TROPHIC CASCADES

Cascading impacts of large-carnivore extirpation in an African ecosystem

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Populations of the world's largest carnivores are declining and now occupy
mere fractions of their historical ranges. Theory predicts that when apex predators
disappear, large herbivores become less fearful, occupy new habitats, and
modify those habitats by eating new food plants. Yet experimental support for
this prediction has been difficult to obtain in large-mammal systems. After the
extirpation of leopards and African wild dogs from Mozambique's Gorongosa
National Park, forest-dwelling antelopes [bushbuck (*Tragelaphus sylvaticus*)]
expanded into treeless floodplains, where they consumed novel diets and
suppressed a common food plant [waterwort (*Bergia mossambicensis*)]. By
experimentally simulating predation risk, we demonstrate that this behavior was
reversible. Thus, whereas anthropogenic predator extinction disrupted a trophic
cascade by enabling rapid differentiation of prey behavior, carnivore restoration
may just as rapidly reestablish that cascade.

The worldwide decline in populations of large mammalian carnivores is a major environmental concern (*1*, *2*), in part because apex predators can exert a defining influence on ecosystems via trophic cascades (*3*). A trophic cascade occurs when predators indirectly affect plants through either of two mechanisms: by consumptively reducing prey abundance (*4*) or by imposing "landscapes of fear" in which prey modify their behavior to reduce predation risk. In landscapes of fear, prey are expected to forego foraging opportunities in resource-rich habitats that are risky, thereby creating spaces where palatable food plants can thrive (*5*, *6*). Accordingly, the extirpation of top carnivores should create "landscapes of fearlessness" where large herbivores seek out the nutritional benefits of previously risky habitats, suppressing food-plant abundance in the process (*5*); conversely, the reestablishment of real or perceived predation risk should reverse this behavior (*7*). Although behaviorally mediated trophic cascades have been documented frequently for relatively small consumer species (*8*–*10*), there are few unequivocal examples involving large mammalian carnivores and herbivores (*11*–*13*). This gap reflects the difficulty of experimentally manipulating predation risk and quantifying its downstream effects at scales relevant to large mammals. Correlative and comparative analyses generally cannot rule out potentially confounding factors, which has fueled debates (*14*–*18*) and prompted calls for stronger mechanistic inference in the study of megafaunal trophic cascades (*19*).

Ecosystems in which top predators have been extirpated present valuable opportunities to test predictions of trophic-cascade theory (*4*, *14*, *20*). In Mozambique's Gorongosa National Park, large-mammal populations were severely reduced during the Mozambican Civil War (1977 to 1992), with >90% declines across all monitored species (*21*–*23*). Large-herbivore populations have subsequently increased, but leopards (*Panthera pardus*), wild dogs (*Lycaon pictus*), and hyenas (*Crocuta crocuta*) were extirpated, while lions (*P. leo*) persisted at low abundance (*23*, *24*) (table S1). In this carnivore-depleted system, we evaluated evidence for a behaviorally mediated trophic cascade by using field manipulations of predator cues and herbivory, GPS telemetry of herbivore movements, spatially explicit wildlife-count data, DNA-based diet analysis, and body-condition measurements.

Gorongosa's central valley (Fig. 1, A and B) encompasses the ~750-km² Lake Urema floodplain and surrounding savanna woodlands (*25*). During the dry season (May to November), the floodplain is a flat, largely treeless landscape, dominated by grasses and forbs and dotted with leguminous subshrubs. Such open habitat is typically avoided by herbivores that rely on crypsis, tree cover, and known escape trails to avoid detection and capture by predators. One such herbivore species is bushbuck (*Tragelaphus sylvaticus*). This midsized antelope is a closed-habitat specialist that is "dependent on thick cover" (*26*) and "concealment to avoid predators" (*27*) and "is not found on open plains or anywhere without sufficient cover to conceal it" (*28*)

from key predators such as leopards and wild dogs (*29*). Previous studies of African ungulates have emphasized the comparative safety of open areas with high visibility (*11*, *13*). For secretive forest browsers such as bushbuck, however, treeless areas should be riskier, and relaxation of predation pressure might embolden individuals to exploit what would otherwise be prohibitively dangerous open habitat. Bushbuck in Gorongosa were historically confined to woodland and thicket habitat (*30*), but in the largely predator-free contemporary environment, we have observed them venturing into the open Urema floodplain.

We quantified these initial observations by fitting GPS collars to 11 bushbuck in 2015 and collecting hourly locations for up to 8 months. The data revealed two broad patterns in habitat use. One subset of individuals was largely confined to densely wooded home ranges; another occupied the sparsely wooded floodplain margin and routinely forayed into treeless floodplain habitat, both at night and throughout the day (Fig. 1, C to E)—behavior never documented prior to predator extirpation (*30*). All available evidence indicates that this habitat shift cannot be explained by competitive interactions. In principle, carnivore extirpation might have increased woodland bushbuck densities, prompting individuals to move into the floodplain to mitigate intraspecific competition, as predicted by ideal free distribution theory (*31*). We explored this possibility by using data from six helicopter counts conducted between 2002 and 2016, when all ungulate populations were recovering from similarly severe war-induced declines (*21*–*23*, *30*). If competition caused the habitat shift, then we would expect floodplain bushbuck densities to be negligible during the earliest counts, to increase only after woodland densities reached some threshold, and to remain lower than woodland densities throughout. To the contrary, floodplain bushbuck density was already ~50% of woodland bushbuck density in 2002, and by 2016, density was ~15% higher in the floodplain (Fig. 1F). Moreover, densities of other ungulates were also highest in the floodplain (*21*). Thus, the observed habitat shift cannot obviously be explained by either intra- or interspecific competition.

To directly test the hypothesis that predator extirpation has promoted the use of open habitat by bushbuck, we experimentally assessed the responses of GPS-collared floodplain (*n* = 7) and woodland (*n* = 5) bushbuck to simulated predator presence in August and September 2016. Habitat affiliations were determined on the basis of capture location and were subsequently verified with reference to GPS-collar locations and diet composition data (figs. S1 and S2). We exposed each individual to both predator and procedural-control cues in separate trials (in randomized order), with collars recording locations every 15 min (*32*). Treatments comprised both auditory and scent cues within the home range of each collared individual (fig. S3). Because both felids and canids prey on bushbuck, we aimed to create generalized hot spots of perceived predation risk by deploying several cues that collectively simulated multiple

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predator species. Playbacks of leopard vocalizations, artificial lion scat, and generic carnivore urine (*32*) were used to mimic predator presence; white noise, locally collected herbivore dung, and saline solution were used as sham cues in procedural-control trials (*12*, *33*). We restricted our analyses to data collected between 17:00 and 06:00, the period encompassing peak bushbuck activity (*32*). For each individual, we quantified two response variables in the 48 hours immediately before and after cue deployment. First, we measured the avoidance of predator and control cues by calculating the distance between each bushbuck GPS point and the sound-cue location. Second, we measured whether

simulated risk caused floodplain individuals to increase their use of tree cover (i.e., shift back toward a more "typical" bushbuck habitat).

Bushbuck strongly avoided predator cues in both habitats but did not avoid sham cues in either habitat (Fig. 2A and table S2). Moreover, predator cues caused a significant increase in the use of tree cover by floodplain but not woodland bushbuck, whereas sham cues did not significantly alter tree-cover use in either habitat (Fig. 2B and table S2). We evaluated the statistical significance of these responses by using mixed-effects models with random intercepts for individuals (table S2). These results were robust to both GPS and habitat classification error (*32*)

(figs. S4 and S5 and table S3). That floodplain bushbuck exhibited more cautious behavior in response to even a brief exposure to simulated risk suggests that some fear of predators has been retained and is primed in more risky open habitats (*7*, *12*). Our design does not enable us to distinguish the roles of specific predator cues in generating these responses, but future studies could test sound and scent cues separately.

Theory often assumes a nutritional opportunity cost of risk avoidance (*5*). We therefore hypothesized that floodplain bushbuck would consume higher-quality diets and exhibit greater size and body condition. We analyzed bushbuck diet composition by using DNA metabarcoding of
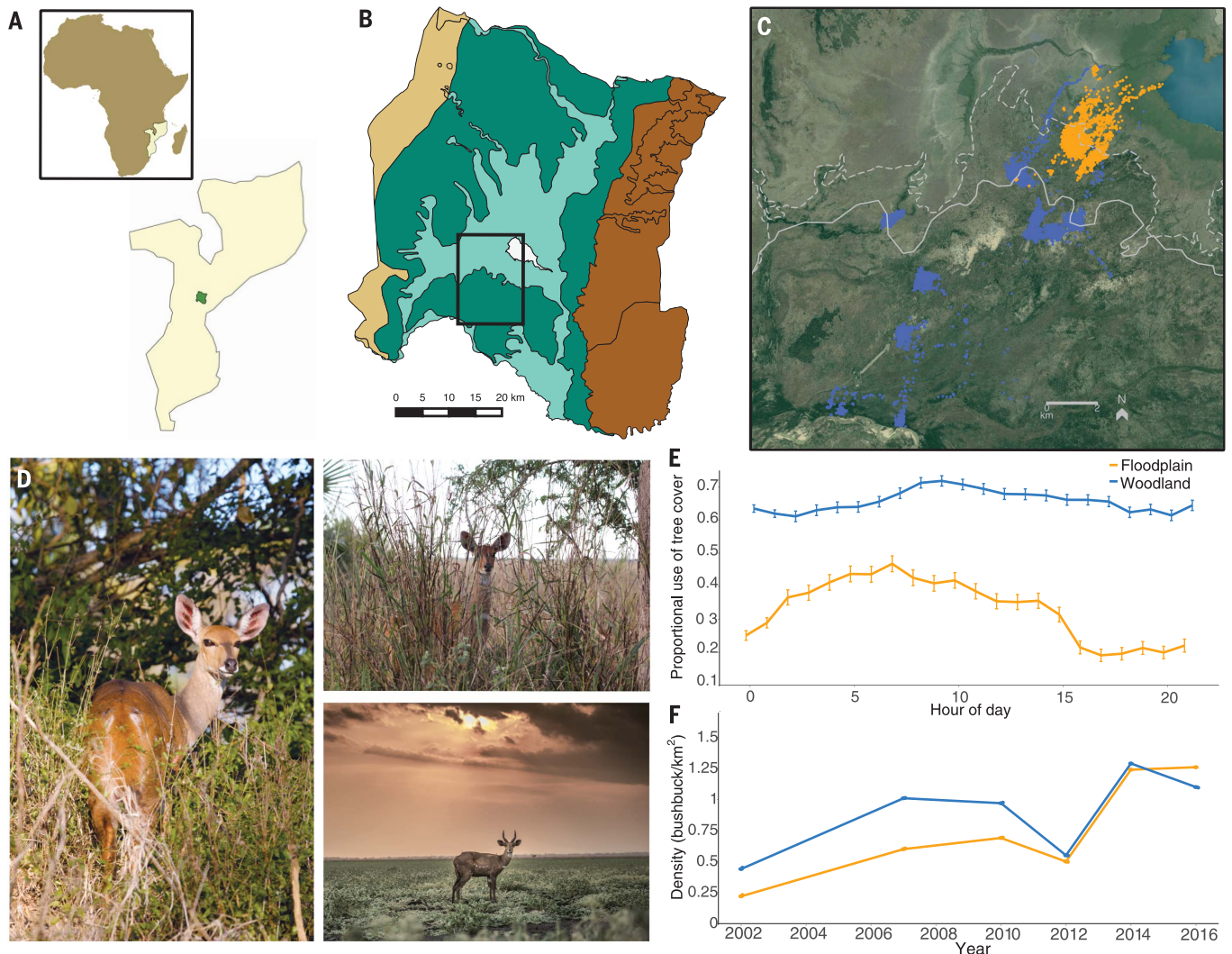

Fig. 1. Differential habitat use by bushbuck. (**A**) Map of Africa and Mozambique, showing the location of Gorongosa National Park (**B**) with its major habitat zones (*25*), from left: the western escarpment, savanna woodland (dark green), floodplain (light green), Lake Urema (white), and eastern escarpment. The boxed area indicates the region shown in the satellite image in (C). (**C**) GPS-collar locations from 11 individuals, three in the floodplain (orange) and eight in the adjoining woodland (blue), from June 2015 to March 2016. The solid line distinguishes the boundary between the floodplain and dense woodland; the dashed line distinguishes the boundary between the sparsely wooded floodplain margin and the treeless floodplain (*25*). (**D**) Bushbuck in woodland (left), the floodplain margin (top right), and the open floodplain (bottom right). (**E**) Use of woody cover by the bushbuck in (C), measured as the proportion of locations falling within a pixel classified as containing tree cover (*32*); error bars show ±1 SE. (**F**) Bushbuck densities in the floodplain and woodland, 2002 to 2016, determined via six aerial helicopter counts (*32*); here, "floodplain" refers conservatively to the treeless area surrounding Lake Urema [dashed line in (C)].

Fig. 2. Responses of floodplain and woodland bushbuck to simulated-predator and procedural-control cues. Average changes in (**A**) the distance from cues (avoidance) and (**B**) the proportional use of tree cover for bushbuck in woodland (*n* = 5) and floodplain (*n* = 7) habitats at night. Each bar (colored by habitat affiliation) represents the average difference between the 48-hour pre-cue period and the 48-hour post-cue period across all collared individuals in each category; error bars show ±1 SE. Shading indicates experimental treatment, with darker bars for the predator cues and lighter bars for the sham cues (see *x*-axis labels). *P* values from generalized linear mixed models are shown above each bar, indicating whether each response differed significantly from zero (see full model results in table S2).
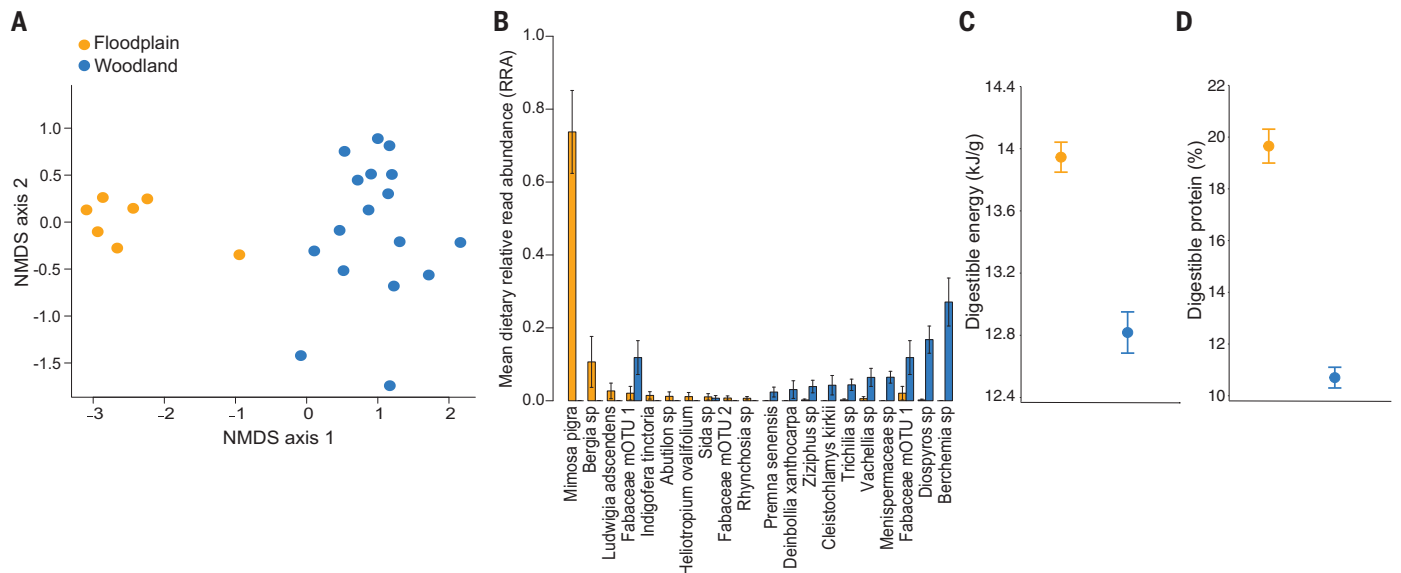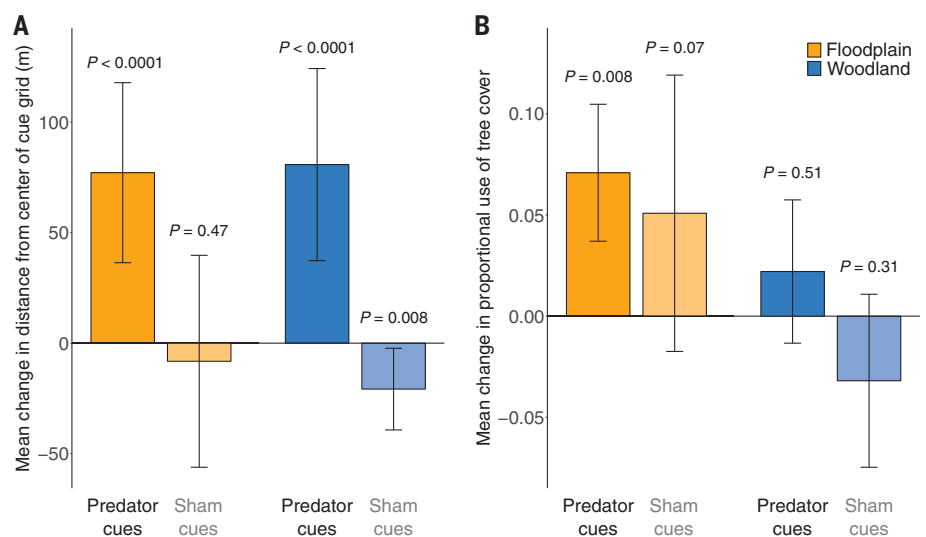

Fig. 3. Differences in composition and nutritional quality of bushbuck diets across habitats. (**A**) Nonmetric multidimensional scaling (NMDS) ordination, based on Bray-Curtis dissimilarities from DNA-metabarcoding data, showing that bushbuck diets clustered within each habitat and diverged between them. The distance between points (*n* = 7 floodplain fecal samples; *n* = 17 woodland fecal samples) reflects compositional dissimilarity. (**B**) The 10 most abundant food-plant taxa for bushbuck in the floodplain (orange bars) and woodland (blue bars). (**C**) Mean digestible energy and (**D**) protein contents of bushbuck diets, revealing higher diet quality in floodplain individuals (Wilcoxon rank sum tests; energy, *W* = 76, *n* = 18 individuals, *P* = 0.0001; protein, *W* = 77, *n* = 18 individuals, *P* < 0.0001). Error bars show ±1 SE.

fecal samples (*32, 34*). We also quantified the digestible-energy and digestible-protein contents of foliage from Gorongosa's most common browse plants. Of the 51 molecular operational taxonomic units (mOTUs) identified in bushbuck diets, 22 matched plant species for which we had nutritional-quality data, and those 22 species accounted for >83% of total diet [measured as the relative read abundance (RRA) of those mOTUs across all samples (*32*)]. We calculated weighted averages of digestible energy and protein in the diet of each sampled bushbuck, using the RRA of each plant species as the weighting factor. We

quantified the body condition of captured individuals by using principal components analysis to reduce multiple measurements of bushbuck morphology (e.g., body mass, body length, fat and muscle thickness, and standardized palpation scores) to two indices of condition: body size and body fat (*32*) (fig. S6 and tables S4 and S5).

We found pronounced differences in diet composition between floodplain and woodland bushbuck (Fig. 3A and fig. S2). The diets of floodplain bushbuck (21 mOTUs) were dominated by the leguminous shrub *Mimosa pigra* (mean RRA = 74% ± 11%) (Fig. 3B and fig. S2), with the forbs

Bergia mossambicensis (Elatinaceae; syn. *B. salaria*) and *Ludwigia adscendens* (Onagraceae; syn. *L. stolonifera*) accounting for an additional 11 and 3% of RRA, respectively. These plants had relatively high crude-protein and digestible-energy contents (fig. S2, C and D). The diets of woodland bushbuck (36 mOTUs) had greater evenness: Two tree species, *Berchemia discolor* and *Diospyros* sp., together accounted for 44% of RRA (Fig. 3B), and both species had lower crude protein and digestible energy than *M. pigra* and most other floodplain food plants (fig. S2, C and D). Overall, floodplain bushbuck diets were 8.8 and 83.6%

Fig. 4. Large herbivores suppress *Bergia mossambicensis*, a common floodplain plant. (**A**) Among all floodplain herbivore species in Gorongosa, only bushbuck consumed substantial quantities of *Bergia*. Shown are the mean changes in (**B**) the percentage of stems browsed and (**C**) the number of leaves per plant on caged versus uncaged *Bergia* plants over a minimum of 16 days (maximum of 18 days). Herbivores also reduced (**D**) the mean number of flowers per plant, (**E**) mean height, and (**F**) mean canopy area at the conclusion of the experiment. For (D) to (F), there was no significant difference in the initial values between caged and uncaged treatment groups. Model results of the effects of herbivore exclusion on each response variable are presented in table S7. Error bars show ±1 SE.
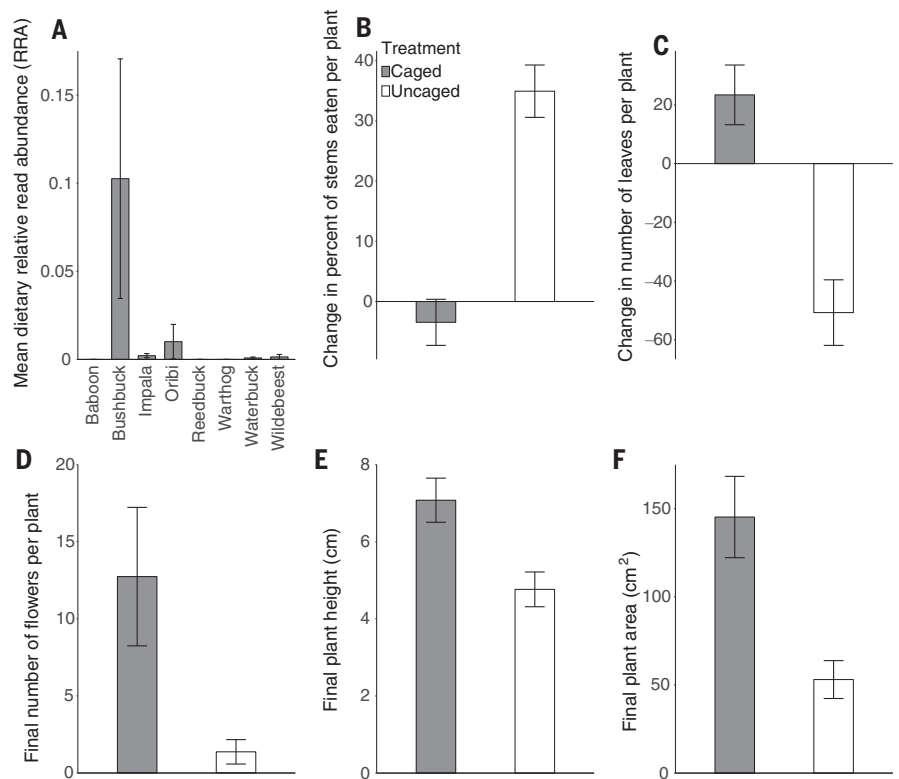
richer in digestible energy and protein, respectively, than woodland bushbuck diets (Fig. 3, C and D). Consistent with these differences in diet quality, floodplain bushbuck were in better condition than woodland bushbuck, with significantly higher scores for the body size [principal component 1 (PC1)] index (table S6). The nutritional value of the most heavily utilized plant, *M. pigra*, may help to promote floodplain use by bushbuck, but this factor alone does not provide a plausible alternative explanation for the long-term shift in habitat use. *M. pigra* was also present on the floodplain before predator extirpation (*30*), with availability similar to that at the time of this study; if this plant drove habitat use independently of predation risk, then bushbuck should have been using the floodplain during the prewar era as well. The relative influences of risk and resource quality on bushbuck habitat use could in principle be quantified by removing *M. pigra* at large scales; we hypothesize that this would reduce carrying capacity but not expel bushbuck from these areas.

In a final experiment, we tested whether the expansion of bushbuck into the floodplain has measurable effects on floodplain plants. Plant community responses to herbivory can be tested by using exclosures, but it would be impossible to parse the effects of bushbuck relative to those of other floodplain ungulates with overlapping diets. Thus, to isolate the effects of bushbuck, we used our diet analysis to identify an indicator plant species consumed almost exclusively by bushbuck. The waterwort *B. mossambicensis* was the second most abundant taxon in the diets of floodplain

bushbuck but was negligible (≤1% RRA) in the diets of all other floodplain herbivores (Fig. 4A). We constructed wire-mesh exclosures around *Bergia* plants in a randomized, paired caged-uncaged design (fig. S7) at two different floodplain sites (with 15 total pairs) (*32*). Before the experiment, and again after a minimum of 16 days, we measured the dimensions of each plant and counted all leaves, flowers, and browsed stems. The percentage of browsed stems per plant increased in uncaged plants during the experiment but did not change in caged plants (Fig. 4B and table S7). Similarly, the mean number of leaves increased in caged plants but decreased in uncaged plants (Fig. 4C). The mean number of flowers, height, and canopy area at the conclusion of the experiment were all significantly greater in caged plants (Fig. 4 and table S7). Thus, the expansion of bushbuck into open habitats was accompanied by strong suppression of growth and reproduction in *Bergia*. Although other floodplain herbivores may have contributed somewhat to this effect, only bushbuck consumed substantial quantities of *Bergia* (Fig. 4A), suggesting that this plant would otherwise find refuge from large-mammal herbivory in the floodplain.

Altogether, our results provide evidence that the extirpation of large carnivores in the wake of the Mozambican Civil War has disrupted a behaviorally mediated trophic cascade. In the absence of apex predators such as leopards and wild dogs, a common ungulate prey species rapidly expanded into a high-risk, high-reward habitat, with concomitant shifts in diet composition and quality,

body size and condition, and the performance of a key food plant. Despite the multidecadal absence of several apex predators in Gorongosa, we found that bushbuck retained a fear of these carnivores (*7, 12*): Experimental imposition of risk cues over just 48 hours shifted habitat use toward patterns that prevailed before carnivore loss (*30*). The next phase of trophic rewilding in Gorongosa involves carnivore reintroductions (*23*), beginning in 2018 with 14 wild dogs, which should eventually enable tests of our prediction that bushbuck will vacate the floodplain. Notably, the relaxation of risk after carnivore extirpation differentially affected individual behavior within a population, leading to bimodality in habitat use and perhaps some degree of reproductive separation, which could amplify preexisting individual variation (*35*). The influence of carnivores on behavioral variation within ungulate populations, and its potential evolutionary significance, remains largely unexplored.

Our study supports the general hypothesis that the loss of top carnivores can convert landscapes of fear into landscapes of fearlessness for large mammalian herbivores, with far-reaching consequences for prey and plant populations (*6*). It further shows that the effects of fear depend on the social, foraging, and antipredator behaviors of the species involved. Whereas recent work has shown that gregarious, flight-dependent grazers and mixed feeders use open, high-visibility habitat for risk avoidance (*11, 13*), our study of a solitary, crypsis-dependent browser reveals an opposing pattern. This distinction was appreciated by early naturalists [(*36*), pp. 32–33]:

It is curious to note the different ideas of safety entertained respectively by the plain and the bush dweller. Let us say a Grant's gazelle and a bushbuck are grazing near each other on the edge of a plain when something occurs to alarm them. The bushbuck lopes quickly back into the bush, sure that in this lies his only chance of safety. The Grant's turns and gallops from the bush as if it were some deadly thing ....

Thus, although generalizations about trophic cascades involving particular species and food chains may be possible on the basis of characteristics such as herbivore size, behavior (*37*, *38*), and predator hunting mode (*39*), we suggest that community-wide cascades may be dampened in diverse African large-mammal assemblages because of the orthogonal responses of different herbivore species to predation risk (*40*).

REFERENCES AND NOTES

1. J. A. Estes *et al.*, *Science* **333**, 301–306 (2011).
2. W. J. Ripple *et al.*, *Science* **343**, 1241484 (2014).
3. J. Terborgh, J. A. Estes, Eds., *Trophic Cascades: Predators, Prey and the Changing Dynamics of Nature* (Island Press, 2010).
4. J. A. Estes, J. F. Palmisano, *Science* **185**, 1058–1060 (1974).
5. J. S. Brown, J. W. Laundre, M. Gurung, *J. Mammal.* **80**, 385–399 (1999).
6. J. W. Laundré, L. Hernandez, W. J. Ripple, *Open Ecol. J.* **3**, 1–7 (2010).
7. J. Berger, J. E. Swenson, I. L. Persson, *Science* **291**, 1036–1039 (2001).
8. B. L. Peckarsky *et al.*, *Ecology* **89**, 2416–2425 (2008).
9. G. C. Trussell, P. J. Ewanchuk, C. M. Matassa, *Ecology* **87**, 2979–2984 (2006).
10. O. J. Schmitz, V. Krivan, O. Ovadia, *Ecol. Lett.* **7**, 153–163 (2004).
11. A. T. Ford *et al.*, *Science* **346**, 346–349 (2014).
12. J. P. Suraci, M. Clinchy, L. M. Dill, D. Roberts, L. Y. Zanette, *Nat. Commun.* **7**, 10698 (2016).
13. E. le Roux, G. I. H. Kerley, J. P. G. M. Cromsigt, *Curr. Biol.* **28**, 2493–2499.e3 (2018).
14. W. J. Ripple, E. J. Larsen, R. A. Renkin, D. W. Smith, *Biol. Conserv.* **102**, 227–234 (2001).
15. D. Fortin *et al.*, *Ecology* **86**, 1320–1330 (2005).
16. M. J. Kauffman, J. F. Brodie, E. S. Jules, *Ecology* **91**, 2742–2755 (2010).
17. B. L. Allen *et al.*, *Biol. Conserv.* **159**, 158–174 (2013).
18. J. Winnie Jr., S. Creel, *Food Webs* **12**, 88–94 (2017).
19. A. T. Ford, J. R. Goheen, *Trends Ecol. Evol.* **30**, 725–735 (2015).
20. J. Terborgh *et al.*, *Science* **294**, 1923–1926 (2001).
21. M. E. Stalmans, T. J. Massad, M. J. S. Peel, C. E. Tarnita, R. M. Pringle, *PLOS ONE* **14**, e0212864 (2019).
22. J. H. Daskin, M. Stalmans, R. M. Pringle, *J. Ecol.* **104**, 79–89 (2016).
23. R. M. Pringle, *Nature* **546**, 91–99 (2017).
24. P. Bouley, M. Poulos, R. Branco, N. H. Carter, *Biol. Conserv.* **227**, 233–242 (2018).
25. M. Stalmans, R. Beilfuss, "Landscapes of the Gorongosa National Park" (Gorongosa National Park, 2008).
26. J. Kingdon, *The Kingdon Field Guide to African Mammals* (Bloomsbury, ed. 2, 2015).
27. R. D. Estes, *The Safari Companion: A Guide to Watching African Mammals* (Chelsea Green, ed. 2, 1999).
28. R. D. Estes, *The Behavior Guide to African Mammals* (Univ. of California Press, 1991).
29. F. G. T. Radloff, J. T. Du Toit, *J. Anim. Ecol.* **73**, 410–423 (2004).
30. K. L. Tinley, thesis, University of Pretoria, Pretoria, South Africa (1977).
31. S. D. Fretwell, H. L. J. Lucas Jr., *Acta Biotheor.* **19**, 16–36 (1969).
32. Materials and methods are available as supplementary materials.
33. A. T. Ford, J. R. Goheen, *J. Mammal.* **96**, 918–926 (2015).
34. J. Pansu *et al.*, *J. Ecol.* 10.1111/1365-2745.13113 (2018).
35. D. I. Bolnick *et al.*, *Am. Nat.* **161**, 1–28 (2003).
36. C. H. Stigand, *The Game of British East Africa* (Horace Cox, 1909).
37. P. J. Jarman, *Behaviour* **48**, 215–267 (1974).
38. K. P. Dial, E. Greene, D. J. Irschick, *Trends Ecol. Evol.* **23**, 394–401 (2008).
39. O. J. Schmitz, *Science* **319**, 952–954 (2008).
40. N. Owen-Smith, *Oikos* **124**, 1417–1426 (2015).
41. J. L. Atkins, R. A. Long, J. Pansu, J. H. Daskin, A. B. Potter, M. E. Stalmans, C. E. Tarnita, R. M. Pringle, Data for "Cascading impacts of large-carnivore extirpation in an African ecosystem," Dryad (2019); https://doi.org/10.5061/dryad.h4r1003.

Science

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Ecosystems feel war's effects
War ravages human lives and landscapes, but nonhuman victims are no less affected. The Mozambican Civil War resulted in the rapid decline of predators in Gorongosa National Park and led to a trophic cascade that shifted prey behaviors and plant communities. Atkins *et al.* monitored this shift and found that the absence of wild dogs and leopards resulted in a change in habitat use and plant consumption by bushbuck, which are forest-dwelling antelopes. Experiments further showed that changes in prey behavior were reversible when signs of predator activity were introduced, supporting the impact of the predator loss. These results confirm patterns seen elsewhere and go further in providing mechanistic detail about the importance of the "landscape of fear" perceived by prey animals.
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RESEARCH

A pack of African wild dogs (*Lycaon pictus*) takes advantage of a rural road in the Gorongosa National Park in Mozambique.

IN *SCIENCE* JOURNALS

Edited by **Michael Funk**

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Science, this issue p. 173

STRUCTURAL BIOLOGY

Bone-cell regulation, fleshed out

One of many medically relevant G protein–coupled receptors, parathyroid hormone receptor-1 (PTH1R) functions in the control of calcium homeostasis and bone physiology. Zhao *et al.* used cryo–election microscopy to observe the structure of PTH1R in a complex with a modified form of parathyroid hormone and stimulatory G protein. The structural model helps explain how parathyroid hormone interacts with its receptor and the molecular basis for receptor activation. —LBR

Science, this issue p. 148

GRAPHENE

Electron hydrodynamics in graphene

Electrons can move through graphene in a manner reminiscent of fluids, if the conditions are right. Two groups studied the nature of this hydrodynamic flow in different regimes (see the Perspective by Lucas). Gallagher *et al.* measured optical conductivity using a waveguide-based setup, revealing signatures of quantum criticality near the charge neutrality point. Berdyugin *et al.* focused on electron transport in the presence of a magnetic field and measured a counterintuitive contribution to the Hall response that stems from hydrodynamic flow. —JS

Science, this issue p. 158, p. 162; see also p. 125

THIN FILMS

Epitaxial films through spin coating

A simple way to coat a surface with a uniform film is by spin coating. The substrate is spun at high speed, and a droplet of solution containing the coating is added at the center, spreads out, and evaporates. This method is used to make polycrystalline inorganic coatings and amorphous films, such as polymers used in lithography. Kelso *et al.* performed spin coating with single-crystal substrates, carefully controlling the thickness of the spreading solution on the basis of its viscosity and the rotation rate. In this way, they achieved epitaxial growth—in which the crystallites are oriented by the substrate—for perovskites, zinc oxide, and sodium chloride. —PDS

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GLACIAL CYCLES

Controlling cooling

On million-year time scales, Earth's climate state is

Science

AAAS

Supplementary Materials for

Cascading impacts of large-carnivore extirpation in an African ecosystem

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This PDF file includes:

Materials and Methods
Figs. S1 to S7
Tables S1 to S8
References

Correction: Since First Release, minor editorial changes have been made throughout for clarity, and two references (originally numbered *78* and *79*) have been removed, as they have been replaced with a more current reference (*21*). Specific percentage estimates of the areal extent of wet-season flooding have also been removed because the available data are insufficient to allow precise estimation of flood extent and interannual variability.

MATERIALS AND METHODS

Study site
Mozambique's Parque Nacional da Gorongosa is located at the southern end of the Great Rift Valley (-18.96°, 34.36°; (*25*)) and encompasses portions of the escarpments to the east and west (Fig. 1). Our study area was located within the Rift Valley, in the southern part of the park, which receives a mean annual rainfall of 700-900 mm (*25*). Lake Urema, which sits near the center of the park, is fed by several in-flowing rivers of the 9300-km^2 Urema catchment (*42*). During the wet season, which typically spans December to May, the lake expands and floods a large portion of the Rift Valley (*25*). During the dry season, this area is a highly productive floodplain, which historically supported vast herds of large-bodied grazers such as buffalo (*Syncerus caffer*), hippo (*Hippopotamus amphibius*), wildebeest (*Connochaetes taurinus*), zebra (*Equus quagga*), and waterbuck (*Kobus ellipsiprymnus*) (*30*). The habitat types within the Rift Valley portion of the park span the continuum of tree cover, from the treeless Urema floodplain-grasslands, through several types of savanna-woodland (*Acacia*, palm, and broadleaf), to closed-canopy forest (*25*).

From 1977-1992, Mozambique endured a devastating civil war. Gorongosa's large-mammal community collapsed, with declines of >90% among all of the major large-herbivore species between 1972 and 1994 (*22*). In the pre-war era, leopard (*Panthera pardus*) and lion (*Panthera leo*) were the most abundant large carnivores; African wild dog (*Lycaon pictus*) and spotted hyena (*Crocuta crocuta*) were also present (*30*). Since 2000, aerial surveys of wildlife counts have been conducted on a regular basis (*21*). The 2016 survey, which coincided with fieldwork for this study, found that total large-herbivore biomass had recovered to approximately 80% of pre-war levels, albeit with very different abundance patterns relative to the pre-war baseline (*21*, *30*). Mid-sized ungulates have displaced the formerly dominant largest-bodied species in relative abundance and biomass. The large-carnivore assemblage has been much slower to recover. The lion population was estimated at ~65 in 2017, down from ~200 in 1972 (*23*). Leopard, wild dog, and hyena remained functionally extirpated, as determined by considerable formal and informal efforts at detection between 2004 and 2017 (*21*, *23*, *30*) (table S1). In recent years, several hyena records have been obtained from camera traps, whereas the first (and only to date) leopard sighting of the post-war era occurred in April 2018, after the conclusion of this study. African wild dogs had not been recorded in Gorongosa since 1972, but in June 2018 one pack of 14 individuals was translocated into the park from South Africa; bushbuck (*Tragelaphus sylvaticus*) have been among the principal prey of this pack since its release.

Study species
Bushbuck are mid-sized (40–60 kg) browsing woodland antelopes. They are largely solitary, typically exhibiting high home-range fidelity and small (< 0.5 km^2) home ranges (*26*, *43*, *44*). Bushbuck are characteristically found in closed, often densely forested or thicketed habitats, where they rely on tree cover, crypsis, and a series of well-known escape trails within their home ranges to avoid detection and capture by predators (*45*). Prior radio-tracking work indicates that bushbuck are more active at night than in the daytime (*46*).

In a detailed study of Gorongosa's ecology from 1968-1972, Tinley (*30*) classified bushbuck as components of the "forest/thicket" fauna; bushbuck were not among the seven large-herbivore

species recorded as utilizing the floodplain, nor were they among the 14 species documented in savannas or grasslands. These assessments from pre-war Gorongosa, based on a large volume of systematic and quantitative observation, are consistent with the canonical characterization of bushbuck across Africa as a secretive woodland species, "essentially dependent on thick cover" (*26*), which "hides in patches of woody vegetation…dependent on concealment to avoid predators" (*27*) and "is not found on open plains or anywhere without sufficient cover to conceal it" (*28*). A study from Uganda's Queen Elizabeth National Park (*44*) found that bushbuck strayed away from thickets into patches of grassland at dusk but invariably returned to thickets before dawn, and upon encountering predators they would "run to a nearby thicket and freeze rather than attempt to escape by speed"; this study concluded that "predation is a primary factor controlling their activities and spatial distribution." Accounts from early 20th-century naturalists reinforce this characterization of bushbuck as exceedingly shy and wary: "they may be seen out of cover just after sunset, when they graze round the edge of the bush-belts" (*36*). Perhaps the most colorful depiction is that of Vasse (*47*), who spent three years hunting in the Gorongosa ecosystem, and described bushbuck behavior as follows:

> "Where numerous warnings have taught it prudence, it will not leave its retreat until the evening twilight…At the time when it cautiously emerges from its retreat in order to feed, a heavy weight may fall on its withers, powerful teeth be implanted in its neck, tearing the jugular vein and carotid artery, and throwing it panting on the ground, the unhappy victim of the leopard."

The Urema floodplain, which is only sparsely wooded at the margins and treeless in the interior, provides none of the predator-protection mechanisms classically relied upon by bushbuck. Individuals in our study that were collared in the floodplain (*n*=10) spent an average of 8% of the days that they were tracked entirely within the treeless interior of the floodplain, and for an additional 31% of days they spent >3 hours in this region. Utilization of wide-open habitats throughout the day is also clear from Fig. 1E and from the locations of bushbuck counted during aerial surveys, which include many individuals occupying the floodplain interior in broad daylight. Only one floodplain bushbuck was tracked through the flood period from December-March 2015-2016; this individual retreated to the wooded area on the margin of the floodplain and remained there until flood waters receded. Thus, our results demonstrate extensive use of the floodplain by bushbuck in Gorongosa—most frequently at night but also during the day—in contrast to (*i*) extensive pre-war studies of the same ecosystem, (*ii*) repeated observational documentation of bushbuck movements throughout Africa, and (*iii*) the well documented predator-avoidance behaviors of this species.

Bushbuck are susceptible to predation by all of the largest African carnivores (*27*), including leopard, lion, wild dog, and hyena (*29, 48, 49*). In a 13-year study from South Africa (*29*), leopards were the primary predator of bushbuck, accounting for 77% of kills; lions and wild dogs accounted for an additional 13% and 5%, respectively. Throughout Africa, bushbuck are significantly preferred prey by both leopards and wild dogs, taken in proportion to availability by hyenas, and significantly avoided by lions (*48, 49*). In records kept by the Gorongosa Lion Project (*24*), bushbuck were not among the 189 kills recorded from 2012–2016. Likewise, human hunting has been effectively eliminated within our study area at the core of the park. Thus, contemporary Gorongosa has been a landscape of vastly reduced risk for bushbuck, owing to (*i*) the anthropogenic extirpation of its main predators; (*ii*) the ~67% reduction in abundance of the only extant large carnivore (lion); and (*iii*) strong anti-poaching enforcement.

Quantifying bushbuck densities across habitat types from 2002 to 2016

Aerial wildlife counts were conducted by trained park personnel. We used data from helicopter surveys in 2002, 2007, 2010, 2012, 2014, and 2016 to calculate the approximate relative densities of bushbuck in floodplain and woodland habitats (counts from helicopter surveys in 2000 and 2001 were excluded because < 100 individuals were recorded, but the relative densities in each habitat in these years were similar to those in 2002). Counts were conducted from a Bell JetRanger with all four doors removed for increased visibility, traveling at a speed of 96 km/hour at an altitude of 50–55 m. Surveys were conducted along parallel strip transects that were 500-m wide (i.e., observers examined 250 m on either side of the helicopter). All animals were individually counted. A count block ranging from 52,800 ha in 2002 to 184,500 ha in 2016 was fully surveyed each count. M.E.S. participated in all surveys from 2010 onwards, and all surveys were flown by the same pilot (M. Pingo, Sunrise Aviation). Survey methodology and results are reported in (*21*). Bushbuck counted during the surveys were classified as either floodplain margin (sparsely wooded habitat), floodplain-grassland (treeless interior), or woodland individuals using a pre-existing habitat classification map (*25*). Density estimates were calculated for each habitat type by dividing the number of bushbuck counted by the total area flown in that habitat. In Fig. 1F, we conservatively report the contrast between woodland and treeless floodplain-grassland; using the more encompassing floodplain definition that includes the sparsely wooded margin, estimated densities of bushbuck in the floodplain are even greater (exceeding those in woodland as of the 2014 survey, and by 58% as of 2016).

Animal capture and GPS telemetry

All animal handling procedures were approved by the Institutional Animal Care and Use Committee at Princeton University (protocols 1958-13 and 2075F-16), and were in accordance with guidelines established by the American Society of Mammalogists (*50*).

For our initial observational study of bushbuck movement behavior, we chemically immobilized 11 adult bushbuck (all females) between June and August, 2015, via darting (thiafentanil, medetomidine, and azaperone) from a vehicle. Three of those individuals were captured in floodplain habitat, whereas the remainder were captured in woodland habitat more typical of bushbuck. These habitat assignations were based on initial capture location and were subsequently verified by overlaying GPS location data on landscape categories defined in a previous remote-sensing analysis (*25*); floodplain bushbuck occurred exclusively within the "floodplain landscape" and frequently utilized the treeless "grassland landscape" surrounding Lake Urema in the interior of the floodplain, whereas woodland bushbuck occurred almost exclusively within dense savanna-woodland (Fig. 1). Each individual was fitted with an Advanced Telemetry Systems iridium GPS collar (model G2110E), programmed to record hourly locations and transmit data to a user-accessible server at midnight each day by satellite uplink. At the end of their battery life, collars were remotely released via satellite and were retrieved without recapturing animals.

To experimentally test our hypothesis that the shift in habitat use by bushbuck was driven by reduced predation pressure, we captured 14 additional adults (five males, nine females) in August 2016 using the same methods. For our experiment, we programmed collars to record locations every 15 minutes to better quantify fine-scale responses of bushbuck to simulated predation risk (see below). GPS locations were filtered to include only the specific experimental periods relevant to each individual. We then screened the remaining GPS locations for points

that might have been inaccurate using an algorithm developed by Bjorneraas et al. (*51*), which identified and removed potentially inaccurate GPS locations by finding deviations from the typical characteristics of the movement pathways in the data. This resulted in a final dataset of 401 ± 18 (mean $\pm$ SD) locations across the two experimental periods (predator cues and procedural-control cues) for each individual. Habitat affiliations were determined as described above for 2015; two of the 2016 individuals occurred within the "floodplain landscape," in *Acacia*-palm savanna with moderate tree cover, but never ventured into the treeless "grassland landscape" (fig. S1) and were therefore classified as woodland bushbuck.

Hypothesis 1: Predator extirpation has promoted utilization of open habitat by bushbuck

Predator-simulation experiments
We obtained useable location data from 12 of the 14 individuals collared in 2016 (five woodland and seven floodplain bushbuck, with two collar failures). In August 2016, we carried out a series of predator-simulation experiments using a combination of auditory (playbacks of leopard vocalizations) and scent (artificial carnivore scat/urine) cues to generically mimic the presence of a suite of historically present large carnivores that are known to prey on bushbuck (*29, 48, 49*). Artificial carnivore scat consisted of commercially available 'Silent Roar' product (Silent Roar Ltd., Ashford, England), a non-toxic nitrogen-based fertilizer soaked in essence of lion feces, which has previously been shown to elicit anti-predator behavior in other African ungulates (*52*). Artificial predator urine was made by diluting 2-phenylethylamine (Sigma-Aldrich, St. Louis, MO, USA); this compound has been identified as a component of carnivore urine (both felids and canids) that elicits innate fear and aversion responses in naïve mammalian prey species (*53*). Thus, our predation-risk treatment did not mimic any one specific predator species, but rather simulated the presence of large mammalian predators in general to create hotspots of perceived predation risk (following precedent in the literature (*7, 54*)).

The recordings of leopard vocalizations were obtained from the Borror Laboratory of Bioacoustics (Ohio State University, Columbus, OH, USA) and the Macaulay Library (Cornell Lab of Ornithology, Ithaca, NY, USA). These calls were compiled into three different playback files that were randomly selected for each trial to avoid pseudo-replication, following (*55*). Each file was six-hours long and consisted of no more than five distinct vocalizations. Individual vocalization bouts lasted 25–55 seconds, and were interspersed by a minimum of 1 h and a maximum of 2.5 h of silence (the specific length was randomized between each vocalization bout to avoid predictability of cue times), again following (*55*). All playbacks were transferred onto a digital-media player and played through a battery-powered portable speaker (Megavox pro 6000, Anchor, Carlsbad, CA, USA) at ~95 dB at a distance of one meter (verified using the SoundMeter app for iOS (*56*) *in situ* after the speakers had been placed in each bushbuck home range each night; see below). To control for any baseline behavioral response of study animals to disturbance—implementation of the experiment exposed animals to increased human activity, foreign sounds, and addition of solids and liquids into the environment—we also deployed procedural-control cues (white noise, saline solution, and herbivore dung collected locally from the dominant ungulate, waterbuck) in the same manner as the predator cues (*54*). In choosing our auditory procedural-control cue, we sought a sound that would be both non-threatening and novel to the experimental animals, because our auditory predator cues—leopard calls—were ecologically novel to bushbuck in Gorongosa. Many commonly used auditory control cues (e.g., baboon vocalizations, running water, bird calls (*54*)) were inappropriate because they are not

novel sounds in Gorongosa. We selected white noise, which has also been used for control treatments in prior studies (*54*), in a conservative attempt to ensure that the control cue would be completely unfamiliar to focal animals.

Each collared bushbuck was exposed to both predator and procedural-control cues, in a randomized order and with at least one week between trials for each individual, enabling us to assess responses to both stimuli. This experiment focused on short-term behavioral responses, both to reduce the likelihood of habituation to cues (*12*, *57*) and to minimize potentially confounding effects of uncontrolled environmental variation within trials in the field. Prior to cue deployment, all bushbuck had spent at least one week with GPS collars attached to allow acclimation. The protocol for cue deployment was as follows. Using GPS-collar data, we identified the area of each bushbuck's home range with the highest intensity of use during the 48 h preceding treatment, and cues were deployed in the central part of this area. The speaker was placed at the center of this deployment zone, and scent cues were placed at 20-m intervals in a 100×100 m grid around the speaker (fig. S3). One of the three distinct playback files was then broadcast from the speaker for six hours, starting at sunset, on two consecutive days. Investigators returned to the cue-deployment areas on the second day of each trial (i.e., 24 hours after initial cue deployment) to replace speakers due to battery-power limitations. We assessed responses of each individual to each cue type in the 48-hour period before versus the 48-hour period after cue deployment. Thus, the experiment (*i*) included before-after contrasts for each cue type within each individual, and (*ii*) enabled a direct post-treatment comparison of the magnitude of bushbuck responses to two equally novel and disruptive sets of cues, only one of which indicated the presence of carnivores.

Quantifying response to simulated predator presence
We analyzed the responses of bushbuck to predator and control cues using experimental period (i.e., the 48 h before versus after cue deployment) as a dichotomous predictor variable. We measured two response variables for each individual: avoidance of cue areas and use of tree cover. These responses enabled us to test the following specific predictions of our central hypothesis: (*i*) predator cues elicit fear but control cues do not, and bushbuck therefore avoid areas where predator cues are deployed; and (*ii*) bushbuck in the floodplain decrease their use of more-risky open habitat, and increase their use of less-risky wooded habitat, when exposed to predator cues (but not control cues).

We compared responses to experimental cues between floodplain and woodland habitat types (as defined above). We restricted our analyses to locations collected between dusk and dawn, because bushbuck are generally more active at night (*27*, *28*, *46*) and because sound cues were deployed at sunset and played only at night (which is appropriate given that leopard activity peaks at night). Proximity to cue-deployment areas was calculated by measuring the linear distance between each individual location and the location of the center of the cue grid (where the speaker was placed). To quantify use of tree cover, we used a supervised classification of 1.8-m resolution orthorectified satellite imagery (WorldView-2, Digital Globe, Longmont, CO, USA) collected in July-August 2010 to classify each pixel as containing either woody vegetation (scored as 1) or open herbaceous vegetation (scored as 0). We assessed the accuracy of the automated classification by comparison with visual classification of 300 randomly selected points in the image (overall accuracy 87%; sensitivity to woody cover 79%; specificity 92%; table S8). This classification was performed by J.H.D., who was blind to the hypotheses of the

study when the analysis was conducted. We then scored each bushbuck GPS location as being in a pixel either with (1) or without (0) tree cover. Our decision to characterize a pixel as 'woody' given the presence of even a single tree or bush was based on the fine-scale nature of the response we aimed to test. During capture attempts on the floodplain, bushbuck typically sought cover, which often consisted of only a single tree; upon reaching the nearest tree, bushbuck would often remain motionless behind it while we approached to <30 m. These observations suggest that even a single tree was likely perceived by bushbuck to be safer than open floodplain habitat. Accordingly, we used a fine-scale (1.8-m resolution), binary classification of woody cover to maximize our odds of detecting fine-scale changes in bushbuck behavior following exposure to either predator or control cues.

To test whether bushbuck exhibited stronger avoidance of cue areas and/or increased use of tree cover in response to predator cues, we used generalized linear mixed-effects models, with random intercepts for individual bushbuck and an autoregressive correlation structure to account for autocorrelation of sequential observations from the same individual (*58*). In total, this resulted in four separate models for each response variable, one for each combination of habitat affiliation and cue type (table S2). We used the *nlme*, *move*, *raster,* and *sp* packages in R (*59–63*) for our analyses.

We conducted two additional analyses to evaluate robustness of our fine-scale woody-cover results to potential sources of error inherent in our data, namely GPS location inaccuracies and imprecision of satellite image classification.

First, to assess sensitivity of our experimental results to GPS location error, we conducted a Monte Carlo simulation analysis. In each simulation run, we first drew a random location from within a 13-m buffer (the estimated location error of the GPS collars) around each bushbuck GPS location. We then iteratively refit our original models of bushbuck responses to predator and control cues (see table S2) to the randomly permuted location data. We repeated this process 10,000 times and quantified the proportion of simulation runs that produced statistically significant results that mirrored those of the original analysis. These simulation results strongly supported our initial conclusions. Floodplain bushbuck significantly increased their use of woody cover at night in response to predator cues in all 10,000 simulation runs (fig. S4). In contrast, <5% of simulations indicated a significant response to control cues by floodplain bushbuck. For woodland bushbuck, <0.01% of simulations indicated a significant response to either predator or control cues (fig. S4).

Second, to evaluate sensitivity of our results to imprecision in the satellite-image classification, we re-analyzed our experimental data using a coarse-grained classification of 'grassland' versus 'non-grassland' habitat (*25*) in lieu of a pixel-level analysis of woody cover. (This coarse-grained classification is the same as that used in our analysis of temporal changes in bushbuck density in Fig. 1F.) All locations for woodland bushbuck were in non-grassland pixels, so we present the results of this analysis for floodplain bushbuck only. This analysis showed that floodplain bushbuck decreased their use of grassland habitat in response to predator cues but not control cues (fig. S5, table S3), consistent with our primary analysis of woody-cover use (Fig. 2). Based on these two sensitivity analyses, we conclude that our experimental results were not an artifact of the fine spatial grain of the woody-cover classification used in the analyses presented in the main text.

Our collared individuals included an approximately equal proportion of males and females in each habitat type (floodplain M:F 4:3; woodland M:F 3:2), meaning that any sex-specific variation in responses is unlikely to bias our conclusions. Due to the very small sex-specific sample sizes, we cannot draw conclusions about sex-specific responses. Although future studies of sex-specific variation in response to predation risk would be of interest, we believe that our results are all the more likely to be robust given they emerged irrespective of any such variation.

Hypothesis 2: Differences in bushbuck habitat use are accompanied by differences in diet composition and quality

Analysis of diet composition
We used fecal DNA metabarcoding to quantify bushbuck diet composition in each habitat type during the dry season of 2016. At the time of capture, in August, we collected one fresh fecal sample directly from each anesthetized individual. Additional samples were collected opportunistically during the 2016 dry season, usually after observing defecation or directly sighting a collared bushbuck and then searching the immediate vicinity. These bushbuck fecal samples were included as part of a broader study of large-herbivore diets in Gorongosa, and the metabarcoding analyses presented here were conducted on a subset of the data made available in association with that study (*34*). Sample processing followed previously published protocols (*34*). Briefly, fecal samples were individually placed in unused plastic bags and immediately stored in a cooler, and were pre-processed on the day they were collected. Pre-processing involved the following steps. We homogenized samples within the collection bag and placed a pea-sized portion into tubes containing silica beads and a cell lysis/DNA preservation buffer (Xpedition Stabilization/Lysis Solution, Zymo Research Corporation, California USA). All samples were subjected to antiviral heat treatment (30 min at 72° C) before importation to the United States, in accordance with the requirements of the US Department of Agriculture. Tubes were then frozen until transport to the United States. DNA was extracted from each sample individually using the Xpedition Soil/Fecal DNA MiniPrep kit (Zymo Reseach Corporation, California, USA), following manufacturer instructions. Established protocols were used to amplify the P6-loop of the *trn*L intron (*64*), a widely used metabarcode for vascular plants (*65–68*). Each primer used contained a unique 8-bp tag (differing by at least 4 nucleotides) at their 5' end to enable PCR products to be multiplexed and sequenced within a single high-throughput sequencing run (*69*). Two-to-three PCR replicates per DNA extract were conducted to monitor reproducibility of results and any effects of variation in sample processing (*68, 70*). Multiple extraction and PCR controls (using nuclease-free water), as well as positive controls, were included in the analysis. PCR products were later purified using a MinElute PCR purification kit (Qiagen, Germantown, Maryland, USA). The sequencing library was prepared using a PCR-free protocol and sequenced on an Illumina HiSeq 2500 platform (2 x 150bp paired-end sequencing) at Princeton's Lewis-Sigler Institute for Integrative Genomics.

Sequence data were curated using the OBITOOLS software package (*71*) to (*i*) align and assemble paired-end reads (*illuminapairedend* command); (*ii*) assign sequences to their original samples using tagged primer information, allowing zero errors on tags and a maximum of two errors on primers (*ngsfilter* command); (*iii*) merge identical sequences (*obiuniq* command); (iv) remove low-quality sequences (those with a low alignment-quality score, those with a length outside of the expected range: 8-180 bp, and those containing ambiguous nucleotides); (*vi*) discard singletons (sequences occurring only once in the dataset); (*vii*) assign remaining

sequences to plant taxa (*ecotag* command) by comparison with two different reference databases, a local plant database comprising 506 vouchered plant specimens of the most common taxa in Gorongosa (*34*)) and a global database generated from the EMBL genetic database (release 130) by *in silico* PCR using the *ecoPCR* program (*72*); (*viii*) detect sequences potentially resulting from amplification or sequencing errors (*obiclean* command with parameters $d = 1$, $r = 0.25$); and (*ix*) convert files into a table of mOTUs × samples (*obitab* command).

By default, sequences were assigned to the local reference database. However, if the local-database assignment score was <98%, and if the global database score was greater than the local database score, then the sequence was re-assigned to the global database. To discard sequences potentially deriving from PCR/sequencing errors, we used information from the *obiclean* program that determines, for each sample, whether a sequence is more likely to be a true sequence ('head') from which others are derived, a sequence that is derived from another ('internal'), or a sequence from which no other sequence is derived and is itself not derived from another ('singleton'). We discarded mOTUs that did not perfectly match a sequence from the local reference database and that, according to their *obiclean* status, were more commonly considered as true sequences ('head' or 'singleton') than errors ('internal') (*68*). We also filtered out putative contaminants by discarding mOTUs that had their maximal average relative read abundance in negative controls. Finally, any mOTU displaying a low similarity (<80% identity) with its closest match in both reference databases was considered to be a chimera and/or a highly degraded sequence and was excluded.

We assessed reproducibility of results using a graph-partitioning approach with the R package *igraph* (*73*). We calculated the Bray-Curtis distance between each pair of PCR products based on their sequence composition, and clustered together those with Bray-Curtis distance <0.3 (an empirical value determined from the distribution of Bray-Curtis distances between replicates and between samples). In the resulting graph, PCR replicates that did not cluster with other replicates from the same DNA extract were considered as outlying PCRs and were discarded (*74*). Next, the number of reads was averaged among the remaining technical replicates of each sample; in an effort to reduce the impact of low-abundance false positives resulting from 'tag-jumps' during the sequencing process, we removed sequences representing <1% of averaged reads.

Prior to analysis, the mOTUs × samples table was rarefied to 4000 reads per sample and converted into relative abundances (i.e., relative read abundance, RRA, the proportional representation of each plant mOTU in each bushbuck fecal sample (*67*)). Considerable evidence indicates that RRA is generally a robust indicator of quantitative consumption patterns when using the *trn*L metabarcoding approach employed here (*67, 75–77*). Because this *trn*L sequence is a chloroplast intron, and because chloroplast density increases in conjunction with leaf-nitrogen concentration, it has been hypothesized that the RRA of a given plant species correlates most strongly with the proportion of protein obtained from that plant species (*76*); in either case, RRA is a valid proxy for diet composition in the context of our study.

Using these dietary data, we constructed a bipartite network of feeding relationships for woodland and floodplain bushbuck (fig. S2B) and calculated the mean RRA of each plant taxon in the diets of floodplain and woodland bushbuck (Fig. 3B). To visualize patterns of compositional dissimilarity between floodplain and woodland bushbuck, we ordinated samples using non-metric multidimensional scaling based on Bray-Curtis distances calculated between each pair of samples (Fig. 3A) (*67, 78*). This ordination does not preserve exact distances

between objects (i.e., samples), but represents as well as possible the ordering relationships among them in two dimensions, such that samples with similar compositions are closer together and compositionally dissimilar samples are farther apart (*78*).

Analysis of diet nutritional quality
To quantify the quality of bushbuck diets, we combined data on diet composition with data on nutritional quality of forage plants. To determine digestible-energy and digestible-protein content of plant taxa consumed by bushbuck, we collected >5 g of the youngest fully opened leaves from at least 3 different individuals of each plant species and pooled those samples for analysis. Pooled samples were dried to constant weight at 60° C, ground in a Wiley Mill with a 1-mm screen, and submitted to Dairy One Cooperative (Ithaca, New York, USA) for analysis of percentage neutral detergent fiber, acid detergent fiber, lignin, ash, and crude protein, along with gross energy. Digestible-energy and -protein contents were then estimated by parameterizing the summative equations of Robbins et al. (*79*, *80*) with the assay results for each plant species.

For each analyzed plant species, we extracted barcode sequences from the local genetic reference database and determined whether the species shared its DNA barcode with any congeneric relative present in the park. For species that were not present in the local DNA reference database, we obtained their barcode from the global reference database and repeated the same analysis. We then cross-checked the assignments between the two databases to refine the assignment and checked for potential multiple hits. For our analyses of diet quality, we only included plant taxa that matched perfectly (100% identity) with plant mOTUs identified in our diet-composition analysis. We obtained nutritional data for 22 of the 51 mOTUs identified in the diet composition analysis, and those taxa represented >83% of overall bushbuck diets based on RRA. In cases when two closely related species shared the same DNA barcode, we either averaged the digestible energy and protein values across those species (when we had nutritional data for both species) or used values from the single species for which we had nutritional data.

We included fecal samples in our analysis of diet quality only when ≥70% of RRA in that sample comprised plant taxa for which we had nutritional data (*n* = 7 floodplain and 11 woodland samples). We calculated weighted averages of digestible energy and protein for each sample using the RRA of each plant taxon as a weighting factor. We then estimated digestible-energy and -protein contents of floodplain versus woodland diets as a weighted average of samples from each habitat type, with the proportion of the diet accounted for in each sample (range: 70–100%) as the weighting factor (i.e., individuals whose diet was more highly resolved had more weight in the final average). We used a Wilcoxon rank-sum two-sample test in the R package *stats* (*81*) to test for differences in diet quality between floodplain and woodland bushbuck.

Analysis of body condition
During captures in 2015 and 2016, we collected a suite of body-condition measurements from each bushbuck. Measurements were based on a validated system for calculating percent ingesta-free body fat of North American ungulates, and included: chest girth, body length, hind foot length, body weight, thickness of the biceps femoris and latissimus dorsi muscles (measured using ultrasonography), maximum rump-fat depth (measured using ultrasonography), and palpitation scores taken at the sacrosciatic ligament, the lumbar vertebrae, the sacrum, the base of the tail and the caudal vertebrae (*82*, *83*). Equations for converting these measurements into an estimate of ingesta-free body fat have so far only been validated for a subset of North American ungulates and, to our knowledge, have never been applied to African ungulates. Thus, in the

absence of a validated model, we used multivariate statistics to develop a body-condition index from the suite of measurements. To improve our ability to identify multivariate, linear combinations of physical measurements that effectively represented variation in animal condition, we increased our sample size (and thus our statistical power) by pooling body-condition metrics from 66 female antelope of the *Tragelaphus* genus (29 bushbuck, 21 kudu and 16 nyala, all closely related and eco-morphologically similar) obtained between 2014 and 2016 during the dry season (from June to August).

We used a Principal Components Analysis in the R *stats* package (*81*) to identify two principal components (i.e., linear combinations of body condition metrics) that explained over 80% of the variation in body condition metrics among all 66 *Tragelaphus* individuals (fig. S6, tables S4 and S5). Principal Component 1 (PC1) served as a proxy for individual body size (most strongly associated with body weight, length, and limb measurements) and Principal Component 2 (PC2) served as a proxy for amount of body fat (most strongly associated with the maximum fat depth measurements obtained via ultrasonography and palpitation scores). We restricted our analysis to females, because males of the *Tragelaphus* genus are larger and more muscular than females, and because we did not have sufficient data to calculate a corresponding index of condition for males. We evaluated whether body condition differed between woodland (*n*=22) and floodplain (*n*=7) bushbuck using generalized linear models in the R package *stats* (*81*) to compare scores for PC1 and PC2 between bushbuck occupying each habitat type. Habitat type was the fixed effect of primary interest, but we also included fixed effects of sample year and the year × habitat-type interaction, because rainfall was lower in 2016 (~300 mm) than in 2014 (~1200 mm) and 2015 (~600 mm) (*84*). We aggregated bushbuck captured in 2014 and 2015 into a single group (assigned to sample year 2015) that represented measurements from higher-rainfall years; individuals from 2016 were labeled separately due to the potential effects of low-rainfall conditions on animal body condition (*84*). The year × habitat-type interaction was non-significant for both PCs, indicating that differences in condition between habitat types were consistent across years (table S6).

Hypothesis 3: Expansion of bushbuck into the floodplain has measurable negative effects on floodplain plants

Herbivore-exclusion experiment
We designed an exclosure experiment that was focused on *Bergia mossambicensis* Wild (syn. *B. salaria* Bremek), the second-most abundant taxon in the diets of floodplain bushbuck. We carefully selected this plant species for a targeted experimental test in light of our diet analysis, which showed that *Bergia* was the only common plant species that comprised a substantial proportion of floodplain-bushbuck diet (mean 11% RRA, maximum 50%) but a negligible proportion of the diet of other floodplain herbivores (Fig. 3B and Fig. 4A). This targeted approach enabled us to isolate the effects of our focal herbivore on a focal plant species, and thus experimentally test for evidence of the hypothesized trophic cascade from carnivores to producers—an element that has been identified as crucial for strong inference about trophic cascades involving megafauna (*19*). In contrast, a community-level exclosure approach would not have enabled us to distinguish the effects of bushbuck from those of more abundant floodplain ungulates (for example, *Mimosa pigra* and *Ludwigia adscendens* syn. *stolonifera*, respectively the first and third most abundant plants in floodplain bushbuck diets, are utilized by

waterbuck and other species (*30*). Using the high-frequency GPS data from 2016, we identified two experimental sites in the floodplain that (*i*) had high *Bergia* abundance and (*ii*) were within areas utilized by collared floodplain bushbuck, to further maximize the likelihood that experimental responses would be driven by bushbuck.

Because *Bergia* is a small forb (~10-15 cm tall, ~10-30 cm canopy width) that often grows in small monospecific clumps, we used clusters of one-to-several plants as sampling units (fig. S7). We located spatially matched pairs of clusters that were qualitatively similar with respect to number of individual plants, size, and condition, and used coin tosses to randomly assign one cluster in each pair to the caged herbivore-exclusion treatment, with the other serving as an uncaged control. Prior to caging, we measured multiple characteristics of each individual plant within each cluster, including height, elliptical canopy area, and the total numbers of leaves, flowers, stems, and browsed stems (i.e., terminal end bitten off). We then set up a temporary exclosure around the treatment cluster using rebar and wire mesh (fig. S7), such that insect herbivores could enter but mammals could not. Each individual plant within each cluster was tagged so that we could measure within-individual change through time. In total, 80 individual plants in 15 caged and uncaged pairs were included, and these were split roughly evenly (42:38 individuals, 8:7 pairs) between the two sites.

After a minimum of 16 days and a maximum of 18 days, all 80 plants were re-measured, and we tested for differences in plant measurements between caged and uncaged plants. The statistical analysis employed, and the formulation of the response variable (i.e., change before versus after caging, or final value at the end of the experimental period) varied among metrics depending on the distribution of the data. All analyses included treatment as a fixed effect and random intercepts for both site and cluster pair to account for potential differences between sites and pseudo-replication resulting from the inclusion of multiple plants within a given cluster. The percentage of browsed stems and the number of leaves per plant were quantified using the absolute change for each individual plant from before to after the experiment, and the effect of herbivore exclusion was analyzed using a generalized linear mixed effects model in the R package *nlme* (*59*). For number of flowers, plant height, and canopy area, we analyzed final values at the end of the experiment, due to non-normality in the change values (importantly, there was no significant difference in the initial values for these metrics between caged and uncaged groups). For plant height (cm) and elliptical area (cm^2), we analyzed log-transformed final values using a generalized linear mixed-effects model in *nlme*. The final number of flowers per plant was highly skewed because only a fraction of plants had flowers; therefore, we used a negative-binomial mixed-effects model in the R package *lme4* (*85*) to analyze the final number of flowers per plant.
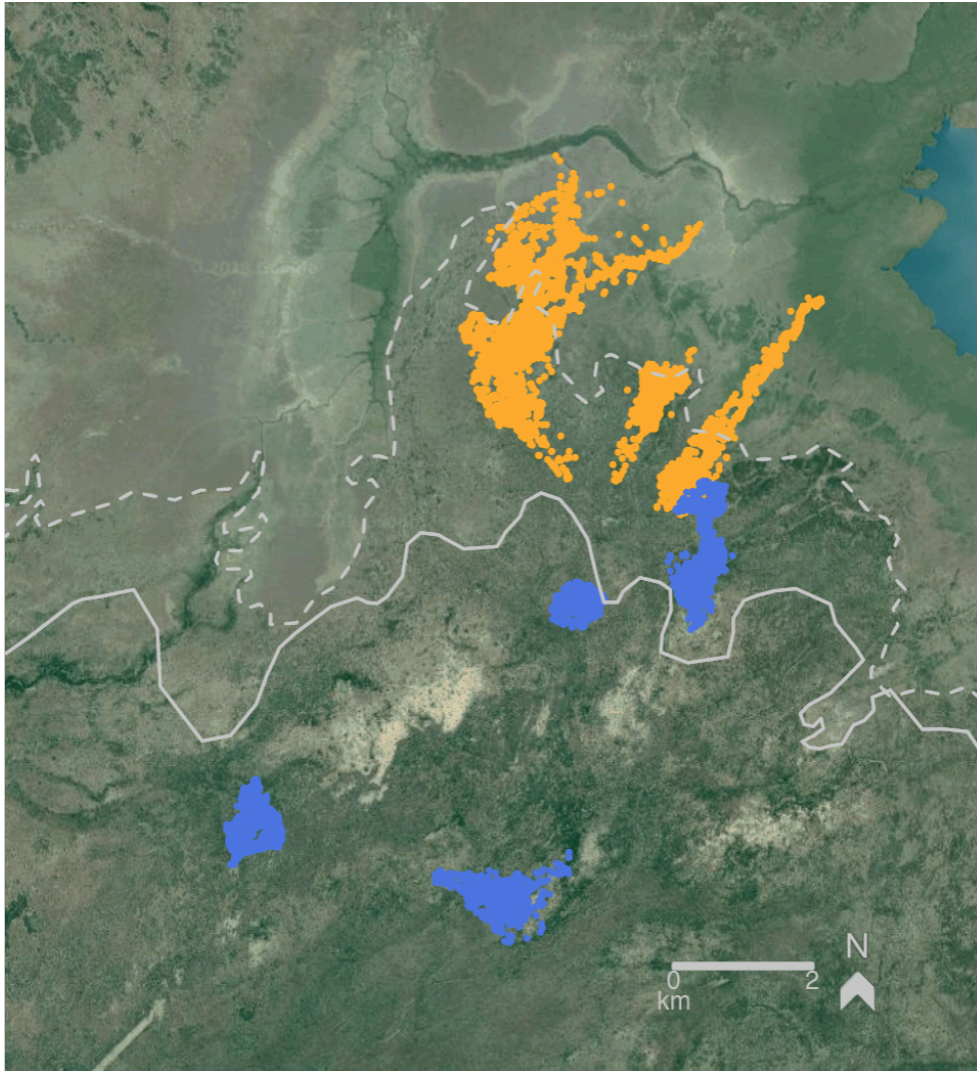
Fig. S1. Differential habitat use by Gorongosa bushbuck, August – September 2016. GPS locations of collared floodplain (orange) and woodland (blue) bushbuck that were subjects of simulated-risk trials in 2016 (*n* = 7 floodplain and 5 woodland). Superimposed solid grey line distinguishes the boundary between the floodplain and dense savanna woodland; dashed grey line distinguishes the boundary between the sparsely wooded floodplain margin and the open, treeless floodplain interior surrounding Lake Urema (top right-hand corner) (*25*).
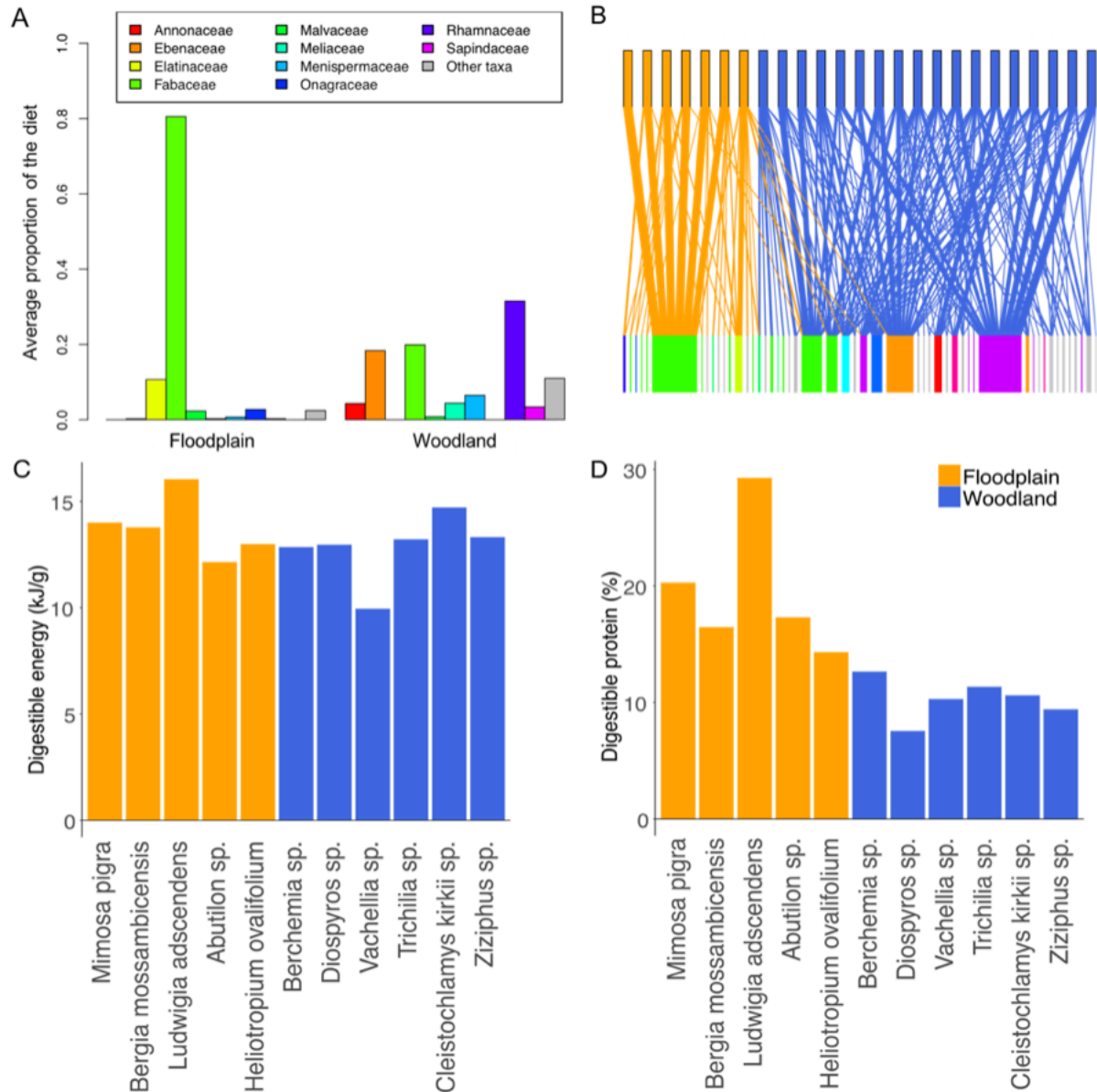
Fig. S2. Bushbuck diet composition in floodplain and woodland habitats. (**A**) Mean representation of the ten most abundant plant families (measured in terms of relative read abundance, RRA) in the diets of floodplain and woodland bushbuck. (**B**) Bipartite network showing composition of each individual bushbuck fecal sample (top bars; orange, floodplain; blue, woodland), with plant mOTUs (bottom bars) colored by plant family per the color scheme in (A). Width of connecting lines reflects the RRA of each mOTU in each sample. (**C**) Digestible energy and (**D**) protein contents of floodplain and woodland bushbuck food plants for which data are available. Plant species are left-to-right in decreasing order of contribution to diet; all species shown are among the top eight forages of bushbuck within each habitat (per Fig. 3B), collectively accounting for 89% and 63% of diet in floodplain and woodland respectively.
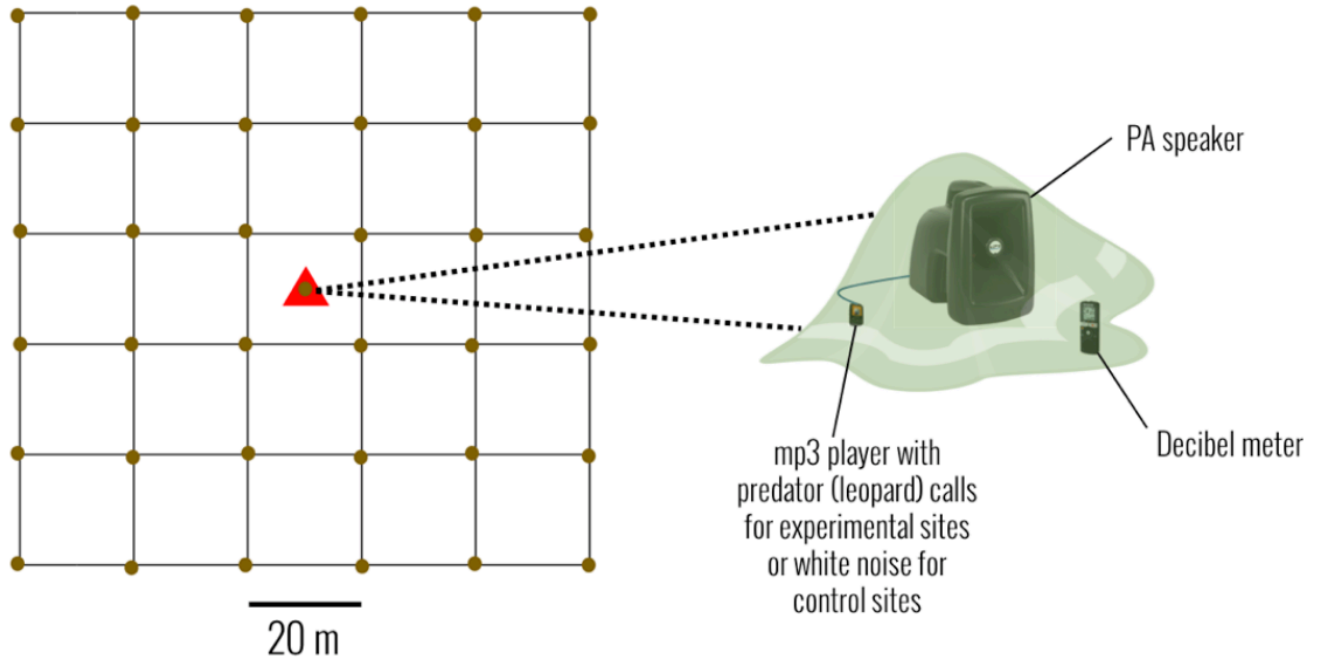
Fig. S3. Schematic representation of the predator-simulation experiment. The red triangle marks the center of the area within each bushbuck's home range that was used most intensively (i.e., had the highest density of GPS-collar locations) by that individual in the 48 hours immediately preceding the deployment of experimental cues. Speakers were placed in this central location. Surrounding the speaker, we delineated a 100 × 100 m grid used for scent-cue deployment (locations indicated by brown circles). Bushbuck home ranges are typically < 0.5 km^2; the grid therefore covered ~ 2% of the overall average home range.
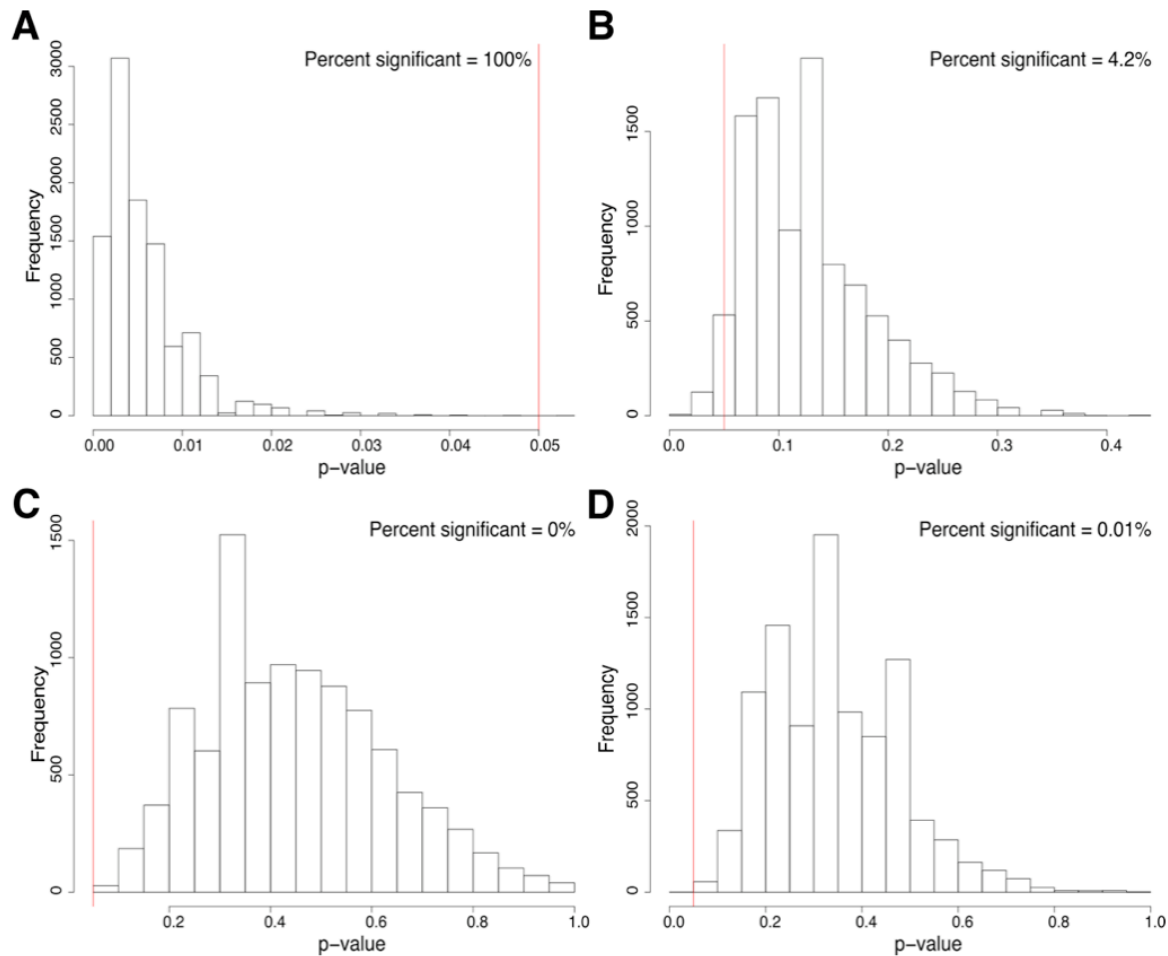
Fig. S4. Sensitivity of experimental results to error in GPS-location data. Frequency distributions of *p*-values estimated by iteratively refitting our models of bushbuck responses (i.e., use of woody cover) to predator and control cues to 10,000 sets of randomly permuted GPS locations in a Monte Carlo simulation analysis. In each simulation run, a location was randomly drawn from within a 13-m (the estimated location error of GPS collars) buffer around each bushbuck GPS location, and the resulting dataset was then used to refit the model. Vertical red lines indicate *p* = 0.05 as a basis for determining the proportion of simulation runs that produced statistically significant results. (**A** and **B**) Results of modeling bushbuck responses to predator and control cues, respectively, in floodplain habitat. (**C** and **D**) Results of modeling bushbuck responses to predator and control cues, respectively, in woodland habitat. This analysis supports our conclusion (Fig. 2, table S2) that floodplain bushbuck shifted towards more wooded habitat in response to predator but not procedural-control cues, and indicates that this result is not an artifact of GPS location error.
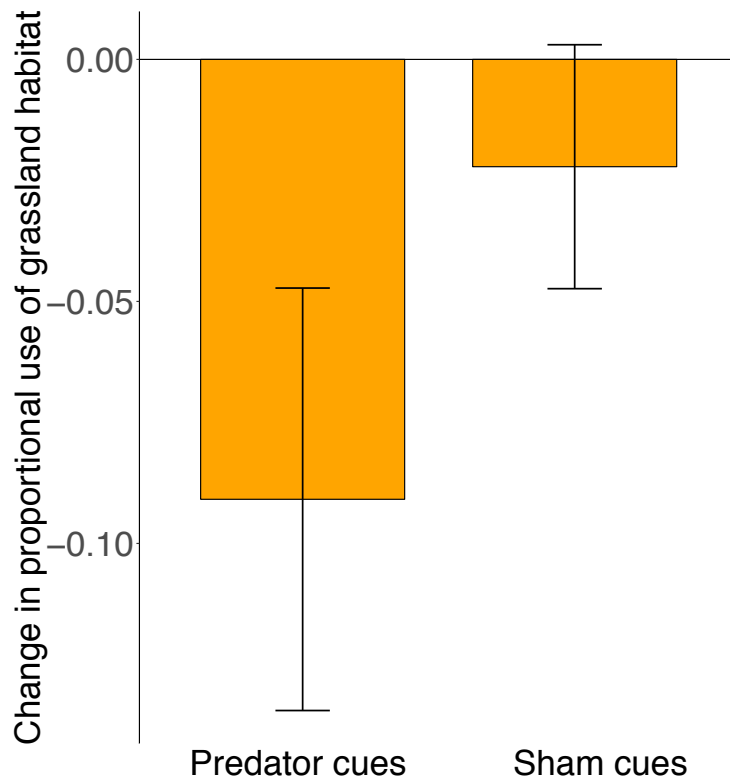
Fig. S5. Sensitivity of experimental results to imprecision in satellite-image classification. Responses of floodplain bushbuck to procedural-control cues (left) and simulated-predator cues (right), using a coarse-grained habitat classification (grassland versus non-grassland) to test the sensitivity of our results. Bars represent the average difference in nighttime use of grassland habitat by floodplain bushbuck ($n = 7$) between the 48 h before and the 48 h after cue deployment; error bars show $\pm$ 1 SE. This analysis supports our conclusion (Fig. 2, table S2) that floodplain bushbuck shifted towards more wooded habitat in response to predator but not procedural-control cues, and indicates that this result is not an artefact of imprecise satellite image classification (see also table S3).
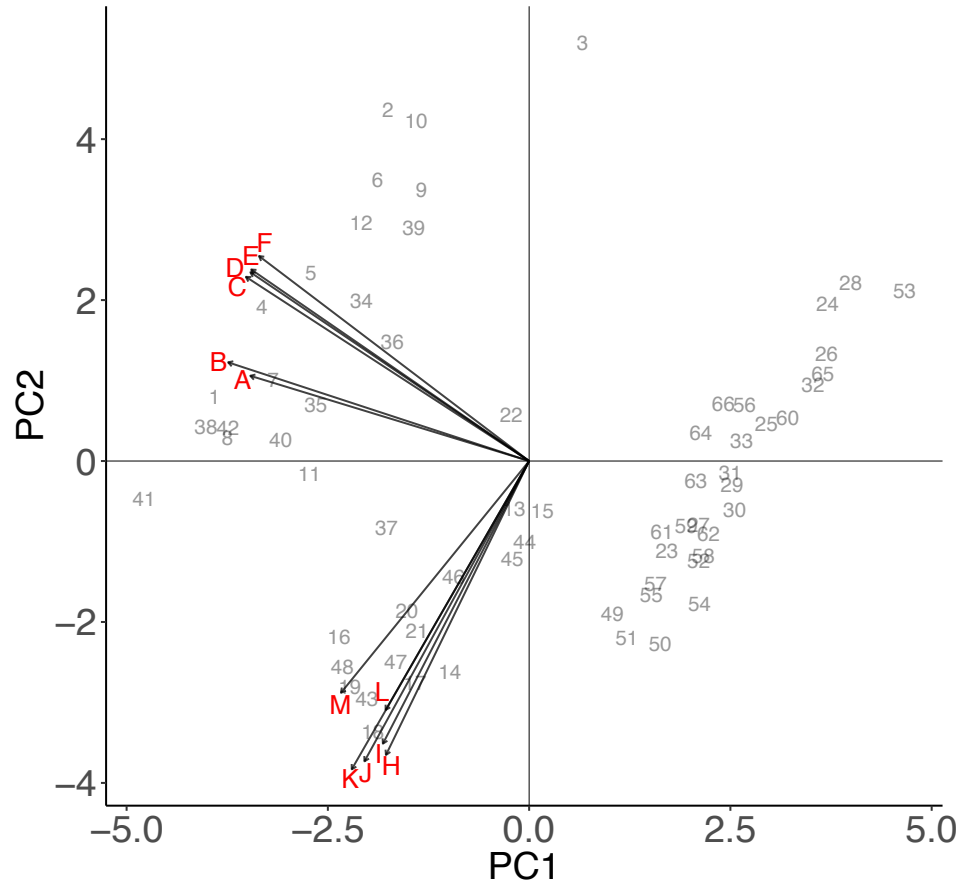
Fig. S6. Principal component analysis on a suite of 12 body condition metrics collected from 66 female *Tragelaphus* antelopes. The body-condition metrics included (as labelled): A) thickness of the *biceps femoris* muscle, B) thickness of the *latissimus dorsi* muscle, C) body mass, D) hind-foot length, E) chest girth, F) body length, palpitation scores taken at H) the sacrum, I) the sacrosciatic ligament, J) the base of the tail, K) the caudal vertebrae and M) the lumbar vertebrae, and L) the maximum rump-fat depth. Each grey number represents an individual antelope, the position of which reflects the score of each individual on the first two principal components. Principal Component 1 (PC1, horizontal axis) and Principal Component 2 (PC2, vertical axis) together explained >80% of the variance in these data. Black arrows show the projections of the original variables. Body-condition metrics that loaded most highly onto PC1 were thickness of the *biceps femoris* and *latissimus dorsi* muscles and measurements of chest girth, body length, hind-foot length, and total body mass. Metrics that loaded most highly onto PC2 were maximum rump-fat depth and palpation scores from the sacrosciatic ligament, lumbar vertebrae, sacrum, base of the tail, and caudal vertebrae. All measurements were made at time of capture.
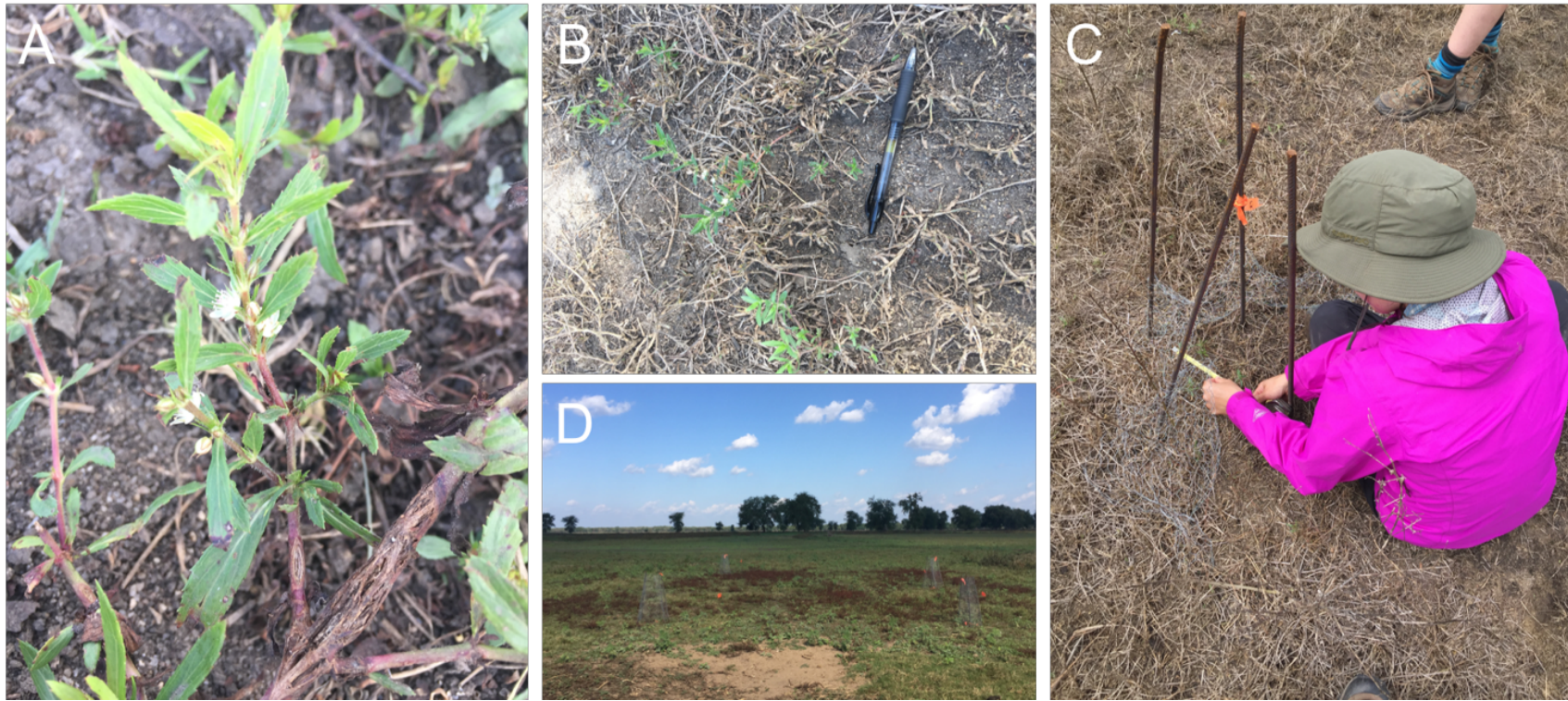
Fig. S7. Herbivore-exclusion experiments with *Bergia mossambicensis* in the floodplain. (**A**) close-up of *Bergia* plant, with small white flowers visible. (**B**) *Bergia* plant with ballpoint pen for scale. (**C**) Temporary exclosures constructed around clusters of *Bergia* plants using iron stakes and wire mesh; here plants are being re-measured after 18 days of herbivore exclusion. (**D**) Experimental setup, with paired clusters of caged and uncaged plants.

Table S1. Abundance information for large carnivores in Gorongosa before (1972) and after (2016) the Mozambican Civil War (*23*, *24*, *30*, *84*)**.** One leopard was observed in April 2018, and one pack of 14 wild dogs were reintroduced into the park in June 2018; both of these events occurred after the conclusion of the present study.

Species	1972	2016	Percent recovery of historical levels
Lion	~200	≥65	~33%
Leopard	"most ubiquitous of the carnivores" (*30*)	0	0%
African wild dog	"rare" but widespread in grassland/savanna (*30*)	0	0%
Spotted hyena	"widespread but uncommon" (*30*)	very rare, perhaps transient	near 0%

Table S2. Effects of predator and procedural-control cues on bushbuck habitat use. Two habitat use metrics – avoidance of cues and use of tree cover – were compared for the 48 h before versus the 48 h after cue deployment for each individual bushbuck. Results shown are from linear (for cue avoidance) and binomial (for tree-cover use) mixed-effects models, with a random intercept included for each individual; coefficients (β) indicate the relative magnitude of the response to each cue type in each habitat at night.

Habitat	Cue type	Avoidance of cues (distance from speaker, m)		Use of tree cover (probability ratio)	
		$\beta \pm$ SE	*P*-value	$\beta \pm$ SE	*P*-value
Floodplain	Predator cues	149.48 ± 23.32	< 0.0001	0.28 ± 0.11	0.008
	Sham cues	27.95 ± 38.64	0.47	0.20 ± 0.11	0.07
Woodland	Predator cues	154.35 ± 17.60	< 0.0001	0.096 ± 0.14	0.51
	Sham cues	-45.00 ± 16.80	0.008	-0.14 ± 0.14	0.31

Table S3. Effects of predator and procedural-control cues on use of grassland habitat by floodplain bushbuck. To test the sensitivity of our experimental results to imprecision in satellite-image classification, we conducted an analysis at a coarser spatial grain than used in the analysis shown in table S2. Specifically, we compared nighttime use of treeless grassland habitat by floodplain bushbuck between the 48 h before and the 48 h after deployment of predator and procedural control cues using a binomial mixed-effects models, with a random intercept included for each individual (see also fig. S5). Coefficients (β) indicate the relative magnitude of the response to each cue type in each habitat at night. This analysis provides additional support for our conclusion (Fig. 2) that floodplain bushbuck shifted towards more wooded habitat in response to predator but not procedural-control cues, and indicates that this result is not an artifact of imprecise satellite image classification (see also fig. S5).

	Use of grassland habitat (probability ratio)	
Trial	$\beta \pm SE$	P-value
Predator	-0.69 ± 0.15	3.38×10^{-6}
Control	-0.112 ± 0.13	0.39

Table S4. **Summary of the principal component analysis of body-condition data from 66 female *Tragelaphus* antelopes, 2014–2016.** Each column (PC1-12) represents a principal component (there are 12 in total as there were 12 body-condition metrics used in the analysis). Over 80% of the variance in these metrics is explained by the first two principal components (50% and 31%, respectively).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Standard deviation	2.47	1.94	0.76	0.67	0.58	0.53	0.46	0.35	0.26	0.18	0.17	0.08
Proportion of Variance explained	0.51	0.31	0.05	0.04	0.03	0.02	0.02	0.01	0.006	0.003	0.002	0.0005
Cumulative Proportion of Variance	0.51	0.82	0.87	0.91	0.94	0.96	0.98	0.99	0.994	0.997	0.999	1

Table S5. **Principal component loadings for each measured body-condition variables from 66 female *Tragelaphus* antelopes in Gorongosa between 2014 and 2016.** Variables associated with body size loaded most heavily on PC1, whereas variables associated with amount of body fat loaded most heavily on PC2.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Max.fat	-0.18	-0.31	0.75	-0.31	-0.39	-0.09	0.22	-0.07	0.00	-0.03	-0.01	0.02
B.femoris	-0.35	0.11	0.01	0.21	0.23	0.42	0.74	0.12	-0.17	-0.03	-0.01	0.02
L.dorsi	-0.38	0.12	-0.03	-0.08	-0.02	-0.03	-0.03	0.39	0.78	0.25	-0.11	0.03
SS.ligament	-0.18	-0.36	0.37	0.55	0.30	0.30	-0.46	0.02	0.01	0.03	-0.02	-0.02
Lumbar.vert	-0.24	-0.29	-0.32	0.56	-0.55	-0.33	0.15	-0.08	0.02	-0.03	0.06	-0.02
Sacrum	-0.18	-0.37	-0.38	-0.36	-0.24	0.63	-0.15	-0.27	0.06	0.04	0.02	-0.01
Base.tail	-0.21	-0.38	-0.11	-0.17	0.56	-0.40	0.16	-0.39	0.15	0.08	0.30	0.00
Caudal.vert	-0.22	-0.39	-0.20	-0.25	0.13	-0.21	-0.10	0.61	-0.40	-0.11	-0.29	0.04
Chest.girth	-0.35	0.24	-0.01	0.00	0.03	-0.11	-0.14	-0.38	-0.08	-0.12	-0.47	0.63
Body.length	-0.34	0.26	0.03	-0.06	-0.11	-0.04	-0.20	0.05	-0.40	0.64	0.43	0.09
Hind.foot	-0.35	0.24	0.01	-0.07	-0.04	0.01	-0.20	0.11	0.02	-0.70	0.52	0.00
Weight	-0.36	0.23	0.02	-0.05	0.02	-0.10	-0.10	-0.26	-0.11	-0.02	-0.36	-0.77

Table S6. Differences in body condition of woodland ($n = 22$) versus floodplain ($n = 7$) bushbuck, measured from 2014 to 2016. Condition scores were calculated using a principal component analysis of 12 body-condition metrics obtained from 66 female *Tragelaphus* antelope (tables S4 and S5). Principal components 1 and 2 together explained >80% of the variation in these data, and loadings reflected generally negative relationships between PC scores and the variables represented by PC1 and PC2 (fig. S6). Betas (β) indicate the magnitude of the difference in the average score for each PC between groups; woodland bushbuck had higher scores for both indices than floodplain bushbuck, which is indicative of poorer condition (e.g., lower mass, less fat). β estimates, standard errors (SE), and *P*-values were estimated from a linear model for each condition score in which the condition (PC) scores were the response variable and the fixed effects were habitat group (floodplain or woodland), year of data collection and an interaction term between habitat × year. (PC 1: $F_{3,25} = 1.55$, PC 2: $F_{3,25} = 1.08$).

	PC 1 (body-size proxy)		PC 2 (body-fat proxy)	
	$\beta \pm SE$	*P*-value	$\beta \pm SE$	*P*-value
Habitat (Woodland)	1.13 ± 0.53	0.044	1.28 ± 0.80	0.12
Year (2016)	1.09 ± 0.66	0.11	1.61 ± 0.98	0.11
Habitat*Year	-1.39 ± 0.85	0.11	-1.45 ± 1.26	0.26

Table S7. Responses of *Bergia mossambicensis* plants to experimental herbivore exclusion. Estimates indicate the average difference in responses variables between caged (no herbivores) and uncaged (open to herbivores) plants after an 18-day experimental period, based on a sample size of 80 individual plants (42 caged, 38 uncaged). For number of flowers, height, and area (which were analyzed using final values at the end of the experiment instead of as change through time), pre-treatment differences between treatment groups were not significantly different (Wilcoxon rank-sum test, all $P > 0.12$). Change in the proportion of stems eaten and number of leaves, along with log-transformed final height and canopy area, were analyzed using generalized linear mixed effects models, with exclosure treatment as the fixed effect and a random intercept for site and exclosure-control pair to account for potential differences between sites and pseudoreplication resulting from the measurement of multiple individual plants within a single caged or uncaged cluster. Final number of flowers was analyzed using a negative-binomial mixed-effects model with the same structure as above, due to a high number of zeroes (i.e., plants with no flowers) that produced overdispersion in the data. Asterisk indicates use of χ^2 in lieu of F test statistic. SE, standard error.

Response variable	Estimate ± SE	F or χ^2	DF	P-value
Change in proportion of stems eaten	-38.53 ± 5.50	49.13	1, 64	< 0.0001
Change in number of leaves	74.19 ± 15.08	24.22	1, 64	< 0.0001
Final number of flowers	2.23 ± 1.07	4.33*	1	0.037
Log of final height (cm)	0.42 ± 0.10	17.49	1, 64	0.0001
Log of final area (cm)	1.23 ± 0.26	22.67	1, 64	< 0.0001

Table S8. Accuracy-assessment criteria for the classification of woody cover in WorldView-2 satellite imagery from 2010 (1.8-m resolution).

Visually classified points	Classed correctly	Classed incorrectly
Woody	89	24
Open	172	15

Overall accuracy: 87%

Sensitivity to woody vegetation: 79%

Specificity to woody vegetation: 92%

References and Notes

1. J. A. Estes, J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R. E. Sinclair, M. E. Soulé, R. Virtanen, D. A. Wardle, Trophic downgrading of planet Earth. *Science* **333**, 301–306 (2011). doi:10.1126/science.1205106 Medline

2. W. J. Ripple, J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, M. P. Nelson, O. J. Schmitz, D. W. Smith, A. D. Wallach, A. J. Wirsing, Status and ecological effects of the world's largest carnivores. *Science* **343**, 1241484 (2014). doi:10.1126/science.1241484 Medline

3. J. Terborgh, J. A. Estes, Eds., *Trophic Cascades: Predators, Prey and the Changing Dynamics of Nature* (Island Press, 2010).

4. J. A. Estes, J. F. Palmisano, Sea otters: Their role in structuring nearshore communities. *Science* **185**, 1058–1060 (1974). doi:10.1126/science.185.4156.1058 Medline

5. J. S. Brown, J. W. Laundre, M. Gurung, The ecology of fear: Optimal foraging, game theory, and trophic interactions. *J. Mammal.* **80**, 385–399 (1999). doi:10.2307/1383287

6. J. W. Laundré, L. Hernandez, W. J. Ripple, The landscape of fear: Ecological implications of being afraid. *Open Ecol. J.* **3**, 1–7 (2010). doi:10.2174/1874213001003030001

7. J. Berger, J. E. Swenson, I. L. Persson, Recolonizing carnivores and naïve prey: Conservation lessons from Pleistocene extinctions. *Science* **291**, 1036–1039 (2001). doi:10.1126/science.1056466 Medline

8. B. L. Peckarsky, P. A. Abrams, D. I. Bolnick, L. M. Dill, J. H. Grabowski, B. Luttbeg, J. L. Orrock, S. D. Peacor, E. L. Preisser, O. J. Schmitz, G. C. Trussell, Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology* **89**, 2416–2425 (2008). doi:10.1890/07-1131.1 Medline

9. G. C. Trussell, P. J. Ewanchuk, C. M. Matassa, The fear of being eaten reduces energy transfer in a simple food chain. *Ecology* **87**, 2979–2984 (2006). doi:10.1890/0012-9658(2006)87[2979:TFOBER]2.0.CO;2 Medline

10. O. J. Schmitz, V. Krivan, O. Ovadia, Trophic cascades: The primacy of trait-mediated indirect interactions. *Ecol. Lett.* **7**, 153–163 (2004). doi:10.1111/j.1461-0248.2003.00560.x

11. A. T. Ford, J. R. Goheen, T. O. Otieno, L. Bidner, L. A. Isbell, T. M. Palmer, D. Ward, R. Woodroffe, R. M. Pringle, Large carnivores make savanna tree communities less thorny. *Science* **346**, 346–349 (2014). doi:10.1126/science.1252753 Medline

12. J. P. Suraci, M. Clinchy, L. M. Dill, D. Roberts, L. Y. Zanette, Fear of large carnivores causes a trophic cascade. *Nat. Commun.* **7**, 10698 (2016). doi:10.1038/ncomms10698 Medline

13. E. le Roux, G. I. H. Kerley, J. P. G. M. Cromsigt, Megaherbivores modify trophic cascades triggered by fear of predation in an african savanna ecosystem. *Curr. Biol.* **28**, 2493–2499.e3 (2018). doi:10.1016/j.cub.2018.05.088 Medline

14. W. J. Ripple, E. J. Larsen, R. A. Renkin, D. W. Smith, Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. *Biol. Conserv.* **102**, 227–234 (2001). doi:10.1016/S0006-3207(01)00107-0

15. D. Fortin, H. L. Beyer, M. S. Boyce, D. W. Smith, T. Duchesne, J. S. Mao, Wolves influence elk movements: Behavior shapes a trophic cascade in Yellowstone National Park. *Ecology* **86**, 1320–1330 (2005). doi:10.1890/04-0953

16. M. J. Kauffman, J. F. Brodie, E. S. Jules, Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade. *Ecology* **91**, 2742–2755 (2010). doi:10.1890/09-1949.1 Medline

17. B. L. Allen, P. J. S. Fleming, L. R. Allen, R. M. Engeman, G. Ballard, L. K.-P. Leung, As clear as mud: A critical review of evidence for the ecological roles of Australian dingoes. *Biol. Conserv.* **159**, 158–174 (2013). doi:10.1016/j.biocon.2012.12.004

18. J. Winnie Jr., S. Creel, The many effects of carnivores on their prey and their implications for trophic cascades, and ecosystem structure and function. *Food Webs* **12**, 88–94 (2017). doi:10.1016/j.fooweb.2016.09.002

19. A. T. Ford, J. R. Goheen, Trophic cascades by large carnivores: A case for strong inference and mechanism. *Trends Ecol. Evol.* **30**, 725–735 (2015). doi:10.1016/j.tree.2015.09.012 Medline

20. J. Terborgh, L. Lopez, P. Nuñez, M. Rao, G. Shahabuddin, G. Orihuela, M. Riveros, R. Ascanio, G. H. Adler, T. D. Lambert, L. Balbas, Ecological meltdown in predator-free forest fragments. *Science* **294**, 1923–1926 (2001). doi:10.1126/science.1064397 Medline

21. M. E. Stalmans, T. J. Massad, M. J. S. Peel, C. E. Tarnita, R. M. Pringle, War-induced collapse and asymmetric recovery of large-mammal populations in Gorongosa National Park, Mozambique. *PLOS ONE* **14**, e0212864 (2019). doi:10.1371/journal.pone.0212864

22. J. H. Daskin, M. Stalmans, R. M. Pringle, Ecological legacies of civil war: 35-year increase in savanna tree cover following wholesale large-mammal declines. *J. Ecol.* **104**, 79–89 (2016). doi:10.1111/1365-2745.12483

23. R. M. Pringle, Upgrading protected areas to conserve wild biodiversity. *Nature* **546**, 91–99 (2017). doi:10.1038/nature22902 Medline

24. P. Bouley, M. Poulos, R. Branco, N. H. Carter, Post-war recovery of the African lion in response to large-scale ecosystem restoration. *Biol. Conserv.* **227**, 233–242 (2018). doi:10.1016/j.biocon.2018.08.024

25. M. Stalmans, R. Beilfuss, "Landscapes of the Gorongosa National Park" (Gorongosa National Park, 2008).

26. J. Kingdon, *The Kingdon Field Guide to African Mammals* (Bloomsbury, ed. 2, 2015).

27. R. D. Estes, *The Safari Companion: A Guide to Watching African Mammals* (Chelsea Green, ed. 2, 1999).

28. R. D. Estes, *The Behavior Guide to African Mammals* (Univ. of California Press, 1991).

29. F. G. T. Radloff, J. T. Du Toit, Large predators and their prey in a southern African savanna: A predator's size determines its prey size range. *J. Anim. Ecol.* **73**, 410–423 (2004). doi:10.1111/j.0021-8790.2004.00817.x

30. K. L. Tinley, thesis, University of Pretoria, Pretoria, South Africa (1977).

31. S. D. Fretwell, H. L. J. Lucas Jr., On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheor.* **19**, 16–36 (1969). doi:10.1007/BF01601953

32. Materials and methods are available as supplementary materials.

33. A. T. Ford, J. R. Goheen, An experimental study on risk effects in a dwarf antelope, *Madoqua guentheri*. *J. Mammal.* **96**, 918–926 (2015). doi:10.1093/jmammal/gyv090

34. J. Pansu, J. A. Guyton, A. B. Potter, J. L. Atkins, J. H. Daskin, B. Wursten, T. R. Kartzinel, R. M. Pringle, Trophic ecology of large herbivores in a reassembling African ecosystem. *J. Ecol.* 10.1111/1365-2745.13113 (2018). doi:10.1111/1365-2745.13113

35. D. I. Bolnick, R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, M. L. Forister, The ecology of individuals: Incidence and implications of individual specialization. *Am. Nat.* **161**, 1–28 (2003). doi:10.1086/343878 Medline

36. C. H. Stigand, *The Game of British East Africa* (Horace Cox, 1909).

37. P. J. Jarman, The social organisation of antelope in relation to their ecology. *Behaviour* **48**, 215–267 (1974). doi:10.1163/156853974X00345

38. K. P. Dial, E. Greene, D. J. Irschick, Allometry of behavior. *Trends Ecol. Evol.* **23**, 394–401 (2008). doi:10.1016/j.tree.2008.03.005 Medline

39. O. J. Schmitz, Effects of predator hunting mode on grassland ecosystem function. *Science* **319**, 952–954 (2008). doi:10.1126/science.1152355 Medline

40. N. Owen-Smith, Mechanisms of coexistence in diverse herbivore-carnivore assemblages: Demographic, temporal and spatial heterogeneities affecting prey vulnerability. *Oikos* **124**, 1417–1426 (2015). doi:10.1111/oik.02218

41. J. L. Atkins, R. A. Long, J. Pansu, J. H. Daskin, A. B. Potter, M. E. Stalmans, C. E. Tarnita, R. M. Pringle, Data for "Cascading impacts of large-carnivore extirpation in an African ecosystem," Dryad (2019); https://doi.org/10.5061/dryad.h4r1003.

42. F. Steinbruch, S. M. Weise, Analysis of water stable isotopes fingerprinting to inform conservation management: Lake Urema Wetland System, Mozambique. *Phys. Chem. Earth* **72–75**, 13–23 (2014). doi:10.1016/j.pce.2014.09.007

43. T. Wronski, A. Apio, M. Plath, C. Averbeck, Do ecotypes of bushbuck differ in grouping patterns? *Acta Ethol.* **12**, 71–78 (2009). doi:10.1007/s10211-009-0058-5

44. P. Waser, Diurnal and nocturnal strategies of the bushbuck Tragelaphus scriptus (Pallas). *Afr. J. Ecol.* **13**, 49–63 (1975). doi:10.1111/j.1365-2028.1975.tb00123.x

45. R. Allsopp, Social biology of bushbuck (Tragelaphus scriptus Pallas 1776) in the Nairobi National Park, Kenya. *Afr. J. Ecol.* **16**, 153–165 (1978). doi:10.1111/j.1365-2028.1978.tb00436.x

46. T. Wronski, A. Apio, M. Plath, Activity patterns of bushbuck (Tragelaphus scriptus) in Queen Elizabeth National Park. *Behav. Processes* **73**, 333–341 (2006). doi:10.1016/j.beproc.2006.08.003 Medline

47. G. Vasse, *Three Years' Sport in Mozambique* (Sir Isaac Pitman and Sons, 1909).

48. M. W. Hayward, G. I. H. Kerley, Prey preferences of the lion (*Panthera leo*). *J. Zool.* **267**, 309–322 (2005). doi:10.1017/S0952836905007508

49. M. W. Hayward, G. Kerley, Prey preferences and dietary overlap amongst Africa's large predators. *S. Afr. J. Wildl. Res* **38**, 93–108 (2008). doi:10.3957/0379-4369-38.2.93

50. R. S. Sikes, W. L. Gannon; The Animal Care and Use Committee of the American Society of Mammalogists, Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* **92**, 235–253 (2011). doi:10.1644/10-MAMM-F-355.1

51. K. Bjørneraas, B. Van Moorter, C. M. Rolandsen, I. Herfindal, Screening Global Positioning System location data for errors using animal movement characteristics. *J. Wildl. Manage.* **74**, 1361–1366 (2010). doi:10.1111/j.1937-2817.2010.tb01258.x

52. Z. Burns, thesis, Princeton University (2010).

53. D. M. Ferrero, J. K. Lemon, D. Fluegge, S. L. Pashkovski, W. J. Korzan, S. R. Datta, M. Spehr, M. Fendt, S. D. Liberles, Detection and avoidance of a carnivore odor by prey. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11235–11240 (2011). doi:10.1073/pnas.1103317108 Medline

54. A. M. Hettena, N. Munoz, D. T. Blumstein, Prey responses to predator's sounds: A review and empirical study. *Ethology* **120**, 427–452 (2014). doi:10.1111/eth.12219

55. K. McComb, C. Packer, A. E. Pusey, Roaring and numerical assessment in contests between groups of female lions, Panthera leo. *Anim. Behav.* **47**, 379–387 (1994). doi:10.1006/anbe.1994.1052

56. C. A. Kardous, P. B. Shaw, Evaluation of smartphone sound measurement applications. *J. Acoust. Soc. Am.* **135**, EL186–EL192 (2014). doi:10.1121/1.4865269 Medline

57. J. Grinnell, K. McComb, Roaring and social communication in African lions: The limitations imposed by listeners. *Anim. Behav.* **62**, 93–98 (2001). doi:10.1006/anbe.2001.1735

58. 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).

59. J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, R Core Team, nlme: Linear and nonlinear mixed effects models (2017).

60. B. Kranstauber, M. Smolla, Move: Visualizing and analyzing animal track data. R package version 2.1.0 (2016).

61. R. J. Hijmans, J. van Etten, raster: Geographic analysis and modeling with raster data (2012).

62. E. J. Pebesma, R. S. Bivand, Classes and methods for spatial data in R. *R News* **5** (2), 9–13 (2005); https://cran.r-project.org/doc/Rnews/.

63. R. S. Bivand, E. Pebesma, V. Gomez-Rubio, *Applied Spatial Data Analysis with R* (Springer, ed. 2, 2013).

64. P. Taberlet, E. Coissac, F. Pompanon, L. Gielly, C. Miquel, A. Valentini, T. Vermat, G. Corthier, C. Brochmann, E. Willerslev, Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.* **35**, e14 (2007). doi:10.1093/nar/gkl938 Medline

65. T. R. Kartzinel, P. A. Chen, T. C. Coverdale, D. L. Erickson, W. J. Kress, M. L. Kuzmina, D. I. Rubenstein, W. Wang, R. M. Pringle, DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8019–8024 (2015). doi:10.1073/pnas.1503283112 Medline

66. P. Taberlet, S. M. Prud'Homme, E. Campione, J. Roy, C. Miquel, W. Shehzad, L. Gielly, D. Rioux, P. Choler, J.-C. Clément, C. Melodelima, F. Pompanon, E. Coissac, Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Mol. Ecol.* **21**, 1816–1820 (2012). doi:10.1111/j.1365-294X.2011.05317.x Medline

67. N. G. Yoccoz, K. A. Bråthen, L. Gielly, J. Haile, M. E. Edwards, T. Goslar, H. Von Stedingk, A. K. Brysting, E. Coissac, F. Pompanon, J. H. Sønstebø, C. Miquel, A. Valentini, F. De Bello, J. Chave, W. Thuiller, P. Wincker, C. Cruaud, F. Gavory, M. Rasmussen, M. T. P. Gilbert, L. Orlando, C. Brochmann, E. Willerslev, P. Taberlet, DNA from soil mirrors plant taxonomic and growth form diversity. *Mol. Ecol.* **21**, 3647–3655 (2012). doi:10.1111/j.1365-294X.2012.05545.x Medline

68. J. Pansu, C. Giguet-Covex, G. F. Ficetola, L. Gielly, F. Boyer, L. Zinger, F. Arnaud, J. Poulenard, P. Taberlet, P. Choler, Reconstructing long-term human impacts on plant communities: An ecological approach based on lake sediment DNA. *Mol. Ecol.* **24**, 1485–1498 (2015). doi:10.1111/mec.13136 Medline

69. J. Binladen, M. T. P. Gilbert, J. P. Bollback, F. Panitz, C. Bendixen, R. Nielsen, E. Willerslev, The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLOS ONE* **2**, e197 (2007). doi:10.1371/journal.pone.0000197 Medline

70. G. F. Ficetola, J. Pansu, A. Bonin, E. Coissac, C. Giguet-Covex, M. De Barba, L. Gielly, C. M. Lopes, F. Boyer, F. Pompanon, G. Rayé, P. Taberlet, Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Resour.* **15**, 543–556 (2015). doi:10.1111/1755-0998.12338 Medline

71. F. Boyer, C. Mercier, A. Bonin, Y. Le Bras, P. Taberlet, E. Coissac, OBITOOLS: A UNIX-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182 (2016). doi:10.1111/1755-0998.12428 Medline

72. G. F. Ficetola, E. Coissac, S. Zundel, T. Riaz, W. Shehzad, J. Bessière, P. Taberlet, F. Pompanon, An in silico approach for the evaluation of DNA barcodes. *BMC Genomics* **11**, 434 (2010). doi:10.1186/1471-2164-11-434 Medline

73. G. Csárdi, T. Nepusz, The igraph software package for complex network research. *InterJournal* **2006**, 1695 (2006).

74. L. Zinger, J. Chave, E. Coissac, A. Iribar, E. Louisanna, S. Manzi, V. Schilling, H. Schimann, G. Sommeria-Klein, P. Taberlet, Extracellular DNA extraction is a fast, cheap

and reliable alternative for multi-taxa surveys based on soil DNA. *Soil Biol. Biochem.* **96**, 16–19 (2016). doi:10.1016/j.soilbio.2016.01.008

75. E. Willerslev, J. Davison, M. Moora, M. Zobel, E. Coissac, M. E. Edwards, E. D. Lorenzen, M. Vestergård, G. Gussarova, J. Haile, J. Craine, L. Gielly, S. Boessenkool, L. S. Epp, P. B. Pearman, R. Cheddadi, D. Murray, K. A. Bråthen, N. Yoccoz, H. Binney, C. Cruaud, P. Wincker, T. Goslar, I. G. Alsos, E. Bellemain, A. K. Brysting, R. Elven, J. H. Sønstebø, J. Murton, A. Sher, M. Rasmussen, R. Rønn, T. Mourier, A. Cooper, J. Austin, P. Möller, D. Froese, G. Zazula, F. Pompanon, D. Rioux, V. Niderkorn, A. Tikhonov, G. Savvinov, R. G. Roberts, R. D. E. MacPhee, M. T. P. Gilbert, K. H. Kjær, L. Orlando, C. Brochmann, P. Taberlet, Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* **506**, 47–51 (2014). doi:10.1038/nature12921 Medline

76. J. M. Craine, E. G. Towne, M. Miller, N. Fierer, Climatic warming and the future of bison as grazers. *Sci. Rep.* **5**, 16738 (2015). doi:10.1038/srep16738

77. B. E. Deagle, A. C. Thomas, J. C. McInnes, L. J. Clarke, E. J. Vesterinen, E. L. Clare, T. R. Kartzinel, J. P. Eveson, Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Mol. Ecol.* **28**, 391–406 (2019). [doi:10.1111/mec.14734](doi:10.1111/mec.14734)

78. D. Borcard, F. Gillet, P. Legendre, *Numerical Ecology with R* (Springer, 2011).

79. C. T. Robbins, S. Mole, A. E. Hagerman, T. A. Hanley, Role of tannins in defending plants against ruminants: Reduction in dry matter digestion? *Ecology* **68**, 1606–1615 (1987). [doi:10.2307/1939852](doi:10.2307/1939852) Medline

80. C. T. Robbins, T. A. Hanley, A. E. Hagerman, O. Hjeljord, D. L. Baker, C. C. Schwartz, W. W. Mautz, Role of tannins in defending plants against ruminants: Reduction in protein availability. *Ecology* **68**, 98–107 (1987). [doi:10.2307/1938809](doi:10.2307/1938809)

81. R Core Team, R: A language and environment for statistical computing (2013); http://www.r-project.org/.

82. R. C. Cook, J. G. Cook, T. R. Stephenson, W. L. Myers, S. M. Mccorquodale, D. J. Vales, L. L. Irwin, P. B. Hall, R. D. Spencer, S. L. Murphie, K. A. Schoenecker, P. J. Miller, Revisions of rump fat and body scoring indices for deer, elk, and moose. *J. Wildl. Manage.* **74**, 880–896 (2010). [doi:10.2193/2009-031](doi:10.2193/2009-031)

83. R. A. Long, R. T. Bowyer, W. P. Porter, P. Mathewson, K. L. Monteith, J. G. Kie, Behavior and nutritional condition buffer a large-bodied endotherm against direct and indirect effects of climate. *Ecol. Monogr.* **84**, 513–532 (2014). [doi:10.1890/13-1273.1](doi:10.1890/13-1273.1)

84. M. Stalmans, M. Peel, "Aerial wildlife count of the Parque Nacional da Gorongosa, Mozambique, October 2016" (Gorongosa National Park, 2016).

85. D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015). [doi:10.18637/jss.v067.i01](doi:10.18637/jss.v067.i01)