



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

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Received: 14 August 2018 / Revised: 29 April 2019 / Accepted: 2 May 2019 / Published online: 14 May 2019
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Abstract The transfer of terrestrial organic matter by terrestrial wild and domestic animals when they urinate and defecate directly in Savanna Rivers has already been studied. However, the eulittoral zone around waterholes receives organic matter during the dry season, which is diluted in the ecosystem when the waterholes returns to its wet season level. In our study, we evaluated this pathway of subsidies in Hwange National Park, Zimbabwe, by estimating dung density in the eulittoral zone at the peak of the dry season. We

also collected dung from different herbivore species during the dry and wet seasons to measure nutrient content and estimate nitrogen and phosphorus leaching rates. Our results show a decrease in carbon:phosphorus and nitrogen:phosphorus ratios in the dry season compared to the wet season. During the dry season, the deposition of total carbon, nitrogen, and phosphorus, mostly due to elephants, is estimated to be 8.65, 0.25, and 0.06 g/m²/day, respectively, while the leaching rates of dissolved inorganic nitrogen and phosphorus are 1.52 mg and 6.59 mg m²/day, respectively. No specific coloured dissolved organic matter signature for dung was identified. We discuss the temporal dynamics of the subsidies as a distinctive feature of the system.

Handling editor: Chris Joyce

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10750-019-3968-x>) contains supplementary material, which is available to authorized users.

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Keywords Eutrophication · Water level fluctuation · Water management · Browsers · Grazers

Introduction

Terrestrial-aquatic subsidies are of major importance for the functioning of aquatic ecosystems. Carbon fluxes from terrestrial to aquatic ecosystems are estimated to be ca. 1.9 billion (10^9) tonnes per year (Cole et al., 2007). Several aquatic systems such as small rivers and oligotrophic lakes depend on these allochthonous inputs to sustain aquatic life (Cole et al., 2006; Rubbo et al., 2006). For other systems, terrestrial inputs contribute significantly to matter and energy fluxes, and in extreme cases, they may dramatically modify the trophic status and functioning of aquatic systems (Chaichana et al., 2010; Earl & Semlitsch, 2013; Sparber et al., 2015; Holgerson et al., 2016; Dutton et al., 2018). For these reasons, continuous efforts have been made to assess terrestrial subsidies to aquatic ecosystems. In a recent study, Gounand et al. (2018) synthesised the spatial flows of carbon connected ecosystems. The median carbon flow from forests and grasslands to lakes and streams due to plants, litter, insects, and amphibians amounted to $164 \text{ g C/m}^2 \text{ year}$, while the flow in the other direction due to insects, fish, and amphibians amounted to $3.27 \text{ g C/m}^2 \text{ year}$. The flow from terrestrial to freshwater ecosystems was three times higher than the gross primary production in freshwater, estimated at $54.6 \text{ g C/m}^2 \text{ year}$ (Gounand et al., 2018).

Terrestrial organic matter enters water through runoff or its active transport by wild and domestic animals. The amount and timing of subsidies brought by wild animals depend on the nature of their activities linked to water (i.e. ontogenetic habitat switch, migration, feeding) (Subalusky & Post, 2019). For instance, wood frogs have biphasic life cycles: they use the aquatic environment for larval development and the terrestrial environment in adult life. When the adults return to breed in wetlands, they provide subsidies to the aquatic ecosystems with carbon, nitrogen, and phosphorus, although the average net flux of these elements is smaller than the subsidies in terrestrial ecosystems related to adult emergence into the terrestrial ecosystems (Capps et al., 2015). Aquatic bird defecation in close vicinity to aquatic ecosystems

is a well-known source of nutrients, which depend on behavioural and migratory patterns, and can be large and highly variable (Post et al., 1998; Kitchell et al., 1999; Chaichana et al., 2010). Herbivores are also active carriers of nutrients when they urinate and defecate in water (Naiman & Rogers, 1997). In temperate ecosystems, different species of now-extinct megaherbivores may have played a dominant role in transferring organic matter from terrestrial to aquatic ecosystems (Moss, 2015). Doughty et al. (2013a, b) estimated and compared the current and former potential phosphorus transfers to aquatic ecosystems due to wild herbivorous mammals. The authors showed that the modern transfer of phosphorus represents 10% of the estimated former transfer in North America, 5% in South America, 45% in Australasia, 18% in Eurasia, and 62% in Africa (Doughty et al., 2013a, b; Moss, 2015). The higher ratio observed in Africa reveals the persistence of large herbivores that continue to subsidise aquatic ecosystems.

The impact and transfer of terrestrial organic matter to aquatic systems has been particularly well studied for megaherbivores in savannas (Gereta & Wolanski, 1998; Wolanski & Gereta, 1999; Masese et al., 2015; Subalusky et al., 2015, 2017; Dutton et al., 2018; Stears et al., 2018). In particular, hippos (*Hippopotamus amphibius* Linnaeus, 1758) forage in savanna grasslands and rest in aquatic ecosystems. This daily feeding migration represents a high input of organic matter as estimated by Subalusky et al. (2015) and causes downstream hypoxia with fish kills (Dutton et al., 2018). Hippo excretion and egestion into the Mara River, Kenya, accounted for 15% of dissolved organic carbon, 27% of total nitrogen, and 29% of total phosphorus loading for the upstream catchment (Subalusky et al., 2015). Masese et al. (2015) also estimated the terrestrial subsidies on the river food web in a section of the Mara River Basin that includes different densities of livestock and hippos. Notable shifts in the source of organic carbon in the river food web were observed according to site and season. For instance, the contribution of C4 producers (i.e. savanna grasses) to aquatic consumers increased along the gradient from the upper forested reaches to those reaches containing hippos, from 18 to 33% during the dry season and from 10 to 58% during the wet season (Masese et al., 2015). One extreme phenomenon is the migration of the Serengeti

wildebeest (*Connochaetes taurinus* Lichtenstein, 1812), as they cross the Mara River in the Maasai Mara National Reserve where massive drownings occur. Another study conducted by Subalusky et al. (2017) estimated the biomass inputs to be 1000 tonnes, representing 107 tonnes of carbon, 25 tonnes of nitrogen, and 13 tonnes of phosphorus. Soft tissues (muscles, viscera, and skin) are decomposed within 50 days, but bones slowly release nutrients over a duration of 7.4 years (Subalusky et al., 2017).

These studies conducted in savannas assessed direct inputs of terrestrial wild and domestic animals when they urinate and defecate in water, or drown in the case of the Serengeti wildebeest. The majority of these studies focus on rivers. In semi-arid ecosystems, the water level of waterholes, lakes, and rivers decreases as the dry season progresses. The area delimited by the maximum water level of the rainy season and the minimum water level of the dry season defines the eulittoral zone (Wetzel, 2001) (Fig. 1). All organic matter that accumulates in this open zone during the dry season will contribute to the nutrient budget of ecosystems when the waterhole returns to its wet season level. In Hwange National Park (HNP), Zimbabwe, wildlife gathers around waterholes during the dry season (Valeix, 2011), and dung accumulates in the eulittoral zone. Elephants represent more than 80–90% of the total biomass of large herbivores at the waterholes (Chamaillé-Jammes et al., 2008, 2016;

Valeix et al., 2007), and their density can range from ca. 75 individuals per day and per waterhole during a rainy year to ca. 350 individuals during a dry year (Valeix et al., 2007). These high densities of elephants suggest high subsidies to the small natural and artificial waterholes found in HNP, which are assessed in the present study. To reach this objective, we assessed dung density around the waterholes during the dry season. We also collected dung from different species of wild and domestic animals to measure their nutrient contents. Using leaching experiments, we then estimated the concentration of nitrogen and phosphorus released in the water. We took advantage of this experiment to look for coloured dissolved organic matter (CDOM) released in water by dung as well as any potential differences between species.

Materials and methods

Study area

Hwange National Park is situated in a semi-arid region in the north-west of Zimbabwe. The rainy season starts in November–December and finishes in March–April. The cold dry season continues until July followed by the hot dry season until November (Chamaillé-Jammes et al., 2006). The vegetation consists of mixed woodland and bushland on Kalahari sand

Fig. 1 A waterhole during the dry season. The dotted double arrow shows the eulittoral zone. The solid double arrow shows the 1-m wide strip around the waterhole that was sampled for dung during the dry season. The white arrows point to undamaged and trampled elephant dung



(Chamaillé-Jammes et al., 2007b). The natural waterholes found in this area are small depressions filled only by rainwater runoff; they are also called “pans” (Fynn et al., 2015) but we refer to them as waterholes. Artificial and natural waterholes are found in the park and adjacent forestry area. In the following, we thus distinguish between natural waterholes, fed only by rainwater, and artificial waterholes, fed by both pumped groundwater and rainwater (Chamaillé-Jammes et al., 2007b). Natural waterholes retain water for only 3 to 6 months after the rainy season. However, the duration depends on their size, the rainfall, and the dryness. In artificial waterholes, water is pumped into a small open-top concrete drinking trough, which is situated at ground level and may be round or rectangular. Water then flows from the trough to the waterhole. The pumps are either solar- or diesel-powered. The former only works during the day, while the latter may run out of petrol. To our knowledge, there are no systematic records on pumping efforts or the presence or absence of water during the dry season. It is therefore difficult to assess artificial water input during the dry season and, more generally, the hydrology of waterholes. During the dry season, artificial waterholes may dry out within 48 h (Chamaillé-Jammes et al., 2016). The water depth in waterholes does not exceed 5 m, while the surface area of permanent waterholes in 2014 ranged from 453 m² in the dry season to 26,788 m² in the rainy season.

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

Estimation of dung density around the waterholes and their composition

To estimate the amount of dung deposited around the waterholes, we monitored three artificial waterholes (Caterpillar, Dom, and Safari Lodge) in November 2015. On the first day, we cleaned a 1-m wide band around the waterhole (Fig. 1). All dung found in this area was moved aside. We also measured the surface of the waterhole as well as the waterhole along with the sampled area by walking around with a Garmin GPSMAP 64; the sampled area was the difference

between the two surfaces. On the second, third, and fourth days, all dung found in this area was weighed and moved aside. We included in this census easily recognisable dung that had been trampled by elephants (Fig. 1). However, in this 1-m wide band, we only found elephant dung. To estimate the amount of dung deposited in this area, we reported the mass in the sampled surface area per waterhole and per day.

Estimation of dung composition during the dry and wet seasons

To estimate the carbon, nitrogen, and phosphorus content of dung and explore whether the composition varied with the seasons and species, we collected dung from different individuals in November 2013, August, October, and November 2014 for the dry season and February and March 2014 for the wet season. We analysed the dung collected during the rainy and dry seasons to detect any differences that would reveal qualitative changes in subsidies. The dung was collected within a radius of 5 m from the waterholes or reservoirs in the park and communal areas. It was then stored in Manila envelopes and dried at ambient temperature. Large dung (i.e. elephants) was broken to speed up the drying process and avoid fermentation. In total, we collected samples from 10 African buffalos (*Syncerus caffer* Sparrman, 1778) (6 dry season/4 wet season), 16 cows (*Bos taurus taurus* Linnaeus, 1758) (10 dry/6 wet), 2 donkeys (*Equus africanus* Heuglin & Fitzinger, 1866) (1 dry/1 wet), 27 elephants (*Loxodonta africana* Cuvier, 1825) (22 wet/5 dry), 5 giraffes (*Giraffa camelopardis* Linnaeus, 1758) (4 dry/1 wet), 12 Burchell's zebras (*Equus quagga* Boddaert, 1785) (7 dry/5 wet), and 2 Cape bushbucks (*Tragelaphus sylvaticus* Pallas, 1766) (1 dry/1 wet). The samples were crushed and then analysed. Organic carbon concentration was measured with the high-temperature combustion method, nitrogen with the Dumas method, and phosphorus with the Olsen method. For each sample, we calculated the carbon:nitrogen (C:N), carbon:phosphorus (C:P), and nitrogen:phosphorus (N:P) ratios.

Dung leaching rates and CDOM

To assess the ammonium, nitrate, and phosphorus leaching rates of dung, we added 2 g of uncrushed dung to 200 mL of deionised water in a 250-mL glass

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

CDOM analyses

Dung releases dissolved organic matter (DOM) into water with nutrients. The optically active part of DOM is known as coloured dissolved organic matter (CDOM). CDOM in water encompasses thousands of compounds (Coble, 2007) that may affect ecosystem processes through light attenuation and nutrient availability (Ishii & Boyer, 2012). For instance, CDOM absorption is several times that of chlorophyll in many coastal areas (Coble, 2007). Monitoring CDOM and their fluctuations allows the study of factors such as land use and climate change, as they affect spatial and temporal variations in CDOM (Ishii & Boyer, 2012). Here, we study CDOM released by dung in order to track the origin of CDOM found in waterholes.

CDOM can be quantified and identified using fluorescence excitation–emission matrices (Stedmon et al., 2003). When excited, a molecule absorbs light at a given wavelength and fluoresces at another wavelength. The position of these two wavelengths and the intensity of responses allow the molecule to be

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

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

For each excitation wavelength, the corresponding emission spectra were decomposed by an expectation–maximization algorithm into an optimised distribution of up to five randomly spaced Gaussian functions. Finally, the optimum composition of the EEM was obtained by grouping all Gaussian functions of one EEM into components. This final step was achieved by an unsupervised procedure based on fuzzy logic criteria that converges to a solution when the number

of components is as small as possible, while satisfying a criterion of mono-modality for each component.

Statistical analyses

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

To analyse the diversity of the CDOM components released by dung as a function of season and species, we used multiple factorial analysis (MFA) on a contingency table (Escofier & Pages, 1994). We explored whether the compounds differed between species and seasons, and whether the identity of the compounds changed during the course of the experiment. The data set consisted of the frequency of each compound for each species and at each time point. The three time points of the experiment were included in the analysis as three groups, which allowed the time effect to be studied. MFA permits the use of qualitative and quantitative variables as well as supplementary variables. The latter do not contribute to the construction of the MFA dimensions, but their correlations to the dimensions can be computed, thus contributing to the interpretation of the dimensions. Consequently, species and season were included in the MFA as qualitative supplementary variables, and all other parameters as quantitative variables. Numerical analysis was carried out using software R (R Development Core Team, 2008), version 3.1.1. MFA was conducted using the FactoMineR package (Husson et al., 2015), version 1.29.

Results

Dung density around the waterholes and nutrient composition

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

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

As we found only elephant dung in the waterhole vicinity during the three consecutive days of the census, we could only estimate carbon, nitrogen, and phosphorus deposition in the eulittoral zone for this species in the dry season. Mean carbon, nitrogen, and

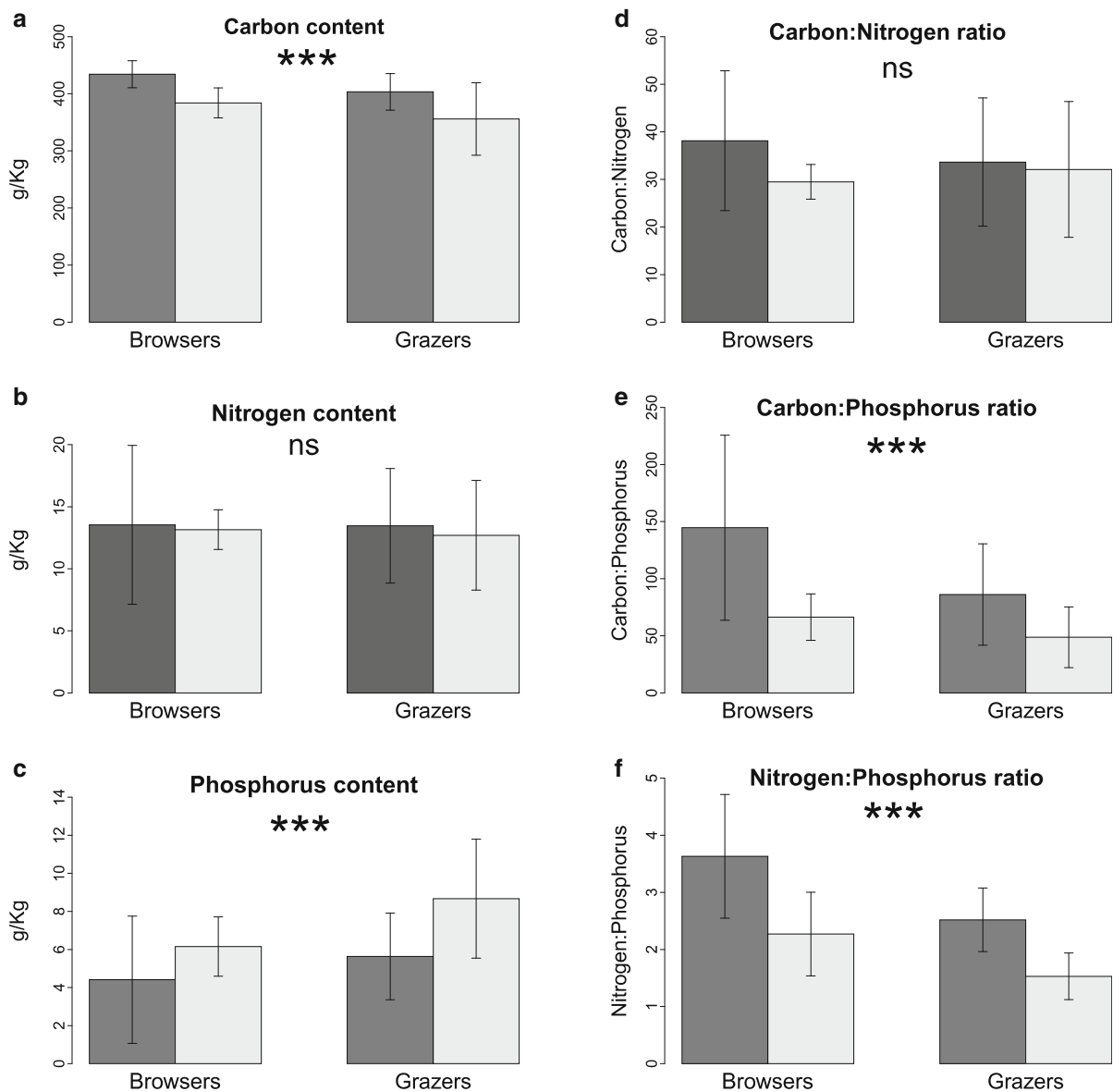


Fig. 2 Dung nutrient contents and nutrient ratios according to guilds and seasons. **a** Carbon content, **b** nitrogen content, **c** phosphorus content, **d** carbon-to-nitrogen ratio, **e** carbon-to-phosphorus ratio, **f** nitrogen-to-phosphorus ratio. Dark bars: dry season; light bars: wet season. The statistical results are given

for the best linear model (guild + season for carbon, phosphorus contents and nitrogen:phosphorus ratio and guild*season for carbon:phosphorus ratio). Significance codes: $P < 0.001$: “***”

phosphorus depositions were respectively $8.00 \text{ g C/m}^2/\text{day}$ (from a minimum of 0.35 to a maximum of $36.9 \text{ g C/m}^2/\text{day}$), $0.24 \text{ g N/m}^2/\text{day}$ (from 0.007 to $2.20 \text{ g N/m}^2/\text{day}$), and $0.07 \text{ g P/m}^2/\text{day}$ (from 0.001 to $0.58 \text{ g P/m}^2/\text{day}$).

Nutrient leaching and estimation of nutrient loading rates

Ammonium, nitrate, and phosphorus leaching did not change over time: the same concentrations were measured in water after 24 h, 48 h, and 72 h. This absence of time effect suggests that the nutrients

Table 1 Results of the linear models on nutrient concentrations and nutrient ratios in dung as a function of guilds and seasons

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

For each variable, the table gives the best model, adjusted r^2 , degrees of freedom (DF), F value, and P value. The guilds are browsers and grazers, and the seasons are wet and dry

released by dung were taken up by microorganisms in water as they were produced. An alternative hypothesis is that all the labile C, N, and P leached out within 24 h, and the remaining material needed to be mineralised. These concentrations differed according to the guilds for ammonium and nitrate (Fig. 3, Table 2). Indeed, ammonium and nitrate concentrations measured in water were significantly lower with browsers. There was no effect for phosphorus concentration.

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

Composition of CDOM

The analysis of CDOM allowed us to identify six compounds characterised by their excitation and emission wavelengths (Fig. 4a, Table 3). The first, identified in elephant dung collected in the dry season and present on t1 of the experiment (Table 4), corresponds to a tyrosine- and tryptophan-like compound (Chen et al., 2003; Ramanujam, 2006), which is an amino acid derived from proteins. The second component found in five samples from a cow and elephants at the three different time points (Table 4)

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

The first two dimensions of the MFA explain 53.04% of the variability (Fig. 4b). The first dimension is defined on the left side by component 4 at the three time points (negative correlation to the first dimension with $P < 0.02$ on t1 and $P < 0.006$ on t2 and t3), and on the right side by component 2 on t3, component 3 on t2 and t3, and component 6 on t1 (positive correlation to the first dimension with $P < 0.005$). Dung collected during the wet season are all characterised by component 3 on t1, and to a lesser extent, on t2, component 2 on t3, and component 6. The wet season is significantly correlated with the first dimension ($P < 0.001$, Fig. 4c), which clearly differentiate dung from the dry and wet seasons (Fig. 4b). The second dimension is characterised on

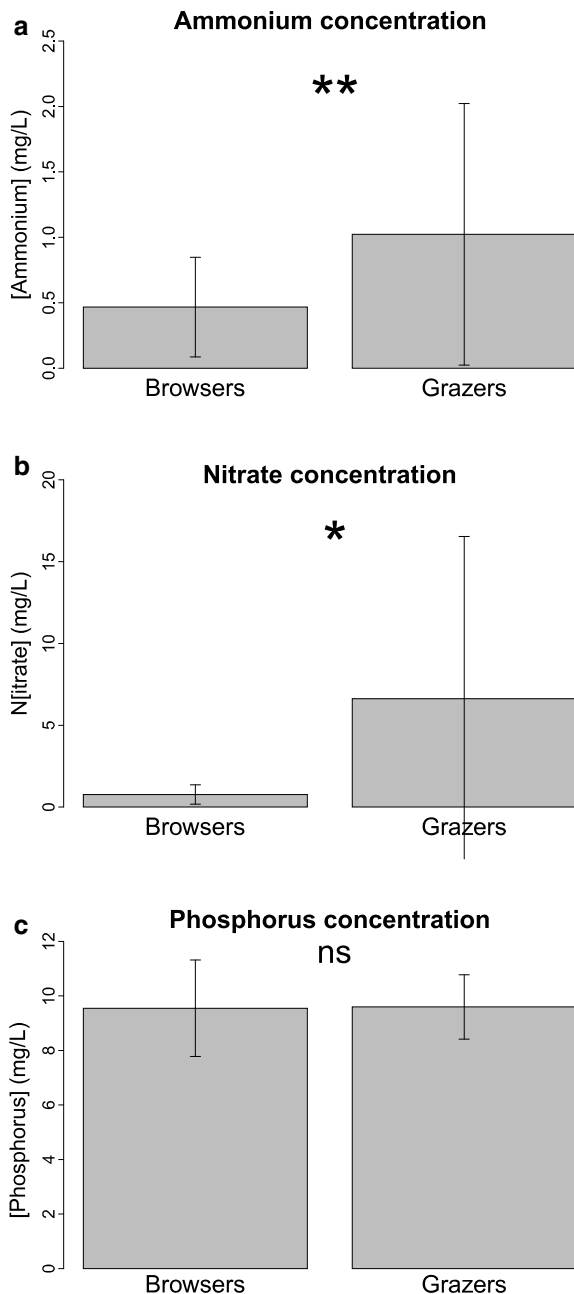


Fig. 3 Dung nutrient leaching rate according to guilds. **a** Ammonium concentration, **b** nitrate concentration, **c** phosphorus concentration. There are no significant effects for time. Significance codes: $0.001 < P < 0.01$: “***”; $0.01 < P < 0.05$: “**”

the lower side by component 5 at the three time points (negative correlation with $P < 0.001$) and on the upper side by component 4 at the three time points and component 3 on t3 (positive correlation with

Table 2 Results of the linear models on nutrient leaching as a function of guilds and time

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

For each variable, the table gives the best model, adjusted r^2 , degrees of freedom (df), F value, and P value

$P < 0.03$). This dimension mostly separates the dung of two zebras in the dry season from the other species, as it only released compound 5 at the three time points. The group representation shows that the dung is separated on the first dimension by the season in which they were collected, and to a lesser extent, on the second dimension by the species identity. This is mostly due to the zebra, which is negatively correlated with the second dimension ($P < 0.03$).

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

Discussion

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

Seasonal effects on dung nutrient and CDOM composition

Our analyses reveal changes in dung nutrient composition between the wet and dry seasons. Indeed, phosphorus content is lower in the dry season, while carbon content is higher. This is reflected in the C:P

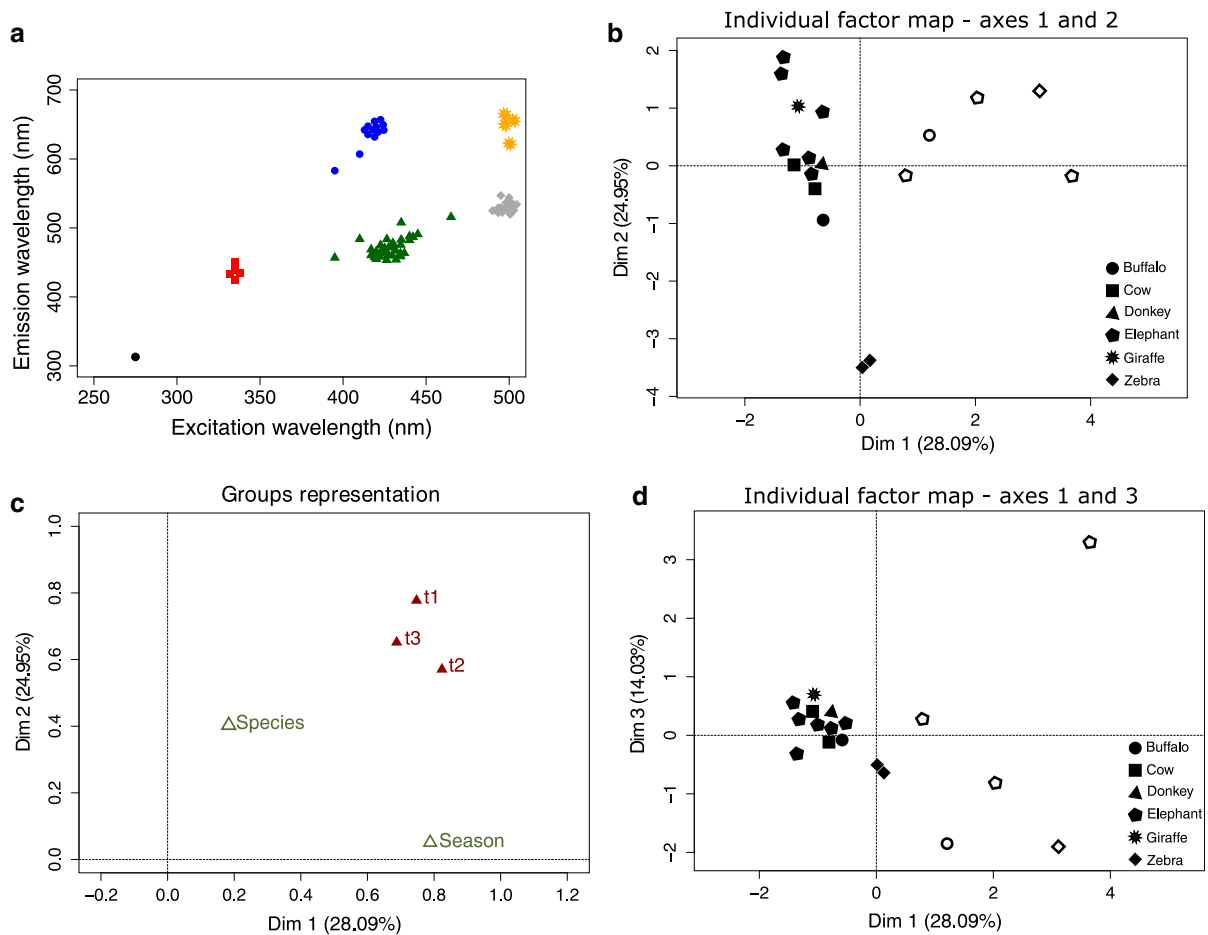


Fig. 4 Results of multiple factorial analysis on coloured dissolved organic matter leaching. **a** Compounds found in water as a function of excitation and emission wavelength. The black dot, red square, blue dot, green triangle, grey diamond, and yellow star represent compounds 1 to 6, respectively. **b** Individual factor map in the first and second dimensions where the open

and closed symbols represent the wet and dry seasons, respectively. Caption: species symbols. **c** Group representations where t1, t2, and t3 represent the time point group. Species and seasons are the qualitative supplementary variables. **d** Individual factor map in the first and third dimensions. See the text for further details

Table 3 Excitation–emission matrix peaks and hypothetical identity of the components

	<i>N</i>	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

N component number of occurrence. *NADH* nicotinamide adenine dinucleotide with hydrogen, *FAD* flavin adenine dinucleotide

and N:P ratios, which both reveal the winter decrease in phosphorus with carbon enrichment. These effects are especially clear for browsers. Grant et al. (1995) showed a seasonal effect of faecal nitrogen and phosphorus concentrations for several herbivores in Kruger National Park, South Africa. Faecal nitrogen and phosphorus concentrations were lower in the dry season than in the wet season. In their study, faecal nitrogen concentration was higher for browsers than for grazers, while phosphorus content was lower for browsers than for grazers. Dung nutrient content results from the resource choice and digestion process. Therefore, dung nutrient content may reveal food quality (Grant et al., 1995; Wrench et al., 1997) with a

Table 4 Contribution in percentage of the total response of compounds 1 to 6 for the 3 time points 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

lower quality during the dry season. The nitrogen concentrations of browsers' dung revealed in our study are in line with the results of Grant et al. (1995), although our phosphorus concentrations are generally higher. This finding is not surprising, because browsers feed on plants that maintain their nutritive value, whereas grazers feed on grass that loses its nutritive value when maturing or becoming dormant during the dry season (Grant et al., 1995).

The composition of CDOM released in water by dung also differs between the dry and wet seasons. The differences essentially relate to the presence of pigment residuals, because the dung collected during the wet season is richer than dung collected during the dry season. In addition to carotenes, vitamin A, FAD (flavin adenine dinucleotides), flavonols, and lipids, wet-season dung has porphyrin-like and humic acid-like components as well as NADH (nicotinamide adenine dinucleotide). This change in pigment residuals may reveal variations in food quality or fauna

diet. There was no species effect on CDOM composition except for zebras during the dry season. These results suggest that even if the different species feed on diverse vegetal sources, the digestion process makes any CDOM differences disappear. Zebra dung collected during the dry season is characterised by component 5 alone, namely FAD, flavonols, and lipids. This difference may indicate changes in zebra diet or the digestive process, or even a sampling effect. However, this analysis relies on a limited number of samples (i.e. few individuals), meaning that these conclusions should be considered with caution. The compounds revealed here might echo diet preferences and individual constraints on nutrition. Our results show that this approach is promising.

CDOM identification

In our short-term experiment, the CDOM analysis reveals unexpected results. The protein-like (tyrosine-

and tryptophane-like) and humic-like substances are the two key fluorescent components found in CDOM decomposition (Coble, 1996; Chen et al., 2003; Stedmon et al., 2003; Hudson et al., 2007). They are respectively identified as soluble microbial and plant structural tissue decomposition by-products, with the latter being recalcitrant DOM (Chen et al., 2003). These CDOM have a negative effect on water transparency and may affect photosynthesis in tropical lakes (Mello Brandao et al., 2018). Here, protein- and humic-like compounds correspond to components 1 and 2, respectively, but they were rarely found in our experiment. One cow (dry season) and four elephants (wet and dry seasons) produced these compounds. As the experiment only lasted 3 days, we were unable to observe the production and accumulation of these by-products, as usually observed in marine and freshwater. However, we measured other components (3 to 6) that are rarely seen in natural water. These pigments and derivatives are easily broken down to colourless products via different intermediate steps for chlorophyll *a* (Moss, 1968; Kogel-Knabner, 2002). Consequently, they do not affect water transparency. All species produced these components with seasonal effects on their relative concentrations. These results suggest that a longer experiment is necessary in order to identify which species provide recalcitrant DOM that affects water transparency and then observe how this changes seasonally. Indeed, dung differs in material granulometry, and straw and woody parts are identified in elephant dung in particular. In this sense, it would be interesting to characterise dung with CDOM given its potential to affect water transparency and identify any potential species signatures.

Dung leaching and terrestrial subsidies

The mean direct deposition of total carbon, nitrogen, and phosphorus in waterhole eulittoral zones during the dry season is estimated to be 8.65, 0.25, and 0.06 g/m²/day, respectively, while the mean leaching rates of dissolved inorganic nitrogen and phosphorus are estimated to be 1.52 mg N/m²/day and 6.59 mg P/m²/day, respectively. The nutrient deposition estimated in our study is only a preliminary assessment, as we considered a low number of samples and only dung found across a 1-m wide surface around the shoreline. The dung deposition is not restricted to this strip but rather to the entire eulittoral zone at all times. During

the peak attendance, elephants wait in line around the waterholes, and we may hypothesise that dung deposition increases with waiting time. In addition, we might expect inter-annual variability in dung deposition, because livestock abundance around the waterholes is linked to rainfall: the drier the year, the higher the abundance of herbivores at waterholes (Chamaillé-Jammes et al., 2016). Between-waterhole differences may also be observed, as different herbivore species seem to prefer certain waterholes, probably due to the neighbouring vegetation (Chamaillé-Jammes et al., 2016), and at least for elephants recently, not because of their sodium concentration (Chamaillé-Jammes et al., 2007a). The leaching rates calculated here may be underestimated, because it was not possible to use bacterial communities from waterholes; we also used deionised water. The sole source of bacteria was found in the dung, and these bacterial communities are less adapted to break down dung than those found in waterholes.

Our results call for several comments. First, dissolved inorganic nitrogen and phosphorus represent 0.04% and 0.28% of the total nutrient deposition, respectively. Only a tiny fraction of the nutrients thus becomes available for aquatic microorganisms. This suggests that a larger part of the dung will decompose in water, particularly when the waterholes become inundated, while the remaining part will be buried in sediments. The leaching rates measured here are based on a short-term experiment with the calculations being made on the first day. Because the dung used in the experiment contains bacteria and other microorganisms, bacterial communities developed, took up nutrients, and modified their concentrations. Therefore, because the modification of nutrient leaching and the adaptation of bacterial communities in the environment are both unknown, it is difficult to assess how much dung will be mineralised in water or buried in the sediment. Second, the comparison of total nutrient deposition to published data shows it to be similar for phosphorus exports in agricultural area, whereas it is clearly inferior for total nitrogen (Wetzel, 2001). Chaichana et al. (2010) estimated the total nitrogen and phosphorus inputs related to birds to be 2 and 0.65 mg/m²/day, respectively, and associated them with the eutrophication of a small pool in Brown Moss, England. Elephant inputs in HNP are much higher but still well below hippo inputs because of the excretion and egestion, which respectively amount to

0.13–0.19 g N/kg hippo/day and 0.01–0.02 g P/kg hippo/day, as described by Subalusky et al. (2015). Hippos spent much more time in and around the waterholes than elephants did. The eutrophication of some waterholes in HNP has been documented with high concentrations of total nitrogen and phosphorus, ammonium, soluble reactive phosphorus, and chlorophyll *a* (Msiteli-Shumba et al., 2018). Cyanobacterial blooms were also reported in a few waterholes in April and November 2013 (Msiteli-Shumba et al., 2017, 2018). This eutrophication is likely due to terrestrial subsidies brought by elephants and, as noted above, these terrestrial subsidies are certainly underestimated.

Subsidy dynamics

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

The temporal dynamics of the subsidies are a distinctive feature of the system studied here. Dung becomes subsidies when the water level rises and incorporates them, or when they are dropped directly into the water. Therefore, the subsidies depend on waterhole hydrology. Waterhole-drying kinetics depend on water volume, rainfall, and evaporation. According to these kinetics, large natural waterholes may receive more subsidies than small ones that dry up more rapidly. The constant flushing of artificial waterholes continues to attract animals, and the waterholes and their eulittoral zone receive dung throughout the year. In natural and artificial waterholes, dung that accumulates in the eulittoral zone is mixed in the water when the rains return. The subsidies are thus temporally available. The final concentration of nutrients brought by these subsidies in waterholes will depend on the water volume and surface of the eulittoral zone in which the dung has accumulated. A large surface area means a large amount of dung, given that the waterhole will attract many species. However, this also implies a high water volume during the rainy season and thus a large dilution compared to small waterholes. It is therefore difficult to predict the final subsidy concentration without an accurate knowledge of waterhole hydrology. This is particularly true for artificial waterholes, which may be filled with an accumulation of organic matter and must be regularly dredged (Florence D. Hulot, personal observation).

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

Acknowledgements The Zimbabwe National Parks and Wildlife Management Authority, Forestry Commission, and Hwange traditional leadership granted access to the study sites. Special thanks go to the rangers, students, and volunteers who participated in the fieldwork, Zoran Cerovic for his help in identifying the CDOM compounds, and Emma Rochelle-Newall, Marion Valeix, Simon Chamaillé-Jammes, and Hervé Fritz for their comments on the initial draft of the manuscript. We are grateful to Chris Joyce and two anonymous reviewers for their helpful comments on an earlier version of this manuscript. This work was conducted within the framework of the Research

Platform “Production and Conservation in Partnership” (RP-PCP). We thank the Agence Nationale de la Recherche for their financial support via the SAVARID 2011 CEPL-003 project.

References

- Blaustein, L. & S. S. Schwartz, 2001. Why study ecology in temporary pools? *Israel Journal of Zoology* 47: 303–312.
- Borrok, D. M. & M. A. Engle, 2014. The role of climate in increasing salt loads in dryland rivers. *Journal of Arid Environment* 111: 7–13.
- Capps, K. A., K. A. Berven & S. D. Tiegs, 2015. Modelling nutrient transport and transformation by pool-breeding amphibians in forested landscapes using a 21-year dataset. *Freshwater Biology* 60: 500–511.
- Chaichana, R., R. Leah & B. Moss, 2010. Birds as eutrophication agents: a nutrient budget for a small lake in a protected area. *Hydrobiologia* 646: 111–121.
- Chamaillé-Jammes, S., H. Fritz & F. Murindagomo, 2006. Spatial patterns of the NDVI-rainfall relationship at the seasonal and interannual time scales in an African savanna. *International Journal of Remote Sensing* 27: 5185–5200.
- Chamaillé-Jammes, S., H. Fritz & R. M. Holdo, 2007a. Spatial relationship between elephant and sodium concentration of water disappears as density increases in Hwange National Park, Zimbabwe. *Journal of Tropical Ecology* 23: 725–728.
- Chamaillé-Jammes, S., H. Fritz & F. Murindagomo, 2007b. Climate-driven fluctuations in surface-water availability and the buffering role of artificial pumping in an African savanna: potential implication for herbivore dynamics. *Austral Ecology* 32: 740–748.
- Chamaillé-Jammes, S., H. Fritz, M. Valeix, F. Murindagomo & J. Clobert, 2008. Resource variability, aggregation and direct density dependence in an open context: the local regulation of an African elephant population. *Journal of Animal Ecology* 77: 135–144.
- Chamaillé-Jammes, S., A. Charbonnel, S. Dray, H. Madzikanda & H. Fritz, 2016. Spatial distribution of a large herbivore community at waterholes: an assessment of its stability over years in Hwange National Park, Zimbabwe. *Plos ONE*. <https://doi.org/10.1371/journal.pone.0153639>.
- Chen, W., P. Westerhoff, J. A. Leenheer & K. Booksh, 2003. Fluorescence excitation—emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science & Technology* 37: 5701–5710.
- Coble, P. G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. *Marine Chemistry* 51: 325–346.
- Coble, P. G., 2007. Marine optical biogeochemistry: the chemistry of ocean color. *Chemical Reviews* 107: 402–418.
- Cole, J. J., S. R. Carpenter, M. L. Pace, M. C. Van de Bogert, J. L. Kitchell & J. R. Hodgson, 2006. Differential support of lake food webs by three types of terrestrial organic carbon. *Ecology Letters* 9: 558–568.
- Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg & J. Melack, 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10: 171–184.
- Doughty, C. E., A. Wolf & Y. Malhi, 2013a. The impact of large animal extinctions on nutrient fluxes in early river valley civilizations. *Ecosphere* 4: 1–17.
- Doughty, C. E., A. Wolf & Y. Malhi, 2013b. The legacy of the *Pleistocene megafauna* extinctions on nutrient availability in Amazonia. *Nature Geoscience* 6: 761–764.
- Dutton, C. L., A. L. Subalusky, S. K. Hamilton, E. J. Rosi & D. M. Post, 2018. Organic matter loading by hippopotami causes subsidy overload resulting in downstream hypoxia and fish kills. *Nature Communications* 9: 1951.
- Earl, J. E. & R. D. Semlitsch, 2013. Spatial subsidies, trophic state, and community structure: examining the effects of leaf litter input on ponds. *Ecosystems* 16: 639–651.
- Escofier, B. & J. Pages, 1994. Multiple factor analysis (AFMULT package). *Computational Statistics and Data Analysis* 18: 121–140.
- Fynn, R. W. S., M. Murray-Hudson, M. Dhliwayo & P. Scholte, 2015. African wetlands and their seasonal use by wild and domestic herbivores. *Wetlands Ecology and Management* 23: 559–581.
- Gereta, E. & E. Wolanski, 1998. Wildlife-water quality interactions in the Serengeti National Park, Tanzania. *African Journal of Ecology* 36: 1–14.
- Gounand, I., C. J. Little, E. Harvey & F. Altermatt, 2018. Cross-ecosystem carbon flows connecting ecosystems worldwide. *Nature Communications* 9: 4825.
- Grant, C. C., H. H. Meissner & W. A. Schultheiss, 1995. The nutritive value of veld as indicated by faecal phosphorus and nitrogen and its relation to the condition and movement of prominent ruminants during the 1992–1993 drought in the Kruger National Park. *Koedoe* 38: 17–31.
- Holgerson, M. A., D. M. Post & D. K. Skelly, 2016. Reconciling the role of terrestrial leaves in pond food webs: a whole-ecosystem experiment. *Ecology* 97: 1771–1782.
- Hudson, N., A. Baker & D. Reynolds, 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. *River Research and Applications* 23: 631–649.
- Husson, F., J. Josse, S. Le & J. Mazet, 2015. FactoMineR: multivariate exploratory data analysis and data mining. R package version 1: 29.
- Ishii, S. K. L. & T. H. Boyer, 2012. Behavior of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review. *Environmental Science & Technology* 46: 2006–2017.
- Kang, C., H.-L. Wu, C. Zhou, S.-X. Xiang, X.-H. Zhang, Y.-J. Yu & R.-Q. Yu, 2016. Quantitative fluorescence kinetic analysis of NADH and FAD in human plasma using three- and four-way calibration methods capable of providing the second-order advantage. *Analytica Chimica Acta* 910: 36–44.
- Kitchell, J. F., D. E. Schindler, B. R. Herwig, D. M. Post, M. H. Olson & M. Oldham, 1999. Nutrient cycling at the landscape scale: the role of diel foraging migrations by geese at the Bosque del Apache National Wildlife Refuge, New Mexico. *Limnology and Oceanography* 44: 828–836.
- Kogel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology & Biochemistry* 34: 139–162.

- Masese, F. O., K. G. Abrantes, G. M. Gettel, S. Bouillon, K. Irvine & M. E. McClain, 2015. Are large herbivores vectors of terrestrial subsidies for riverine food webs? *Ecosystems* 18: 686–706.
- Mello Brandao, L. P., L. S. Brighenti, P. A. Staehr, E. Asmala, P. Massicotte, D. Tonetta, F. A. Rodrigues Barbosa, D. Pujoni & J. F. Bezerra-Neto, 2018. Distinctive effects of allochthonous and autochthonous organic matter on CDOM spectra in a tropical lake. *Biogeosciences* 15: 2931–2943.
- Moss, B., 1968. Studies on the degradation of chlorophyll a and carotenoids in freshwaters. *New Phytologist* 67: 49–59.
- Moss, B., 2015. Mammals, freshwater reference states, and the mitigation of climate change. *Freshwater Biology* 60: 1964–1976.
- Msiteli-Shumba, S. M., S. Kativu & F. D. Hulot, 2017. Influence of environmental variables on plankton community composition in permanent and temporal pools in and around Hwange National Park, Zimbabwe. *Transactions of the Royal Society of South Africa* 72: 266–279.
- Msiteli-Shumba, S. M., S. Kativu, B. Utete, E. Makuwe & F. D. Hulot, 2018. Driving factors of temporary and permanent shallow lakes in and around Hwange National Park, Zimbabwe. *Water SA* 44: 269–282.
- Naiman, R. J. & K. H. Rogers, 1997. Large animals and system level characteristics in river corridors. *Bioscience* 47: 521–529.
- Post, D. M., J. P. Taylor, J. F. Kittell, M. H. Olson, D. E. Schindler & B. R. Herwig, 1998. The role of migratory waterfowl as nutrient vectors in a managed wetland. *Conservation Biology* 12: 910–920.
- Pringle, R. M., 2017. How large herbivores subsidize aquatic food webs in African Savannas. *Proceedings of the National Academy of Sciences of the United States of America* 114: 7489–7491.
- Ramanujam, N., 2006. Fluorescence spectroscopy in vivo. In Meyers, R. A. (ed.), *Encyclopedia of Analytical Chemistry*. Wiley, New York: 1–37.
- Rehman, A. U., A. G. Anwer, M. E. Gosnell, S. B. Mahbub, G. Liu & E. M. Goldys, 2017. Fluorescence quenching of free and bound NADH in HeLa cells determined by hyperspectral imaging and unmixing of cell autofluorescence. *Biomedical Optics Express* 8: 1488–1498.
- Rubbo, M. J., J. J. Cole & J. M. Kiesecker, 2006. Terrestrial subsidies of organic carbon support net ecosystem production in temporary forest ponds: evidence from an ecosystem experiment. *Ecosystems* 9: 1170–1176.
- Sparber, K., C. Dalton, E. de Eyto, E. Jennings, D. Lenihan & F. Cassina, 2015. Contrasting pelagic plankton in temperate Irish lakes: the relative contribution of heterotrophic, mixotrophic, and autotrophic components, and the effects of extreme rainfall events. *Inland Waters* 5: 295–310.
- Stears, K., D. J. McCauley, J. C. Finlay, J. Mpemba, I. T. Warrington, B. M. Mutayoba, M. E. Power, T. E. Dawson & J. S. Brashares, 2018. Effects of the hippopotamus on the chemistry and ecology of a changing watershed. *Proceedings of the National Academy of Sciences of the United States of America* 115: E5028–E5037.
- Stedmon, C. A., S. Markager & R. Bro, 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry* 82: 239–254.
- Subalusky, A. L., C. L. Dutton, E. J. Rosi-Marshall & D. M. Post, 2015. The hippopotamus conveyor belt: vectors of carbon and nutrients from terrestrial grasslands to aquatic systems in sub-Saharan Africa. *Freshwater Biology* 60: 512–525.
- Subalusky, A. L., C. L. Dutton, E. J. Rosi & D. M. Post, 2017. Annual mass drownings of the Serengeti wildebeest migration influence nutrient cycling and storage in the Mara River. *Proceedings of the National Academy of Sciences of the United States of America* 114: 7647–7652.
- Subalusky, A. L. & D. M. Post, 2019. Context dependency of animal resource subsidies. *Biological reviews* 94: 517–538.
- Tshipa, A., H. Valls-Fox, H. Fritz, K. Collins, L. Sebele, P. Mundy & S. Chamailé-Jammes, 2017. Partial migration links local surface-water management to large-scale elephant conservation in the world's largest transfrontier conservation area. *Biological Conservation* 215: 46–50.
- Valeix, M., 2011. Temporal dynamics of dry-season water-hole use by large African herbivores in two years of contrasting rainfall in Hwange National Park, Zimbabwe. *Journal of Tropical Ecology* 27: 163–170.
- Valeix, M., S. Chamailé-Jammes & H. Fritz, 2007. Interference competition and temporal niche shifts: elephants and herbivore communities at waterholes. *Oecologia* 153: 739–748.
- Wetzel, R. G., 2001. *Limnology. Lake and River Ecosystems*, 3rd ed. Academic Press, New York.
- Wolanski, E. & E. Gereta, 1999. Oxygen cycle in a hippo pool, Serengeti National Park, Tanzania. *African Journal of Ecology* 37: 419–423.
- Wrench, J., H. Meissner & C. Grant, 1997. Assessing diet quality of African ungulates from faecal analyses: the effect of forage quality, intake and herbivore species. *Koedoe* 40: 125–136.

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