MANAGEMENT

Nutritional profiles of some preferred food grasses of the greater one-horned rhinoceros before and after grassland burning in Manas National Park, Assam, India

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Abstract
The nutritional variations of food plants play a significant role in the health and population dynamics of wildlife. The body conditions of vulnerable greater one-horned (GOH) rhinoceros (*Rhinoceros unicornis*) in Manas National Park (MNP), Assam, India deteriorate noticeably during the late monsoon to winter, and improve after routine grassland burning, when rhinos feed on the newly emerged leaves and young shoots of grasses. To investigate this, we analysed the nutritional parameters of the leaves of five grass species preferred by rhinos, including three tall grasses (*Saccharum spontaneum*, *S.narenga* and *Imperata cylindrica*) and two short grasses (*Cynodon dactylon* and *Axonopus compressus*), before and after grassland burning. Leaf samples were collected in triplicate from five different sites in MNP before (September) and after (March) grassland burning. Biochemical analyses showed the highest crude protein content in *S. narenga* before and after grassland burning. *S. spontaneum* and *S. narenga* had the highest and second highest fat contents, respectively, after grassland burning. Crude fibre content in *S. narenga* increased significantly ($p < 0.05$) after grassland burning, but not in the other two tall grasses. The total ash content and the acid-insoluble ash content increased significantly ($p < 0.05$) in post-burning samples of all five grasses. The short grasses *A. compressus* and *C. dactylon* showed the highest calcium and phosphorus contents, respectively, after grassland burning, highlighting their significance in the diets of GOH rhinos along with tall grasses with higher proximate nutrient contents, and their contribution to the improved health status of rhinos after grassland burning. This study will be helpful for improved management of rhino health and their habitats, essential to maintaining the progress made in MNP since the 2008 to 2022 GOH rhino reintroductions under the Rhino Vision 2020 initiative.

Additional Keywords: Mega-herbivores, proximate analysis, macronutrient, minerals, grassland management

Résumé

Les variations nutritionnelles des plantes alimentaires jouent un rôle significatif dans la santé et les dynamiques de population des animaux sauvages. Le rhinocéros indien (*Rhinoceros unicornis*), espèce vulnérable présente dans le parc national de Manas (Assam, Inde), voit son état de santé se dégrader sensiblement entre la fin de la mousson et l'hiver. Après les brûlages systématiques des prairies, qui permettent aux rhinocéros de se nourrir des nouvelles pousses d'herbes et des jeunes feuilles tendres, leur condition s'améliore nettement. Afin d'étudier cette situation, nous avons examiné, avant et après brûlage, les paramètres nutritionnels sur les feuilles de cinq espèces d'herbes ayant les faveurs des rhinocéros : trois espèces d'herbes hautes (*Saccharum spontaneum*, *S. narenga* et *Imperata cylindrica*) et trois espèces d'herbes basses (*Cynodon dactylon* et *Axonopus compressus*). Les échantillons ont été collectés en trois exemplaires sur cinq sites différents dans le parc national de Manas, en septembre (avant brûlage) et en mars (après brûlage). Les analyses biochimiques ont montré que, de tous les échantillons, *S. narenga* contenait le plus de protéines brutes avant et après brûlage. *S. spontaneum* et *S. narenga* avaient respectivement les deux teneurs les plus élevées en matières grasses, après brûlage. Le taux de fibres brutes contenu dans *S. narenga* avait nettement augmenté ($p < 0.05$) après brûlage, contrairement aux deux autres espèces d'herbes hautes. La teneur totale en cendres et la teneur en cendres insolubles dans l'acide se sont significativement accrues ($p < 0.05$) dans tous les échantillons après brûlage. Les herbes basses *A. compressus* et *C. dactylon* ont montré, respectivement, les taux les plus élevés en calcium et en phosphore après brûlage. Ces éléments illustrent par là même leur importance dans le régime alimentaire des rhinocéros indiens — de même que celle des herbes hautes ayant des teneurs immédiates en nutriments plus élevées — ainsi que leur contribution à l'amélioration de l'état de santé des rhinocéros après le brûlage des zones herbeuses. Cette étude représentera un outil intéressant pour une meilleure gestion sanitaire des rhinocéros et de leur habitat, composante essentielle à la continuité des progrès effectués dans le parc national de Manas depuis qu'a été initié en 2008 le programme Vision 2020, pour la réintroduction du rhinocéros indien.

Mot-clés supplémentaires: Méga-herbivores, analyse immédiate, macronutriments, minéraux, gestions des prairies

Introduction

The nutritional profiles of food plants have a significant effect on locomotion, activity patterns, demography, and population dynamics of wild animals, especially mega-herbivores (Hazarika and Saikia 2012). Specialist mega-herbivores are affected by habitat modification to a greater extent than generalist mega-herbivores, as they are not able to switch to foods that are more readily available during periodic shortages of their preferred food when they cannot meet their nutritional targets (Felton et al. 2009). The greater one-horned (GOH) rhinoceros (*Rhinoceros unicornis Linnaeus*, 1758) is an iconic but globally threatened megaherbivore. GOH rhinos are browsers and grazers, eating a very wide range of plant species (Devi 2022). They once existed across the northern Indian sub-continent including parts of Nepal, Bangladesh, and Bhutan, but their distribution is now fragmented and restricted to a few protected areas in India and Nepal (Thapa et al. 2013; Rookmaaker et al. 2016; Ellis and Talukdar 2019). Following the successful implementation of conservation strategies, the GOH rhino population is recovering, and recent estimates show that there are more than 3,500 individuals in the wild (Ellis and Talukdar 2019; Pant et al. 2020). In Manas National Park (MNP), Assam, India, the species was extirpated during a period of civil unrest in the late 1990s and early 2000s (Barman et al. 2014; Dutta et al. 2017). The rhino population has now been re-established in MNP following a successful reintroduction programme initiated by Rhino Vision 2020 (IRV2020) in 2008.The MNP is well known for its rare and endemic wildlife. However, wildlife habitats are being degraded by the invasion of alien plant species (Das et al. 2019; Nath et al. 2019), causing food-related problems in populations of many wildlife species, especially mega-herbivores which require large amounts of food per day. Given the requirement for grassland habitats by GOH rhinos and the significance of the nutrient content of their diet for their health and reproduction, it is essential to understand the nutrient dynamics of their preferred food grasses (Hazarika and Saikia 2012).

Since GOH rhinos prefer riverine grassland and savannah grassland for food and shelter, controlling the invasion of grassland by pioneer trees and invasive weed species is an important strategy for the maintenance of rhino habitat (Sinha et al. 2022). GOH rhinos show a seasonal preference for alluvial plain grasslands but also feed in adjacent swamps and forests (Laurie 1978; Jnawali 1995; Devi et al. 2022). Their diet consists mainly of grasses but also includes some fruits, leaves, shrubs, and tree branches, as well as cultivated crops (Hazarika et al. 2012). GOH rhinos avoid eating sharp-edged, woody, hard, and thorny plant parts. During dry seasons GOH rhinos exhibit preferential rather than non-selective grazing. Preferential grazing involves searching for species of choice like *C. dactylon* and *Andropogon sp.*, and tender shoots of *S. spontaneum*, *Mikania micrantha*, etc. (Fig. 1), whereas non-selective feeding involves eating whatever is within the reach including *Arundo donax*, *Phragmites karka*, etc. (Sinha and Ghosh 2000). GOH rhinos are equipped with hypsodont dentition, and they consume mainly grass all year around. At MNP, grasses constitute 87%, and aquatic and woodland species only 13% of the total annual diet of rhinos (Dutta et al. 2016). However, during the monsoon season and receding monsoon, preferred tall-grass species mature, grow rank, and become less palatable, causing the rhinos at MNP to become semi-browsers (Sinha and Ghosh 2000). During this period, rhinos browse on tree twigs, leaves, and fruits of the small plants such as *Bombax ceiba*, *Butea monosperma*, *Careya arborea*, *Dillenia pentagyna*, *Gmelina arborea* and *Macaranga denticulata* (Dutta et al. 2016).

Applying the standard body condition scoring system for rhinos (Heidegger et al. 2016), experienced frontline field staff and researchers at MNP have observed that, during late monsoon to winter, a noticeable deterioration of the body condition occurs in the rhino population. It is hypothesized that this may be due to a reduction in the nutritional content of food

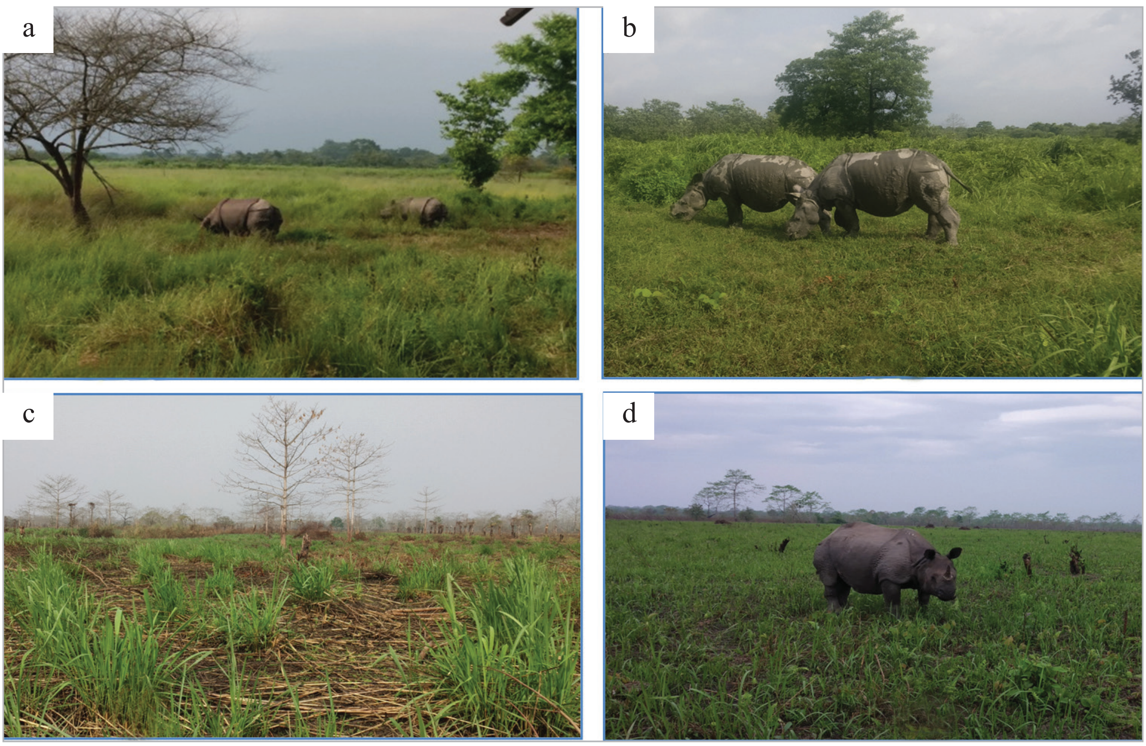

Figure 1. (a) GOH rhinos grazing in their natural grassland habitat in MNP; (b) rhinos grazing in the short grasses in MNP; (c) tender leaves and twigs of tall grasses which emerge after grassland burning (early-monsoon); (d) rhinos grazing on tender leaves and twigs of tall grasses following grassland burning.

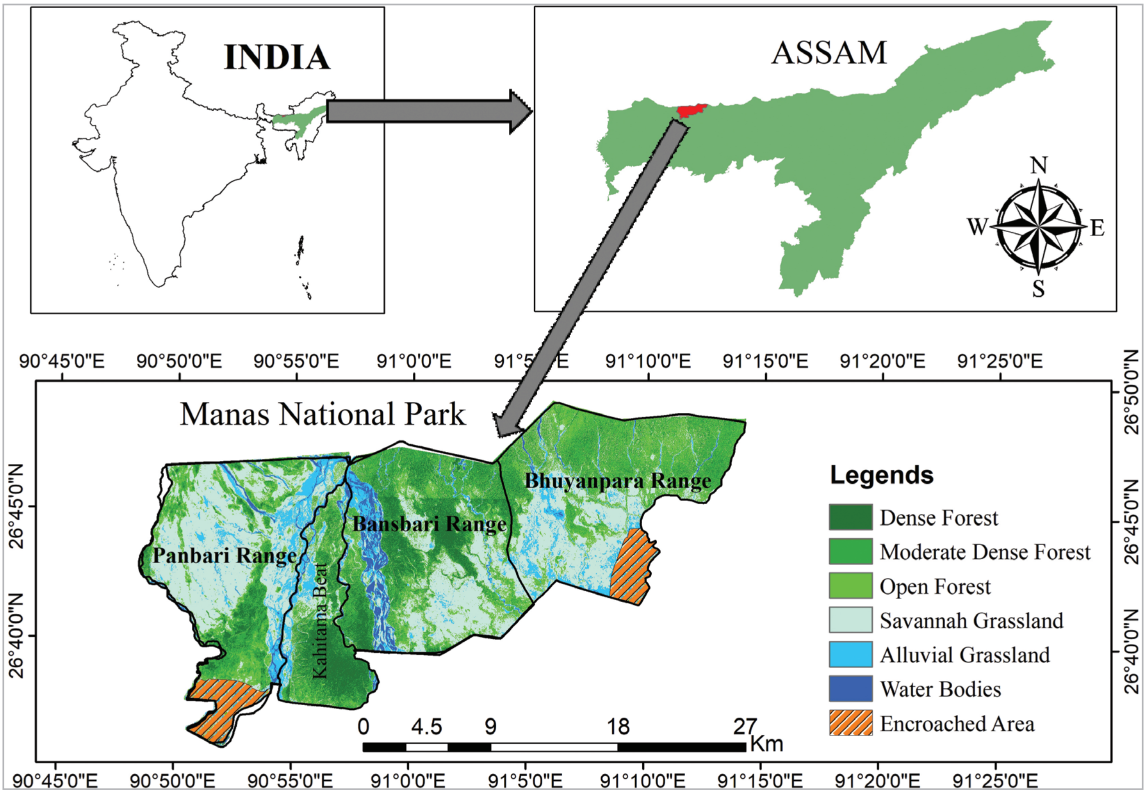

Figure 2. Map of Manas National Park in Assam, India.

plants. The deterioration of rhino condition occurs before annual grassland burning carried out by the Forest Department. Rhino condition recovers after annual burning, when the animals feed on newly emerged leaves and young shoots of tall grasses and on new growth that continues until the following early monsoon period. However, detailed and comparative analyses of nutritional profiles of the food plants of free-ranging rhinos in MNP before and after burning are lacking. To fill this data gap, we analysed the nutritional parameters of the leaf blades of five grass species preferred by rhinos and compared the parameters before and after burning. The species analysed included three tall grasses, namely *S. spontaneum L.*, *S. narenga Hack.*, *Imperata cylindrica L Raeusch* and two short grasses, namely *C. dactylon (L.)* and *A. compressus (Sw.)*. Our purpose was to evaluate whether there were significant variations in nutritional profiles of those grasses before and after burning that could account for the differences in the body condition status of rhinos.

Methods

Study area

The MNP falls under the administrative jurisdiction of the Chirang and Baksa Districts of the north-eastern state of Assam in India (26°35′–26°50′N, 90°45′–91°15′E) and is located at the intersection of the Indo-Malayan, Indo-Gangetic, and Indo-Bhutan biogeographic realms. It is a strategic conservation area that occupies 500 km^2 in the southern foothills of the eastern Himalayas (Wikramanayake et al. 2001) and forms the central area of the Manas Tiger Reserve (2,837 km^2) (Fig. 2). The MNP is bound to the north by Bhutan's international border, to the south by several heavily populated settlements, to the east by the Daodhara Reserve Forest, and to the west by the First Addition to MNP. The MNP is located on both sides of the Manas River, in the eastern Duars (floodplains) at the foothills of the Himalayas, and is divided into three zones known as the Panbari (Western), Bansbari (Central) and Bhuyanpara (Eastern) ranges. The altitude varies from 50m asl on the Southern border to 250m asl in the hills of Bhutan.

MNP has a tropical monsoon climate. The average yearly rainfall is 3,430 mm and the typical annual temperature range is between 10° and 37°C, while the humidity can reach up to 76%. The monsoon (June–September) is the hottest and wettest period of the year, while winter (December–February) is characterized by chilly temperatures and fog. The pre-monsoon season (March–May) and the retreating monsoon (October–November) are transitional periods (Barthakur 1986). The MNP is famous for its rich faunal diversity, including species such as the tiger (*Panthera tigris*), pygmy hog (*Sus salvanius*), golden langur (*Trachypithecus geei*), hispid hare (*Caprolagus hispidus*), Bengal florican (*Houbarogsis bangalensis*) and white-winged duck (*Cairina scutula*). The MNP consists of a mix of moist mixed deciduous and semi-evergreen forests, confined mainly to the northern and extreme south-west sections of the Park (Sarma et al. 2008), and extensive savannah grasslands. The latter are dominated by tall species such as *Saccharum porphyrocoma, Imperata cylindrica, Phragmites karka, S. spontaneum, A. donax, Themeda arundinacea, S. procerum* and *Vetiveria zizanioides*, interspersed with trees such as *Bombax ceiba* and *Dillenia pentagyna* (Fig. 1).

Sample collection

Five abundant grass species, namely *S. spontaneum, S.narenga, I. cylindrica, C. dactylon* and *A. compressus*, which are the preferred food grasses of rhinos (Dutta 2016), were collected in triplicate from five different sites in the central Bansbari Range using stratified random sampling (Birnie-Gauvin et al. 2017). Samples of mature grasses were collected in September (pre-burning). Following burning, which took place between mid-January and mid-February, the grasses regrew (up to 0.3 to 0.5 m tall) and samples of the resprouted leaf blades were collected in March (post burning). Collected samples, in batches of about 500 g, were dried in a hot air oven and then ground and subjected to different biochemical analyses to assess their organic and inorganic nutrient content.

Biochemical analyses

Total nitrogen was estimated using the micro-Kjeldahl method as described by the Association of Official Analytical Chemists (AOAC 1985). Crude protein was calculated as Kjeldahl N × 6.25 (based on the assumption that nitrogen constitutes 16.0% of protein). The contents of crude fat, crude fibre, total ash, acid-insoluble ash, and minerals were also estimated using the methods described by AOAC (1985).

For calcium (Ca), approximately 35 ml ash solution was first digested with concentrate HCl and methyl red indicator was added. Then an ammonium oxalate solution was gradually added, followed by ammonium hydroxide to make the contents alkaline. The solution was then boiled and, after settlement of the precipitate, filtered into a beaker. The filter paper was carefully transferred to the same beaker and washed. After that, 10 ml H_2SO_4 was added, and the contents of the beaker were titrated against a potassium permanganate solution (N/10 $KMnO_4$). Calcium was determined using the following formula:

$$1 \text{ ml N/10 } KMnO_4 = 0.002 \text{ g Ca}$$

For the estimation of phosphorus (P) content, about 25 ml ash solution was treated with HNO_3 concentrate and ammonium molybdate. After settlement, the content was filtered, and the precipitate was washed with 2% HNO_3 followed by a KNO_3 solution. The filter paper was transferred along with the precipitate to the same beaker and N/10 NaOH was added. Subsequently, phenolphthalein and standard alkali were added and titrated against N/10 HCl. Phosphorous was determined using the following formula:

$$1 \text{ ml N/10 NaOH} = 0.000135 \text{ g P}$$

Chemicals used in biochemical analyses are shown in Table 1.

Table 1. Table of chemicals used in authors' analysis, and formulas.

Sulphuric acid	H_2SO_4
Potassium permanganate	$KMnO_4$
Nitric acid	HNO_3
Potassium nitrate	KNO_3
Sodium hydroxide	NaOH
Hydrochloric acid	HCl
Ammonium molybdate	$(NH_4)2MoO_4$

Statistical analyses

All determinations were performed in triplicate and the values were expressed as mean ± 1 standard deviation, calculated using MS-Excel. Least significant difference (LSD) and Tukey's significance tests ($p = 0.05$) were performed using the statistical software SPSS.

Results and discussion

Protein content

S. narenga, which is strongly preferred by rhinos at MNP, had the highest crude protein content compared to all other grass species both before (13.38 ± 0.45%) and after burning (8.73 ± 0.03%). Among the short grasses, crude protein content was higher in *C. dactylon* compared to *A. compressus* in the monsoon period. However, after burning, protein content of *C. dactylon* decreased significantly, and that of *A. compressus* increased significantly, while remaining lower than that of *S. narenga*. Protein is an essential macro-nutrient for all life forms and is a necessary part of the protoplasm in all cells (Schaefer 1946). Low protein content in the diet adversely affects health and may cause anorexia, slow growth rate, decreased feed efficiency, low birth weight, lower milk production, and other disorders. During the monsoon (June–September) and retreating monsoon (October–November), when the tall-grass species mature and become less palatable, rhinos are observed to increase the time spent grazing and expand their range size to maintain their nutrient intake and diet (Laurie 1978; Dutta et al. 2017). The range of crude protein content (2.63 ± 0.01% to 13.38 ± 0.45%) in the fodder plant samples from MNP was lower but similar to the range of 6–15% found in the fodder plants of rhinos in Pobitora Wildlife Sanctuary in Assam by Deka et al. (2002). Differences in crude protein content of fodder at the two sites might be due to soil types or other ecological factors, or to variations in the protein content of grasses at different growth stages. The significant increase in the crude protein content of *A. compressus* after burning corroborates the results of Dutta et al. (2016), as well as the year-round preference of rhinos for *C. dactylon*, which had the second highest protein content (11.31 ± 0.70%) before burning (Table 2). *S. spontaneum*, *S. narenga* and *C. dactylon* were preferred by rhinos in MNP, Kaziranga NP and Bardia NP due to their significantly higher crude protein content compared to other grasses, rendering them more nutritious in the monsoon season (Dutta et al. 2016; Jhala et al. 2021).

Fat content

In addition to contributing to body weight, dietary fat provides essential fatty acids and/or fat-soluble vitamins, which are crucial components in the synthesis of various hormones, including the steroid hormone that plays an essential role in reproductive cycles in both males and females (Hariyadi 2016). The results of the LSD and Tukey's significance tests indicated that the fat content of all five grasses did not change significantly following burning. The fat content after burning was the highest in the tall grass *S. spontaneum* (3.16 ± 0.05%), among the species studied, followed by *S. narenga* (2.349 ± 0.006%). This suggests that these newly emerging tall grass species play an important role in restoring the health of rhinos after burning (Table 2).

Fibre content

Fibre is a necessary component of the diet to maintain normal physiological functions in the digestive tract (Gunstone et al. 1986). Although the crude fibre content in *S. narenga* was the lowest (34.5 ± 0.31%) among the tall grasses in the pre-burn period, it increased significantly after burning (36.48 ± 0.21%), unlike *S. spontaneum* and *I. cylindrica*, which both showed significantly lower crude fibre content after burning (Table 2). This increase in fibre content will contribute to the restoration of the health of rhinos that feed on the emerging leaves of *S. narenga* after burning.

The high contribution of *Saccharum spp.* in the rhino diet is probably due to these species' constant sprouting throughout the year (Lehmkuhl 1994) and their high standing biomass (Jnawali 1995). The burning of tall grasslands from mid-January to early February is followed by a lush regrowth of *S. narenga* and other tall grasses, which dominate the tall grassland for about two to three months before reaching maturity in the monsoon season. The tender leaves rich in protein, fat and fibre content emerge from the charred stems of grasses and become available only after routine burning (Laurie 1978).

Table 2. Proximate composition of rhino food grasses before and after grassland burning in MNP.

Species	Type of grass (short/tall)	Crude protein (%)		Crude fat (%)		Crude fibre (%)	
		Before	After	Before	After	Before	After
S. spontaneum	Tall	6.12 ± 0.88	6.55 ± 0.05	3.54 ± 1.04	3.16 ± 0.05	52.5 ± 0.30	32.87 ± 0.02
S. narenga	Tall	13.38 ± 0.45	8.73 ± 0.03	2.5 ± 0.06	2.35 ± 0.006	34.5 ± 0.31	36.48 ± 0.21
I. cylindrica	Tall	6.01 ± 0.40	5.24 ± 0.003	1.1 ± 0.05	1.07 ± 0.006	47.5 ± 0.58	40.3 ± 0.1
C. dactylon	Short	11.31 ± 0.70	5.25 ± 0.006	0.87 ± 0.15	0.39 ± 0.007	15 ± 0.69	7.93 ± 0.02
A. compressus	Short	2.62 ± 0.01	5.69 ± 0.006	0.84 ± 0.002	1.50 ± 0.01	49.5 ± 0.06	32.69 ± 0.01

Table 3: Ash and mineral composition of rhino food grasses before and after grassland burning in MNP.

Species	Total ash (%)		Acid insoluble ash (%)		Calcium (%)		Phosphorus (%)	
	Before	After	Before	After	Before	After	Before	After
S. spontaneum	4.47 ± 0.06	20.97 ± 0.01	2.25 ± 0.25	12.69 ± 0.02	0.203 ± 0.006	0.145 ± 0.001	1.897 ± 0.025	0.863 ± 0.006
S. narenga	7.3 ± 0.1	20.38 ± 0.07	2.3 ± 0.2	13.21 ± 0.01	0.4 ± 0.03	0.067 ± 0.006	1.323 ± 0.057	0.896 ± 0.005
I. cylindrica	9.07 ± 0.08	31.54 ± 0.05	2.45 ± 0.05	14.96 ± 0.05	0.133 ± 0.035	0.148 ± 0.002	1.350 ± 0.026	0.907 ± 0.010
C. dactylon	8.42 ± 0.12	25.61 ± 0.02	3.97 ± 0.06	23.83 ± 0.12	0.107 ± 0.021	0.207 ± 0.006	0.41 ± 0.010	1.322 ± 0.002
A. compressus	3.51 ± 0.02	6.06 ± 0.006	2.04 ± 0.006	5.0 ± 0.001	0.087 ± 0.002	0.216 ± 0.003	0.93 ± 0.001	1.074 ± 0.005

Ash and mineral content

The total ash content and the acid insoluble ash content increased significantly in post-burning samples of all five grasses. The ash content was the highest in *I. cylindrica* both before and after burning. Regarding the mineral elements, *S. narenga* was found to contain the highest Ca content (0.4 ± 0.03%) before burning, while *A. compressus* showed the highest Ca content (0.216 ± 0.003%) after burning. The Ca content decreased significantly in two tall grasses, that is, *S. narenga* and *S. spontaneum*, while there was no significant difference in calcium content of the other tall grass *I. cylindrica*. The P content was the highest in *S. spontaneum* (1.897 ± 0.025%) before burning, and in *C. dactylon* (1.322 ± 0.002%) after burning (Table 3). The P content decreased significantly in all three tall grasses and increased significantly in two short grasses after burning. Total ash content and Ca content increased significantly in the new shoots of *A. compressus* after burning, possibly explaining why this is one of the short grasses most preferred by rhinos in MNP, even though this species low in crude protein.

The total ash of plants usually represents the inorganic mineral content of the plants, including elements such as Ca, Na, K, Cl, and P that are essential for a healthy growth of animals (Tambe and Kadam 2010). Mineral elements are an essential part of a mammal's diet for the body's physiological functions and metabolic processes, including reproduction. Mineral deficiencies, including subclinical ones, can result in significant reductions in bone density and formation, hormone synthesis, immune function, and the creation of blood, as well as affecting heart function (Fisher 2008; Kumar et al. 2021). Minerals are important bioactive molecules and also cofactors of enzymes. Ca is essential for strengthening bones, skeleton, and teeth, as well as affecting many functions such as milk production, maintenance of cell membranes, and metabolism of enzymes and hormones (Pandey 2011). Short grasses showed higher Ca and P contents after burning, while the content of these two minerals decreased in tall grasses after burning. This highlights the significant contribution of these short grasses to the observed improvement of the health status of this megaherbivore after routine burning.

Conclusion

The presence of grassland habitat is crucial for GOH rhinos in the wild and therefore for all PAs with GOH rhino populations, where habitat restoration activities are prioritized. Moreover, the nutritional profiles of grassland food plants have significant effects on the health and population dynamics of wild animals. This study advances our knowledge of the nutritional dynamics of GOH rhinos' diets due to variations in the nutritional profiles of their preferred food plants before and after burning. Biochemical analyses showed the highest crude protein content in *S. narenga* before and after burning, while *S. spontaneum* and *S. narenga* had the highest and second highest fat contents, respectively, after burning. The crude fibre content in *S. narenga* increased significantly after burning, unlike in the other two tall grasses. Total ash content and the acid insoluble ash contents increased significantly in post-burning samples of all the five grasses. Short grasses *A. compressus* and *C. dactylon* showed the highest Ca and P contents, respectively, after burning, highlighting their importance in the diet of GOH rhinos along with tall grasses with higher proximate nutrient content, and their contribution to the improvement of the health status of rhinos after burning.

The results of this study provide baseline information for implementing measures to improve the habitat quality and availability of the preferred grasses of rhinos in the MNP. However, more in-depth studies are needed to correlate the rhinos' body condition scores with the nutritional profiles of preferred food plants before and after grassland burning. The influence of other associated factors, including the nutritional profiles of other less preferred food plants ingested by rhinos should also be considered. Furthermore, rhino parasitic load/infection in different seasons also impact on the health of rhinos, and would be worth investigating in a follow-up study. Studying the above three parameters in combination with the nutritional profiles of preferred food plants in two different seasons of the year would provide an even more detailed picture of factors affecting the health of rhinos in MNP.

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