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Effect of high population density of eastern black rhinoceros, a mega-browser, on the quality of its diet

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Abstract

High density of herbivore populations can lead to intense foraging competition and depletion of food consequently lowering diet quality and population performance. We tested for the effects of the density of eastern black rhinoceros (***Diceros bicornis michaeli***) in nine ***in situ*** populations of 0.01–0.7 individuals per km^2 density range on the quality of their diet while controlling for plant available moisture and plant available nutrients. We used faecal calcium, phosphorus, copper and zinc concentrations as proxy indices for dietary quality from 473 fresh faecal samples obtained from 77 adult animals ***in situ***, after determining a positive faeces-diet mineral correlation in feeding trials with black rhinoceros in zoos. Some populations surpassed 70%–80% of their estimated maximum stocking densities expected to cause impact on forage. However, we did not find significant correlation between rhino population density and dietary quality, as measured via faecal mineral nutrient content. This suggests that black rhinoceros may have sufficient behavioural plasticity to adjust their diet to cover their nutritional requirements when density increases. Instead, 1-month lagged plant available moisture, reflecting precipitation over the 4 weeks preceding each sampling effort, significantly explained the mineral concentrations in the faeces. By contrast, plant available nutrients had no effect.

KEYWORDS
black rhinoceros, diet quality, faecal minerals, plant available nutrients and moisture, population density

Résumé

Une forte densité de populations d'herbivores peut entraîner une intense compétition pour le fourrage et un épuisement de nourriture, ce qui réduit la qualité du régime alimentaire et les performances de la population. Nous avons testé les effets de la densité du rhinocéros noir de l'Est (*Diceros bicornis michaeli*) dans neuf populations *in situ* d'une densité de l'ordre de 0,01 à 0,7 individu par km^2 sur la qualité de leur alimentation tout en contrôlant l'humidité et les nutriments disponibles pour les plantes. Nous avons utilisé les concentrations fécales de calcium, de phosphore, de cuivre et de zinc comme indices indirects de la qualité de l'alimentation sous la base de 473 échantillons de matières fécales fraîches provenant de 77 animaux adultes *in situ*, après avoir déterminé une corrélation minérale fécale-régime positive dans des essais d'alimentation avec des rhinocéros noirs dans des zoos. Certaines populations ont dépassé 70 à 80% de leur densité maximale de peuplement estimée pour avoir un impact sur le fourrage. Cependant, nous n'avons pas trouvé de corrélation significative entre la densité de la population de rhinocéros et la qualité de l'alimentation, telle que mesurée par la teneur en nutriments minéraux fécaux. Cela démontre que les rhinocéros noirs peuvent avoir une plasticité comportementale suffisante pour ajuster leur alimentation afin de couvrir leurs besoins nutritionnels lorsque la densité augmente. Au lieu de cela, l'humidité disponible des plantes décalée d'un mois, reflétant les précipitations au cours des 4 semaines précédant chaque effort d'échantillonnage, a expliqué de manière significative les concentrations de minéraux dans les fèces. En revanche, les nutriments disponibles pour les plantes n'avaient aucun effet.

1 | INTRODUCTION

A high density of herbivores can lead to a decline in the quantity of forage and subsequently to a decline in their quality due to the selectivity of the feeding habits of herbivores. A persistent high density of herbivores leads to a low-quality diet for grazers (e.g. Owen-Smith, 2008; Van Wieren & Bakker, 2008) and browsers alike (e.g. Simard et al., 2008; Van der Waal et al., 2003), which then may induce population decline (Caughley, 1976; Ellis & Swift, 1988; Klein, 1968; Scogland, 1985; Sinclair et al., 1985). More so, plant available moisture (PAM)—the water in the soil that can potentially be absorbed by plants in a soil horizon—and plant available nutrient (PAN)—the sum of exchangeable cations in the soil—may each affect quantity and quality of forage differentially (e.g. Mistry, 2011; Solbrig et al., 1992). The significance of PAM and PAN in understanding population dynamics has been well studied for many grazer populations (Ahrestani et al., 2011; Illius & O'Connor, 2000; Ludwig et al., 2008; Olff et al., 2002; Prins & Van Langevelde, 2008; Treydte et al., 2009) but less so for pure browsers with exception of a study for black rhinoceros (*Diceros bicornis*) (Okita-Ouma et al., 2020) and for moose (*Alces alces*) (e.g. Andersen, 1991; Ayotte et al., 2006; Ellis, 2016; Kielland & Bryant, 1998; Murray et al., 2006) but not in relation to their population density. The aim of the present study was to test the relevance of PAM and PAN measures, alongside population density, on populations of a browser species.

Under high precipitation (i.e. high PAM), an abundance of browse material is expected (Olff et al., 2002; Sankaran et al., 2008) but also a higher browse quality is found as shown in experiments (Barbosa et al., 2014; Singh & Singh, 2004). This is in contrast to the effect of increased PAM on grasses, because nitrogen (Olff et al., 2002) and phosphorus (Breman & de-Wit, 1983) in the leaves of grasses decrease with increasing PAM. When the soil nutrient status is high (i.e. high PAN), both a higher abundance and a higher quality of grasses are expected (Olff et al., 2002). For browse, however, one expects only a higher quantity (Polis, 1999; Tilman, 1984) but insignificant changes in quality (Le Houérou, 1980). These lack of changes in browse leaf nutrient quality may be due to the fact that woody plants differ widely in their response to differences in soil nutrients (Le Houérou, 1980). Indeed, scaling effects are very different in woody species as compared to grassy ones (van der Waal et al., 2011). For instance, plant species in high fertility sites grow faster and are of higher tissue nutrient concentrations compared to plants in low fertility sites (Chapin, 1980). Moreover, it has been suggested that plants that are adapted to infertile soils store more nutrients in storage organs (e.g. roots system) than plants in fertile sites (Bryant et al., 1983). These stored nutrients can be readily used in conditions of stress (e.g. high herbivory or density pressure, fire, nutrient shortage) to support growth or other important physiological processes (Chiariello & Roughgarden, 1984). Variation in the quality of leaves of woody plants, especially from thorny *Vachellia* and *Senegalia* species, tends to be less marked than variation in nutritional quality of grasses in African savannahs (Owen-Smith, 2008; Tomlinson et al., 2016) Therefore, by considering PAM and PAN, it is possible that population density may not significantly impact the nutritional quality of forage over time for mega-browsers (i.e. a browsing animals exceeding 1,000 kg in body mass), particularly the hindgut fermenters such as the black rhinoceros.

In this study, we disentangled those variables that affect forage quantity and quality in order to determine the effects of population density on the diet of the Eastern black rhinoceros (*Diceros bicornis michaeli*) by measuring faecal mineral nutrient concentrations in nine local populations of contrasting densities and varying PAM and PAM. The black rhinoceros has a digestive system with hindgut fermentation that can process plants of low nutritional quality and high concentrations of secondary compounds such as *Euphorbia* spp (e.g. Goddard, 1968, 1970; Hall-Martin et al., 1982; Hitchins, 1979; Luske et al., 2009; Mukinya, 1977). Its tolerance for low-quality browse and its twig-removing feeding behaviour suggests that it can have significant impact on its habitat, especially in enclosed areas at high densities. For example, Luske et al. (2009) reported South-central

TABLE 1 Characteristics of the study areas including size, densities, rainfall, vegetation descriptions and other factors likely to impact on population performance between 1993 and 2010

Study area	Available area (km^2)	Density in 2010 (animals/km^2)	% density to max. stocking density (*K*) estimates (Adcock et al., 2007)	Geographic location	Mean annual Rainfall (mm ± *SD*) 2000–2011	Vegetation and soils (Sombroek et al., 1982)	Other factors on population performance
Aberdare Nat. Park (Aberdare)	70	0.1	9.9%	36°25′E–37°00′E and 0°05′S–0°45′S	607 ± 149	Montane forest, undergrowth of mixed species of bushland, bamboo forests, grass tussocks on the moorlands. Soils are composed of humic nitosols, andosols and montemorillonites	Ring-fenced; significant poaching impact on population decline
Lake Nakuru Nat. Park (Nakuru)	144	0.5	97.3%	36°20′E–36°25′E and 0°50′S–1°00′S	755 ± 169	Open grassland, *Vachellia* and *Senegalia*, Tarchonanthus bush land, deciduous and Euphorbia forests and riverine bush land. Soils are composed of lacustrine and riverine sediments, volcanic ash, volcanics, very fine clay, silt, gravel and Elementaita tuff cones	Ring-fenced; 35 black rhinos in total translocated out during the period
Masai Mara Nat. Reserve (Mara)	1510	0.02	28.9%	34°45′E–35°25′E and 1°13′S–1°45′S	911 ± 147	Grassland with isolated scrublands and woodlands especially along drainage lines. Soils are composed of planosols, vertisols, luvisol, rankers, regosols and phaeozems	Not fenced; Contiguous with Serengeti N.P. in Tanzania
Nairobi Nat. Park (Nairobi)	117	0.6	176.9%	36°23′E–36°28′E and 2°18′S–2°20′S	540 ± 151	Deciduous forest, riverine thorn forests, shrubs and grasslands. Soils are composed of Nairobi phonolite lava and tuff, Athi tuff, Kapiti phonolite and lake bed deposits with chert, Mbagathi phonolite and Nairobi trachytes	Ring-fenced except for a 20 km stretch for wildebeest migration; 67 black rhinos in total translocated out during the period
Ngulia Rhino Sanctuary (Ngulia)	92	0.7	172.8%	38°06′E–38°10′E and 3°01′S–3°06′S	385 ± 146	*Commiphora*, *Vachellia and Senegalia* woodland with scattered baobab trees. Soils are composed of Rhodic ferrosols, chromic luvisols, fluvisols and Eutric regosols	Ring-fenced; Expanded from 63 92 km^2 in 2007; 255 elephants, 200 buffaloes and 12 black rhinos translocated out in 2006 to reduce competition (Okita-Ouma et al., 2008)

(Continues)

TABLE 1 (Continued)

Study area	Available area (km^2)	Density in 2010 (animals/km^2)	% density to max. stocking density (*K*) estimates (Adcock et al., 2007)	Geographic location	Mean annual Rainfall (mm ± *SD*) 2000–2011	Vegetation and soils (Sombroek et al., 1982)	Other factors on population performance
Ol Jogi Pyramid (Ol Jogi)	50	0.4	133.8%	37°00′E–37°05′E and 0°15′N–0^{0}20′N	459 ± 113	Mosaic of grassland, *Vachellia* & *Senegalia* woodland and shrubs. Soils are composed of vertisols, chromic luvisols/typic haplustalfs, fluvisols, chromic Cambisols, typic ustropepts, chromic luvisols/typic haplustalfs, Eutric cambisols, and lithosols/Lithic Usropepts/ ustrothents	Ring-fenced; 30 black rhinos translocated out; 9 black rhinos died from disease (Ndeereh et al., 2012)
Ol Pejeta Conservancy (Ol Pejeta)	300	0.3	97.7%	36°40′E–37°00′E and 0°02′S–0°07′N	557 ± 115	Mosaic of grassland, *Vachellia and Senegalia* woodland, *Euclea* shrub and riverine woodland grassland. Soils are composed of verto-luvic phaeozems, luvic phaeozems, chromic vertisols, haplic phaeozems and dystric histosols and gleyic cambisols	Ring-fence designed to allow migration of other species. Expanded from 93 300 km^2 and 27 black rhinos translocated in 2007; Vegetation damage and competition from giraffes and elephants (Birkett, 2002)
Tsavo East National Park (Tsavo East)	1800[a]	0.02	<5%	38°10′E–39°25′E and 1°50′S–3°30′S	414 ± 157	Semi-arid vegetation mainly *Vachellia and Senegalia -Commiphora* woodland. Similar soil composition as in Ngulia	Not fenced. Population established in 1994 and supplemented in 1999. Population significantly declined through poaching (Mulama, 2002; Ngene et al., 2011)
Tsavo West National Park Intensive Protection Zone (Tsavo West IPZ)	1200[a]	0.01	<5%	37°45′E–38°45′E and 2°40′S–4°02′S	426 ± 141	Similar vegetation type as Tsavo East NP. Similar soil composition as in Ngulia	Population established in 2008 with a founder of 20 animals from Ngulia Rhino Sanctuary, Nakuru NP and Nairobi NP

Note: The mean annual rainfall is from January to December of 2000 to 2011. The shortened names for the study areas as used in the main text are in brackets.

[a] The estimated area used by black rhinoceros inside the *ca.* 11,000 km^2 Tsavo East NP and *ca.* 9,000 km^2 Tsavo West NP based on home range data.

black rhinoceros' (*Diceros bicornis minor*) significant impact on 213 plants they monitored in the Great Fish Reserve in South Africa. Biomass decreased by an average of 38% over the 3 years of monitoring, 19% of the plants died, reproductively active plants and densities decreased, while the protected *Euphorbia bothae* showed no compensatory growth in response to browsing.

Here, we used concentrations of faecal mineral concentrations in black rhinoceros as an indirect indicator of its diet quality, as has been done in some wild mammals (Grant et al., 1995; Hodgson et al., 1996; Kohn & Wayne, 1997; Prins & Beekman, 1989; Putman, 1984; Wrench et al., 1997). Faecal nitrogen (N) and phosphorus (P), for example, have been widely used to discern dietary differences between (Grant et al., 1995; Prins, 1989; Robbins et al., 1987) and quality within (Van der Waal et al., 2003) individual herbivores. Other methods available for dietary assessments involve chemical analyses of food plants (Muya & Oguge, 2001; Oloo et al., 1994) by backtracking the browsing path of a mega-herbivore and collecting samples of eaten plants (Helary et al., 2009, 2012; Van Lieverloo et al., 2009). However, backtracking is labour-intensive, and often it is not easy to collect sufficient quantities and representative parts of plants temporally and spatially for chemical analyses. Errors also occur in ascribing a browsed plant to the specific animal species under study. Faecal analyses are therefore sometimes preferred over backtracking, as it is less prone to errors associated with plants selection and subsequent mixing of these species in the gut and subsequently in the faecal samples.

We analysed calcium (Ca), phosphorus (P), copper (Cu) and zinc (Zn). As a first step to our understanding of differential population density effects on diet quality in a PAM-PAN space, we tested the premise that diet and faecal mineral concentration are correlated. We hypothesised that after controlling for the effects of PAM and PAN, mineral concentrations as measured in faeces would be low at high population densities, reflecting a low diet quality. Our second hypothesis was that mineral concentrations in the faeces would be high for populations located in high PAM and high PAN space. Using generalised linear mixed models (GLMMs), we explored these relationships and verified whether this approach is sensitive enough to detect black rhinoceros density effects on the nutritional status for the animals.

2 | METHODS

2.1 | Zoo study

We analysed correlations in the faeces-diet mineral concentrations using data from feeding trials with seven male and eight female adult black rhinos in three zoological institutions and in three unpublished feeding trials from Clauss et al. (2007). Not all minerals were analysed in all cases resulting in different sample sizes for different minerals. We did not consider the relationships between ingested and faecal mineral concentration as directly causal, but as modified by digestibility. Digestibility depends on the amount of diet ingested, faeces excreted and endogenous/metabolic losses and different proportions of faecal and urinary excretion for different minerals. It may vary depending on whether the animal is in a state of maintenance where it only has to replace concurrent losses of a mineral, or in a state of growth or reproduction where minerals are used to build up new body tissue. Nevertheless, the analysed minerals served to underscore our assumption that, there exists a correlation between mineral concentrations of diets and faeces.

2.2 | Field study

We selected nine different locations with black rhinoceros populations in Kenya (Table 1). We based selection criteria on the various densities of local rhinoceros populations listed in the Table, and in the variation of PAM and PAN among these sites. Data collection, processing and calculation of PAM and PAN followed methods described in Okita-Ouma et al. (2020).

2.3 | Field sample collection and preparation

We collected fresh (<18 h old) faecal material in the field with 473 faecal samples from 77 individual adult black rhinoceroses comprising 37 males and 40 females (mean = 5; Range = 1–18 samples per animal). We avoided samples contaminated with urine or rainwater whereas we removed by hand visible contaminants such as soil particles and insects. We sampled between 06:00 and 12:00 h from July 2010 to November 2011 in nine populations with varying population densities (Table 1) and varying levels of PAM and PAN. Some of the densities were significantly beyond their estimated maximum stocking densities (Adcock et al., 2007) to have an impact on the habitat. We only analysed faecal samples that we could confidently earmark to known individuals. Individual animals were identified by dedicated rhino monitoring personnel using ear-notches, body marks, home ranges and remote measures such as camera traps and radio tracking (Mulama et al., 2005).

While in the field, we thoroughly mixed by hand each sample on a disposable polythene sheet surface of 50 cm by 50 cm. This was to ensure that sub-samples collected for analyses were representative of the faecal matter content in each of the 473 samples. We put about 30 g of the hand-mixed sub-samples into three airtight sampling bottles, for backup purposes, and immediately placed them in an iced cooler box then stored them in a −20°C freezer in the field. The samples were then transported to the industrial laboratories of the Ministry of Mines and Geology in Nairobi for chemical analyses. We followed standardised protocols for plant material (Plank, 1992) to prepare the sub-samples and to analyse them for chemical content (see Appendix 1A for the procedures). The precision at 95% confidence level of measures as obtained from the standard parallelism curves was as follows: ±0.05 ppm for Ca; ±0.05 ppm for P, ±0.002 ppm for Cu; and ±0.005 ppm for Zn (*F* test, all $p > 0.05$).

2.4 | Statistical analyses

We assessed correlations between the plotted dietary and faecal Ca, P, Cu and Zn concentrations for the feeding trials with the zoo animals using Spearman's correlation procedure; additionally, we performed linear regression analyses (in all cases, residuals of regressions had a normal distribution), providing 95% confidence intervals (95% CI) for parameter estimates. We determined faecal Ca, P, Cu and Zn concentrations from the field study through generalised linear mixed modelling (GLMM) (Goldstein, 2003; Rasbash et al., 2012) since these data consisted of repeated measurements and were hierarchically structured, namely observations within rhinos (level 1) and between rhinos (level 2). This allowed us to fit random effects and partition variance within response variables (i.e. the different minerals) within and between rhinos prior to the addition of fixed effects. Broadly, this was equivalent to fitting explanatory variables in generalised linear models. We used MLwiN (version 2.26) (Rasbash et al., 2005) to fit the GLMM models.

Our response variables were Ca, P, Cu and Zn mineral concentrations measured in the faeces. The measurements of these minerals were $\log_{10}$ transformed to normalise their error distribution. In the first step of model exploration, the nine study areas were entered as explanatory variables (categorical fixed effects), as was PAM (and its 1- and 2-month lag preceding faeces deposition) and PAN. We assessed significance of individual explanatory variables using the Wald χ^2 statistic and checked quadratic fits of PAM for significance. To assess curvilinearity, we fitted 'Area' as a categorical term within the fixed effects before fitting constituents of PAM (temperature and rainfall) separately as estimated from satellite data (LSASAF, 2010; Tchuenté et al., 2011) lagging these variables by up to 3 months prior to the collection of faecal samples and the squares of these variables. From a plant eco-physiological perspective, we considered that cumulative rainfall and temperature effects would likely have impact on the nutrient content of plants, hence these initial tests of lagged rainfall and temperature. After these preliminary model explorations, we found that the most consistent and parsimonious modelling was when temperature and rainfall were dropped and replaced by PAM and its square without any lags. PAM takes into account other variables in addition to rainfall and temperature that could affect nutrient uptake by plants, such as humidity, solar radiation and wind speed (Blaney & Criddle, 1962; Penman, 1948).

Our second step in building the model was determining the effects of PAM and its quadratic fits on the variability of concentration of faecal minerals. We found high co-linearity between PAM and its lagged values ($r > 0.6$). This high co-linearity made it difficult to evaluate the consequence of adding each variable to changes in model log-likelihood; hence, we decided that PAM_Lag1 (i.e. over the 1-month [4 weeks] preceding sampling) was ecologically the most realistic variable to explore further. This was also borne out by comparing models with PAM, PAM_Lag1 (1-month lagged) and PAM_Lag2 (2-months lagged): the addition of PAM_Lag1 caused the greatest decline in model variance.

Our third step of the analyses was exploring whether there were any effects of the density of rhinos on faecal mineral concentration after first controlling for PAM and PAN. We added 'Density' as a continuous variable; it ranged from 0.01 to 0.7 rhinos/km^2.

3 | RESULTS

3.1 | Zoo study

Data on dietary and faecal mineral concentration from feeding trials with black rhino in zoos indicated that an increase in dietary mineral concentrations was correlated with an increase in faecal mineral concentrations. Correlations between dietary and faecal mineral concentrations were significant for the four chemical elements (Spearman Ca: $r_s = 0.718$, $p < 0.001$, $n = 50$; P: $r_s = 0.715$, $p < 0.001$, $n = 36$; Cu: $r_s = 0.734$, $p < 0.001$, $n = 50$; and Zn: $r_s = 0.769$, $p < 0.001$, $n = 36$). The 95% CI for the slopes of the regression lines included 1 for Zn were higher than 1 in the case of P and Cu and lower than 1 in the case of Ca (Figure 1).

3.2 | Field study

3.2.1 | PAM-PAN space

Figure 2 summarises the results of the nine black rhinoceros study on a PAM-PAN space with a Table underneath listing the values of PAM (mm/day) and PAN (mEq/100 g) at 95% CI.

3.2.2 | Faecal analyses

Faecal mineral concentration differed significantly between study areas (fitting the model *Ln Mineral Concentration ~ Area*; chi-square χ^2 values for Area each $df = 8$ (Ca: $\chi^2 = 36$, $p < 0.0001$, P: $\chi^2 = 59$, $p < 0.0001$, Cu: $\chi^2 = 170$, $p < 0.0001$, Zn: $\chi^2 = 383$, $p < 0.0001$). Concentrations of all measured minerals in samples from Aberdare NP had consistently higher values than those of any other area. Exploring the remaining areas, Cu was highest in Nairobi while Zn was highest in Nakuru and Nairobi. Cu and Zn were lowest in Tsavo East and Ol Pejeta (Figure 3). Faeces of black rhinoceroses from the field study had higher concentrations of both Ca and P compared to those measured in the zoo study (mean ± SD); Ca = 10.1 ± 3.9 compared to 3.9 ± 1.6 g/kg DM and P = 13.2 ± 8.3 compared to 6.9 ± 1.9 g/kg DM. The microminerals Cu and Zn had lower concentrations in the faeces of free-ranging animals than in zoo animals; Cu = 12.2 ± 6.3 compared to 19.2 ± 5.8 mg/kg DM and Zn = 51.6 ± 37.6 compared to 104.1 ± 44.0 mg/kg DM.

The addition of the 1-month lag period of PAM (PAM_Lag1) to the models of mineral concentration significantly reduced the variance observed in Ca, P, Cu and Zn. The parameter estimates

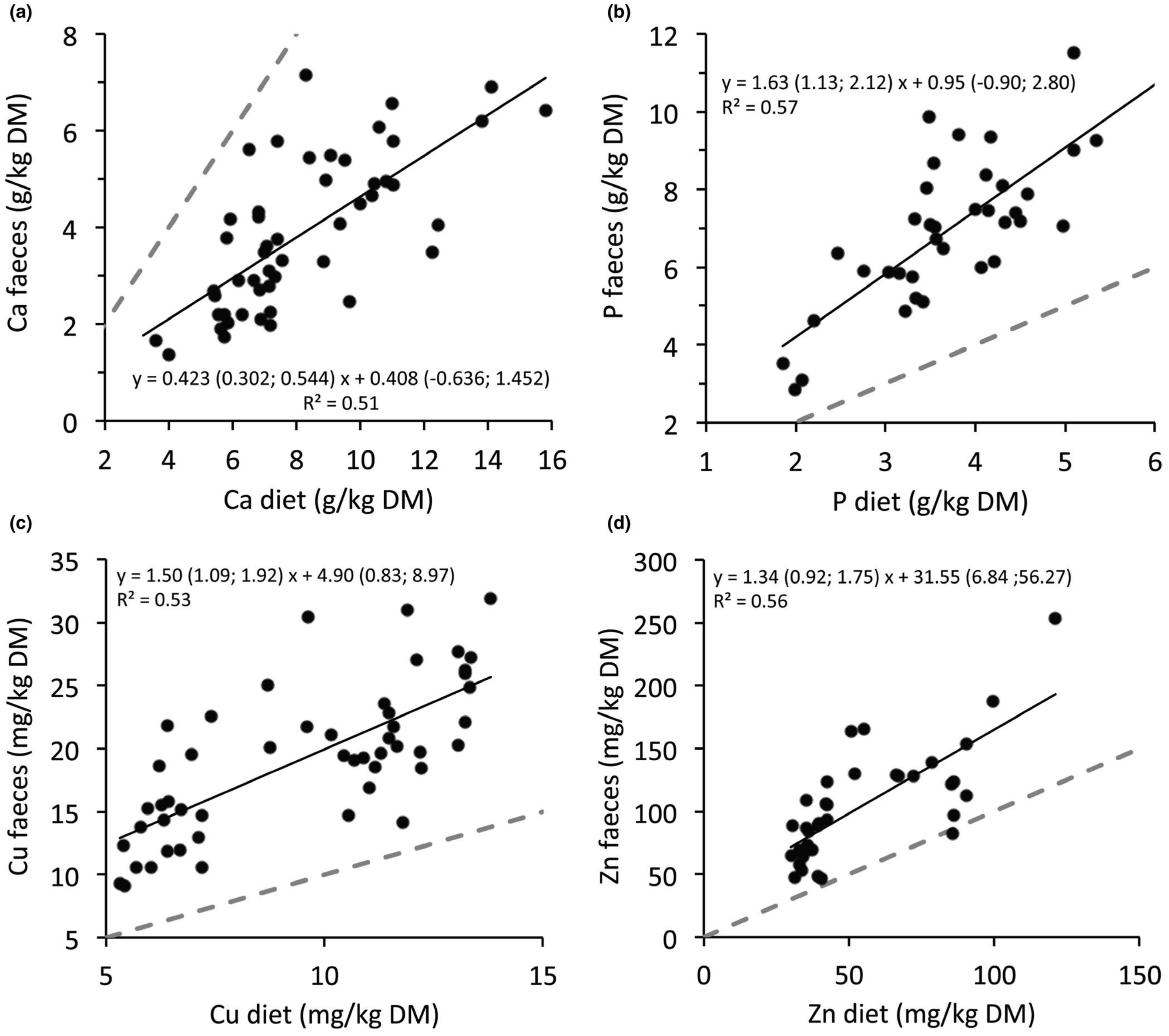

FIGURE 1 Relationship between dietary and faecal mineral concentrations for (a) calcium (Ca), (b) phosphorus (P), (c) copper (Cu) and (d) zinc (Zn) from feeding trials with 15 black rhinoceroses (*Diceros bicornis*) in zoos. The dotted line indicates $y = x$ to visualize if concentrations are higher in diet or in faeces. The bold line represents regression equations (in brackets for each parameter estimate the 95% CI)

indicate that each of these minerals increased in the faeces with increasing values in PAM_Lag1, strongly suggesting higher mineral availability in the vegetation as moisture increased ($p < 0.0001$; Table 2). PAN did not explain faecal mineral content and was not a significant co-variable in any of the four GLMMs (Table 2). Similarly, we did not find a significant relationship ($p > 0.05$) between density and any mineral content measured in the faeces when controlling for effects of PAM and PAN.

4 | DISCUSSION

Our reasoning that faecal mineral concentrations are a reliable proxy for dietary quality hinged on the assumption that there was a significant positive correlation between faecal and dietary mineral concentrations. We tested this assumption with data sets collected from captive black rhinoceros held in zoos. These feeding trials showed that an increase in the faecal mineral concentration was correlated with an increase in dietary mineral concentrations, with over 50% of the variance explained in this correlation across all four minerals investigated (P: 57%, Zn: 56%, Cu: 53% and Ca: 51%; Figure 1). In all cases except for Zn, the faecal concentrations were significantly different from the dietary concentrations. Faecal Ca falls below the $y = x$ line, which can be accounted for by high proportions of Ca being excreted in urine in rhinos (Clauss et al., 2007) as in many other hindgut fermenters (Hagen et al., 2015), thus explaining lower faecal than dietary concentrations (Figure 1a). For Cu or Zn, the loss of the organic fraction of the diet due to digestion explains

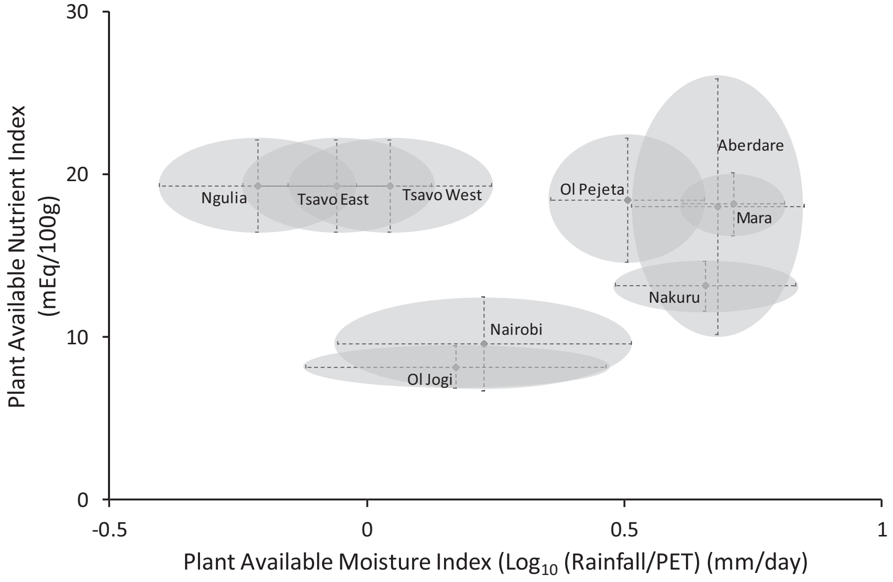

FIGURE 2 Nine black rhino conservation areas in Kenya plotted on a PAM-PAN plane with values summarised in the Table underneath the graph. The dotted lines give the 95% confidence intervals. PET, potential evapotranspiration

PAM (mm day^{-1}) (*n* = 10)	PAN (mEq/100g)	PAM (mm day^{-1}) (*n* = 10)	PAN (mEq/100g)	PAM (mm day^{-1}) (*n* = 10)	PAN (mEq/100g)
Aberdare: 0.7 ± 0.2	18.0 ± 7.9 (*n* = 23)	Nairobi: 0.2 ± 0.2	9.6 ± 2.9 (*n* = 28)	Ol Pejeta: 0.5 ± 0.1	18.4 ± 3.8 (*n* = 19)
Mara: 0.7 ± 0.1	18.2 ± 1.9 (*n* = 43)	Ngulia: - 0.2 ± 0.2	19.3 ± 2.8 (*n* = 179)	Tsavo East: -0.06 ± 0.2	19.3 ± 2.8 (*n* =179)
Nakuru: 0.7 ± 0.2	13.2 ± 1.5 (*n* = 47)	Ol Jogi: 0.2 ± 0.3	8.2 ±1.3 (*n* = 17)	Tsavo West: 0.04 ± 0.2	19.3 ± 2.8 (*n* = 179)

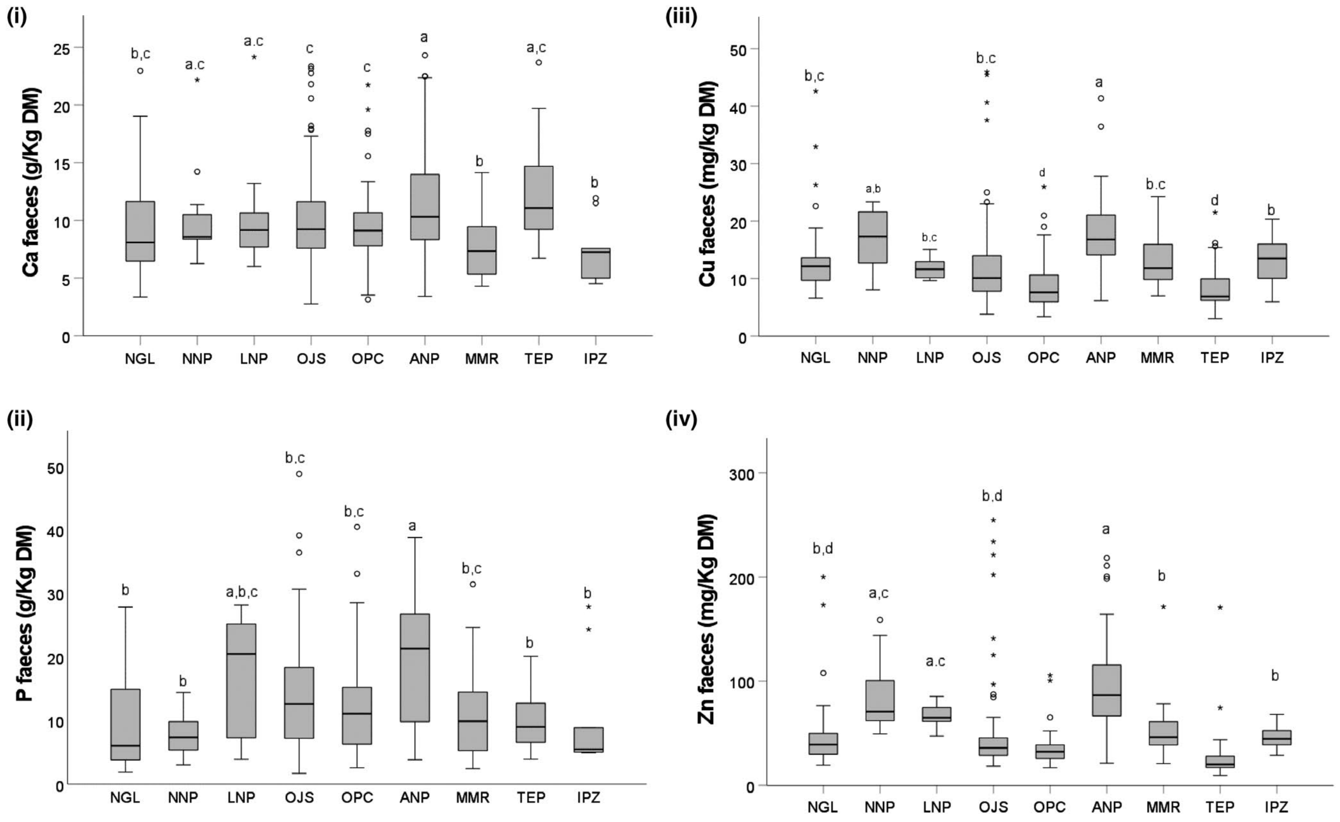

FIGURE 3 Box plots illustrating differences between study areas† in concentration of (i) calcium (Ca), (ii) phosphorus (P), (iii) copper (Cu) and (iv) zinc (Zn) as measured from faeces of black rhinoceroses in the field. The study areas are arranged in descending order (L–R) of their population density as at 2010. The same superscript letters above the 95% CI bars indicate not significantly different mineral concentration between study areas. The study areas that do not share the same letter above the 95% CI error bars had significantly different faecal mineral concentration as assessed by Wald χ^2 statistic at $p \leq 0.05$ (Appendix 1B). For example, Zn in ANP, LNP and NNP are not significantly different, but Zn levels in ANP and IPZ do differ significantly. ANP, Aberdare N. Park; IPZ, Tsavo West N. Park Intensive Protection Zone; LNP, Lake Nakuru N. Park; MMR, Masai Mara N. Reserve; NGL, Ngulia Sanctuary; NNP, Nairobi N. Park; OJS, Ol Jogi Pyramid; OPC, Ol Pejeta Conservancy; TEP, Tsavo East N. Park

TABLE 2 Results of generalised linear mixed models exploring the variability in faecal mineral concentration in faeces of black rhinoceros in relation to plant available nutrient (PAN), plant available moisture (lag PAM— lagged by 1 month to reflect rainfall over the 4 weeks preceding sampling) and black rhinoceros density in each of the nine study areas in Kenya 2010/2011

Effects	Parameter Estimate	SE	Δ deviance	Δ *df*	*p*
Calcium					
Intercept	9.218	0.132	4852.74	1	<0.0001
Area	–	–	36.70	8	<0.0001
Density	−0.024	0.044	0.29	1	NS
PAN	−0.005	0.013	0.161	1	NS
Lag PAM	0.085	0.027	9.86	1	0.002
Phosphorus					
Intercept	9.787	0.223	1764.61	1	<0.0001
Area	–	–	59.80	8	<0.0001
Density	−0.009	0.028	0.11	1	NS
PAN	0.010	0.011	0.86	1	NS
Lag PAM	0.184	0.047	15.28	1	<0.0001
Copper					
Intercept	2.646	0.193	118.39	1	<0.0001
Area	–	–	170.51	8	<0.0001
Density	−0.116	0.061	3.61	1	NS
PAN	−0.009	0.018	1.49	1	NS
Lag PAM	0.141	0.029	23.16	1	<0.0001
Zinc					
Intercept	4.195	0.256	265.04	1	<0.0001
Area	–	–	383.60	8	<0.0001
Density	−0.118	0.080	2.16	1	NS
PAN	−0.026	0.024	1.16	1	NS
Lag PAM	0.171	0.031	30.18	1	<0.0001

the higher concentration of these minerals in the faeces than in the diet (Figure 1b–d); for P, microbes that grow in the hindgut of the animals and that are lost via faeces are an important additional source. The linear correlations and the reasonably high-explained variances between diet and faecal mineral concentrations in the zoo study gave sufficient confidence to use faecal minerals for black rhinoceros as a measure of diet quality.

Additionally, concentration of the measured minerals in faecal samples from the field study increased with increase in PAM_Lag1. This correlation between PAM and mineral concentration strongly suggested that increase in moisture resulted in either higher mineral availability or a higher concentration of digestible nutrients in the browse plants (Barbosa et al., 2014; Singh & Singh, 2004). Diet quality has been shown to relate to plant mineral concentrations (e.g. Hall-Martin et al., 1982; Hodgson et al., 1996; Howery & Pfister, 1990; Wrench et al., 1997). For these reasons, we interchangeably refer to faecal minerals as diet minerals or diet quality in this discussion.

4.1 | PAM-PAN influence on diet quality

One-month lag of PAM consistently explained the variability of mineral concentration in faecal samples from the field study, reflecting growth of woody plants in relation to earlier precipitation in preceding months (Gaye & Edmunds, 1996; Ludwig et al., 2004; Priyadarshini et al., 2016; Rutherford, 1984; Taylor & Howard, 1996). In line with our hypothesis, the results were consistent with the reported increase in quality (Bell, 1982; Huntley, 1982) of woody plants with increase in rainfall in savannah ecosystems receiving annual rainfall between 200 and 1000 mm. Singh and Singh (2004) and Barbosa et al. (2014) showed that generally the concentration of micro- and macro-nutrients in leaves and twigs of woody plants increased with increase in water availability. This may explain why the Aberdare, with its high annual rates of precipitation and thus the highest PAM, also had the highest concentration of macro- and micro-minerals.

In Nairobi, black rhinoceros showed high concentrations of Cu in their faeces while Nairobi and Nakuru had higher faecal Zn concentrations than in the rest of the study areas. The rhinoceroses in Aberdare had the highest Cu and Zn levels in their faecal samples compared to those in other study areas. Because the soils of these three different areas are of volcanic origin (McCall, 1967; Onyancha et al., 2011; Sombroek et al., 1982), one could perhaps expect high concentrations of these minerals in rhinoceros diets. However, low concentrations of Cu in soils and a subsequent low concentration of Cu in two browse plants (i.e. yellow-barked acacia—*Vachellia xanthophloea* and Sodom apple—*Solanum incanum*) have been reported for Nakuru (Maskall & Thornton, 1989). Earlier, Chamberlain (1959) and Pinkerton (1967) reported that the soil characteristics of volcanic ash and pumice derived from Menengai caldera explain the low concentration of Cu in soils of Nakuru and its surroundings for wheat crops. Based on these earlier studies, mineral supplementation by providing saltlicks for animals in Nakuru (e.g. KWS, 1993; Okita-Ouma et al., 2007) was done for some time but could not be sustained beyond early 2000 for financial reasons. At the time of sample collection, no mineral supplementation occurred at any of the sites.

Maskall and Thornton (1989) also reported low Cu levels in the serum of impala (*Aepyceros melampus*) to be aggravated by the high molybdenum levels in the vegetation of Nakuru. Therefore, dietary quality for black rhinoceros in Nakuru may after all not be critically deficient of trace elements as reported earlier (Jonyo et al., 1988; Maskall & Thornton, 1989). Our results may therefore also mean that mineral concentration in different browse plants is not determined by soil fertility as assayed in chemical laboratories. The latter possible interpretation of our results was demonstrated by Le Houérou (1980) when he showed no significant differences in phosphorus concentration in legume browse between East and West African soils; West African soils are overwhelmingly deficient in phosphorus compared to East African soils. This lack of a clear relationship between soil mineral concentration and mineral concentration in browse material explains why we did not a find significant effect of PAN on faecal mineral concentrations.

While PAN was of little statistical value in helping explain the relationship in black rhinoceros diet quality through faecal minerals, PAM appears to have a much higher explanatory power. Tsavo East as compared to Aberdare, Nakuru and Nairobi is located in an area of low PAM (Figure 2) and consequently had the lowest Cu and Zn in dietary browse of black rhinoceros as measured through faecal mineral analyses (Figure 3).

Both Ca and P occurred at much higher faecal concentrations in the field than in the zoo study. For Ca, this is expected, because browse usually contains higher levels of Ca than the diet items usually fed to zoo animals (Clauss & Hatt, 2006). For P, this cannot be explained in the same manner, as P levels in browse are not higher than diet items fed to zoo animals (Clauss & Hatt, 2006). Therefore, we can only speculate that this might be an effect of increased microbial activity in free-ranging animals with higher microbial P excretion. In contrast, micro-minerals Cu and Zn had lower concentrations in faecal samples of free-ranging animals than in faecal samples of zoo animals. It is evidently not possible to extrapolate from faecal concentrations on the amount of mineral ingested. Yet, when using the regression line for Cu (Figure 1), this average faecal Cu corresponds to a dietary concentration of approximately 5 mg/kg DM, which is distinctively lower than the 10 mg/kg DM maintenance recommended for horses (NRC, 2007). These findings tentatively support previous speculations by Maskall and Thornton (1989) that free-ranging black rhinoceros might experience marginal dietary Cu concentrations. Similarly for Zn, such an extrapolation exercise yields hypothetical dietary concentrations of approximately 15 mg/kg DM, which again is distinctively lower than the 40 mg/kg DM maintenance recommended for horses (NRC, 2007). While a critical deficiency appears unlikely in the wild given that animals have lived in those habitats for decades, these indications of a marginal provision may support concepts of variations in micronutrient levels affecting reproductive success and hence population growth across habitats.

It was not clear why faecal samples in Ol Pejeta and in Tsavo East had the lowest Cu despite both being in volcanic soils (Sombroek et al., 1982) of high PAN. We attribute this to the lack of a definite pattern between minerals in browse plants and PAN. This is supported by the contrasting Cu levels in browse plants compared to Cu levels in blood serum of impala in Nakuru (Maskall & Thornton, 1989). More so, similar P levels in legume browse in East and West Africa soils have been reported despite the significantly different levels of P in the soils of these two regions (Le Houérou, 1980). Additionally, heterogeneity of soil characteristics even when the soils are of similar origin can result in significant differences in mineral patches (Nyandat & Ochieng', 1976).

Trace elements such as Zn and Cu have been reported to play important roles in reproduction and fertility in rats (e.g. Bedwal & Bahuguna, 1994; Gunn & Gould, 1958; Yousofvand et al., 2013), while (Koen, 1988) and Vermeulen et al. (2000) have suggested that low levels of these elements could have led to a decline in the Knysna elephant population in combination with anthropogenic factors and demographic stochasticity (Moolman et al., 2019). The role of these elements as important modifiers of fertility or reproduction in domestic horses—the closest relative of rhinoceroses—has not been demonstrated (NRC, 2007). We can therefore only infer from the studies in rats and elephant that the higher dietary Cu and Zn in Nairobi and Nakuru than in other areas, in addition to overall resource availability, could partly explain why black rhinoceros in these two populations reproduced optimally (Okita-Ouma et al., 2009; Okita-Ouma et al., 2020). This could also mean that the low densities recorded by Goddard (1969) in a very large section of Tsavo East were probably associated with the lower concentration of trace elements as observed by us. The Aberdare population is not discussed in this context of fertility and reproduction because its population was adversely affected by poaching (Okita-Ouma & Wandera, 2006).

4.2 | Density effects on diet quality

Density effects on the quality of dietary plants may be revealed only after controlling for variation in precipitation (Owen-Smith, 1990) and also nutrient availability. In our study though, after controlling for PAM and nutrients, we did not find that the density of black rhinoceros explained any statistically significant component of the variances observed in the concentration of dietary minerals ($p > 0.05$ in all instances). This may mean four things: (a) There was no density dependence in any of the populations we studied. (b) Consequently, the densities studied did not affect the quality of browse plants. (c) The physiological ability of black rhinoceros to process plants of low nutritional quality such as *Euphorbia* spp with high concentrations of secondary compounds (e.g. Goddard, 1968, 1970; Hall-Martin et al., 1982; Hitchins, 1979; Luske et al., 2009; Mukinya, 1977) could have overridden density feedbacks. (d) Black rhinoceroses have sufficient behavioural plasticity to adjust their diet so that it covers their requirements when density increases.

5 | CONCLUSION

Our results for the four minerals P, Cu, Ca and Zn do not show any effect of rhino density, and we hypothesise that there would be no effect of rhino density on other nutritional parameters. It was not feasible to assess metabolic faecal N in this study to strengthen an evaluation of the nutritional value of the diet for mega-herbivores and help discern any large dietary differences. We therefore recommend that any future research into density and diet quality of a mega-browser include faecal nitrogen measurements. However, we suspect that the N/P ratio would not be affected by rhino density, and hence, we posit that this mega-browser does not modify its forage quality due to higher densities. Large browsers may thus have a very different effect as compared to large grazers including possible sufficient behavioural plasticity to adjust their diet to cover nutritional requirements at high densities.

Our findings underscore the use of faecal analyses over chemical analyses of individual plants in determining quality of diet for browsers. We strongly recommend this approach in assessing habitat quality as it eliminates the uncertainties and errors associated with habitat quality assessments that are based on the analyses of more than 220 plant species browsed on by black rhinoceros.

It was highly evident that areas of high PAM had higher levels of faecal minerals and sustained higher densities of black rhinoceros than areas of low PAM. This reinforced the fact that a black rhinoceros (a mega-browser) is controlled by bottom-up processes on food resources.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this study.

AUTHOR CONTRIBUTIONS

B.O-O. designed the study with the guidance of H.H.T.P. R.P carried out the generalised linear mixed models analyses. M.C provided, analysed and interpreted the zoo feeding trials data. B.O-O. collated and analysed the field rhino data, plant available moisture and nutrient. B.O-O. led the writing of this article with input from H.H.T.P, M.C. and R.P. All co-authors commented on and approved the final version of this article.

DATA AVAILABILITY STATEMENT

All data used in this research is available on request from Kenya Wildlife Service, University of Wageningen and University of Zurich. Data especially on individual locations may be shared only by the written permission from the Kenya Wildlife Service due to the sensitivity surrounding conservation of a critically endangered species.

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APPENDIX 1A

PROCEDURES FOR FAECAL MINERAL ANALYSES

20–30 g of fresh faecal samples were sun-dried for 5 days then oven-air dried at 80°C for 24 h. A subsample of 2.5 g of oven-dried sample was ground and weighed into a 50 ml beaker and mixed with 3 ml distilled water to make slurry which was then digested with 15 ml concentrated hydrochloric acid and 5 ml concentrated nitric acid. After the reaction ceased, the beaker was heated on a hot plate while being agitated from time to time until 5ml of solution remained. 10 ml of distilled water was added and then allowed to settle. The cooled and settled digested solution was filtered using hardened ash-free filter paper grade no. 541, and then washed thoroughly with distilled water to the mark of 50 ml volumetric flask. The solutions were subjected to flame photometer for determination of Ca (Sherwood, 2005) and atomic absorption spectrometer (AAS) for determination of Cu and Zn (Levenson, 2001).

For chemical analysis of P, 1.0 g of oven-air dried faecal sample was added to 1 ml of concentrated sulphuric acid and 5 ml of concentrated nitric acid and left to react until the solution became colourless (Plank, 1992). 20 ml distilled water and 0.05 ml of phenolphthalein indicator was added then neutralised with 1 N NaOH to Ph = 7. The solution was transferred into a 100-ml volumetric flask and distilled water added to the mark. The standard solutions were treated likewise. 10 ml of digested samples, blank and standard solutions were pipetted into 100 ml beakers and 10 ml of molybdovanadate added to the solution. 25 ml of distilled water was then added into each beaker, mixed and allowed to stand for at least 5 min. The percentage transmittance for each solution was then determined at 430 nm in a UV visible spectrometer using reagent blank as the reference blank solution (Plank, 1992).

APPENDIX 1B

Differences between study areas[†] in concentration of Ca, P, Cu and Zn mineral nutrients as measured from analyses of faeces of black rhinoceros collected from nine study areas[†]. For each nutrient in the table, the signs given in each cell reading down the column compares whether the area in the row cell is significantly greater or less than the area given in the column header. For example, Cu concentrations in the faeces from IPZ were significantly less than mean concentration of Cu in faeces collected from ANP. In addition, mean Cu concentrations from TEP (or OPC) were greater than mean Cu in IPZ. Statistical significance (assessed using the Wald chi-square) is indicated by $p \leq 0.05$ *; $p \leq 0.01$ **; $p \leq 0.001$ ***; $p \leq 0.0001$ ****; and ns, not significant.

	ANP	IPZ	LNP	MMR	NGL	NNP	OJS	OPC
Ca								
ANP								
IPZ	–**							
LNP	ns	–*						
MMR	–****	ns	–*					
NGL	–*	ns	ns	–*				
NNP	ns	–*	ns	–*	ns			
OJS	–*	–*	ns	–**	ns	ns		
OPC	–*	–*	ns	–**	ns	ns	ns	
TEP	ns	–***	ns	–****	–**	ns	–**	–**
P								
ANP								
IPZ	–**							
LNP	ns	ns						
MMR	–****	ns	ns					
NGL	–****	ns	+**	ns				
NNP	–****	ns	+**	ns	ns			
OJS	–****	ns	ns	ns	–***	–**		
OPC	–****	ns	ns	ns	–**	–*	ns	
TEP	–****	ns	–*	ns	ns	ns	ns	ns
Cu								
ANP								
IPZ	–*							
LNP	–**	ns						

(Continues)

(Continued)

	ANP	IPZ	LNP	MMR	NGL	NNP	OJS	OPC
MMR	−***	ns	ns					
NGL	−****	ns	ns	ns				
NNP	ns	ns	−*	−*	−*			
OJS	−****	ns	ns	+*	+*	+****		
OPC	−****	+**	+**	+****	+****	+****	+****	
TEP	−****	+**	+**	+****	+****	+****	+***	ns
Zn								
ANP								
IPZ	−****							
LNP	ns	−*						
MMR	−****	ns	+*					
NGL	−****	ns	+**	ns				
NNP	ns	−***	ns	−****	−****			
OJS	−****	ns	+****	+**	ns	+****		
OPC	−****	+*	+****	+****	+***	+****	+**	
TEP	−****	+****	+****	+****	+****	+****	+****	+***

† Abbreviations: ANP, Aberdare National Park; IPZ, Tsavo West National Park Intensive Protection Zone; LNP, Lake Nakuru National Park; MMR, Masai Mara National Reserve; NGL, Ngulia Rhinoceros Sanctuary; NNP, Nairobi National Park; OJS, Ol Jogi Ranch.