



Animal body size distribution influences the ratios of nutrients supplied to plants

Elizabeth le Roux^{a,1} , Laura S. van Veenhuisen^{b,c}, Graham I. H. Kerley^a , and Joris P. G. M. Cromsigt^{a,b,c}

^aCentre for African Conservation Ecology, Department of Zoology, Nelson Mandela University, Port Elizabeth 6031, South Africa; ^bDepartment of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden; and ^cCopernicus Institute of Sustainable Development, Utrecht University, 3584 CB Utrecht, The Netherlands

Edited by James A. Estes, University of California, Santa Cruz, CA, and approved July 27, 2020 (received for review February 25, 2020)

Nutrients released through herbivore feces have the potential to influence plant-available nutrients and affect primary productivity. However, herbivore species use nutrients in set stoichiometric ratios that vary with body size. Such differences in the ratios at which nutrients are used leads to differences in the ratios at which nutrients are deposited through feces. Thus, local environmental factors that affect the average body size of an herbivore community (such as predation risk and food availability) influence the ratios at which fecal nutrients are supplied to plants. Here, we assess the relationship between herbivore body size and the nitrogen-to-phosphorus ratios of herbivore feces. We examine how shifts in the average body size of an herbivore community alter the ratios at which nitrogen and phosphorus are supplied to plants and test whether such differences in the stoichiometry of nutrient supply propagate through plants. We show that dung from larger-bodied herbivores contain lower quantities of phosphorus per unit mass and were higher in N:P ratio. We demonstrate that spatial heterogeneity in visibility (a proxy for predation risk and/or food availability) and rainfall (a proxy for food availability), did not affect the overall amount of feces deposited but led to changes in the average body size of the defecating community. Feces deposited in areas of higher rainfall and reduced visibility originated from larger herbivores and were higher in N:P ratios. This indicates that processes that change the size distribution of herbivore communities, such as predation or size-biased extinction, have the potential to alter the nutrient landscape for plants.

ecosystem stoichiometry | consumer-driven biogeochemical cycling | megaherbivores | landscape of fear

Animal waste products (feces and urine) are an important source of nutrients to plants (1–3) and an important driver of biogeochemistry (4). Nutrients from animal waste affect plant primary production through both the overall amount of nutrients deposited and the ratios of different nutrients (stoichiometry) in the feces. Stoichiometry refers to the relative abundances of different elements, and organisms require nutrients in a set stoichiometric ratio, which means that excess of one nutrient will be of no use when another is limiting (5). However, there are few studies that expressly investigate how the stoichiometry of animal feces influence the stoichiometry of elements available to plants (5).

The stoichiometric ratios required by organisms vary among species, and this variation is often linked to body size. Nitrogen is an important constituent of protein, and as protein synthesis is one of the major metabolic processes, nitrogen requirement is expected to scale to body size in the same way as metabolic rate, which in herbivorous mammals is with an allometric exponent of 0.75 (6, 7). In vertebrates, skeletal investment increases disproportionately with body size. Bone is phosphorus rich with very low N:P ratios (~0.8:1) compared to other body tissues (8). As such, in vertebrates, phosphorus requirement would be expected to scale to body size with an exponent greater than 1, following skeletal allometry (exponent of 1.1; ref. 9). Thus, larger mammals are expected to require less nitrogen and more phosphorus per unit body mass relative to smaller mammals, and body N:P is thus expected to decrease as body size increases (5). For

example, the N:P requirement in mammal consumers may range from 24:1 in a 10-g vertebrate to ~10:1 in a 1,000-kg vertebrate (8). Such differences in the body stoichiometric balance will in turn alter the balance of nutrients released through feces. At constant food N:P, retaining less nitrogen and more phosphorus would cause feces from large mammals to be richer in nitrogen and lower in phosphorus relative to that of small-bodied mammals.

Factors that affect the average body size of the mammal community within a particular area are therefore predicted to influence the ratios of local fecal nutrient input. Three important features, food quality and quantity and predation risk, strongly shape how herbivores of different body size use the landscape (10). Smaller herbivores require relatively less forage but of higher digestive quality, whereas larger herbivores can tolerate low-quality food, provided that it is of sufficient quantity (11, 12). For example, areas with high amounts of rainfall are generally characterized by higher food abundance yet lower food nutritional quality (13), and tend to be populated by communities dominated by larger mammals (13). Body size also influences the number of predators to which an herbivore is vulnerable (14). Smaller herbivores are vulnerable to more predators (14), while intermediate-sized herbivores may also be vulnerable if they fall within the predator's preferred prey size range (15). At the upper extreme of the body size gradient, megaherbivores (species that weigh more than 1,000 kg) are practically invulnerable to predation as adults (16). Vulnerable herbivore species will select the parts of the landscape where perceived predation risk is low (17).

Significance

Herbivores influence nutrient cycling by depositing feces across the landscape. Where herbivores go in the landscape is governed by factors such as food requirements and vulnerability to predation, traits that are related to body size. We show that mammals that differ in body size not only use the landscape differently but also differ in the amount of nitrogen relative to phosphorus that they release through their feces. This ensures that plants that grow in areas used by predominantly larger herbivores (such as areas of higher predation risk or areas of greater food availability) will receive lower amounts of fecal phosphorus relative to fecal nitrogen, potentially impacting plant nutrient availability. This finding is noteworthy considering the extinction bias toward larger animals.

Author contributions: E.I.R., G.I.H.K., and J.P.G.M.C. designed research; E.I.R. and L.S.v.V. performed research; E.I.R. and L.S.v.V. analyzed data; E.I.R., G.I.H.K., and J.P.G.M.C. wrote the paper; and G.I.H.K. and J.P.G.M.C. supervised the work.

The authors declare no competing interest.

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¹To whom correspondence may be addressed. Email: elizabeth.liza.le.roux@gmail.com.

This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2003269117/-DCSupplemental>.

First published August 24, 2020.

How the perception of predation risk varies spatially will depend on the herbivore's antipredatory strategy. Herbivores live in a landscape of fear (17–20) where spatial heterogeneity in vegetation density alters lateral visibility and influences the perception of risk (21). To reduce predation risk, grazing herbivores select for habitat with increased visibility (18, 19, 21–23), where predators' hunting success is lower due to fewer ambush opportunities (24). Conversely, some browsers may rely more on crypsis to avoid being detected by predators and instead depend on low visibility to provide concealment (20, 23).

While forage quantity and quality, predation risk, and antipredatory response may result in local variation in the average body size of the herbivore community, this does not necessarily lead to differences in the overall herbivore biomass density. Larger species occur at lower densities, which ensures that, for a given level of productivity, overall herbivore biomass should remain roughly equivalent (25, 26). If so, the absolute amounts of fecal material deposited should also remain constant, as shown by le Roux et al. (19). However, differences in the N:P stoichiometric ratios between the dung of large and small animals mean that changes in the average body size of the herbivore community will cause a shift in the stoichiometric ratio of nutrients deposited. This shift in N:P ratio can in turn feed back into the plant community, potentially altering plant productivity, plant species composition, decomposition dynamics, and many other aspects of ecosystem functioning (e.g., ref. 27).

Here, we assess the relationship between herbivore body size and fecal stoichiometry and examine the potential impact of average herbivore body size within a local community on ecosystem N:P stoichiometry. To do this, we quantified fecal N:P stoichiometric ratios of herbivores that differed in body size and compared herbivore visitation, fecal input, and grass and soil N:P stoichiometry across areas that varied in food quality and quantity, and lateral visibility. We predicted that areas of abundant, low-quality food and/or low visibility should attract relatively more large-bodied herbivores, and thus feces of higher N:P ratios should accumulate in these areas. We predicted that this higher N:P stoichiometry of fecal input would be associated with higher grass and soil N:P content.

We focus on defecation, partly due to the difficulty of measuring urine release in the wild, but also because nearly all waste phosphorus is released through defecation (5). Multiple dynamic processes other than herbivore fecal inputs influence nitrogen loss or accumulation. For example, nitrogen can accumulate in soils through nitrogen fixation or be lost through volatilization during fires (3). Thus, we expect phosphorus to be more closely coupled to fecal inputs than nitrogen. It has been suggested that the provision of animal waste should boost predominantly P-availability and drive ecosystems toward N-limitation (3).

Our study was performed in a South African protected savanna (Hluhluwe-iMfolozi Park, Fig. 1) that contains a diverse predator community and a mammal herbivore community that spans a 10-fold gradient in body mass. To test herbivores' impact on biogeochemistry, we used grazing lawns, a system that represents a close link between grazers, grass, and nutrients where herbivores' contribution to biogeochemistry has been well established (28). Grazing lawns are herbivore-maintained patches of short-statured, stoloniferous grass communities that are a frequently used resource hot spot for a wide range of ungulate short grass grazers that vary in body size from impala (*Aepyceros melampus*) to white rhino (*Ceratotherium simum*) (28). The persistence of these lawns depends on intense grazing affording the lawn grass species a competitive edge over taller grass species, and as such, they represent intensely grazed islands in and among a surrounding matrix of less often grazed tall grass habitat. Grazing lawns are considered a very high-quality grazing resource, in part because regular grazing prevents the buildup of structural carbohydrates and stimulates compensatory growth, which ensures that fresh

growth is frequently available, providing the nutritional incentive for repeated grazing (28). Compensatory growth may place high nutritional demands on lawn grasses (29), a demand that is proposed to be sustained by the continual input of herbivore feces and urine (30). Furthermore, grazing lawns vary widely in lateral visibility, ranging from completely open to surrounded by, and interspersed with, dense woody cover (31).

We selected 15 grazing lawns that varied widely in rainfall and surrounding visibility (Fig. 1 and *SI Appendix*, Fig. S1) and demarcated a 5 × 5-m plot in each lawn, within which we monitored herbivore presence over a 10-mo period using camera traps. We quantified the accumulation of herbivore fecal material in each grazing lawn plot over the same period. We considered rainfall as an indication of food quantity and grazing lawn size as an indication of the availability of high-quality food that, because of the short stature of the grasses, is low in quantity (*Materials and Methods*). Because of our focus on grassy systems, we felt it justifiable to consider lateral visibility as a proxy for predation risk, i.e., higher visibility interpreted as lower perceived risk [see the previous studies that established this link (18, 19, 21, 24)]. However, we acknowledge that certain browsing species may instead consider low visibility as the safer habitat and that the woody cover that leads to lower visibility may also be a food source for browsers. Thus, lateral visibility may impact the average herbivore body size within a local community through both changes in predation risk and food availability. From the perspective of the grazing lawn plot, we related lawn visibility, lawn size, and rainfall to 1) total herbivore presence (i.e., visitation and time spent) per lawn; 2) the average body size of each lawn's herbivore visitors; 3) how much fecal material accumulated on each lawn; and 4) the average body size of each lawn's herbivore defecators. We quantified fecal nutrient content for each defecator and related fecal nutrient deposition and the average body size of the defecators to the nitrogen and phosphorus content and the N:P stoichiometric ratio of lawn grass leaves and lawn soils. We also compared the extent to which the grass and soil nitrogen and phosphorus content in each lawn plot differed from that of a nearby tall grass control plot (i.e., a plot comparable in tree/shrub cover and slope, within 60 m away from the grazing lawn plot yet outside the lawn within the tall-grass surrounds; see photo *Insets* in Fig. 1). Grazing lawns have been shown to be elevated in foliar nutrients compared to surrounding tall grass (28), and thus we tested how the absolute nutrient concentration and the degree of nutrient elevation of lawn grass and soils relates to the fecal material accumulation and the average body weight of the defecator. As an additional sensitivity check, we reran all our analyses on a separate fecal nutrient dataset for the same species recorded in a savanna game reserve in Kenya (32) and report these results in *SI Appendix*.

Specifically, we predicted that 1) as herbivore body size increases, the phosphorus content of their fecal material will decrease and the N:P ratio will increase; 2) the herbivore visitation and dung accumulation on grazing lawns of lower visibility, higher rainfall, and smaller size will be, on average, from larger herbivore species; and 3) despite local community body size differences, overall visitation, and therefore overall dung accumulation, would be comparable across grazing lawn plots; but that 4) the greater the average body size of the defecating community, a) the higher the N:P ratio of the total fecal nutrient input and b) the higher the N:P ratio of the soil and grass leaf material.

Results

Fecal nitrogen content only varied with body size (log-transformed) among mixed feeders [which in our dataset consisted of impala, nyala (*Tragelaphus angasii*) and elephant (*Loxodonta africana*); Fig. 2 and *SI Appendix*, Table S1], and not for the other feeding guilds. In contrast, fecal phosphorus decreased with body size (log-transformed), regardless of the herbivore's foraging strategy

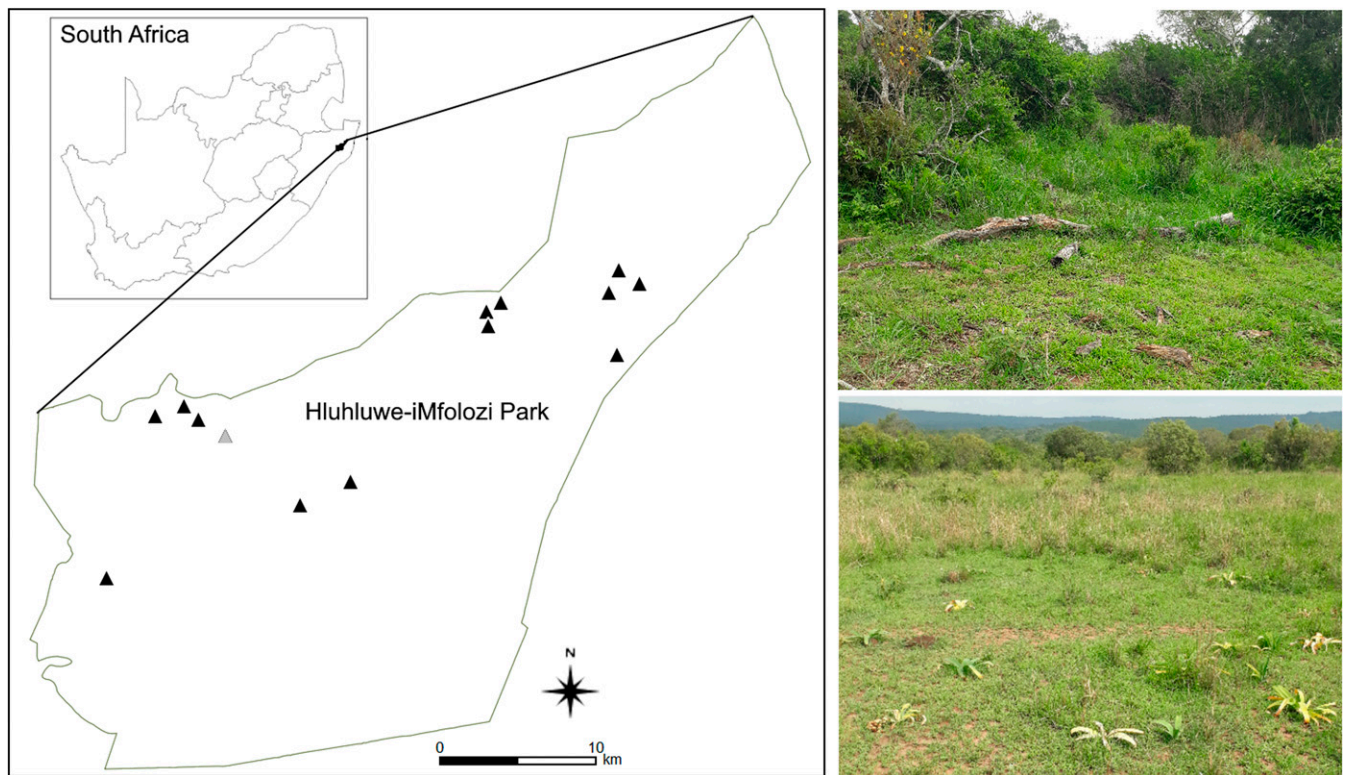


Fig. 1. Location of Hluhluwe-iMfolozi within South Africa and schematic of plot distribution. The gray symbol indicates two plots that were just 55 m apart and thus too close to display separately on the map. The photo insets show a low-visibility (Top) and a high-visibility (Bottom) grazing lawn. The Bottom photo shows the clear division between the grazing lawn and the surrounding tall grass layer in which the control plot was placed. Tall grass areas appropriate for placing control plots (i.e., comparable in tree/shrub cover and slope) were within 20 m of the lawn plot (apart from one site where the nearest suitable control was located 60 m away from the lawn plot). The entire grazing lawn within which the lawn plot was demarcated ranged greatly in size with the largest lawn being 6.5 times larger than the smallest lawn.

(however, the browser category was omitted from these analyses as the dataset only contained one species, giraffe, *Giraffa camelopardalis*). Correspondingly, the fecal N:P ratio increased with body size (log-transformed; Fig. 2 and *SI Appendix, Table S1*).

The total amount of fecal material (in kilograms dry mass) that accumulated on each plot was independent of visibility, rainfall, or lawn extent (*SI Appendix, Table S2*). However, the average weight of the herbivore community that visited a grazing lawn increased with decreasing visibility and increasing rainfall (Fig. 3 and *SI Appendix, Table S3*); i.e., on average, larger species visited lawns of lower visibility and lawns that received more rainfall. Similarly, as visibility decreased, the average weight (log-transformed) of the herbivore community that defecated on each grazing lawn increased (Fig. 3 and *SI Appendix, Table S4*); i.e., the fecal material that accumulated on less visible grazing lawns originated on average from larger herbivores. This log-linear relationship indicates that the increase in average body weight is particularly stark at the lawns with the lowest visibility. These relationships with surrounding visibility remained unchanged, regardless of whether visibility was measured at impala eye height (~90 cm) or at observer eye height (~156 cm). Lawn extent did not influence the average weight of the herbivore community visiting or depositing dung on lawns (*SI Appendix, Tables S3 and S4*).

The observed increase in average body size of the local mammal community, in response to higher rainfall and reduced visibility, significantly increased the N:P ratio of fecal input (Table 1 and Fig. 4). Replacing the measured fecal nutrient values with those obtained from Sitters et al. (32) did not change the interpretations (*SI Appendix, Table S5*). An increase in the average body size of the defecators was associated with an increase in the total amount

of fecal nitrogen and a decrease in the total amount of fecal phosphorus deposited on each lawn plot (Table 1). However, according to AICc (Akaike information criterion corrected for small sample sizes), defecator body size did not explain variation in total fecal nitrogen and phosphorus input any better than a null model.

The nutrient content of lawn soils and the nitrogen content of lawn grass leaves did not vary with a change in average body size of the defecators (*SI Appendix, Table S6*). While an increase in average defecator body size was associated with a significant increase in lawn grass leaf N:P ratio, the model fit was poor with the difference in AICc over a null model being less than 2.

The leaf material of the grasses growing inside the grazing lawns was elevated in nitrogen and phosphorus relative to the leaf material of the grasses growing in the adjacent tall-grass control plot (nitrogen: $t = 4.33$, $df = 14$, $P < 0.001$; phosphorus: $t = 1.92$, $df = 14$, $P = 0.074$; *SI Appendix, Fig. S2*). However, as predicted, the degree of phosphorus elevation was less pronounced in the lawn grass leaves that received fecal material from predominantly larger herbivores, compared to the lawn grass leaves where the defecating herbivore was on average smaller (estimate = -0.0005 , $SE = 0.0002$, $P = 0.018$; *SI Appendix, Table S6*), although again the model fit was poor and the AICc difference over a null model was less than 2.

Discussion

In agreement with our predictions, spatial heterogeneity in visibility and in forage quantity (rainfall) led to local herbivore communities that varied in average body size. Grazing lawns in areas of higher rainfall and grazing lawns of lower visibility were visited by, on average, larger-bodied species and the fecal material

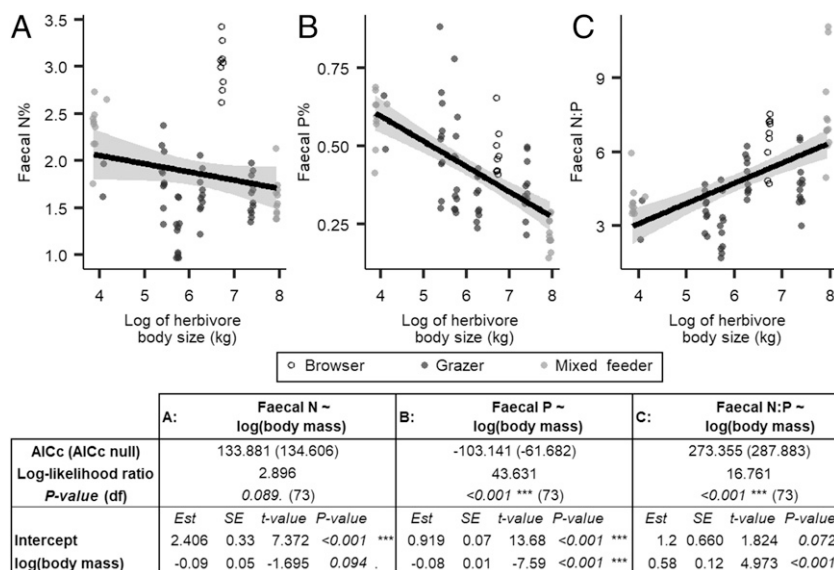


Fig. 2. Fecal nitrogen (A), fecal phosphorus (B), and fecal N:P ratio (C) in relation to the log of herbivore body size (in kilograms). Points are categorized according to feeding type. The browser category contains only a single species, giraffe. The trendlines were produced using `geom_smooth` [ggplot2 package (33)] of a single-term linear model fit to all herbivores regardless of feeding strategy. The table *Inset* shows test statistics and coefficient estimates for generalized least-squares models of all feeding types combined, with heteroscedasticity corrected in the model of fecal N:P. Test statistics for models specific to each feeding type can be found in *SI Appendix, Table S1*. As an indication of overall model fit, the AICc statistic of each model is presented alongside the AICc of each model's corresponding null model (intercept-only model). The log-likelihood ratio statistic and associated P value for each model compared to the null model are also given with degrees of freedom reported in parentheses. Significance codes: ***<0.001 and ". " 0.1.

that accumulated on lawns of lower visibility originated from, on average, larger-bodied species. The consequence of this variation in average community body size is that despite fecal material having been deposited in relatively equal amounts across all grazing lawns, the deposition of particular nutrients was uneven. Because the feces from larger-bodied herbivores contained lower quantities of phosphorus per unit mass and were higher in N:P ratio, lawns on which predominantly larger herbivores defecated received fecal material that was higher in N:P ratios.

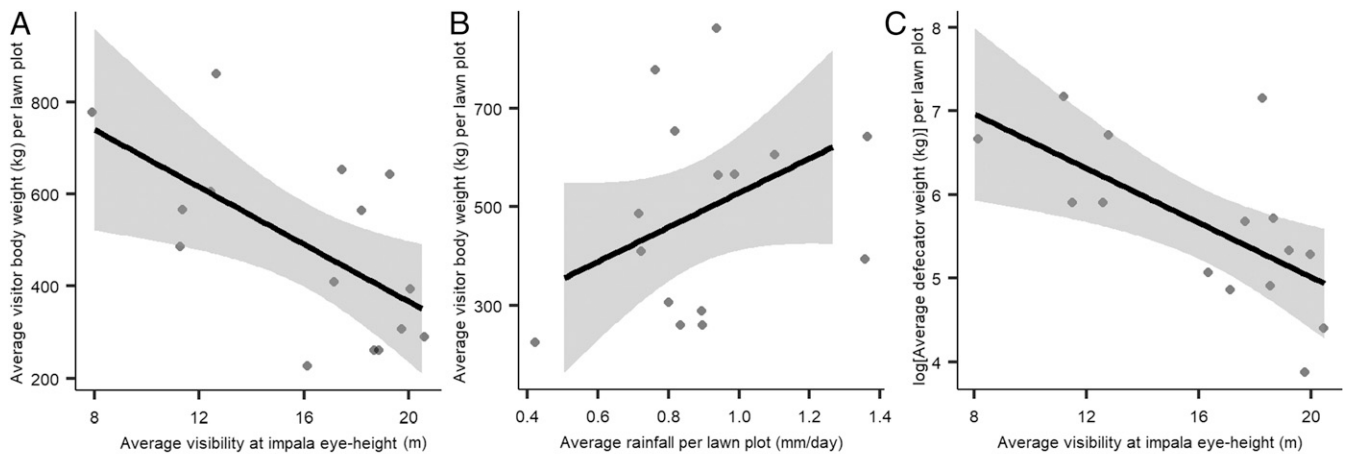
The fecal nutrient dataset used in this study had low sample size for certain species (*SI Appendix, Table S7*), and it was only collected in a single season. However, in recognition of this shortcoming, we substituted all fecal nutrient data with a published dataset collected in Saadani National Park, Tanzania, over multiple years and multiple seasons (32). Although we were unable to evaluate the changes in fecal N and P for mixed feeders (as the dataset from ref. 32 only contained two mixed feeders), the relationship between grazer fecal N and P with body size remained the same. We ran the analyses relating fecal nutrient input to defecator body size using this substitute dataset, and all relationships and conclusions remained unchanged.

While the average body size of the local herbivore community strongly influenced the N:P stoichiometric ratio of fecal input to a grazing lawn, it was a poor predictor of grass N:P ratio, despite a significant relationship. This is likely because fecal input is just one factor of many that may impact stoichiometric balances. For example, in savannas, the abundance of nitrogen-fixing *Vachellia* species may play prominent roles in nitrogen replenishment while frequent fires may drive losses in volatile nitrogen, more so than phosphorus (3). Moreover, the release of nitrogen through animal feces is said to be very diet dependent and thus the relative ratios of browsers vs. mixed feeders vs. grazers will be an important factor in the stoichiometry of nutrient release. With browser dung being generally higher in nitrogen content compared to that of grazers (32) (see Fig. 24 for the elevated fecal nitrogen content of giraffe), nitrogen may play a more prominent role in determining the N:P ratio of browser dung. In addition, browsing species are also unlikely to congregate in open areas as an

antipredatory response and may instead perceive denser vegetation as safer (20, 23). By limiting our study to using grazing lawns, we neglected the browser influence, an omission that should be remedied in future studies.

Certain legacy effects in our study site may also account for the tenuous relationship between the average body size of the herbivore community and grass N:P content. While Hluhluwe-iMfolozi Park (HiP) currently contains high densities of megaherbivores, their impact on ecosystem stoichiometry has been greatly disrupted over the past century. Elephants have been reintroduced in HiP in 1981, after almost a hundred-year absence (34). White rhino populations recovered from near extinction at the beginning of the 1900s, giraffes were reintroduced during the mid-1900s, and hippopotamuses (*Hippopotamus amphibius*) occur in very low numbers (34). Thus, the long-term stoichiometric pattern may not yet be fully recovered. In view of these constraints (i.e., various alternative pathways of grass nutrient control, long-term disruptions to consumer-driven nutrient movement, and the small sample size of our study), a weak grass N:P response is perhaps unsurprising. Future work should endeavor to quantify the relative contribution of herbivore nutrient release to the stoichiometry of plant nutrient availability in comparison to that of other potential nutrient sources. We propose measuring environmental variables that link explicitly to both the direct and indirect nutrient transfer pathways (e.g., fecal inputs, litterfall, density of nitrogen-fixing plant species) and analyzing these variables through path analyses that can separate the relative contributions of each. This will require sample sizes much higher than what we present here.

If stoichiometric differences in herbivore fecal N:P ratios translate into plant nutrient limitation, the consequences may be far-reaching, manifesting in plant productivity, phenology, and species composition. For example, somewhat speculatively, in areas where smaller herbivores create conditions of low N:P ratios and plants are driven toward nitrogen limitation, the growth of particular species such as nitrogen fixers may be promoted, gradually shifting plant species composition. This may be a fruitful area of investigation. Another avenue for future research may be to investigate the stoichiometric consequences of direct predation on predominantly



| | Average visitor body weight ~ std(Visibility) + Std(Rain) (graph panel A & B) | | | | log(Average defecator body weight) ~ std(Visibility) (graph panel C) | | | |
|-----------------------------|---|-----------|----------------|----------------|--|-----------|----------------|----------------|
| AICc (AICc null) | 204.076 (208.638) | | | | 42.928 (49.000) | | | |
| Log-likelihood ratio | 13.047 | | | | 9.891 | | | |
| P-value (df) | 0.0015 ** (12) | | | | 0.0017 ** (13) | | | |
| | <i>Est</i> | <i>SE</i> | <i>t-value</i> | <i>P-value</i> | <i>Est</i> | <i>SE</i> | <i>t-value</i> | <i>P-value</i> |
| Intercept | 486.882 | 36.074 | 13.497 | <0.001 *** | 5.624 | 0.218 | 25.752 | <0.001 *** |
| std(Visibility) | -125.116 | 37.373 | -3.348 | 0.006 ** | -0.647 | 0.107 | -6.046 | <0.001 *** |
| std(Lawn extent) | - | - | - | - | - | - | - | - |
| std(Rain) | 92.272 | 37.373 | 2.469 | 0.030 * | - | - | - | - |

Fig. 3. The relationship between the average visitor body weight (in kilograms) and the average visibility measured at impala eye height (A) and the average rainfall received per lawn plot (B). C shows the relationship between the average defecator body weight (log-transformed) and visibility measured at impala eye height. The trendlines were produced using `geom_smooth` [ggplot2 package (33)] of a linear model fit to the single explanatory term. Note the difference in y axis scaling between A and B. The table *Inset* shows test statistics and coefficient estimates for generalized least-squares models that include a spatial correlation term. For modeling purposes, all explanatory variables are standardized by subtracting the mean and dividing by the SD. As an indication of overall model fit, the AICc statistic of each model is presented alongside the AICc of each model's corresponding null model (intercept-only model). The log-likelihood ratio statistic and associated P value for each model compared to the null model are also given with degrees of freedom reported in parentheses. The full model selection steps and associated test statistics can be found in *SI Appendix, Tables S3 and S4*. Significance codes: ***<0.001, **<0.01, and *<0.05.

smaller species leading to compositional shifts in the herbivore community toward proportionally more large species. We might speculate that shifting the herbivore community to a dominance of larger body sizes may lead to a greater demand for phosphorus by the herbivore trophic level as a whole, which may propagate through the food web to lower the availability of phosphorus at the primary producer level, driving plants toward P-limitation. This has been

shown in lake experiments where shifts from a community dominated by high body N:P species to one dominated by low body N:P species caused primary producers to shift from being limited by the availability of N to being limited by the availability of P (35).

These findings gain further relevance when seen within the context of past megafaunal extinctions. Between ~50,000 y ago and the present, ~50% of large mammal species (>44 kg) went

Table 1. Test statistics and coefficient estimates for generalized least-squares models testing the relationship between the average defecator body weight (in kilograms) and total fecal nitrogen input (A), total fecal phosphorus input (B), and the nitrogen-to-phosphorus ratio of total fecal input (C)

| | Dataset: Outlying point excluded (n = 14) | | | | | | | | Dataset: Full (n = 15) | | | |
|---------------------------------|--|-----------|----------------|----------------|--|-----------|----------------|----------------|---|-----------|----------------|----------------|
| | A: Total fecal N input ~ average defecator body weight | | | | B: Total fecal P input ~ average defecator body weight | | | | C: N:P ratio of fecal input ~ average defecator body weight | | | |
| AICc (AICc null) | 147.109 (147.203) | | | | 105.172 (104.097) | | | | 22.198 (48.212) | | | |
| Log-likelihood ratio | 4.138 | | | | 2.970 | | | | 29.832 | | | |
| P value (df) | 0.042* (12) | | | | 0.085 . (12) | | | | <0.001*** (13) | | | |
| | <i>Est</i> | <i>SE</i> | <i>t value</i> | <i>P value</i> | <i>Est</i> | <i>SE</i> | <i>t value</i> | <i>P value</i> | <i>Est</i> | <i>SE</i> | <i>t value</i> | <i>P value</i> |
| Intercept | 52.895 | 12.866 | 4.111 | 0.001** | 15.643 | 3.089 | 5.065 | <0.001*** | 3.782 | 0.138 | 27.369 | <0.001*** |
| Average defecator body size, kg | 0.026 | 0.012 | 2.128 | 0.055 . | -0.006 | 0.003 | -2.436 | 0.031* | 0.002 | 0.000 | 9.055 | <0.001*** |

Responses A and B were tested using a subset dataset excluding an influential outlier, and response C did not have an outlier and as such was tested using the full dataset. As an indication of overall model fit, the AICc statistic of each model is presented alongside the AICc of each model's corresponding null model (intercept-only model). The log-likelihood ratio statistic and associated P value for each model compared to the null model are also given with degrees of freedom reported in parentheses. Significance codes: ***<0.001, **<0.01, *<0.05, and "." 0.1.

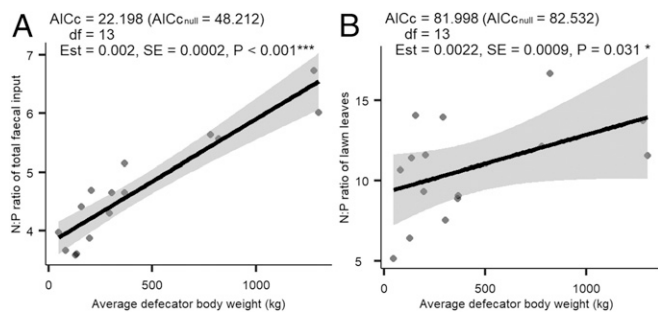


Fig. 4. The relationship between average defecator body weight (in kilograms) and the N:P ratio of total faecal input (A) and the N:P ratio of lawn grass leaves (B). The trendlines were produced using `geom_smooth` [`ggplot2` package (33)] of a linear model fit to the single explanatory term. The coefficient estimates are presented above each panel and were obtained using single-term generalized least-squares models with a spatial correlation. As an indication of overall model fit, the AICc statistic of each model is presented alongside the AICc of each model's corresponding null model (intercept-only model). df, degrees of freedom. Significance codes: ***<0.001 and *<0.05. Estimates of the relationships between defecator body size and total N and P fecal input and total N and P grass content can be found in Table 1 and *SI Appendix*, Table S6.

extinct (36). This extinction differs from previous mass extinctions in that it is skewed toward larger species (37), and thus globally, the remaining mammal communities are made up of smaller-bodied species than they have been for at least the last 30 to 40 million years (38). At the start of the Pleistocene, the mean body mass of the world's mammal communities was $\gg 100$ kg, yet this has collapsed to ≤ 10 kg [with the exception of a few protected areas in Africa and Asia (38)]. The consequences of this dramatic body size downgrading for ecosystem processes is of much current interest and has been frequently discussed in recent literature (39–43). We hope that this work will contribute to these debates by showing that the extinction of predominantly large animals may have altered stoichiometric balances. This insight is crucial at a time when body size downgrading of mammalian communities continues, and the ecological consequences of this complete global megafaunal collapse are unclear.

Materials and Methods

The study site was situated in the megaherbivore-rich HiP in KwaZulu-Natal, South Africa. It is a mesic to semiarid savanna with a strong rainfall gradient from north to south, ranging from an average of 968 mm pa (± 36 SE) to 691 mm pa (± 30 SE) (44). The vegetation ranges from open grasslands to closed *Vachellia* and broadleaved woodlands (45). Grazing lawns form a prominent feature of the HiP landscape, and at the time of this study, grazing lawns covered an estimated 6.9% of the surface area of the reserve, and up to 20 to 30% in the southern sections (31).

Grazing Lawn Selection. We selected 15 grazing lawns varying in lateral visibility (*SI Appendix*, Fig. S1) and demarcated a 5 × 5-m plot within each. The average distance between grazing lawn plots was 14.75 km (SD = 6.13 km), ranging between 55 m and 35 km (Fig. 1). We quantified visibility surrounding each plot using a 1.6-m-tall Nudds' density board (46) vertically divided into 0.2-m sections. For every 0.2-m section of the board, we recorded the distances at which approximately half of the section was no longer visible, along transects into the eight cardinal and intercardinal directions from the center of the plot and up to a distance of 20 m. We averaged measurements across all transects per plot. We performed this visibility measurement in July 2014 and March 2015 to account for any seasonal variation in visibility. Visibility measurements were made with the observer viewing from an eye height level of ~90 cm, reflecting the average eye height of impala (*Aepyceros melampus*), the dominant prey species in our study (34). We repeated the measurements with the observer standing upright (eye height, ~156 cm) and tested with every analysis whether visibility measured at a higher height yielded a different result.

Prior to lawn plot selection, we quantified grass cover and identified grass species and selected plots that were comparable in grass cover and that were

made up of between 70 and 100% prostrate-growing, lawn-type species, including *Dactyloctenium australe*, *Digitaria longiflora*, *Sporobolus nitens*, *Urochloa mosambicensis*, *Panicum coloratum*, and *Sporobolus ioclados* (see ref. 28). We quantified surrounding lawn extent along 50-m transects in each of the eight cardinal and intercardinal directions. This lawn extent estimate was missing for one of the 15 lawn sites.

Herbivore Presence and Fecal Nutrient Input. Between May 2014 and February 2015, we recorded herbivore presence in each lawn by placing a motion-triggered Bushnell Trophy trap camera in the corner of each lawn plot, set to take photos at 1-s intervals when triggered with no refractory period set. This ensured that the more time an animal spent on the plot, the more photos were taken, thus combining information on the number of animals visiting and the duration of the visit. Due to occasional animal interference, cameras did not function continuously throughout this period, but we recorded the number of days the camera was functional to correct for this difference in effort.

From the photos, we identified the herbivore species and quantified the number of individuals within the 5 × 5-m plot. We recorded rainfall and species-specific dung accumulation within the 5 × 5-m plots on a roughly 2- to 3-wk rotation. We counted the number of dung piles and crushed these in situ to avoid recounting. We calculated total dung input (in kilograms) per species by multiplying the dung pile count with an average dung pile weight. Average dung pile weight was estimated by collecting five dung pile samples per species, drying at 60 °C for 48 h and measuring the dry weight. We estimated the total input of nitrogen and phosphorus (in grams) to each lawn plot over the 10-mo monitoring period using species-specific measurements of dung nutrient content from dung samples collected in 2018 (see next section). With the exception of gray duiker (*Sylvicapra grimmia*) and waterbuck (*Kobus ellipsiprymnus*), fecal nutrient measurements were available for all species that defecated on the lawn plots. We used published estimates (32) of fecal nutrient content for gray duiker and used wildebeest (*Connochaetes taurinus*) fecal nutrient content for waterbuck. While dietary differences between waterbuck and wildebeest are likely to influence their dung nutrient profile, this is unlikely to influence our results, as only two piles of waterbuck dung were recorded over the 10-mo period.

Fecal Nutrient Content Measurements. Between January and April 2018, we collected fresh dung (no older than a day) from all mammalian herbivores that were recorded on the lawn plots (a sample size summary is presented in *SI Appendix*, Table S7). Sampling locations were spread across the park and dung samples of the same species found in the same location within a time span of 7 d were combined into composite samples. The dung was dried at 60 °C for 48 h, ground, and stored for later analysis of nutrient content by an external laboratory (KZN Agricultural Research Laboratories). Nitrogen content was estimated using the Dumas (dry combustion) method using a Leco CNS 2000 Analyzer, and phosphorus content was estimated by inductively couple plasma optical emission spectrometry.

Grass and Soil Nutrient Content Measurements. During May 2014, we collected five soil and five grass leaf samples from randomly selected locations within each lawn plot and within a tuft grass-dominated control plot located in the vicinity of each lawn. While the nutrient measurements were taken at the start of the herbivore use and dung accumulation monitoring, we assume that the results of our 10-mo monitoring of herbivore presence and dung deposition reflected the long-term responses to rainfall and visibility gradients and therefore reflect long-term, cumulative impact on nutrient status. Control plots were delineated outside the lawn area such that they contain predominantly tall bunch grass species, yet within close proximity to the lawn plot (within 20 m for all sites apart from one where the closest suitable control area was located ~60 m away). The control plots were comparable to the lawn plots in terms of slope and canopy cover. The soil samples were collected to a depth of 10 cm and divided into a top soil (0 to 5 cm) subsample and a deeper soil (5 to 10 cm) subsample. Each subsample was analyzed separately. The grass samples were hand plucked and included all leaf biomass from the mixture of the species growing within ~5 cm from each of the soil sampling locations (i.e., five locations randomly spread across each plot). The species mixture ensured that the sample reflected the nutrient availability within the plot regardless of species. For soil and leaf material, the five samples were combined into a composite sample [dry weight averages: lawn grass \bar{x} = 3 g, control grass \bar{x} = 4 g, lawn soils \bar{x} = 437 g (0–5 cm); \bar{x} = 493 g (5–10 cm), control soils \bar{x} = 425 g (0–5 cm); \bar{x} = 469 g (5–10 cm)], dried at 60 °C, and stored for subsequent analysis of nutrient content by KZN Agricultural Research Laboratories (see previous section).

Statistical Analysis. To investigate variation in dung nutrient content with varying body size, we applied three single-term linear models, regressing fecal nitrogen percentage, fecal phosphorus percentage and nitrogen-to-phosphorus ratio against log-transformed body size. These models were run on all herbivore data combined as well as on subsets of mixed-feeding species and grazing species. We corrected heteroscedasticity in the models with grazer fecal nitrogen, grazer fecal phosphorus, and mixed-feeder fecal phosphorus as response [using the weights function from the nlme package (47)].

We modeled four response variables on the lawn plots: 1) herbivore presence; 2) total fecal material accumulation [total dung accumulation in kilograms (dry matter) across the entire 10-mo period]; 3) average body size of all herbivores present on each grazing lawn plot (see Eq. 1); and 4) average body size of all herbivores that defecated on each plot (see Eq. 2).

We calculated an estimate of total herbivore presence per grazing lawn plot (response 1) by summing the number of photos taken of each species and the number of individuals in each photo (combining information on visitation and length of stay). To correct for effort, we divided this value by the number of days in which the camera was functional (to account for variability in measurement effort due to animal interference).

We calculated the average body size of the herbivores that were present on each grazing lawn plot (response 3) by multiplying the number of photos taken of each species per plot by the average female body weight of that species (taken from ref. 16), summing this value across all species and dividing the total value by the total number of herbivore photos taken per plot (across all species):

$$\frac{\sum_{i=1}^n (P_i \times aBW_i)}{\text{sum}(P)}, \quad [1]$$

where i denotes herbivore species and P_i and aBW_i are the species-specific number of photos recorded and average female body weight, respectively. P represents the total number of herbivore photos taken at that lawn.

Similarly, we calculated the average body size of the herbivores that defecated on each grazing lawn plot (response 4) by multiplying the number of dung piles deposited by each species per plot by the average female body weight of that species, summing this value across all species and dividing the total value by the total number of dung piles deposited per plot (across all species):

$$\frac{\sum_{i=1}^n (DP_i \times aBW_i)}{\text{sum}(DP)}, \quad [2]$$

where i denotes herbivore species and DP_i and aBW_i are the species-specific number of dung piles deposited and average female body weight, respectively. DP represents the total number of dung piles deposited on that lawn plot (regardless of species).

We applied generalized least-squares models from the package nlme (47) and modeled each response (herbivore presence, dung accumulation, average visitor body size, and average defecator body size) as a function of 1) surrounding lawn extent, 2) rainfall, and 3) surrounding visibility. All explanatory variables were standardized prior to modeling by subtracting the mean of each variable and dividing by the SD. All variance inflation factors

were below 2, and as such there were no concerns over multicollinearity in these models. We incorporated a spatial exponential correlation structure, allowing the correlation to decay exponentially with distance between lawns. We obtained a best adequate model through backward selection based on AICc, removing the least significant term at each step until all remaining parameters were significant at the 0.05 level (48). Herbivore presence (response 1) and herbivore dung accumulation (response 2) each contained a single outlying point. All model interpretations were verified with and without this point included. We log-transformed the average herbivore defecator body weight response to improve model fit.

We then tested whether the total fecal phosphorus input per plot (in grams), the total fecal nitrogen input per plot (in grams), and the total fecal nitrogen to phosphorus ratio changed in relation to the average body size of the herbivore defecators using single-term generalized least-squares models with the spatial exponential correlation structure. Model results presented for the total fecal P and fecal N input have a single outlier removed; however, its omission did not change our interpretation.

To analyze lawn nutrient status relative to the surrounding tall grass control plot, we used paired sample t tests to compare absolute nutrient contents in lawn samples to absolute nutrient contents in control samples. Because lawn plots were widely distributed across areas likely to differ in a number of conditions that would influence elemental profiles (Fig. 1), we also calculated a proportional difference in nutrients (for both soils and grass leaves) between lawn and control:

$$\text{diffN} = \frac{N_{\text{lawn}} - N_{\text{control}}}{N_{\text{control}}}. \quad [3]$$

We tested whether lawn nutrients (N, P, and N:P ratio in grass leaf material, soil at 0- to 5-cm depth, and soil at 5- to 10-cm depth) changed in relation to the average body size of the defecator. We again applied generalized least-squares models with the spatial exponential correlation structure. We used the same procedure to see whether the extent of lawn nutrient enhancement [diffN, diffP, and diffN:P (see Eq. 3) in grass leaf material, soil at 0- to 5-cm depth and soil at 5- to 10-cm depth] changed in relation to the average body size of the defecator. To reduce skewness we log transformed the following variables: $P_{\text{grass leaves}}$, $P_{\text{soil 0-5 cm}}$, $P_{\text{soil 5-10 cm}}$, $N:P_{\text{soil 0-5 cm}}$, $N:P_{\text{soil 5-10 cm}}$, $\text{diffP}_{\text{soil 0-5 cm}}$, $\text{diffP}_{\text{soil 5-10 cm}}$, $\text{diffN:P}_{\text{soil 0-5 cm}}$, and $\text{diffN:P}_{\text{soil 5-10 cm}}$.

Throughout all modeling procedures, we routinely checked for heteroscedasticity and validated models graphically. All analyses were performed in R (49).

Data Availability. Data files and the analysis script have been deposited in the Mendeley Data repository (DOI: [10.17632/jwc4rr6kwr.3](https://doi.org/10.17632/jwc4rr6kwr.3)) (50).

ACKNOWLEDGMENTS. We thank Ezemvelo KwaZulu-Natal Wildlife for logistical and technical support and the many students and volunteers who assisted in data collection. We thank three anonymous reviewers for their insightful comments on earlier drafts of this manuscript. The research was financially supported by a Marie Curie grant held by J.P.G.M.C. (Grant PCIG10-GA-2011-304128). E.I.R. was supported by the Claude Leon Foundation, the South African National Research Foundation, and the Nelson Mandela University.

1. S. J. McNaughton, F. F. Banyikwa, M. M. McNaughton, Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science* **278**, 1798–1800 (1997).
2. R. D. Bardgett, D. A. Wardle, Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* **84**, 2258–2268 (2003).
3. P. G. Cech, T. Kuster, P. J. Edwards, H. Olde Venterink, Effects of herbivory, fire and N_2 -fixation on nutrient limitation in a humid African savanna. *Ecosystems* (*N. Y.*) **11**, 991–1004 (2008).
4. O. J. Schmitz *et al.*, Animals and the zoogeography of the carbon cycle. *Science* **362**, eaar3213 (2018).
5. J. Sitters *et al.*, The stoichiometry of nutrient release by terrestrial herbivores and its ecosystem consequences. *Front. Earth Sci.* **5**, 32 (2017).
6. V. Geist, On the relationship of social evolution and ecology in ungulates. *Am. Zool.* **14**, 205–220 (1974).
7. C.-A. Darveau, R. K. Suarez, R. D. Andrews, P. W. Hochachka, Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166–170 (2002).
8. J. J. Elser, D. R. Dobberfuhl, N. A. MacKay, J. H. Schampel, Organism size, life history, and N:P stoichiometry. Toward a unified view of cellular and ecosystem processes. *Bioscience* **46**, 674–684 (1996).
9. W. W. Reynolds, Skeleton weight allometry in aquatic and terrestrial vertebrates. *Hydrobiologia* **56**, 35–37 (1977).
10. J. G. C. Hopcraft, T. M. Anderson, S. Pérez-Vila, E. Mayemba, H. Olf, Body size and the division of niche space: Food and predation differentially shape the distribution of Serengeti grazers. *J. Anim. Ecol.* **81**, 201–213 (2012).
11. R. H. V. Bell, “The use of the herb layer by grazing ungulates in the Serengeti” in *Animal Populations in Relation to Their Soil Resources*, A. Watson, Ed. (Blackwell Scientific Publications, 1970), pp. 111–123.
12. P. J. Jarman, The social organisation of antelope in relation to their ecology. *Behaviour* **48**, 215–267 (1974).
13. H. Olf, M. E. Ritchie, H. H. T. Prins, Global environmental controls of diversity in large herbivores. *Nature* **415**, 901–904 (2002).
14. A. R. E. Sinclair, S. Mduma, J. S. Brashares, Patterns of predation in a diverse predator-prey system. *Nature* **425**, 288–290 (2003).
15. E. le Roux *et al.*, Top-down limits on prey populations may be more severe in larger prey species, despite having fewer predators. *Ecography* **42**, 1115–1123 (2019).
16. N. Owen-Smith, *Megaherbivores: The Influence of Very Large Body Size on Ecology*, (Cambridge University Press, 1988).
17. J. W. Laundré, L. Hernández, W. J. Ripple, The landscape of fear: Ecological implications of being afraid. *Open Ecol. J.* **3**, 1–7 (2010).
18. A. T. Ford *et al.*, Large carnivores make savanna tree communities less thorny. *Science* **346**, 346–349 (2014).
19. E. le Roux, G. I. H. Kerley, J. P. G. M. Crowsigt, Megaherbivores modify trophic cascades triggered by fear of predation in an African savanna ecosystem. *Curr. Biol.* **28**, 2493–2499.e3 (2018).
20. J. L. Atkins *et al.*, Cascading impacts of large-carnivore extirpation in an African ecosystem. *Science* **364**, 173–177 (2019).
21. C. Riginos, J. B. Grace, Savanna tree density, herbivores, and the herbaceous community: Bottom-up vs. top-down effects. *Ecology* **89**, 2228–2238 (2008).

22. C. C. Ng'weno, A. T. Ford, A. K. Kibungei, J. R. Goheen, Interspecific prey neighborhoods shape risk of predation in a savanna ecosystem. *Ecology* **100**, e02698 (2019).
23. T. O. Otieno *et al.*, Human- and risk-mediated browsing pressure by sympatric antelope in an African savanna. *Biol. Conserv.* **232**, 59–65 (2019).
24. J. P. Elliott, I. M. Cowan, C. S. Holling, Prey capture by the African lion. *Can. J. Zool.* **55**, 1811–1828 (1977).
25. J. Damuth, Population density and body size in mammals. *Nature* **290**, 699–700 (1981).
26. M. J. Coe, D. H. Cumming, J. Phillipson, Biomass and production of large African herbivores in relation to rainfall and primary production. *Oecologia* **22**, 341–354 (1976).
27. S. J. Moe *et al.*, Recent advances in ecological stoichiometry: Insights for population and community ecology. *Oikos* **109**, 29–39 (2005).
28. G. P. Hempson *et al.*, Ecology of grazing lawns in Africa. *Biol. Rev. Camb. Philos. Soc.* **90**, 979–994 (2015).
29. T. P. Craig, The resource regulation hypothesis and positive feedback loops in plant–herbivore interactions. *Popul. Ecol.* **52**, 461–473 (2010).
30. N. T. Hobbs, Modification of ecosystems by ungulates. *J. Wildl. Manage.* **60**, 695 (1996).
31. J. P. G. M. Cromsigt *et al.*, “The functional ecology of grazing lawns: How grazers, termites, people, and fire shape HIP’s savanna grassland mosaic” in *Conserving Africa’s Mega-Diversity in the Anthropocene: The Hluhluwe-IMfolozi Park Story*, J. P. G. M. Cromsigt, N. Owen-Smith, S. Archibald, Eds. (Cambridge University Press, 2017), pp. 135–160.
32. J. Sitters, M.-J. Maechler, P. J. Edwards, W. Suter, H. Olde Venterink, Interactions between C:N:P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores. *Funct. Ecol.* **28**, 776–786 (2014).
33. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*, (Springer, 2016).
34. E. Le Roux *et al.*, “Temporal changes in the large herbivore fauna of Hluhluwe-IMfolozi Park” in *Conserving Africa’s Mega-Diversity in the Anthropocene: The Hluhluwe-IMfolozi Park Story*, J. P. G. M. Cromsigt, S. Archibald, N. Owen-Smith, Eds. (Cambridge University Press, 2017), pp. 80–109.
35. R. W. Sterner, J. J. Elser, D. O. Hessen, Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry* **17**, 49–67 (1992).
36. A. D. Barnosky, Colloquium paper: Megafauna biomass tradeoff as a driver of Quaternary and future extinctions. *Proc. Natl. Acad. Sci. U.S.A.* **105** (suppl. 1), 11543–11548 (2008).
37. J. A. Estes *et al.*, Trophic downgrading of planet Earth. *Science* **333**, 301–306 (2011).
38. F. A. Smith, R. E. Elliott Smith, S. K. Lyons, J. L. Payne, Body size downgrading of mammals over the late Quaternary. *Science* **360**, 310–313 (2018).
39. F. Keesing, T. P. Young, Cascading consequences of the loss of large mammals in an African savanna. *Bioscience* **64**, 487–495 (2014).
40. Y. Malhi *et al.*, Megafauna and ecosystem function from the Pleistocene to the Anthropocene. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 838–846 (2016).
41. C. E. Doughty *et al.*, Global nutrient transport in a world of giants. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 868–873 (2016).
42. C. E. Doughty, A. Wolf, Y. Malhi, The legacy of the Pleistocene megafauna extinctions on nutrient availability in Amazonia. *Nat. Geosci.* **6**, 761–764 (2013).
43. A. Wolf, C. E. Doughty, Y. Malhi, Lateral diffusion of nutrients by mammalian herbivores in terrestrial ecosystems. *PLoS One* **8**, e71352 (2013).
44. R. A. Howison, H. Olff, N. Owen-Smith, J. P. G. M. Cromsigt, S. Archibald, “The abiotic template for the Hluhluwe-IMfolozi Park’s landscape heterogeneity” in *Conserving Africa’s Mega-Diversity in the Anthropocene: The Hluhluwe-IMfolozi Park Story*, J. P. G. M. Cromsigt, N. Owen-Smith, S. Archibald, Eds. (Cambridge University Press, 2017), pp. 33–55.
45. P. M. Brooks, I. A. W. MacDonald, “The Hluhluwe-Umfolozi Reserve: An ecological case history” in *Management of Large Mammals in African Conservation Areas*, R. N. Owen-Smith, Ed. (Haum Educational Publishers, 1983), pp. 51–57.
46. T. D. Nudds, Quantifying the vegetation structure of wildlife cover. *Wildl. Soc. Bull.* **5**, 113–117 (1977).
47. J. Pinheiro, D. Bates, S. DebRoy, D. Sakar, R Core Team, nlme: Linear and nonlinear mixed effects models. R package (Version 3.1–128, 2016). <https://CRAN.R-project.org/package=nlme>. Accessed 29 February 2020.
48. A. F. Zuur, E. N. Ieno, N. J. Walker, A. A. Saveliev, G. M. Smith, *Mixed Effects Models and Extensions in Ecology with R*, (Springer, 2009).
49. R Development Core Team, *R: A Language and Environment for Statistical Computing*, (R Foundation for Statistical Computing, 2015).
50. E. le Roux, L. S. van Veenhuisen, G. I. H. Kerley, J. P. G. M. Cromsigt, Data from “Animal body size distribution influences the ratios of nutrients supplied to plants.” Mendeley. <http://dx.doi.org/10.17632/jwc4r6kvr.3>. Deposited 10 August 2020.