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1 **A first assessment of megaherbivore subsidies in artificial**
2 **waterholes in Hwange National Park, Zimbabwe**

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20

21 **Abstract**

22 The transfer of terrestrial organic matter by terrestrial wild and domestic animals when they urinate
23 and defecate directly in savanna rivers has already been studied. However, the eulittoral zone
24 around ponds receives organic matter during the dry season, which is diluted in the ecosystem when
25 the water body returns to its wet season level. In our study, we evaluated this pathway of subsidies
26 in Hwange National Park, Zimbabwe, by estimating dung density in the eulittoral zone at the peak of
27 the dry season. We also collected dung from different herbivore species during the dry and wet
28 seasons to measure nutrient content and estimate nitrogen and phosphorus leaching rates. Our
29 results show a decrease in carbon:phosphorus and nitrogen:phosphorus ratios in the dry season
30 compared to the wet season. During the dry season, the deposition of total carbon, nitrogen, and
31 phosphorus, mostly due to elephants, is estimated to be 8.65, 0.25, and 0.06 g/m²/day, respectively,
32 while the leaching rates of dissolved inorganic nitrogen and phosphorus are 1.52 mg and 6.59 mg
33 m²/day, respectively. No specific coloured dissolved organic matter signature for dung was identified.
34 We discuss the temporal dynamics of the subsidies as a distinctive feature of the system.

35

36 **Keywords**

37 Eutrophication, water level fluctuation, water management, browsers, grazers

38

39 **Introduction**

40 Terrestrial-aquatic subsidies are of major importance for the functioning of aquatic ecosystems.
41 Carbon fluxes from terrestrial to aquatic ecosystems are estimated to be *ca.* 1.9 billion (10^9) tonnes
42 per year (Cole et al. 2007). Several aquatic systems such as small rivers and oligotrophic lakes depend
43 on these allochthonous inputs to sustain aquatic life (Cole et al. 2006; Rubbo et al. 2006) . For other
44 systems, terrestrial inputs contribute significantly to matter and energy fluxes, and in extreme cases,
45 they may dramatically modify the trophic status and functioning of aquatic systems (Chaichana et al.
46 2010; Dutton et al. 2018; Earl and Semlitsch 2013; Holgerson et al. 2016; Sparber et al. 2015). For
47 these reasons, continuous efforts have been made to assess terrestrial subsidies to aquatic
48 ecosystems. In a recent study, Gounand et al. (2018) synthesised the spatial flows of carbon
49 connected ecosystems. The median carbon flow from forests and grasslands to lakes and streams
50 due to plants, litter, insects, and amphibians amounted to 164 g C/m².year, while the flow in the
51 other direction due to insects, fish, and amphibians amounted to 3.27 g C/m².year. The flow from
52 terrestrial to freshwater ecosystems was three times higher than the gross primary production in
53 freshwater, estimated at 54.6 g C/m².year (Gounand et al. 2018).

54

55 Terrestrial organic matter enters water through runoff or its active transport by wild and domestic
56 animals. The amount and timing of subsidies brought by wild animals depend on the nature of their
57 activities linked to water (i.e., ontogenic habitat switch, migration, feeding, etc.). For instance, wood
58 frogs have biphasic life cycles: they use the aquatic environment for larval development and the
59 terrestrial environment in adult life. When the adults return to breed in wetlands, they provide
60 subsidies to the aquatic ecosystems with carbon, nitrogen, and phosphorus, although these fluxes
61 are smaller than the nutrient fluxes related to the adult emergence in terrestrial ecosystems (Capps
62 et al. 2015). Aquatic bird defecation in close vicinity to aquatic ecosystems is a well-known source of
63 nutrients, which depend on behavioural and migratory patterns, and can be large and highly variable
64 (Chaichana et al. 2010; Kitchell et al. 1999; Post et al. 1998). Herbivores are also active carriers of

65 nutrients when they urinate and defecate in water (Naiman and Rogers 1997). In temperate
66 ecosystems, different species of now-extinct megaherbivores may have played a dominant role in
67 transferring organic matter from terrestrial to aquatic ecosystems (Moss 2015). Doughty et al.
68 (2013a,b) estimated and compared the current and former potential phosphorus transfers to aquatic
69 ecosystems due to wild herbivorous mammals. The authors showed that the modern transfer of
70 phosphorus represents 10% of the estimated former transfer in North America, 5% in South America,
71 45% in Australasia, 18% in Eurasia, and 62% in Africa (Doughty et al. 2013a; Doughty et al. 2013b;
72 Moss 2015). The higher ratio observed in Africa reveals the persistence of large herbivores that
73 continue to subsidise aquatic ecosystems.

74

75 The impact and transfer of terrestrial organic matter to aquatic systems has been particularly well
76 studied for megaherbivores in savannas (Dutton et al. 2018; Gereta and Wolanski 1998; Masese et al.
77 2015; Stears et al. 2018; Subalusky et al. 2015; Subalusky et al. 2017; Wolanski and Gereta 1999). In
78 particular, hippos (*Hippopotamus amphibius*) forage in savanna grasslands and rest in aquatic
79 ecosystems. This daily feeding migration represents a high input of organic matter as estimated by
80 Subalusky et al. (2015) and causes downstream hypoxia with fish kills (Dutton et al. 2018). Hippo
81 excretion and egestion into the Mara River, Kenya, accounted for 15% of dissolved organic carbon,
82 27% of total nitrogen, and 29% of total phosphorus loading for the upstream catchment (Subalusky
83 et al. 2015). Masese et al. (2015) also estimated the terrestrial subsidies on the river food web in a
84 section of the Mara River Basin that includes different densities of livestock and hippos. Notable
85 shifts in the origin of the terrestrial organic carbon were observed according to the seasons and
86 species. For instance, the contribution of C4 producers (i.e., savanna grasses) increased from 18 to
87 33% during the dry season and from 10 to 58% during the wet season in the upper forested and river
88 reaches that received hippo inputs compared to the C4 producer areal cover in riparian vegetation
89 (Masese et al. 2015). One extreme phenomenon is the migration of the Serengeti wildebeest
90 (*Connochaetes taurinus*), as they cross the Mara River in the Maasai Mara National Reserve where

91 massive drownings occur. Another study conducted by Subalusky et al. (2017) estimated the biomass
92 inputs to be 1,000 tonnes, representing 107 tonnes of carbon, 25 tonnes of nitrogen, and 13 tonnes
93 of phosphorus. Soft tissues (muscles, viscera, and skin) are decomposed within 50 days, but bones
94 slowly release nutrients over a duration of 7.4 years (Subalusky et al. 2017).

95

96 These studies conducted in savannas assessed direct inputs of terrestrial wild and domestic animals
97 when they urinate and defecate in water, or drown in the case of the Serengeti wildebeest. The
98 majority of these studies focus on rivers. In semi-arid ecosystems, the water level of ponds, lakes,
99 and rivers decreases as the dry season progresses. The area delimited by the maximum water level of
100 the rainy season and the minimum water level of the dry season defines the eulittoral zone (Wetzel
101 2001) (Figure 1). All organic matter that accumulates in this open zone during the dry season will
102 contribute to the nutrient budget of ecosystems when the water body returns to its wet season level.
103 In Hwange National Park (HNP), Zimbabwe, wildlife gathers around pans during the dry season
104 (Valeix 2011), while dung pills up in the eulittoral zone. Elephants represent more than 80-90% of the
105 total biomass of large herbivores at the waterholes (Chamaillé-Jammes et al. 2016; Chamaillé-
106 Jammes et al. 2008; Valeix et al. 2007), and their density can range from *ca.* 75 individuals per day
107 and per waterhole during a rainy year to *ca.* 350 individuals during a dry year (Valeix et al. 2007). The
108 presence of elephants suggests high subsidies to the small natural and artificial waterholes found in
109 HNP, which are assessed in the present study. To reach this objective, we assessed dung density
110 around the waterholes during the dry season. We also collected dung from different species of wild
111 and domestic animals to measure their nutrient contents. Using leaching experiments, we then
112 estimated the concentration of nitrogen and phosphorus released in the water. We took advantage
113 of this experiment to look for coloured dissolved organic matter (CDOM) released in water by dung
114 as well as any potential differences between species.

115

116

117 **Material and methods**

118 **Study area**

119 Hwange National Park is situated in a semi-arid region in the north-west of Zimbabwe. The rainy
120 season starts in November-December and finishes in March-April. The cold dry season continues
121 until July followed by the hot dry season until November (Chamaillé-Jammes et al. 2006). The
122 vegetation consists of mixed woodland and bushland on Kalahari sand (Chamaillé-Jammes et al.
123 2007b). The waterholes found in this area are small depressions filled only by rainwater runoff;
124 hereafter, we refer to them as pans (Fynn et al. 2015). Artificial and natural pans are found in the
125 park and adjacent forestry area. In the following, we thus distinguish between natural pans, fed by
126 rainwater, and artificial pans, fed by both pumped groundwater and runoff (Chamaillé-Jammes et al.
127 2007b). Natural pans retain water for only 3 to 6 months after the rainy season. However, the
128 duration depends on their size, the rainfall, and the dryness. In artificial pans, water is pumped into a
129 small open-top concrete drinking trough, which is situated at ground level and may be round or
130 rectangular. Water then flows from the trough to the pan. The pumps are either solar- or diesel-
131 powered. The former only works during the day, while the latter may run out of petrol. To our
132 knowledge, there are no systematic records on pumping efforts or the presence or absence of water
133 during the dry season. It is therefore difficult to assess artificial water input during the dry season
134 and, more generally, the hydrology of pans. During the dry season, artificial pans may dry out within
135 48 h (Chamaillé-Jammes et al. 2016). The water depth in pans does not exceed 5 m, while the surface
136 area of permanent pans in 2014 ranged from 453 m² in the dry season to 26,788 m² in the rainy
137 season.

138 The eulittoral zone is the area between the maximum water level in the rainy season and the
139 minimum water level in the dry season (Wetzel 2001) (Fig. 1). During the rainy season, the perimeter
140 of the pans is at its maximum. As the surface water shrinks with dryness, the pan perimeter
141 decreases and the eulittoral zone appears.

142

143 **Estimation of dung density around the pans and their composition**

144 To estimate the amount of dung deposited around the pans, we monitored three artificial pans
145 (Caterpillar, Dom, and Safari Lodge) in November 2015. On the first day, we cleaned a 1 m wide band
146 around the pan (Fig. 1). All dung found in this area was moved aside. We also measured the surface
147 of the pan as well as the pan along with the sampled area by walking around with a Garmin GPSMAP
148 64; the sampled area was the difference between the two surfaces. On the second, third, and fourth
149 days, all dung found in this area was weighed and moved aside. We included in this census easily
150 recognisable dung that had been trampled by elephants (Fig. 1). However, in this 1 m wide band, we
151 only found elephant dung. To estimate the amount of dung deposited in this area, we reported the
152 mass in the sampled surface area per pan and per day.

153

154 **Estimation of dung composition during the dry and wet seasons**

155 To estimate the carbon, nitrogen, and phosphorus content of dung and explore whether the
156 composition varied with the seasons and species, we collected dung from different individuals in
157 November 2013, August, October, and November 2014 for the dry season and February and March
158 2014 for the wet season. We analysed the dung collected during the rainy and dry seasons to detect
159 any differences that would reveal qualitative changes in subsidies. The dung was collected within a
160 radius of 5 m from the pans or reservoirs in the park and communal areas. It was then stored in
161 Manila envelopes and dried at ambient temperature. Large dung (i.e., elephants) was broken to
162 speed up the drying process and avoid fermentation. In total, we collected samples from 10 African
163 buffalos (*Syncerus caffer*) (6 dry season/4 wet season), 16 cows (*Bos taurus taurus*) (10 dry/6 wet), 2
164 donkeys (*Equus africanus*) (1 dry/1 wet), 27 elephants (*Loxodonta africana*) (22 wet/5 dry), 5 giraffes
165 (*Giraffa camelopardis*) (4 dry/1 wet), 12 Burchell's zebras (*Equus quagga*) (7 dry/5 wet), and 2 Cape
166 bushbucks (*Tragelaphus sylvaticus*) (1 dry/1 wet). The samples were crushed and then analysed.
167 Organic carbon concentration was measured with the high-temperature combustion method,

168 nitrogen with the Dumas method, and phosphorus with the Olsen method. For each sample, we
169 calculated the carbon:nitrogen (C:N), carbon:phosphorus (C:P), and nitrogen:phosphorus (N:P) ratios.
170

171 **Dung leaching rates and CDOM**

172 To assess the ammonium, nitrate, and phosphorus leaching rates of dung, we added 2 g of uncrushed
173 dung to 200 mL of deionised water in a 250 mL glass jar. The jar lids were left slightly open to allow
174 gas exchanges with the atmosphere. The jars were kept in the dark at a temperature of *ca.* 20°C.
175 Water was sampled at 24 h (t1), 48 h (t2), and 72 h (t3) to follow the changes in ammonium, nitrate,
176 and phosphorus concentrations. Because water sampling is a strong perturbation, we used three
177 separate jars for the measurement of each dung sample at t1, t2, and t3. The 18 dung samples used
178 in the experiment comprised 9 elephants (3 collected during the wet season and 6 during the dry
179 season), 3 zebras (1 wet/2 dry), 2 buffalos (1 wet/1 dry), 2 cows (1 wet/1 dry), 1 donkey (dry), and 1
180 giraffe (dry). At each time point, we sampled 5 mL of water filtered through a Whatman GF/F filter.
181 The filtered water was stored in amber glass bottles and frozen for CDOM analyses (see below). The
182 remaining water content of the jars was filtered through a Whatman GF/C filter and analysed for
183 ammonium, nitrate, and orthophosphate concentrations. We used the Hach-Lange DR-6000
184 spectrophotometer and Hach-Lange kits according to the corresponding procedures for these
185 quantitative analyses. The detection limits for ammonium, nitrate, and orthophosphate are 0.01,
186 0.01, and 0.02 mg/L, respectively.

187

188 **CDOM analyses**

189 Dung releases dissolved organic matter (DOM) in the water with nutrients. The optically active part
190 of DOM known as coloured dissolved organic matter (CDOM) can be quantified and identified using
191 fluorescence excitation–emission matrices (Stedmon et al. 2003). When excited, a molecule absorbs
192 light at a given wavelength and fluoresces at another wavelength. The position of these two
193 wavelengths and the intensity of responses allow the molecule to be characterised and quantified.

194 Scanning water samples reveals the presence of different components, with each one being
195 characterised by its excitation and emission wavelength (Stedmon et al. 2003). Compound
196 quantification is conducted by measuring the peak height at its characteristic excitation and emission
197 wavelength combination. To identify and quantify the CDOM freed in water by wild and domestic
198 herbivores, fluorescence measurements were conducted with a Cary Eclipse Agilent® fluorescence
199 spectrophotometer. The excitation wavelengths spanned from 250 to 500 nm with an interval of 5
200 nm, and the emission wavelength from 260 to 700 nm with an interval of 1 nm. Prior to analyses, the
201 samples were refiltered through a 0.2 µm disposable filter (Millipore). Each sample was measured in
202 a quartz cuvette that was rinsed three times with deionised water before use. The pure water blank
203 was run with Milli-Q water. For each sample, we collected an excitation-emission matrix, and the
204 identification of DOM fluorescent components was based on the position of excitation-emission
205 maxima. The types of components found in each sample can be interpreted as a fingerprint (Coble
206 2007).

207

208 Fluorescence measurements produce matrices from which the wavelengths corresponding to the
209 components (peaks) can be extracted. The height of the peaks allows the components to be
210 quantified relative to each other. For the spectra analysis, we used Matlab (Matworks) to develop an
211 unsupervised method to extract the components from the spectra obtained for the various excitation
212 wavelengths. All emission spectra obtained for the range of excitation wavelengths were assembled
213 into one bi-dimensional spectrum, i.e. excitation emission matrix (EEM). The impact of Rayleigh
214 scattering was eliminated by truncating the spectra. The Raman peak of water was eliminated by
215 subtracting the blank spectrum of water from each EEM.

216

217 For each excitation wavelength, the corresponding emission spectra were decomposed by an
218 expectation-maximization algorithm into an optimised distribution of up to five randomly spaced
219 Gaussian functions. Finally, the optimum composition of the EEM was obtained by grouping all

220 Gaussian functions of one EEM into components. This final step was achieved by an unsupervised
221 procedure based on fuzzy logic criteria that converges to a solution when the number of components
222 is as small as possible, while satisfying a criterion of mono-modality for each component.

223

224 **Statistical analyses**

225 Due to the small number of samples for some species, we analysed the results at the guild level for
226 the dung composition and leaching rates. We thus separated browsers (elephants and giraffes) from
227 grazers (buffalos, bushbucks, cows, donkeys, and zebras). We used linear regressions to determine
228 species and seasonal differences for dung nutrient content and species, and temporal differences for
229 dung nutrient leaching rates. For dung nutrient content, we used the Akaike information criterion
230 (AIC) to select the best model from the following: a full model in which guilds interact with seasons; a
231 model with additive effects of the guilds and seasons; and a model with either guilds or seasons
232 (Appendix Table A1). For the nutrient leaching rates, prior to analyses, ammonium and nitrate
233 concentrations were respectively log and square-root transformed to decrease overdispersion. We
234 again compared the full model where guilds and time interact to simpler models and selected the
235 model with the lowest AIC value (Appendix Table A2).

236

237 To analyse the diversity of the CDOM components released by dung as a function of season and
238 species, we used multiple factorial analysis (MFA) on a contingency table (Escofier and Pages 1994).
239 We explored whether the compounds differed between species and seasons, and whether the
240 identity of the compounds changed during the course of the experiment. The data set consisted of
241 the frequency of each compound for each species and at each time point. The three time points of
242 the experiment were included in the analysis as three groups, which allowed the time effect to be
243 studied. MFA permits the use of qualitative and quantitative variables as well as supplementary
244 variables. The latter do not contribute to the construction of the MFA dimensions, but their
245 correlations to the dimensions can be computed, thus contributing to the interpretation of the

246 dimensions. Consequently, species and season were included in the MFA as qualitative
247 supplementary variables, and all other parameters as quantitative variables. Numerical analysis was
248 carried out using software R (R Development Core Team, 2008), version 3.1.1. MFA was conducted
249 using the FactoMineR package (Husson et al. 2015), version 1.29.

250

251

252 **Results**

253 **Dung density around the ponds and nutrient composition**

254 The dung found in the sampling surface was exclusively elephant dung. The monitoring of dung
255 density around the three study pans on three sample days showed a strong variability. The mean
256 density was 18.89 g/m²/day (std: 26.04 g/m²/day). The density ranged from 1.01 g/m²/day at Safari
257 Lodge on the third day to 79.8 g/m²/day at Caterpillar on the first day.

258

259 Carbon concentration in the dung varied with the guilds and seasons (Fig. 2, Table 1), ranging from
260 251 g/kg for buffalo dung sampled during the wet season to 486 g/kg for elephant dung sampled
261 during the dry season. Browsers showed a higher carbon concentration than grazers did, while there
262 was a seasonal effect with a higher carbon concentration in the dry season (Fig. 2a, Table 1). Carbon
263 concentrations were homogeneous within guilds. Nitrogen concentration did not change with the
264 guilds or seasons (Fig. 2b). Phosphorus concentration ranged from 1.4 g/kg for elephant dung
265 collected during the dry season to 15.9 g/kg for cow dung collected during the wet season. Contrary
266 to carbon concentration, phosphorus concentration was higher during the wet season and in grazer
267 dung (Fig. 2c, Table 1). For browsers, elephant dung had a lower phosphorus concentration than
268 giraffe dung, while for grazers, zebra dung had a lower phosphorus concentration than other grazer
269 species (Appendix, Fig. 1A). There was no seasonal or guild effect for the C:N ratio (Figure 2d). C:P
270 and N:P ratios were significantly higher during the dry season and in browser dung (Fig. 2e and f).
271 Mean C:P and N:P ratios for browser dung respectively decreased from 144.7 and 3.6 during the dry
272 season to 66.4 and 2.3 during the wet season. For browsers, elephant dung had a higher N:P ratio
273 than giraffe dung, especially in the wet season (Appendix, Fig. 1A). For grazers, mean C:P and N:P
274 ratios decreased from 86.2 and 2.5 during the dry season to 48.7 and 1.5 during the wet season.

275

276 As we found only elephant dung in the pan vicinity during the three consecutive days of the census,
277 we could only estimate carbon, nitrogen, and phosphorus deposition in the eulittoral zone for this

278 species in the dry season. Mean carbon, nitrogen, and phosphorus depositions were respectively
279 8.00 g C/m²/day (from a minimum of 0.35 to a maximum of 36.9 g C/m²/day), 0.24 g N/m²/day (from
280 0.007 to 2.20 g N/m²/day), and 0.07 g P/m²/day (from 0.001 to 0.58 g P/m²/day).

281

282 **Nutrient leaching and estimation of nutrient loading rates**

283 Ammonium, nitrate, and phosphorus leaching did not change over time: the same concentrations
284 were measured in water after 24 h, 48 h, and 72 h. This absence of time effect suggests that the
285 nutrients released by dung were taken up by microorganisms in water as they were produced. An
286 alternative hypothesis is that all the labile C, N, and P leached out within 24 hours, and the remaining
287 material needed to be mineralised. However, these concentrations differed according to the guilds
288 for ammonium and nitrate (Fig. 3, Table 2). Indeed, ammonium and nitrate concentrations measured
289 in water were significantly lower with browsers. There was no effect for phosphorus concentration.

290

291 We estimated the nutrient loading rate for elephants alone, as we found only elephant dung in the
292 pan vicinity during the three consecutive days of the census. The mean (std) quantities of
293 ammonium, nitrate, and phosphorus released in water during our experiment were 0.0817 (0.2019),
294 0.0765 (0.0530), and 0.7838 (0.2039) g/kg, respectively. Weighted by the mean density of the
295 elephant dung found around the pans, ammonium, nitrate, and phosphorus leaching rates were 1.54
296 (3.81), 1.45 (1.0), and 16.49 (3.85) mg/m²/day, respectively. This corresponds to a mean of 1.52 mg
297 N/m²/day and 6.59 mg P/m²/day.

298

299 **Composition of CDOM**

300 The analysis of CDOM allowed us to identify six compounds characterised by their excitation and
301 emission wavelengths (Fig. 4a, Table 3). The first, identified in elephant dung collected in the dry
302 season and present on t1 of the experiment (Table 4), corresponds to a tyrosine- and tryptophan-like
303 compound (Chen et al. 2003; Ramanujam 2006), which is an amino acid derived from proteins. The

304 second component found in five samples from a cow and elephants at the three different time points
305 (Table 4) corresponds to humic acid-like components and nicotinamide adenine dinucleotide with
306 hydrogen (NADH) (Chen et al. 2003; Ramanujam 2006; Rehman et al. 2017). The third and sixth
307 compounds correspond to porphyrin-like molecules (Ramanujam 2006) (Table 3). These heterocyclic
308 organic compounds (three pyrroles and one pyrroline) form chlorophyll with a magnesium ion. They
309 were found in dung leachates from elephants, a zebra, and a buffalo, mostly collected during the wet
310 season (Table 4). The fourth compound corresponds to carotenes and vitamin A, a derivative of
311 carotene (Ramanujam 2006). The fifth compound corresponds to flavin adenine dinucleotides (FAD),
312 flavonols, and lipids (Kang et al. 2016; Ramanujam 2006) (Table 3). Flavonols are plant pigments. The
313 fourth and fifth components were found in almost all dung leachates at all time points with the
314 exception of the dung of two zebras collected during the dry season, which only produced the fifth
315 component (Table 4).

316

317 The first two dimensions of the MFA explain 53.04% of the variability (Fig. 4b). The first dimension is
318 defined on the left side by component 4 at the three time points (negative correlation to the first
319 dimension with $p < 0.02$ on t1 and $p < 0.006$ on t2 and t3), and on the right side by component 2 on
320 t3, component 3 on t2 and t3, and component 6 on t1 (positive correlation to the first dimension
321 with $p < 0.005$). Dung collected during the wet season are all characterised by component 3 on t1,
322 and to a lesser extent, on t2, component 2 on t3, and component 6. The wet season is significantly
323 correlated to the first dimension ($p < 0.001$, Fig. 4c), which clearly differentiate dung from the dry
324 and wet seasons (Fig. 4b). The second dimension is characterised on the lower side by component 5
325 at the three time points (negative correlation with $p < 0.001$) and on the upper side by component 4
326 at the three time points and component 3 on t3 (positive correlation with $p < 0.03$). This dimension
327 mostly separates the dung of two zebras in the dry season from the other species, as it only released
328 compound 5 at the three time points. The group representation shows that the dung is separated on
329 the first dimension by the season in which they were collected, and to a lesser extent, on the second

330 dimension by the species identity. This is mostly due to the zebra, which is negatively correlated to
331 the second dimension ($p < 0.03$).

332

333 The third dimension represents 14.03% of the variability (Fig. 4d) and is defined by the components
334 released by the dung of two elephants collected during the wet season in opposition to the other
335 individuals. Component 6 produced by one elephant's dung on t1 and component 2 produced by two
336 elephants' dung on t3 are positively correlated with the third dimension ($p \leq 0.001$), while
337 components 3 and 6 produced on t3 by the dung of another elephant, a zebra, and a buffalo are
338 negatively correlated ($p \leq 0.007$).

339

340

341 **Discussion**

342 Our study documents the carbon, nitrogen, and phosphorus contents in herbivores' dung and the
343 potential terrestrial subsidies delivered to plants when herbivores defecate in close vicinity. To our
344 knowledge, this is the first attempt to assess terrestrial subsidies in waterholes located in a savanna.

345

346 **Seasonal effects on dung nutrient and CDOM composition**

347 Our analyses reveal changes in dung nutrient composition between the wet and dry seasons. Indeed,
348 phosphorus content is lower in the dry season, while carbon content is higher. This is reflected in the
349 C:P and N:P ratios, which both reveal the winter decrease in phosphorus with carbon enrichment.
350 These effects are especially clear for browsers. Grant et al. (1995) showed a seasonal effect of faecal
351 nitrogen and phosphorus concentrations for several herbivores in Kruger National Park, South Africa.
352 Faecal nitrogen and phosphorus concentrations were lower in the dry season than in the wet season.
353 In their study, faecal nitrogen concentration was higher for browsers than for grazers, while
354 phosphorus content was lower for browsers than for grazers. Dung nutrient content results from the
355 resource choice and digestion process. Therefore, dung nutrient content may reveal food quality
356 (Grant et al. 1995; Wrench et al. 1997) with a lower quality during the dry season. The nitrogen
357 concentrations of browsers' dung revealed in our study are in line with the results of Grant et al.
358 (1995), although our phosphorus concentrations are generally higher. This finding is not surprising,
359 because browsers feed on plants that maintain their nutritive value, whereas grazers feed on grass
360 that loses its nutritive value when maturing or becoming dormant during the dry season (Grant et al.
361 1995).

362

363 The composition of CDOM released in water by dung also differs between the dry and wet seasons.
364 The differences essentially relate to the presence of pigment residuals, because the dung collected
365 during the wet season is richer than dung collected during the dry season. In addition to carotenes,
366 vitamin A, FAD (flavin adenine dinucleotides), flavonols, and lipids, wet-season dung has porphyrin-

367 like and humic acid-like components as well as NADH (nicotinamide adenine dinucleotide). This
368 change in pigment residuals may reveal variations in food quality or fauna diet. There was no species
369 effect on CDOM composition except for zebras during the dry season. These results suggest that
370 even if the different species feed on diverse vegetal sources, the digestion process makes any CDOM
371 differences disappear. Zebra dung collected during the dry season is characterised by component 5
372 alone, namely FAD, flavonols, and lipids. This difference may indicate changes in zebra diet or the
373 digestive process, or even a sampling effect. However, this analysis relies on a limited number of
374 samples (i.e., few individuals), meaning that these conclusions should be considered with caution.
375 The compounds revealed here might echo diet preferences and individual constraints on nutrition.
376 Our results show that this approach is promising.

377

378 **CDOM identification**

379 In our short-term experiment, the CDOM analysis reveals unexpected results. The protein-like
380 (tyrosine- and tryptophane-like) and humic-like substances are the two key fluorescent components
381 found in CDOM decomposition (Chen et al. 2003; Coble 1996; Hudson et al. 2007; Stedmon et al.
382 2003). They are respectively identified as soluble microbial and plant structural tissue decomposition
383 by-products, with the latter being recalcitrant DOM (Chen et al. 2003). These CDOM have a negative
384 effect on water transparency and may affect photosynthesis in tropical lakes (Mello Brandao et al.
385 2018). Here, protein- and humic-like compounds correspond to components 1 and 2, respectively,
386 but they were rarely found in our experiment. One cow (dry season) and four elephants (wet and dry
387 seasons) produced these compounds. As the experiment only lasted 3 days, we were unable to
388 observe the production and accumulation of these by-products, as usually observed in marine and
389 freshwater. However, we measured other components (3 to 6) that are rarely seen in natural water.
390 These pigments and derivatives are easily broken down to colourless products via different
391 intermediate steps for chlorophyll *a* (Kogel-Knabner 2002; Moss 1968). Consequently, they do not
392 affect water transparency. All species produced these components with seasonal effects on their

393 relative concentrations. These results suggest that a longer experiment is necessary in order to
394 identify which species provide recalcitrant DOM that affects water transparency and then observe
395 how this changes seasonally. Indeed, dung differs in material granulometry, and straw and woody
396 parts are identified in elephant dung in particular. In this sense, it would be interesting to
397 characterise dung with CDOM given its potential to affect water transparency and identify any
398 potential species signatures.

399

400 **Dung leaching and terrestrial subsidies**

401 The mean direct deposition of total carbon, nitrogen, and phosphorus in pan eulittoral zones during
402 the dry season is estimated to be 8.65, 0.25, and 0.06 g/m²/day, respectively, while the mean
403 leaching rates of dissolved inorganic nitrogen and phosphorus are estimated to be 1.52 mg N/m²/day
404 and 6.59 mg P/m²/day, respectively. The nutrient deposition estimated in our study is only a
405 preliminary assessment, as we considered a low number of samples and only dung found across a 1
406 m wide surface around the shoreline. The dung deposition is not restricted to this strip but rather to
407 the entire eulittoral zone at all times. During the peak attendance, elephants wait in line around the
408 pans, and we may hypothesise that dung deposition increases with waiting time. In addition, we
409 might expect inter-annual variability in dung deposition, because livestock abundance around the
410 pans is linked to rainfall: the drier the year, the higher the abundance of herbivores at waterholes
411 (Chamaillé-Jammes et al. 2016). Between-pan differences may also be observed, as different
412 herbivore species seem to prefer certain pans, probably due to the neighbouring vegetation
413 (Chamaillé-Jammes et al. 2016), and at least for elephants recently, not because of their sodium
414 concentration (Chamaillé-Jammes et al. 2007a). The leaching rates calculated here may be
415 underestimated, because it was not possible to use bacterial communities from waterholes; we also
416 used de-ionised water. The sole source of bacteria was found in the dung, and these bacterial
417 communities are less adapted to break down dung than those found in waterholes.

418

419 Our results call for several comments. First, dissolved inorganic nitrogen and phosphorus represent
420 0.04% and 0.28% of the total nutrient deposition, respectively. Only a tiny fraction of the nutrients
421 thus becomes available for aquatic microorganisms. This suggests that a larger part of the dung will
422 decompose in water, particularly when the pans become inundated, while the remaining part will be
423 buried in sediments. The leaching rates measured here are based on a short-term experiment with
424 the calculations being made on the first day. Because the dung used in the experiment contains
425 bacteria and other microorganisms, bacterial communities developed, took up nutrients, and
426 modified their concentrations. Therefore, because the modification of nutrient leaching and the
427 adaptation of bacterial communities in the environment are both unknown, it is difficult to assess
428 how much dung will be mineralised in water or buried in the sediment. Second, the comparison of
429 total nutrient deposition to published data shows it to be similar for phosphorus exports in
430 agricultural area, whereas it is clearly inferior for total nitrogen (Wetzel 2001). Chaichana et al.
431 (2010) estimated the total nitrogen and phosphorus inputs related to birds to be 2 and 0.65
432 mg/m²/day, respectively, and associated them with the eutrophication of a small pool in Brown
433 Moss, England. Elephant inputs in HNP are much higher but still well below hippo inputs because of
434 the excretion and egestion, which respectively amount to 0.13-0.19 g N/kg hippo/day and 0.01-0.02
435 g P/kg hippo/day, as described by Subalusky et al. (2015). However, hippos spent much more time in
436 and around the waterholes than elephants did. The eutrophication of some pans in HNP has been
437 documented with high concentrations of total nitrogen and phosphorus, ammonium, soluble reactive
438 phosphorus, and chlorophyll a (Msiteli-Shumba et al. 2018). Cyanobacterial blooms were also
439 reported in a few pans in April and November 2013 (Msiteli-Shumba et al. 2018; Msiteli-Shumba et
440 al. 2017). This eutrophication is likely due to terrestrial subsidies brought by elephants and, as noted
441 above, these terrestrial subsidies are certainly underestimated.

442

443 **Subsidy dynamics**

444 Nutrient transport and the associated allochthonous fluxes from terrestrial to aquatic ecosystems
445 have been modified with changes in wildlife migration due to the building of dams and walls or
446 climate change (Pringle 2017). In HNP, the creation of artificial pans as a management tool has led to
447 an increase in elephant density (Chamaillé-Jammes et al. 2008). Based on body mass, large animals
448 are disproportionately important in the spread of nutrients (Doughty et al. 2013a; Tshipa et al. 2017).
449 The provision of water year-round probably causes an increase in nutrient fluxes to aquatic
450 ecosystems. In addition, the change in dung nutrient composition between the wet and dry seasons
451 has implications at the landscape level, because herbivore migration and displacements vary
452 between the seasons and especially in terms of faecal nutrients. The need for water is less marked
453 during the wet season, as this resource can be found almost everywhere. The partial migration of
454 individual elephants during the dry season can reach up to 260 km. Therefore, phosphorus-rich
455 elephant dung, produced during the wet season, is dispersed throughout the savanna. During the dry
456 season, wild and domestic animals gather around pans (Chamaillé-Jammes et al. 2016; Valeix 2011).
457 This implies that artificial pans, which provide water during the dry season, receive more subsidies
458 than natural pans produced during the dry season, particularly in terms of phosphorus-poor elephant
459 dung. In short, our results show that in this system, both the quantity and quality of the subsidies
460 change with the season.

461

462 The temporal dynamics of the subsidies are a distinctive feature of the system studied here. Dung
463 becomes subsidies when the water level rises and incorporates them, or when they are dropped
464 directly into the water. Therefore, the subsidies depend on pan hydrology. Pan-drying kinetics
465 depend on water volume, rainfall, and evaporation. According to these kinetics, large natural pans
466 may receive more subsidies than small ones that dry up more rapidly. The constant flushing of
467 artificial pans continues to attract animals, and the pans and their eulittoral zone receive dung
468 throughout the year. In natural and artificial pans, dung that accumulates in the eulittoral zone is
469 mixed in the water when the rains return. The subsidies are thus temporally available. The final

470 concentration of nutrients brought by these subsidies in pans will depend on the water volume and
471 surface of the eulittoral zone in which the dung has accumulated. A large surface area means a large
472 amount of dung, given that the pan will attract many species. However, this also implies a high water
473 volume during the rainy season and thus a large dilution compared to small pans. It is therefore
474 difficult to predict the final subsidy concentration without an accurate knowledge of pan hydrology.
475 This is particularly true for artificial pans, which may be filled with an accumulation of organic matter
476 and must be regularly scooped (Florence D. Hulot, personal observation).

477

478 Providing water from the water table during the dry season may increase water salinity and
479 conductivity from saltwater (Borrok and Engle 2014; Msiteli-Shumba et al. 2018), while the loss of
480 temporary pans endangers species adapted to these ecosystems (Blaustein and Schwartz 2001). The
481 enrichment of pans and the necessity to scoop them add to the side effects of surface-water
482 provision as a management tool in semi-arid areas.

483

484

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494

495

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645

646

647 **Table 1. Results of the linear models on nutrient concentrations and nutrient ratios in dung as a**
 648 **function of guilds and seasons.** For each variable, the table gives the best model, adjusted r^2 ,
 649 degrees of freedom (DF), F-value, and p -value. The guilds are browsers and grazers, and the seasons
 650 are wet and dry.

Nutrient or ratio	Model	Adjusted r^2	DF	F-value	p-value
Carbon	Guild + season	0.27	2, 68	26.81	<0.0001
Nitrogen	Guild	0.01	1, 69	0.27	0.61
Phosphorus	Guild + season	0.23	2, 68	11.67	<0.0001
Carbon:Nitrogen	Season	0.02	1, 69	2.111	0.15
Carbon:phosphorus	Guild * season	0.32	3, 67	12.03	<0.0001
Nitrogen:phosphorus	Guild + season	0.54	2, 68	41.75	<0.0001

651
 652
 653

654 **Table 2. Results of the linear models on nutrient leaching as a function of guilds and time.** For each
655 variable, the table gives the best model, adjusted r^2 , degrees of freedom (DF), F-value, and p -value.

656

Nutrient	Model	Adjusted r^2	DF	F-value	p-value
Ammonium	Guild	0.13	1, 48	8.22	<0.01
Nitrate	Guild	0.12	1, 48	7.194	0.01
Phosphorus	Guild	0.02	1, 48	0.012	0.91

657

658

659 **Table 3. Excitation-emission matrix peaks and hypothetical identity of the components.** N:
 660 component number of occurrence. NADH: nicotinamide adenine dinucleotide with hydrogen; FAD:
 661 flavin adenine dinucleotide.

662

	N	λ_{ex} (nm)	λ_{em} (nm)	Component
1	1	275.0	313.0	Tyrosin and tryptophan-like
2	5	335.0	437.2	Humic acid-like and NADH
3	13	416.1	636.5	Porphyrin-like
4	48	427.6	473.4	Carotenes and vitamin A
5	50	500.0	533.2	FAD, flavonols, lipids
6	4	500.0	647.5	Porphyrin-like

663

664

665 **Table 4. Contribution in percentage of the total response of compounds 1 to 6 for the 3 time points**
 666 **for each sample identified according to the season and species.**

	Species	t1						t2						t3					
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Dry	Buffalo	0	0	0	44.9	55.1	0	0	0	0	37.3	62.7	0	0	0	0	43.6	56.4	0
	Cow	0	9.5	0	54.3	36.2	0	0	0	0	52.8	41.9	5.3	0	0	0	58.3	41.7	0
	Cow	0	0	0	47.2	52.8	0	0	0	0	62.7	37.3	0	0	0	0	52.3	47.7	0
	Donkey	0	0	0	58.8	41.2	0	0	0	0	54.7	45.3	0	0	0	0	54.5	45.5	0
	Elephant	0	0	16.6	60	23.4	0	0	0	0	66.6	33.4	0	0	0	0	74.9	25.1	0
	Elephant	0	0	0	56.1	43.9	0	0	0	0	58.5	41.5	0	0	0	0	56.5	43.5	0
	Elephant	0	0	0	55	45	0	0	0	0	51.4	48.6	0	0	0	0	59.3	40.7	0
	Elephant	0	0	0	100	0	0	0	0	0	58.8	47.4	0	0	0	0	100	0	0
	Elephant	0	0	0	53.2	46.8	0	0	25	0	40.6	34.4	0	0	0	0	74.6	25.4	0
	Elephant	21.2	0	0	38.1	40.7	0	0	0	0	100	0	0	0	0	9.7	70	20.3	0
	Giraffe	0	0	0	100	0	0	0	0	0	60.9	39.1	0	0	0	0	59.5	40.5	0
	Zebra	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0	100	0
	Zebra	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0	100	0
Wet	Buffalo	0	0	36.5	31	32.5	0	0	0	0	37.3	62.7	0	0	0	21	45.5	20.1	13.4
	Elephant	0	0	29.8	43.6	26.6	0	0	0	28.4	42.5	29.1	0	0	0	28.8	40.9	30.3	0
	Elephant	0	0	37.7	27.1	35.2	0	0	7	0	38.2	54.8	0	0	13.4	0	46.4	40.2	0
	Elephant	0	0	22.5	43.8	19.2	14.5	0	0	37.1	29.9	33	0	0	24.9	0	0	75.1	0
	Zebra	0	0	36.1	35.4	28.5	0	0	0	31.7	35.9	32.4	0	0	0	27.2	26.1	25.1	21.6

667

668

669 **Figures**

670 **Figure legends**

671 **Figure 1. A pan during the dry season.** The dotted double arrow shows the eulittoral zone. The solid
672 double arrow shows the 1 m wide strip around the pan that was sampled for dung during the dry
673 season. The white arrows point to undamaged and trampled elephant dung.

674

675 **Figure 2. Dung nutrient contents and nutrient ratios according to guilds and seasons.** a. Carbon
676 content; b. nitrogen content; c. phosphorus content; d. carbon-to-nitrogen ratio; e. carbon-to-
677 phosphorus ratio; f. nitrogen-to-phosphorus ratio. Dark bars: dry season; light bars: wet season. The
678 statistical results are given for the best linear model (guild + season for carbon, phosphorus
679 contents and nitrogen:phosphorus ratio and guild*season for carbon:phosphorus ratio).
680 Significance codes: $p < 0.001$: “***”.

681

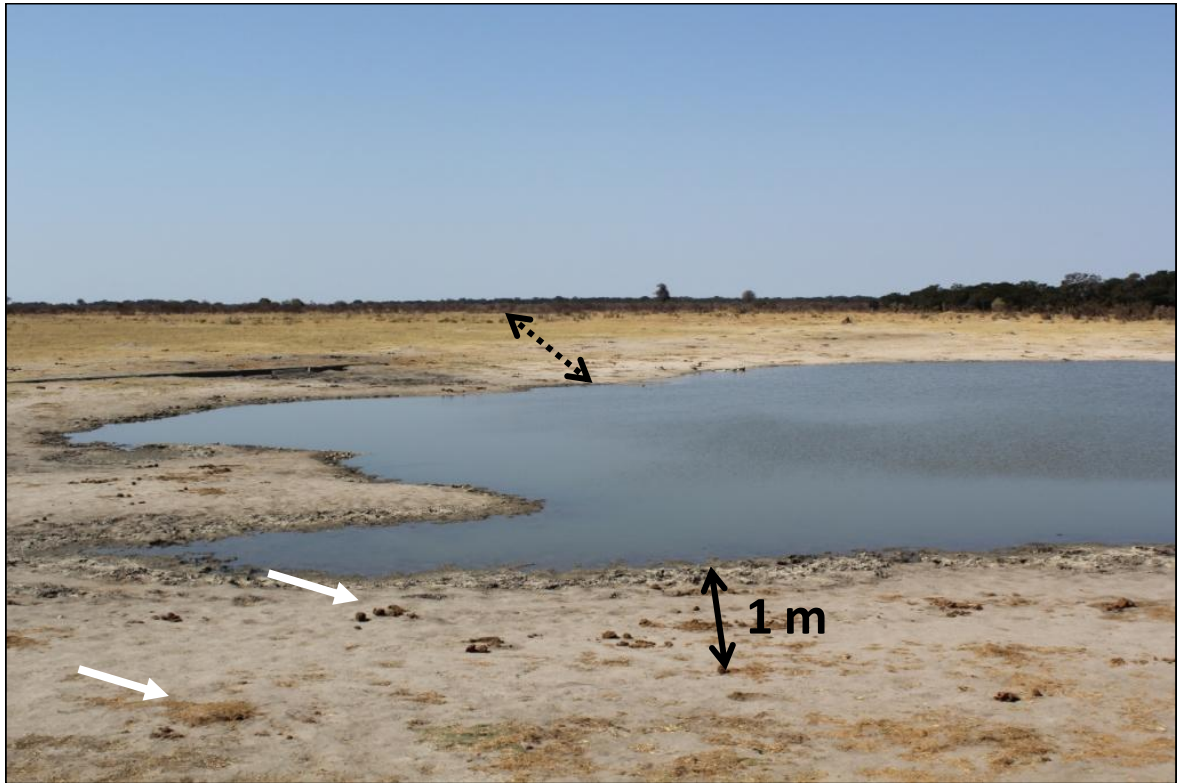
682 **Figure 3. Dung nutrient leaching rate according to guilds.** a. Ammonium concentration; b. nitrate
683 concentration; c. phosphorus concentration. There are no significant effects for time. Significance
684 codes: $0.001 < p < 0.01$: “***”; $0.01 < p < 0.05$: “*”.

685

686 **Figure 4. Results of multiple factorial analysis on coloured dissolved organic matter leaching.** a.
687 Compounds found in water as a function of excitation and emission wavelength. The black dot, red
688 square, blue dot, green triangle, grey diamond, and yellow star represent compounds 1 to 6,
689 respectively. b. Individual factor map in the first and second dimensions where the open and closed
690 symbols represent the wet and dry seasons, respectively. Caption: species symbols. c. Group
691 representations where t1, t2, and t3 represent the time point group. Species and seasons are the

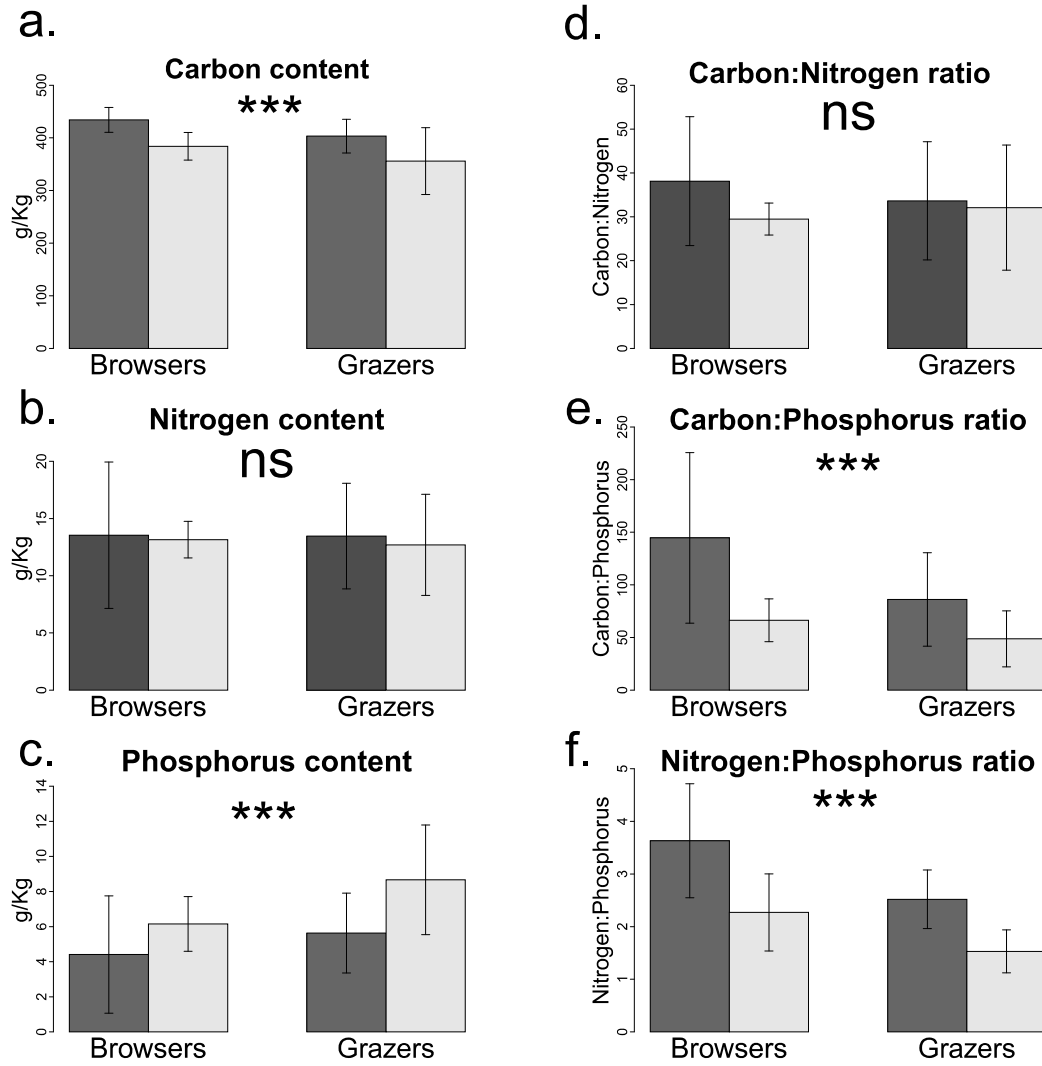
692 qualitative supplementary variables. d. Individual factor map in the first and third dimensions. See
693 the text for further details.
694

695 **Figure 1**
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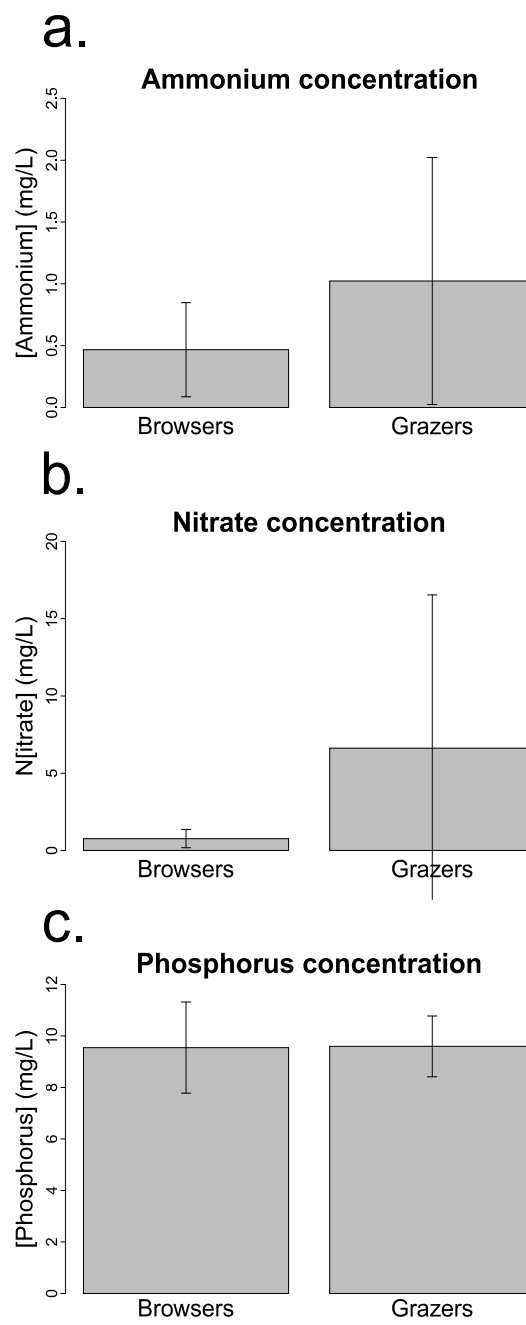
702 **Figure 2**
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708 **Figure 3**

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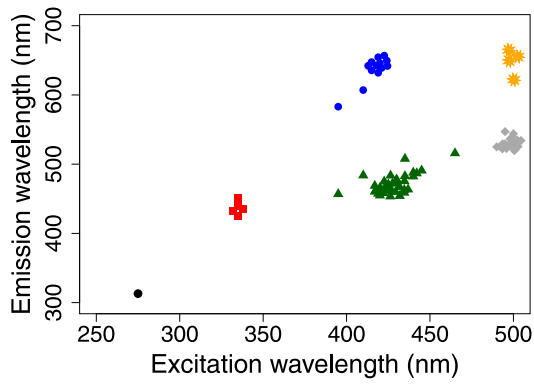
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714 **Figure 4.**

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717 a.



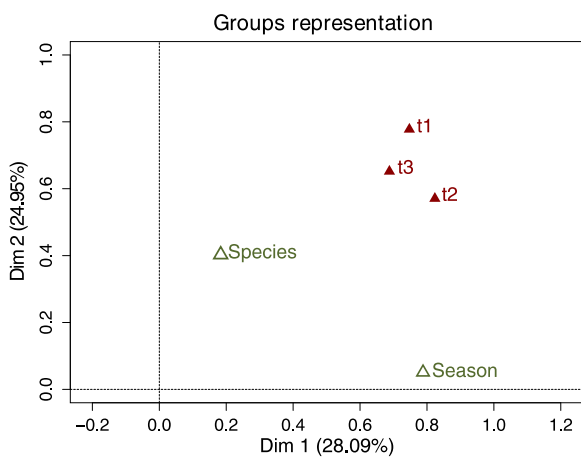
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c.



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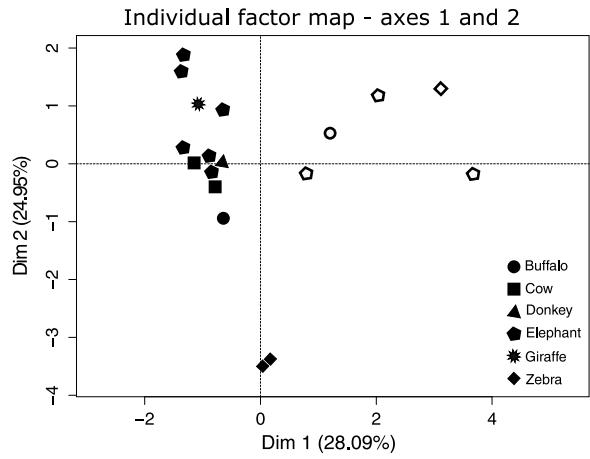
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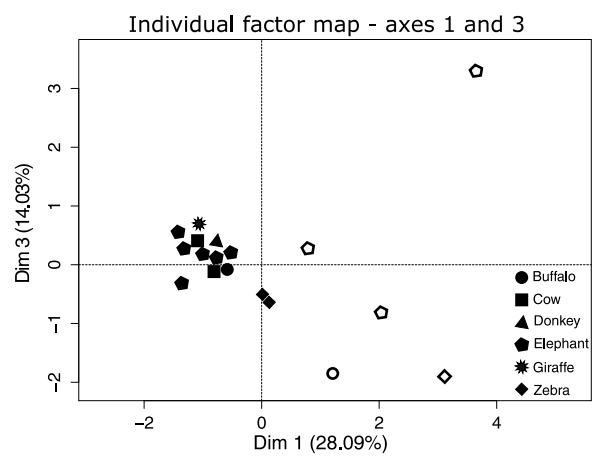
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b.



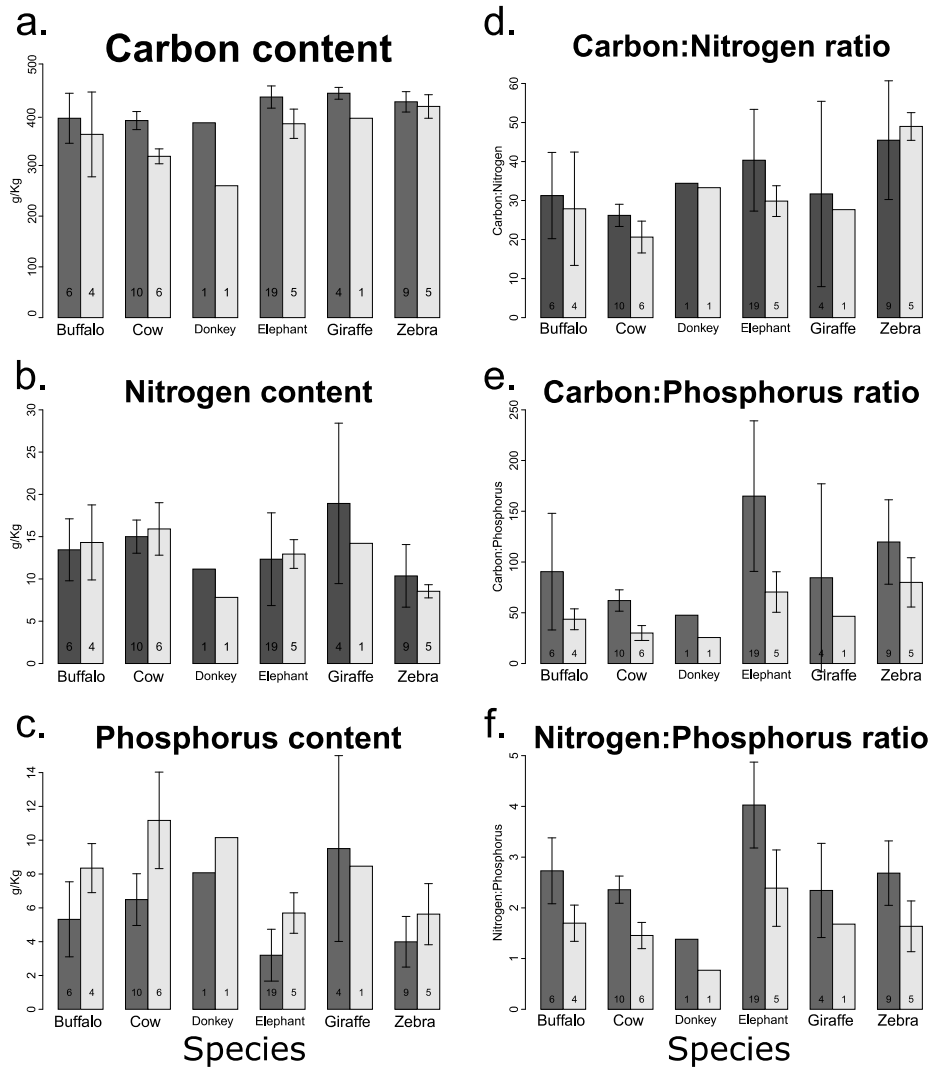
d.



727

Appendix

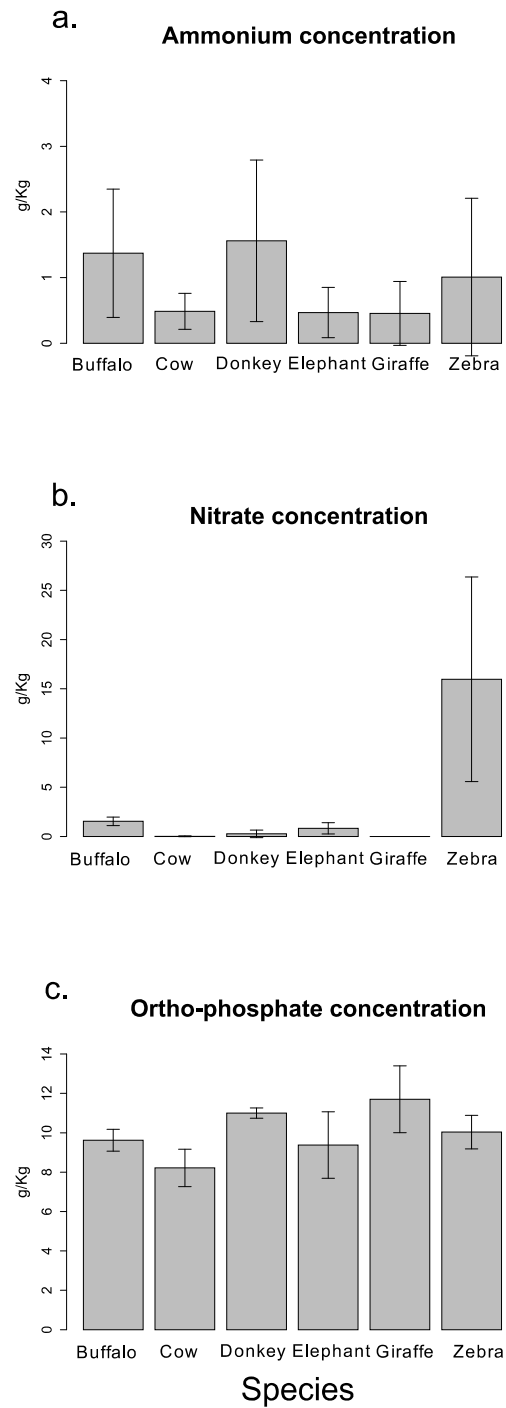
728 **Figure 1A. Dung nutrient contents and nutrient ratios according to species and seasons.** a. Carbon
729 content; b. nitrogen content; c. phosphorus content; d. carbon-to-nitrogen ratio; e. carbon-to-
730 phosphorus ratio; f. nitrogen-to-phosphorus ratio. Dark bars: dry season; light bars: wet season. The
731 numbers in the bars correspond to the number of samples.



732

733

734 **Figure A2. Dung nutrient leaching rate according to species.** a. Ammonium concentration; b. nitrate
735 concentration; c. phosphorus concentration.



736

737

738 **Table A1. Comparison of the linear models on nutrient concentrations and nutrient ratios in dung**
 739 **as a function of guilds and seasons. Only significant models are shown. The best model is in bold.**

740

Nutrient or ratio	Model	DF	AIC
Carbon	Guild * season	5	725.3484
	Guild + season	4	723.4279
	Guild	3	742.4301
	Season	3	733.0074
Phosphorus	Guild * season	5	353.3271
	Guild + season	4	352.1731
	Guild	3	364.1287
	Season	3	354.9158
Carbon:phosphorus	Guild * season	5	778.1912
	Guild + season	4	778.4024
	Guild	3	789.3099
	Season	3	789.4132
Nitrogen:phosphorus	Guild * season	5	167.2580
	Guild + season	4	166.5873
	Guild	3	193.0952
	Season	3	193.6852

741

742

743

744 **Table A2. Comparison of the linear models on nutrient leaching as a function of guilds and time**

745

Nutrient	Model	DF	AIC
Ammonium	Guild * season	7	54.59159
	Guild + season	5	54.70016
	Guild	3	53.75769
	Season	4	60.86247
Nitrate	Guild * season	7	180.7183
	Guild + season	5	176.8023
	Guild	3	172.9792
	Season	4	181.8176
Phosphorus	Guild * season	7	193.6069
	Guild + season	5	190.1101
	Guild	3	188.1276
	Season	4	188.2698

746

747