHA	SSM
Herbage Ti <sup>2</sup> content, µg/g DM <sup>3</sup>	9.2	9.2	9.2	9.2	9.2	-	-	-
Fecal Ti content, µg/g DM	346 <sup>a</sup>	313 <sup>ab</sup>	232 <sup>b</sup>	336 <sup>a</sup>	257 <sup>b</sup>	153	0.035	0.032
Soil Ti content, µg/g DM	2815	2815	2815	2841	2790	-	-	-
DMD <sup>4</sup>	0.71 <sup>b</sup>	0.73 <sup>ab</sup>	0.73 <sup>a</sup>	0.71 <sup>b</sup>	0.74 <sup>a</sup>	0.02	0.033	<0.001
SIR, % DM intake	3.75 <sup>a</sup>	3.14 <sup>ab</sup>	2.12 <sup>b</sup>	3.58 <sup>a</sup>	2.43 <sup>b</sup>	1.90	0.015	0.013
Daily SIA, g DM/100 kg BW <sup>5</sup>	71.8 <sup>a</sup>	66.2 <sup>a</sup>	52.4 <sup>a</sup>	71.0 <sup>a</sup>	55.9 <sup>a</sup>	41.7	0.260	0.130
Daily SIA, g DM/kg BW <sup>0.75</sup>	2.6	2.4	1.9	2.6	2.0	1.5	0.252	0.119

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

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

<sup>2</sup>Ti = titanium.

<sup>3</sup>DM = dry matter.

<sup>4</sup>DMD = dry matter digestibility.

<sup>5</sup>BW = body weight.

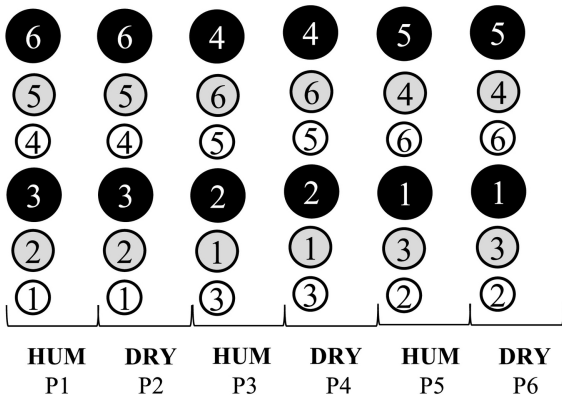
## HIGHLIGHTS

- Soil intake can be a major route for cattle exposure to environmental contaminants
- Accurate estimates of contaminated soil intake are required for risk assessment
- Herbage offer reduction and soil surface moisture increase both amplify soil intake
- Grazing practices are to be adapted to limit exposure to soil-bound pollutants

ACCEPTED MANUSCRIPT

Experimental design for  
the 1<sup>st</sup> group (bulls 1 to 6)

Periods 1 (P1) to 6 (P6)



**3** ADLIB DHA for bull 3

**2** HIGH DHA for bull 2

**1** LOW DHA for bull 1

Experimental design for  
the 2<sup>nd</sup> group (bulls 7 to 12)

Periods 7 (P7) to 12 (P12)

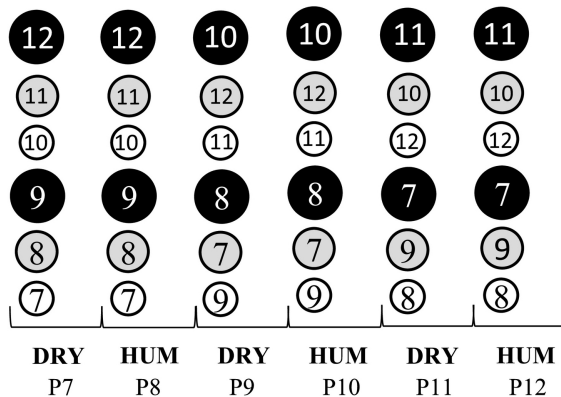


Figure 1



Figure 2



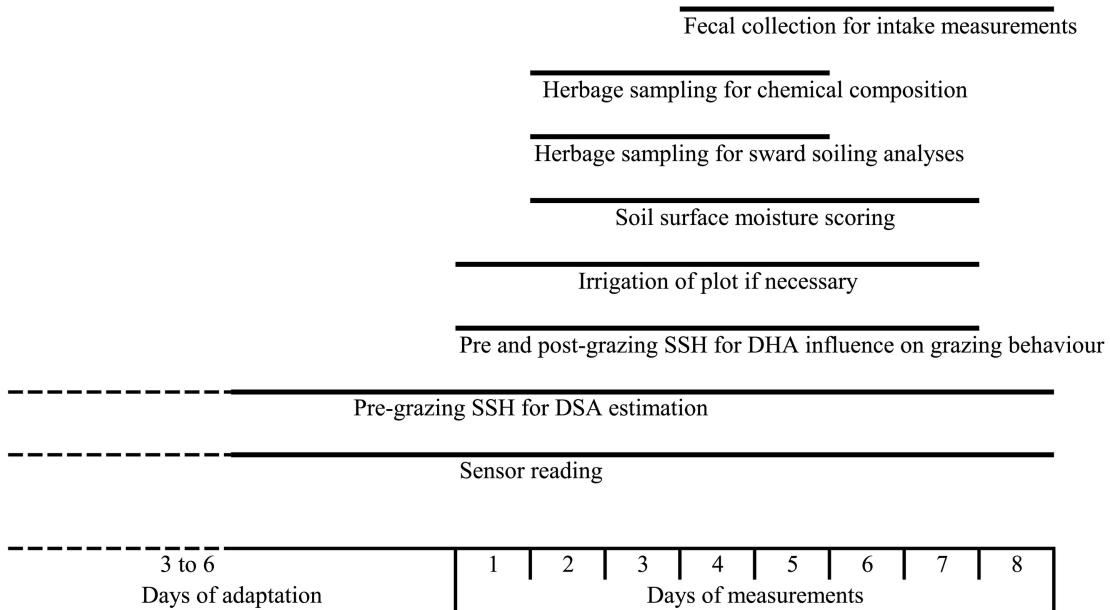


Figure 3

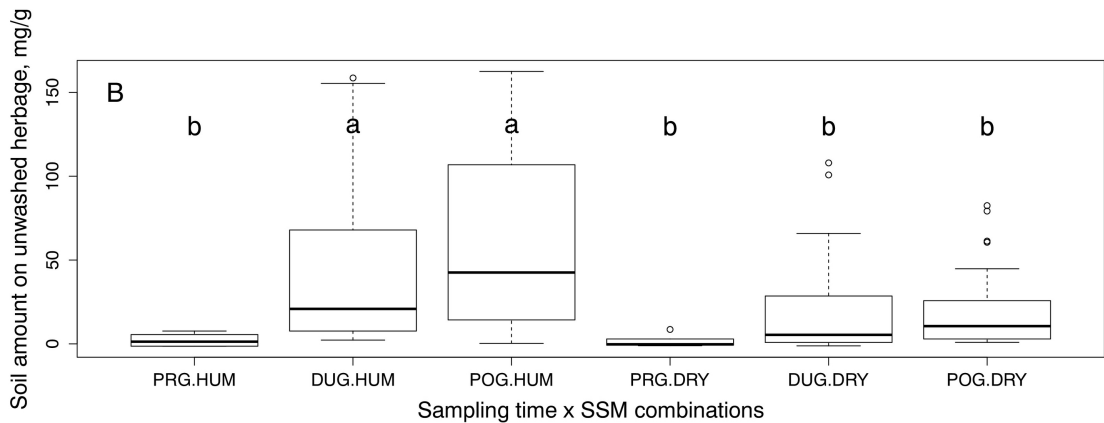
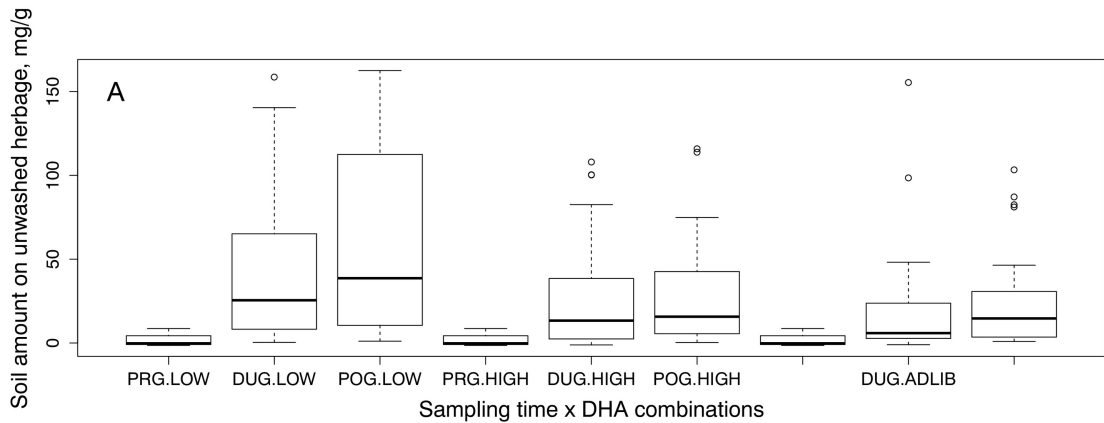


Figure 4

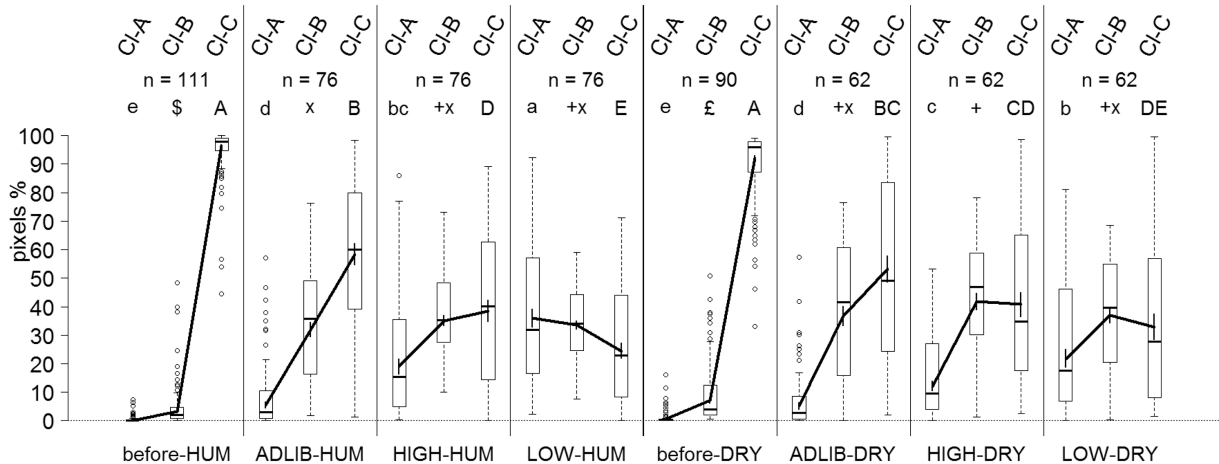


Figure 5