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Research Article

Supplementation of White-tailed deer increases the size of the assemblages and the presence of predators in the area

Fernando X. Plata-Pérez*[1], Diana P. Urbina-Flores[2], Brenda Duana Hernández[1], José A. Martínez-García[1], Germán D. Mendoza-Martínez[2], Oscar A. Villarreal-Espino-Barros[3], Pedro A. Hernández-García[4], Alejandra Caballero Zamora[1]

*[1]Universidad Autónoma Metropolitana, Unidad Xochimilco, Departamento de Producción Agrícola y Animal. Calzada del hueso no 1100. Col. Villa Quietud. Alcaldía Coyoacán, CP 04970, CdMx. México
[2]Universidad Autónoma Metropolitana, Unidad Xochimilco, Doctorado en Ciencias Agropecuarias, Calzada del hueso no 1100. Col. Villa Quietud. Alcaldía Coyoacán, CP 04970, CdMx. México
[3]Benemérita Universidad Autónoma de Puebla. Facultad de Medicina Veterinaria y Zootecnia. Carretera Tecamachalco-Cañada Morelos Km. 7.5, El Salado, CP 75460 Tecamachalco, Pue. México
[4]Universidad Autónoma del Estado de México. Medicina Veterinaria y Zootecnia, Centro Universitario UAEM Amecameca. Carretera Amecameca km 2.5 Col. Centro. C.P. 56900, Estado de México, México
*Email: ppfx2221@correo.xoc.uam.mx

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Abstract

The use of supplements to increase the body and antler size in *Odocoileus virginianus* (white-tailed deer) is rampant; however, the impact of this strategy on the size of surrounding species assemblages needs to be better understood. This work aimed to determine the effect of the multi-nutrient block (MNB) for white-tailed deer on the richness and abundance of birds and mammals and the presence of predators. We realized this work in Santa Cruz Nuevo, Puebla, Mexico, from 2017 to 2019. A completely randomized design with two treatments and eight replicates per treatment was used, for which 16 completely randomized sites were chosen, within which a camera trap was installed, and the animals' visits to the site were recorded. Plots of 50 m^2 were established, and two MNBs (protein and minerals) were placed in the central part of eight squares. The other eight sites served as control treatments. The response variables were animal richness and relative abundance. The results showed that animal richness was higher in the MNBs sites (10.6 species; $P < 0.05$) and lower (7 species) in the control. The relative abundance of the sites with MNBs was higher for predators: coyote (*Canis latrans*), bobcat (*Lynx rufus*), puma (*Puma concolor*), and mammals less than 10 kg (rabbits, rodents and other omnivores). However, there were no significant differences in the relative abundance of white-tailed deer ($P= 0.1568$). In conclusion, the presence of the MNBs modifies the relative abundance of predators but does not increase the presence of white-tailed deer.

Keywords*:* *Odocoileus virginianus*, relative abundance index, supplementation, camera traps

Introduction

In Mexico, the white-tailed deer (*Odocoileus virginianus*) is the principal wild ruminant for hunting, and it is highly valued in rural communities (Villarreal Espino-Barros et al., 2008). Various management strategies have been promoted to maintain the nutritional condition of white-tailed deer populations and their body condition in critical seasons. One such strategy is supplementation, which improves the body condition of animals and stimulates population growth, thereby mitigating the shortage of food sources in critical situations (Jones et al., 2014); it can also be used to attract or concentrate wildlife for recreational activities such as ecotourism or hunting (Garshelis et al., 2020) or move wildlife away from specific areas or crop types (Muthoka et al., 2023). In Europe, farm managers promote hunting through the use of perimeter fences and supplementary feeding as strategies to increase the species' abundance (Carpio et al., 2021). However, the spread of diseases is one of the issues caused by supplementation. According to a review conducted by Murray et al. (2016), 115 studies that examined the relationship between wildlife health and supplementary feeding found that it can increase the risk of pathogen transmission through host contact and lead to pathogen accumulation in feeders and surrounding areas. In addition, most of the food supplied was found to be a source of immunosuppressive contaminants.

Sets of species that develop from deterministic processes such as predation, competition, and species' differential responses to their environment are known as "assemblages." Natural and anthropogenic disturbances can alter these assemblages by modifying the biotic and abiotic characteristics of the habitat (Le Borgne et al., 2018). It is recommended that assemblage comparisons be conducted with similar sampling completeness levels. To understand the concept of sample completeness, Chao & Jost (2012b) reviewed the concept of sample coverage, which measures the percentage of individuals of each species in the sample compared to the total number of individuals (Ramirez & Gutiérrez-Fonseca, 2016).

The effects on the spread of diseases caused by strategies to increase the presence of wildlife that can be observed or hunted have been extensively studied (Murray et al., 2016); in contrast, little research has been conducted on the impact of supplements on the species that coexist (assemblages) with the white-tailed deer. Therefore, the purpose of this study was to determine the effect of multi-nutrient block (MNB) supplementation on the number of animal species (richness), the relative abundance of white-tailed deer, and the presence of certain predators in the area.

Materials and methods

Study Area

The study was carried out in the Management Unit for the Conservation of Wildlife (MUCW), located in the community of Santa Cruz Nuevo in the municipality of Totoltepec de Guerrero, Puebla. This municipality belongs to the so-called Mixteca Poblana (18°17'44" N, 97°48'35" W). The predominant climates are semi-humid with summer rains, an annual average temperature between 22 and 25 °C, and an annual rainfall between 759 and 950 mm. Additionally, the area has sub-humid temperate climates with summer rains, with an annual average temperature of 17 to 19 ºC and rainfall of 650 to 700 mm (Rzedowski, 2006). This region is part of the hydrological region of the Balsas River (Villarreal-Espino et al., 2011; Instituto Nacional de Estadística y Geografía [INEGI], 2021) and presents a marked seasonality. The slope ranges from 20 to 70%, and the soil depth ranges from 0 to 25 cm (INEGI, 2021). In a completely random manner, 16 sites were selected within an area of 4150 ha. Plots of 50 m^2 were established (5 x 10 m). Of these sites, eight quadrants were chosen at random. In the center of each quadrant, two types of MNBs were placed: one energy protein and one mineral (Trophy Maker® and Min Plex VE12®). The other sites served as control sites. To observe the effects of treatments on wildlife, camera trap stations with a single camera were established in all quadrants. Vegetation sampling and animal capture through camera traps were carried out from 2017 to 2019. The minimum distance between cameras where plots were close together was 500 m (O'Brien, 2011).

Photo collection, camera cleaning, and battery replacement were done every three to four months (Díaz-Pulido & Payán Garrido, 2012). We utilized Bushnell Trophy Cam HD trap cameras with passive infrared motion detectors (0.3-second velocity). The cameras were fixed and active for 24 hours. The programming of the equipment was adjusted according to the conditions of the focal field. Medium sensitivity was utilized in locations with difficult access, thick vegetation, and challenging clearance. Otherwise, biomass was removed in the accessible areas to keep the focal field free, and sensitivity was high. The cameras were set to capture three pictures per exposure in open field areas and two in areas with high vegetation. Each camera trap was mounted on stable supports, such as trees or dry logs, approximately 50 to 60 cm above ground level. Successive captures of the same species within an interval of fewer than 5 minutes were considered a single event, while captures of different species that appeared within two continuous sequences of less than 5 minutes were considered independent.

Wildlife visits to the sites were recorded, and the free software Wild.ID (Mandujano & Morteo-Montiel, 2018) was used to facilitate the observation and ordering of photo folders derived from the sampling. The independent variables considered were the estimation of the relative abundance index (RAI) for each species, which was calculated as the number of photo-capture events independent of each animal divided by the sum of the total sampling effort of all camera traps multiplied by 100 (Gronwald & Russell, 2021). The richness of animal species was determined by the number of species found in the sampling plots and the Shannon diversity index per site, including herbivorous and omnivorous mammals as well as mesopredators and carnivores (Alvarenga et al., 2018). On the other hand, habitat variables associated with the presence of deer included escape cover (EC%). For this project, the escape cover was defined as the percentage of vegetation capable of concealing a subject 15 m away (Griffith & Youtie, 1988). In contrast, the basal cover was defined as the percentage of plant species that cover the soil (CB%; Huruba et al., 2018), and elevation was also taken into account (AMSL). These variables were related to the presence of MNBs, white-tailed deer, and other animals, such as predators or prey, using a pairwise correlation.

Data analysis

A completely randomized design with two treatments (control and MNB) was used to analyze the abovementioned variables. The response variables were animal richness and the Shannon index. Since some of the variables mentioned lacked a normal distribution, their values were analyzed using nonparametric tests (the Wilcoxon test). The chi-square test was used to determine whether there were statistical differences between the treatments. Statistical analyses were performed using JMP 8.0 software**.** According to Chao et al. (2020), in an assemblage, it is impossible to detect all species; therefore, some species present in the area cannot be identified. As a result, the specific richness of an area depends on the sampling effort. Rarefaction curves were estimated based on the completeness of the sample to analyze this variable (Chao & Jost, 2012a). For rarefaction analysis by sample coverage, we used the iNEXT program (iNterpolation/EXTrapolation; Chao et al., 2014), following the authors' recommendations. This program is available at https://chao.shinyapps.io/iNEXTOnline/. To evaluate the effect of MNBs over time and estimate extrapolation, we conducted 500 bootstraps, which allowed us to graphically visualize the influence of prolonged periods of supplementation on the diversity of species inhabiting the MUCW. Due to the possibility of behavioral differences between birds and mammals, rarefaction curves and statistical analyses were performed separately.

Results

The characteristics of the sampling sites concerning the landscape are presented in Table 1. As expected in a completely random model, no differences in habitat characteristics were found among landscapes. With a total sampling effort of 1077 photo-trapping nights, we obtained approximately 6,000 animal photographs, including 2,188 mammal sightings and 718 bird sightings. The total number of sightings by species is shown in Table 3. The MNBs increased ($p < 0.05$) the number of species (richness), the total number of individuals identified in the area, and the Shannon index of animal diversity (Table 2). In total, 13 species of birds and 17 species of mammals were identified. While both male and female white-tailed deer (*Odocoileus virginianus*) were identified, for most species, the sex was not determined. Therefore, it was considered that photographs of the same species captured within an hour of observation at the same photo-trapping station belonged to the same individual or individuals and were excluded from the analysis.

Table 1. Plots' landscape characteristics, species richness, and Shannon index for the MNBs and control treatments.

	MNBs	Control	EE	P
Escape cover, %	46.6	47.0	6.37	0.95
Basal cover, %	22.21	28.0	5.71	0.473
Vegetal richness, number of species	12.11	11.50	1.15	0.721
Shannon index, vegetal diversity	2.14	2.02	0.116	0.448

Table 2. Number of sightings by species between treatments.

Variable	MNBs	Control	EE	P
Animal richness, number of species (mammals)	4.17	2.68	0.45	0.05
Number of individuals	91.17	25.10	16.26	0.05
Shannon index, animal diversity	1.22	0.78	0.10	0.05

Table 3 shows the relative abundance index for each treatment. The MNBs tended (P = 0.067) to increase the presence of the *Lynx rufus* (bobcat), the *Peromyscus spp* (mouse), and the *Geococcyx velox* (roadrunner) and increased (P = 0.017) the presence of the *Canis latrans* (coyote) and some predatory birds such as *Cathartes* (buzzard); however, they had an inverse effect on *Buteo jamaicensis* (sparrowhawk) and *Nasua narica* (coati). On the other hand, the *Oryctolagus cuniculus* (rabbit), *Urocyon cinereoargenteus* (grey fox), *Rattus novaeguineae* (rat), and *Peromyscus spp* (mouse) had the highest relative abundances, both in the areas with MNBs and in the controls. No significant differences were seen in the frequency of observations of *O.*

virginianus (white-tailed deer; P = 0.196). Also, Table 3 shows the correlation between some species of predators and prey. It can be observed that the cougar has a direct correlation with the white-tailed deer, as does the gray fox with the rabbit. At the same time, the relationship between mesopredators (bobcat and coyote) is similar to the one between skunks and raccoons, so the data suggest species sympatry and predator-prey relationships.

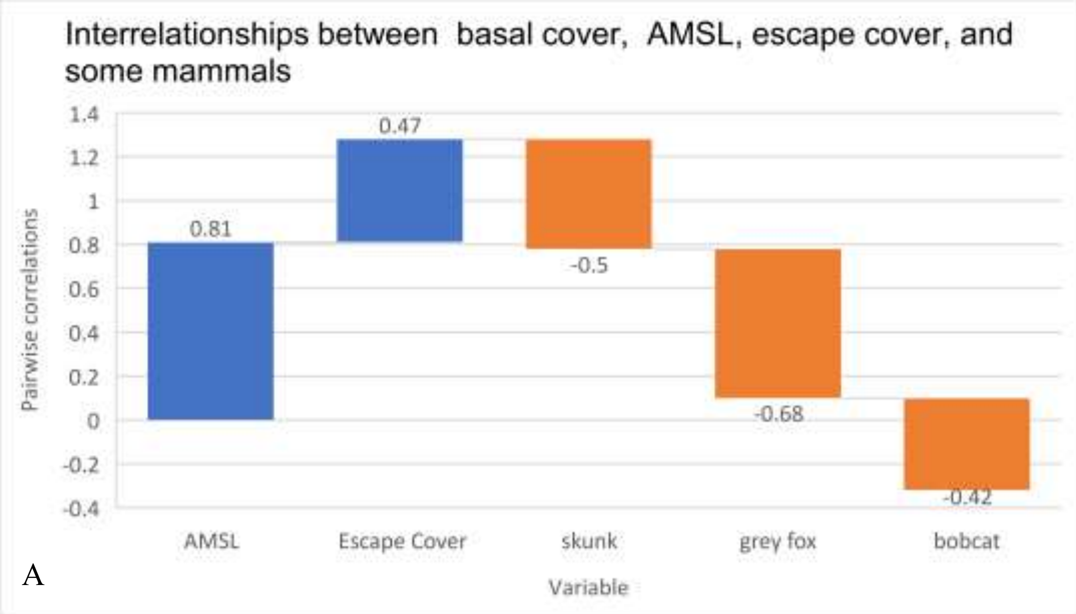
Table 3. The number of individuals and animal species present in areas with and without MNBs.

	Mammals							
	N*	MNBs	Control	EE	LCI	LCS	X2	P
Oryctolagus cuniculus	824	106.4	23.07	43.04	15.15	197.65	0.63	0.42
Rattus novaeguineae	366	46.49	0	42.19	-42.94	135.92	1.34	0.24
Peromyscus mekisturus	354	41.6	0.48	20.07	-0.95	84.17	3.61	0.05[t]
Urocyon cinereoargenteus	347	65.73	21.81	41.91	-23.12	154.58	1.1	0.29
Bos primigenius	154	13.61	10.58	9.06	-5.59	32.81	0.69	0.40
Odocoileus virginianus	105	3.65	7.631	3.14	-3.01	10.31	1.67	0.19
Canis lupus familiaris	66	13.94	0.41	13.94	-15.61	43.5	0.35	0.55
Procyon lotor	54	3.32	6.19	1.61	-0.08	6.73	2.11	0.14
Mephitis mephitis	52	4.57	3.82	1.52	1.35	7.79	0.42	0.51
Canis latrans	50	5.88	0.81	1.42	2.86	8.89	5.67	0.01*
Linx Rufus	34	2.77	2.88	1.48	-0.36	5.91	3.36	0.067[t]
Didelphis virginiana	9	1.07	0	0.73	-0.49	2.62	0.86	0.35
Homo sapiens	6	0.08	0.95	0.08	-0.09	0.25	5.29	0.02*
Bassariscus astutus	5	0.92	0.13	0.72	-0.61	2.46	0.03	0.95
Nasua narica	5	0.47	0.6	0.47	-0.53	1.47	4.02	0.04*
H. yagouaroundi	3	0.8	0	0.61	-6.97	8.56	0.79	0.86

Puma concolor	2	0.08	0.13	0.08	-0.09	0.26	0.35	0.551
Spilogale angustifrons	2	0	0.26	0	0	0	2.43	0.12
				Birds				
Zenaida asiatica	344	34.74	6.5	15.53	2.88	66.61	0.25	0.62
Toxostoma curvirostre	125	14.94	0.9	8.25	-1.99	31.87	0.76	0.38
Geococcyx velox	87	8.71	1.97	1.95	4.7	12.72	2.51	0.11[t]
Leptotila verreauxi	41	1.92	3.66	1.65	-1.46	5.3	0.71	0.4
Chondestes grammacus	34	3.76	0.3	2.86	-2.11	9.62	0	0.94
Buteo jamaicensis	25	0	48.14	20.1	-41.24	41.24	3.94	0.05*
Aphelocoma californica	15	5.23	0	3.49	-1.93	12.4	1.09	0.3
Cathartes aura	13	1.55	0	0.45	0.62	2.49	4.58	0.03*
Zenaida macroura	11	0.45	1.04	0.47	-0.52	1.41	1.27	0.26
Haemorhous mexicanus	9	0.3	0.85	0.43	-0.59	1.19	0.27	0.6
Columbina inca	7	0	0.85	0.36	-0.73	0.73	1.9	0.17
Ortalis poliocephala	6	0.46	0	0.29	-0.14	1.07	1.09	0.3
Caracara cheriway	2	0.23	0	0.13	-0.04	0.5	1.09	0.3

*** N = total number of animals**

Figure 1 shows the effect of the interrelation between the different types of cover, the altitude, and the mammal species they affect. Here it can be observed that the basal cover, altitude, and escape cover have a strong interrelation (Fig. 1A) and that the escape cover positively or negatively (Fig. 1B) affects some predator species and other mammalian species; however, not all species present in the area are affected by these variables.



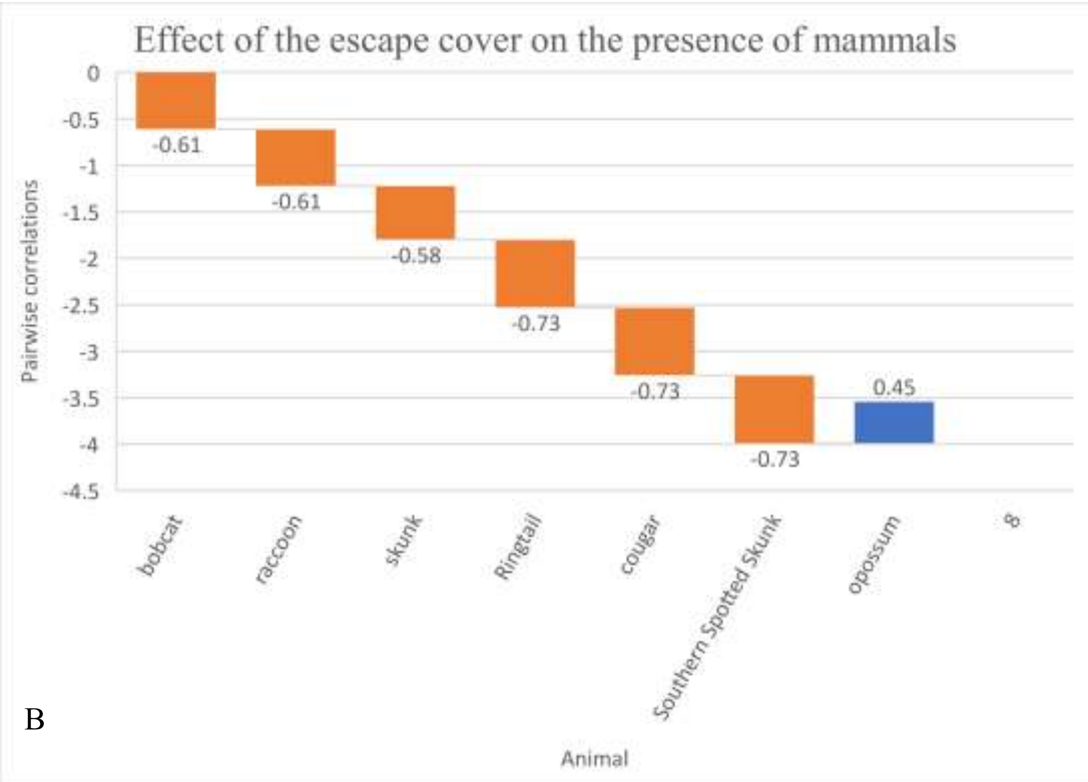


Figure 1. Effect of some environmental variables on the presence of some mammals

The rarefaction curve derived from mammalian diversity analysis shows (Fig. 2A) that species richness increases over time according to the presence of MNBs; in addition, the accumulation/extrapolation curve defines the expected diversity and abundance of a finite sample of size m; in this case, extrapolation shows that higher values can be achieved than those observed, so diversity is expected to increase concerning increased sightings. The comparison between the control treatment and MNBs was made with sample coverages close to 1 (Fig. 2B), and the number of species (Fig. 2C) increased due to the inclusion of MNBs. Like that observed in mammals, Figure 2D shows that the completeness of the birds' sample is almost 1; however, MNBs show only a tiny increase in observed bird species (Fig. 2E), which suggests that MNBs are not so important for stimulating the presence of birds.

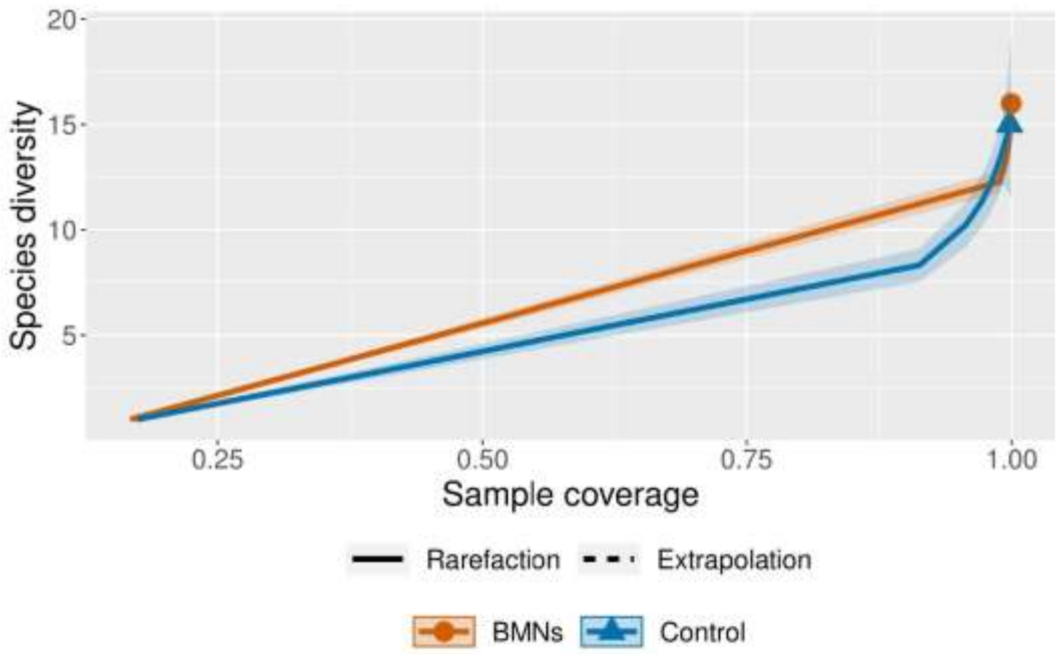


Figure 2A. The rarefaction curve derived from mammalian diversity analysis shows that species richness increases over time according to the presence of MNBs.

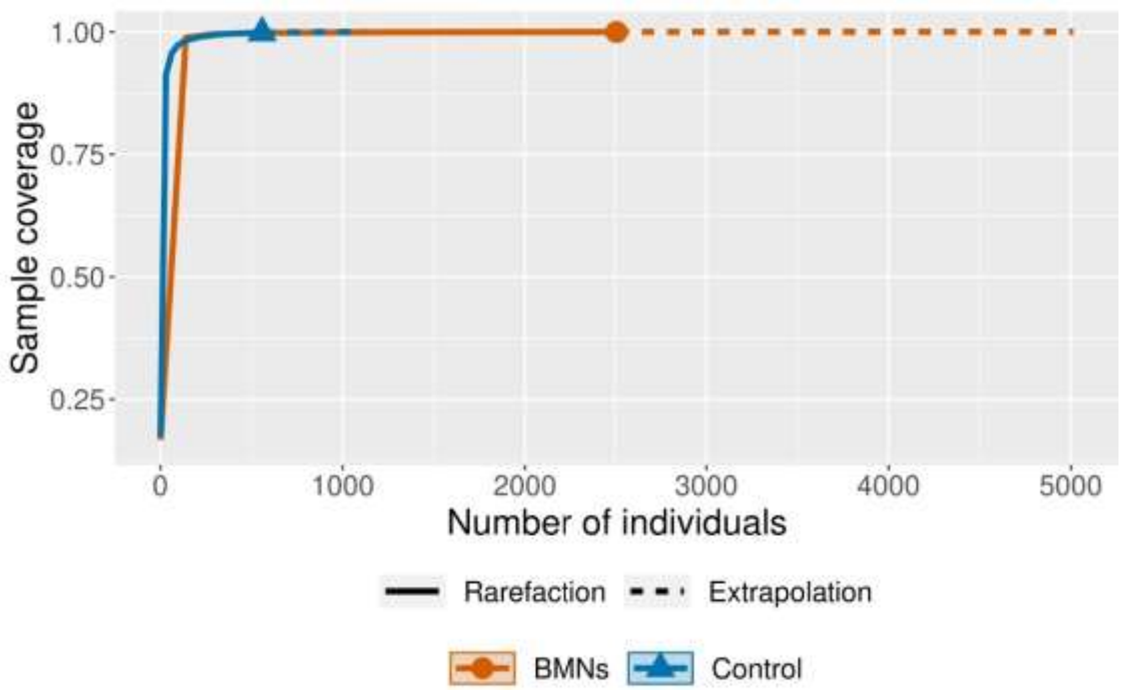


Figure 2B. The comparison between the control treatment and MNBs was made with sample coverages close to 1.

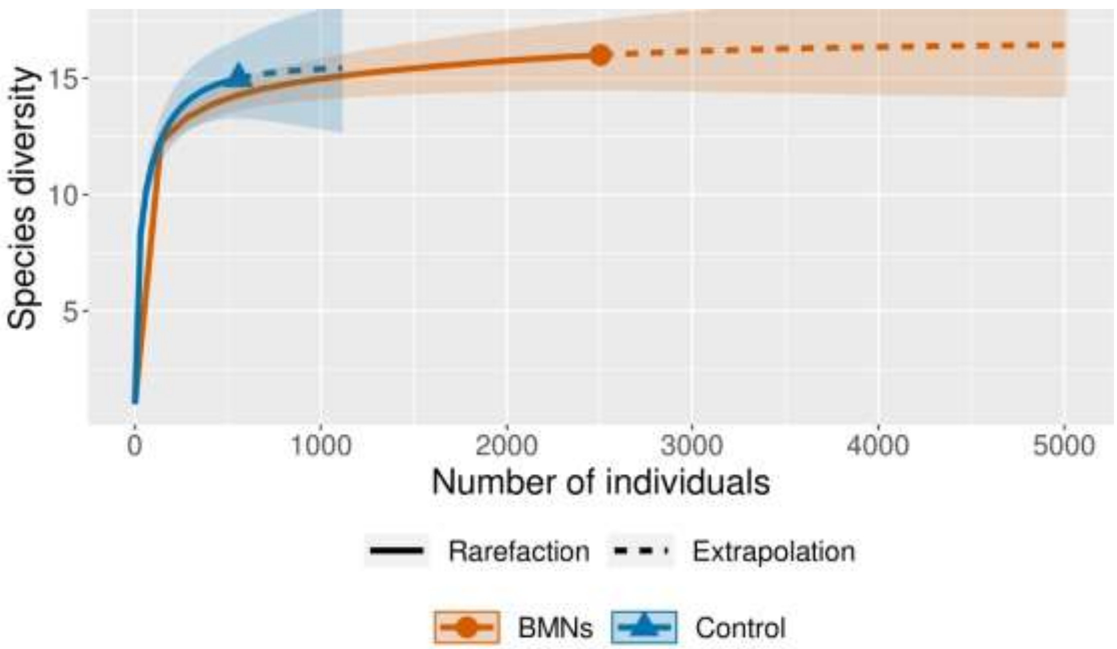


Figure 2C. The number of species increased due to the inclusion of MNBs.

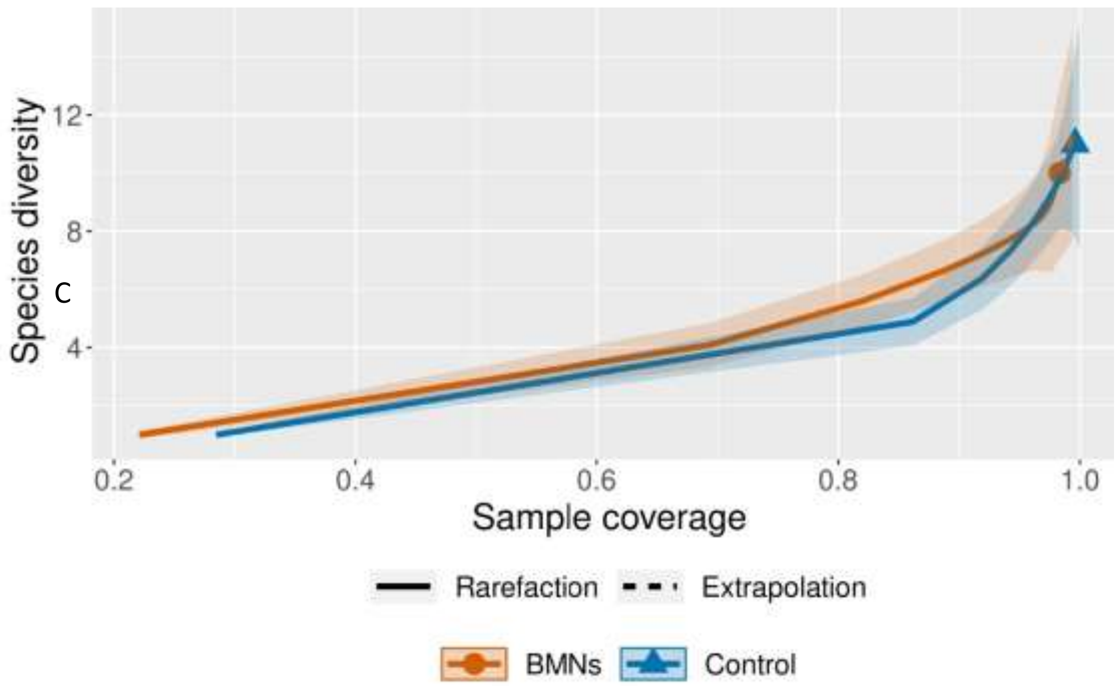


Figure 2D. shows that the completeness of the birds' sample is almost 1.

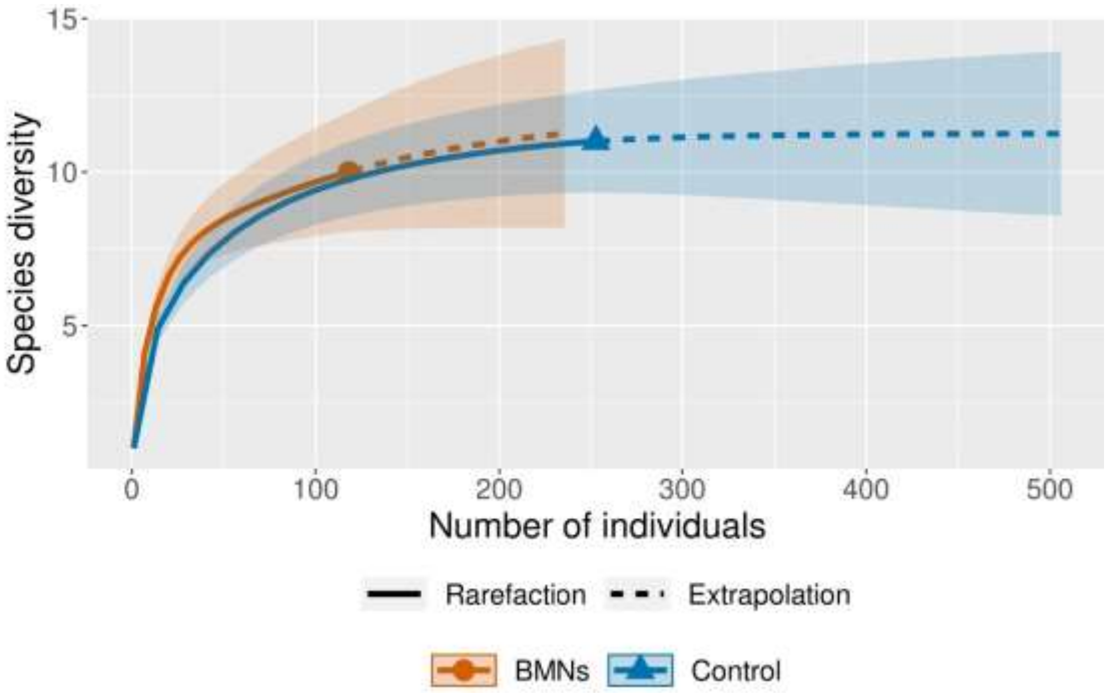


Figure 2E. Unlike mammals, MNBs show only a tiny increase in bird observed species, which suggests that MNBs are not so crucial for stimulating this presence.

Discussion

The number of individuals and observed species that visited the MNBs was greater than that of the control areas; these results are relatively similar to those observed by (Armenteros et al., 2021), who recently evaluated the number of species (not considered to be hunted; "non-game species") who approached feeders and troughs in a protected area in northwestern Spain. Their results showed that, as in this study, the number of individuals observed increased, due mainly to the more significant presence of birds and rodents. Under the conditions of this work, the total number of individuals observed was higher in areas with MNBS than in the control. In the particular case of rodents, their olfactory ability to identify foods with high nutritional value has been demonstrated; the availability of such foods makes rodents increase their presence in the area (Price & Banks, 2017). In contrast, (Moseley et al., 2011) reported that neither the white-tailed deer density nor supplementation to this species modified the abundance of rodents in areas close to this management; however, in this work, the increase of *Peromyscus mekisturus* (mouse) in some of the sites with MNBs was very high, and although statistically, only a trend in its increase is observed (P = 0.057), this trend could be explicitly explained (in the photographs it is observed) by the consumption of protein energy block. Because of this trend in the increase in mice in the areas with MNBs, we observed a similar increase in *Lynx rufus* (bobcat); according to Sánchez-González et al. (2018), in San Luis Potosí the *Peromyscus spp* is one of the main components of the diet of this species. Although the diet of none of the species evaluated in this work was analyzed, it can be assumed that the abundance of mice in the area is also an essential part of the bobcat diet. The increase in the presence of coyotes also may be related to the increased presence of *Peromyscus spp* (mouse). Articles published by (Byerly et al., 2018) show that this species consumes between 16 and 29% of rodents in the Utah desert, US, depending on the time of year. Birds' ability to identify foods with high nutritional density and a particular preference for sugar have been demonstrated (Schaefer et al., 2008). Because the protein energy block contained molasses, it is to be expected that the population of certain types of birds would increase. Also, many of the bird species in these areas are granivorous, and since the MNBs contained corn grain, a more critical presence was expected. In addition, *Cathartes aura* was found in the area, which can be explained by the fact that MNBs contained meat meal as a source of protein; the smell of it can be confused with that of a corpse. Comparative genomic studies have demonstrated the development of olfactory receptors and the presence of genes positively associated with the |immune system that strengthen *Cathartes aura*'s immune defense against the invasion of abundant pathogens in carrion carcasses (Zhou et al., 2019). As a consequence of the increase of individuals and species in the area, the SI increased; this is a logical consequence of the increase in the number of species in the area. If a mathematical review of this index is made, it becomes evident that the value increases proportionally to the number of species; for example, an area with ten individuals and one species has an SI smaller than an area with ten individuals of different species.

Rarefaction curve. Rarefaction and extrapolation aim to make appropriate comparisons between samples that may be incomplete, standardizing sample size and providing helpful information for size varieties, ensuring that samples of equal integrity are compared over a range of coverages (Chao et al., 2021). A total of 31 species were found; in the areas with MNBs, a total of 28 species, while in the control areas, only 24 species; in this work, the sample coverage is almost one (Figure 2A). For that reason, the possibility of finding more species (of birds or mammals) is reduced. Although in Puebla, the presence of 161 species of mammals is recorded (Camacho Rico et al., 2011), due to the characteristics of the work, identification of the various species of *Peromyscus* was not possible. Moreover, in Puebla, at least fifteen subspecies of it are reported. Additionally, the order Soricomorpha and the Pilosa were absent in their totality. However, at least one species was identified from the other orders reported for this state. The higher number of species in the MNBs is an interrelated phenomenon that can be explained by the rise in rodents and birds that increases in the presence of their predators. As a consequence of the presence of these, other species move away. Papers published by this research group showed that the deer kept away from the coyote (Villarreal-Espino-Barros et al., 2012), and the fact that this work does not approach the MNBs confirms that the smell of predators keeps it away from the coyotes (Bytheway et al., 2013).

The rarefaction curve shows that the number of individuals estimated with re-sampling (Figure 2B) and species (Figure 2C) increased by the effect of inclusion in the MNB areas. It is generally accepted that rarefaction curves estimate the expected number of species for a given sample size; based on a hypergeometric distribution model, this size is expected to be equal to or less than that of the actual sample (Zou et al., 2023). These curves have been widely used to compare the biodiversity of incompletely sampled communities represented by samples of different sizes. Sometimes, depending on the analysis method (by sample completeness or sample size), they have conflicting results (Shimadzu, 2018); however, under the conditions of this work, both sampling methods show that MNBs increase the number of individuals and observed species.

The presence of protein and mineral blocks modifies the frequency and relative abundance of wild species, such as predators and small mammals, but does not increase the presence of white-tailed deer.

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