

RESEARCH ARTICLE

Resource partitioning in a novel herbivore assemblage in South America

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Abstract

- Human-induced species declines and extinctions have led to the downsizing of large-herbivore assemblages, with implications for many ecosystem processes. Active reintroduction of extirpated large herbivores or their functional equivalents may help to reverse this trend and restore diverse ecosystems and their processes. However, it is unclear whether resource competition between native and non-native herbivores could threaten restoration initiatives, or to what extent (re) introduced species may influence local vegetation dynamics.
- To answer these questions, we investigated the diets of a novel South American herbivore assemblage that includes resident native species, reintroduced native species and introduced non-native species. We examined plant composition, diet breadth and the overlap between species to describe the local herbivory profile and the potential for resource competition.
- Using DNA metabarcoding on faecal samples ($n=465$), we analysed the diets of the herbivore assemblage in the Rincón del Socorro rewilding area of Iberá National Park, Argentina. We compared the species richness of faecal samples, the occurrence of plant families/growth forms and the compositional similarity of samples (inter- and intraspecifically).
- Our results indicate species-level taxonomic partitioning of plant resources by herbivores in this system. Differences in sample richness, composition and diet breadth reflected a diverse range of herbivory strategies, from grazers (capybara) to mixed feeders/browsers (brocket deer, lowland tapir). Differences in diet compositional similarity (Jaccard) revealed strong taxonomic resource partitioning. The two herbivores with the most similar diets (Pampas deer and brocket deer) still differed by more than 80%. Furthermore, all but one species (axis deer) had more similar diet composition intraspecifically than compared to the others.
- Overall, we found little evidence for resource competition between herbivore species. Instead, recently reintroduced native species and historically introduced non-natives are likely expanding the range of herbivory dynamics in the

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ecosystem. Further research will be needed to determine the full ecological impacts of these (re)introduced herbivores. In conclusion, we show clear differences in diet breadth and composition among native, reintroduced and non-native herbivore species that may be key to promoting resource partitioning, species coexistence and the restoration of ecological function.

KEYWORDS

diet overlap, DNA metabarcoding, exotic species, herbivore ecology, novel ecosystems, resource partitioning, rewilding, species coexistence

1 | INTRODUCTION

Large herbivores play key functional roles in terrestrial ecosystems, yet human impacts have fundamentally changed the composition of herbivore communities in the Anthropocene (Pringle et al., 2023). Species extinctions, extirpations and the introduction of non-natives have all contributed to the rise of novel ecosystems, that is ecosystems with composition or functioning that deviate from historical baselines (Hobbs et al., 2009). These changes have been driven by both historical and contemporary human influences, from the Late Pleistocene megafauna extinctions in which up to 80% of large species (≥ 45 kg) went extinct in some regions (Stuart, 2015) to the ongoing decline and homogenisation of plant and animal communities (IPBES, 2019). One important dimension of ongoing biodiversity loss is the rapid turnover in the identity and abundance of species (McKinney & Lockwood, 1999). Humans have contributed to this biotic turnover by causing declines in native species (Ripple et al., 2015) and by introducing non-native herbivores into terrestrial ecosystems across the world (Spear & Chown, 2009; Wallach et al., 2018). Now there is also growing interest in the reintroduction of lost megafauna for ecosystem restoration (Svenning et al., 2016). Given the substantial biotic changes involved, it is important to understand the ecological dynamics in ecosystems characterised by novel herbivore assemblages, including the dynamics of resource competition and species coexistence.

Sympatric herbivore species share potentially limiting food resources; therefore, according to ecological theory, they must partition these resources in some way to enable their coexistence (Levine & Hart, 2020; Tilman, 1982), especially when food availability is low (Gordon & Illius, 1989). There are many different mechanisms for resource partitioning among herbivores, including spatiotemporal segregation of feeding, selective feeding according to plant traits and physiological differences between animals, for example ruminant versus non-ruminant (Dickie & Serrouya, 2022). Some of these mechanisms concern coarse differences among species, for example the dichotomy between grazers (grass eaters) and browsers (non-grass eaters). Yet, recent insights based on faecal eDNA show that the diet of herbivores within these broad groups can be highly variable, with the differences in plant species composition within groups sometimes as great as between them (Kartzinel et al., 2015). This finding highlights how a high degree of diet specialisation, although hard to

detect, may be important for promoting coexistence among sympatric herbivores (Pansu et al., 2022). Although there is some plasticity in these plant–herbivore interactions, human activities have nonetheless strongly impacted competition and coexistence dynamics, for example, through predator removal, hunting and habitat destruction (Ripple et al., 2015). Humans may also affect herbivore diets and coexistence by introducing new competitors, in the form of non-native herbivores, into terrestrial ecosystems (Vázquez, 2002).

Non-native herbivores have been introduced into ecosystems for a variety of reasons, often related to hunting (subsistence and recreational), and are an important reason why faunal communities are becoming more homogenous across the world. Although they may have been present for decades or even centuries, non-native herbivores are often considered as separate from the local fauna and unwanted invaders that should be eradicated or ignored (Wallach et al., 2018). Indeed, non-native herbivores can negatively affect local ecosystems by impairing forest regeneration (Gürtler et al., 2023; Vázquez, 2002) or competing with native species (Spear & Chown, 2009). Feral domestic pigs (*Sus scrofa domestica*), for example, have been in South America since European colonisation over 500 years ago (Oliver et al., 1993) and may compete with native Pampas deer (Pérez Carusi et al., 2009) and peccaries (Galetti et al., 2015; but see Oliveira-Santos et al., 2011; Desbiez et al., 2009). In contrast to reported negative effects, however, there are also potential benefits associated with herbivore introductions—such as the replacement of ecological functions that were lost due to the extinction of large herbivores in the Late Pleistocene (Durigan et al., 2022; Lundgren et al., 2020).

The reintroduction of extirpated large herbivores is an increasingly popular conservation practice, one that is often associated with rewilding principles. Rewilding is a process-driven management approach that aims to restore complex, self-sustaining ecosystems and promote biodiversity by encouraging linked ecological processes (Perino et al., 2019). Herbivore (re)introductions, as part of ‘trophic rewilding’ (Svenning et al., 2016), can re-establish extirpated species and can restore lost animal trait combinations with their associated ecosystem processes (e.g. removing woody plants and dispersing seeds; Fricke et al., 2022; Gordon et al., 2023). Given South America’s recent and severe history of defaunation, trophic rewilding has been identified as a promising restoration strategy (Di Bitetti et al., 2022; Galetti et al., 2017; Root-Bernstein et al., 2017). In recent years, rewilding

initiatives have been underway to restore functional large vertebrate populations on the continent (Genes et al., 2019; Zamboni et al., 2017). Yet, how do (re)introduced herbivores interact with resident herbivore populations and with the plant community? Depending on population density and resource availability, there is potential for resource competition in these modified assemblages. Furthermore, it is important to understand how each species contributes to the expansion, or modification, of herbivory, an important ecosystem function. Recent advances in molecular methods that can describe species diets in high detail are expanding our understanding of herbivore coexistence, competition and functional roles in Anthropocene communities (Taberlet et al., 2018; Thomassen et al., 2023).

DNA metabarcoding is a molecular analysis technique used to identify species from environmental samples by extracting, amplifying and sequencing specific genetic markers (Pompanon et al., 2012). This method can uncover cryptic differences between herbivore diets by identifying the plant material in faecal samples (Iacolina et al., 2020; Kartzinel et al., 2015; Pansu et al., 2022). Using DNA metabarcoding, we aimed to investigate the diets of the novel herbivore assemblage in the Rincón del Socorro rewilding area in northern Argentina—a site that features five native and two non-native herbivores, and recent reintroductions of three historically extirpated species. We were interested in the following research questions: (1) are there dietary differences between herbivores that support species coexistence in this assemblage? and (2) what are the implications of dietary overlap for competition between species and species groups (native, non-native, reintroduced) and for the expansion/modification of the assemblage-wide herbivory niche?

We expected to find differences between herbivores in the richness and identity of plants consumed that reflect their morphological and ecological differences (Clauss et al., 2013). We expected that diet taxonomic diversity would be positively correlated with body size because large herbivores have greater access to different plant species (i.e. they are taller and have larger home ranges; Jetz et al., 2004), and because they have lower nutrient and energy requirements per unit of mass (they can therefore be less selective; Demment & Van Soest, 1985). We anticipated that non-native and reintroduced native herbivores are either expanding dietary space (Lundgren et al., 2020) or overlapping with, and thus possibly competing with, ecologically similar resident native species (Spear & Chown, 2009). Together, our research questions will help to reveal the diet structure of a modern herbivore assemblage and shed light on the broader ecological implications of non-native and reintroduced herbivores in the Anthropocene.

2 | METHODS

2.1 | Study site and species

The Rincón del Socorro rewilding area lies in the south-eastern part of the Iberá National Park in the province of Corrientes, Argentina (Figure S1). The area encompasses 124 km² of gallery forests, palm

savannas, wooded savannas and grasslands (Zamboni et al., 2017). The area has been protected since 2002 and hosts an assemblage of native large herbivores, including capybara (*Hydrochoerus hydrochaeris*), marsh deer (*Blastocerus dichotomus*), gray brocket deer (*Subulo gouazoubira*), greater rhea (*Rhea americana*) and plains vizcacha (*Lagostomus maximus*). Three native mammals that were previously extirpated from the area—collared peccary (*Pecari tajacu*), lowland tapir (*Tapirus terrestris*) and Pampas deer (*Ozotoceros bezoarticus*)—have been reintroduced into the area in recent years (Zamboni et al., 2017). Alongside the native herbivores, there are established populations of two non-native species: feral pigs (*Sus scrofa*) and axis deer (*Axis axis*). These 10 species, split into three groups: native (never extirpated), reintroduced (native but previously extirpated) and non-native (historical introductions), make up the current herbivore assemblage (Figure 1, Table S1).

2.2 | Sample collection and DNA processing

Fresh faecal samples were collected in the field during May and October 2018 from all major habitat types. We collected approximately 1 cm³ of dung (sampling different parts of the faeces) into a sterile 20 mL scintillation tube filled with silica gel desiccant (~1–3 mm, with orange indicator gel, Merck KGaA, Germany; Spitzer et al., 2019; Taberlet et al., 2018). The samples were stored in these tubes at room temperature until they were processed. In total, 568 samples were collected in the field. Faecal samples were analysed by DNA metabarcoding to identify the plant (Viridiplantae) species present. Full PCR amplification and sequence processing methods are provided in the Supporting Information. Animal DNA was also used to verify the source of the faecal samples. Capybara and plains vizcacha could not be confirmed with these methods because of low host DNA or a lack of amplification with the primer. However, these two species have distinct faeces that were easily differentiated from those of other species. To minimise the chance that samples came from the same animal, we sampled widely across the site, temporally separating any collections within the same area.

Several steps were taken to minimise uncertainty in our DNA matching. We used similarity thresholds to filter the GenBank matches to our amplified DNA sequences. The query coverage per subject and percentage of identical matches had to each exceed 98% for inclusion in the analysis. DNA matches to ambiguous species were kept at the genus level (18% of taxa, 12% of total reads after quality filtering). To limit sample contamination, we excluded any sequence detections that had fewer than 25 reads in a sample, or which constituted less than 0.5% of the total sequence reads per that sample (this cutoff is recommended by Drake et al., 2022, to balance the removal of false positives and the retention of target reads). We also identified and removed plant species that were more than 1000 km outside of their known range (native or introduced) using range maps downloaded from the BIEN plant database (Enquist et al., 2016). We assessed 63 species that were missing BIEN ranges using GBIF occurrence records (GBIF, 2023) and known ranges from



FIGURE 1 Mammalian herbivore assemblage of Rincón del Socorro reserve: (a) capybara; (b) feral pig; (c) axis deer; (d) Pampas deer; (e) greater rhea; (f) collared peccary; (g) plains vizcacha; (h) brocket deer; (i) tapir; (j) marsh deer. Label colours show groups: green = native (never extirpated); blue = reintroduced native; orange = introduced non-native. Photographs used under licence CC BY-NC 4.0: (a, e, f, i, j) Jens-Christian Svenning; (b) NASA, public domain; (c) Norberto Muzzachiodi; (d) Bennett Hennessey; (g) Nicolas Olejnik; (h) Diego Kondratzky.

Plants of the World Online (POWO, 2023). Lastly, we removed faecal samples that contained only one identified plant species, as these likely represent degraded faecal samples. After quality filtering, 465 of 568 faecal samples and 134 of 180 plant species remained.

2.3 | Data analysis

To compare herbivore diets, we calculated the richness of plant species found in faecal samples and investigated plant composition at the level of species, family and growth form. Plant richness per sample (alpha diversity) was compared using the Kruskal–Wallis rank sum test (null hypothesis of equal median richness across species) and pairwise comparisons using Dunn's post hoc rank sum test (Siegel & Castellan, 1988). To investigate the relationship between body mass and sample richness, we used least squares linear regression on the richness of faecal samples versus body mass. Body mass data was acquired from the HerbiTraits dataset (version 1.2, Lundgren et al., 2021), complemented by Cirignoli and Lartigau (2019) for plains vizcacha. We assessed the trait composition of herbivore diets by considering the growth forms of plant species consumed (i.e. their physical structure; Engemann et al., 2016) and the occurrence of plants that were nitrogen-fixing species (which are typically more nutritious; taxonomic orders: Cucurbitales, Fabales, Fagales and Rosales).

Using the presence/absence of plant species, we calculated Jaccard distances between samples and conducted a principal coordinates analysis (PCoA) to visualise differences in composition. We used permutational multivariate analysis of variance (PERMANOVA; McArdle & Anderson, 2001) on the original Jaccard distances to test for differences in diet composition between the herbivore species and species groups. This non-parametric method compares

the variance within and between classes (here species or herbivore groups), using permutations to assess the significance of differences ($n = 999$). We also applied PERMANOVA to test the significance of all pairwise combinations, adjusting p values with the Bonferroni correction for multiple tests. To convey the results of these tests, we calculated the mean Jaccard similarity within and between all species or groups. We also calculated the mean similarity of each herbivore species to the entire rest of the assemblage to illustrate overall dietary uniqueness.

We used one-tailed Mantel tests (Mantel, 1967) to compare the compositional similarity of faecal samples (Jaccard distances) with the geographic distances between samples to understand how habitat use affects diet, and whether there was spatial autocorrelation among samples. For each animal species, we used permutations to test the significance of any correlation between sample composition and geographic distance. We interpreted all p value estimates according to the evidence thresholds set out in Muff et al. (2022). All analyses were conducted in R version 4.2.3 (R Core Team, 2023) using the specialist packages: *vegan* (Oksanen et al., 2022); *pgirmess* (Giraudoux, 2023); *BIEN* (Maitner, 2023); and *iNEXT* (Chao et al., 2014; Hsieh et al., 2016).

3 | RESULTS

Across the 10 herbivore diets, we detected 143 plant species after quality filtering. We found that herbivore faecal samples had an overall median richness of five plant species, although richness varied among herbivores (Kruskal–Wallis, $\chi^2_{(9)} = 103.7$, $p < 0.001$; Figure 2). The plains vizcacha reached the highest levels of sample richness, with up to 18 plant species found in one sample—38% more than any other herbivore. Because the plains vizcacha also

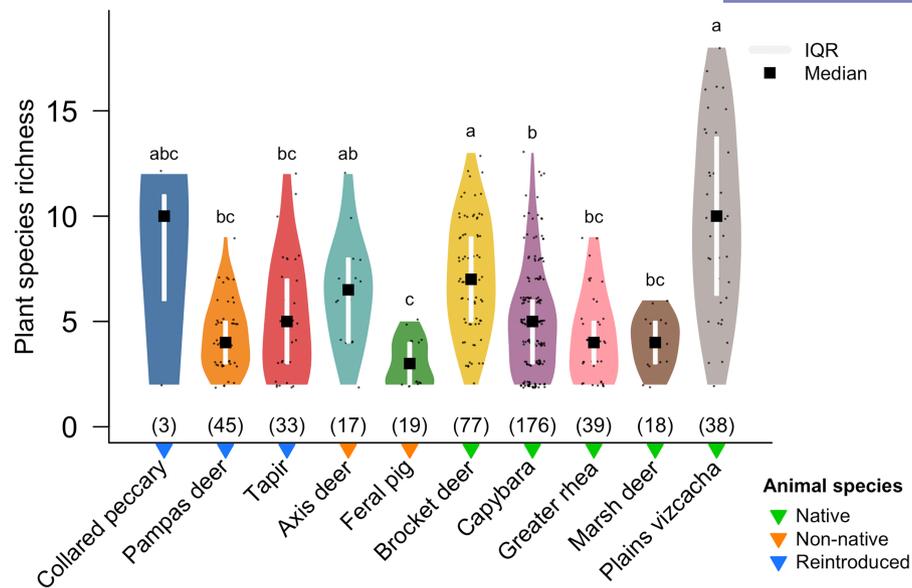


FIGURE 2 Herbivore diet diversity as plant species richness of faecal samples. Richness was compared across species using the Kruskal-Wallis test and Dunn's post hoc test. Species that do not share letters are significantly different at $p=0.05$. Numbers in brackets are the number of faecal samples per animal species after data cleaning.

had a large variance in sample richness, pairwise comparisons group it with other herbivores with rich diets: the axis deer, brocket deer and collared peccary. The collared peccary was poorly sampled compared to the other herbivore species, resulting in large uncertainty; nonetheless, two of the three samples collected for collared peccary have more than double the plant richness of the most diverse sample ($n=5$) of the feral pig. Indeed, feral pigs appear to have one of the least diverse diets in terms of sample plant richness.

We found very strong evidence that faecal sample richness decreased with body mass (linear regression, $F_{(1, 452)}=89.66$, $R^2=0.17$, $p<0.001$; Figure S2). When body mass doubled, faecal sample richness declined by approximately one plant species. If the plains vizcacha is excluded, as it is very small and had very high sample richness, the linear model's explanatory power decreases from 17% to 5% and the effect of body mass is approximately halved ($F_{(1, 414)}=21.37$, $R^2=0.05$, $p<0.001$). For most herbivores, sample-based species accumulation curves showed that there were likely many plant species we were unable to detect at the current sample size (Figure S3). This limitation prevents comparisons of diet gamma diversity across species. For capybara, which had the best sampled diet, species accumulation began to plateau at around 85 species in total.

Poaceae was the most prevalent plant family found; it occurred in nearly 70% of faecal samples. The next most common plant families—Malvaceae and Fabaceae—occurred in roughly 40% of samples (Figure S4). The greater rhea had the greatest proportion of its diet made up of plant families, which accounted for less than 10% of occurrences, indicating a diverse diet at the family level (Figure 3a). In contrast, capybara had the greatest contribution from one plant family, as Poaceae accounted for two thirds of species detections. Pampas deer also subsisted on very few plant families; Malvaceae, Poaceae and Asteraceae made up 85% of occurrences. Several

herbivores appeared to specialise on plant families consumed very little by the others, for example marsh deer with Onagraceae (29%) and Alismataceae (10%), and tapir with Loranthaceae (15%) and Bignoniaceae (15%).

Clear differences between herbivores are also evident when considering the growth form of plants consumed (Figure 3b). Marsh deer were the only species with obligate aquatic plants in their diet; meanwhile, the diet of the plains vizcacha comprised entirely of forbs and graminoids. More than 70% of the species consumed by capybara were graminoids. A relatively high proportion of trees and shrubs were consumed by all deer species except the Pampas deer. Tapir consumed the greatest proportion of woody plants, including trees, shrubs and climbing plants (most were woody lianas). Two fleshy-fruit bearing plants were dominant in tapir faecal samples: *Dolichandra cynanchoides* (13-cm flat oblong fruits) and *Tripodanthus acutifolius* (0.7-cm ovoid berries) were each found in 79% of samples, 30% more than the next most common species. The highest consumption of nitrogen-fixing species was by the greater rhea (26% of species occurrences; Figure S5), followed by the tapir, plains vizcacha and capybara (~10% each). The marsh deer is the only herbivore that did not consume any nitrogen-fixing plant species.

The ordination of faecal samples according to Jaccard differences explained 19.1% of the variance in the first two dimensions (Figure 4). Multivariate differences among samples largely concerned variation in the occurrence of Poaceae versus a diverse collection of other plant families, including Malvaceae and Asteraceae. The distribution of herbivore species in this dietary space varied. Tapir and most of the deer species tended towards the upper right corner, meanwhile capybara samples mostly contained Poaceae and therefore occupied the lower left side of the ordination space. Pampas deer stood out from most other species when considering

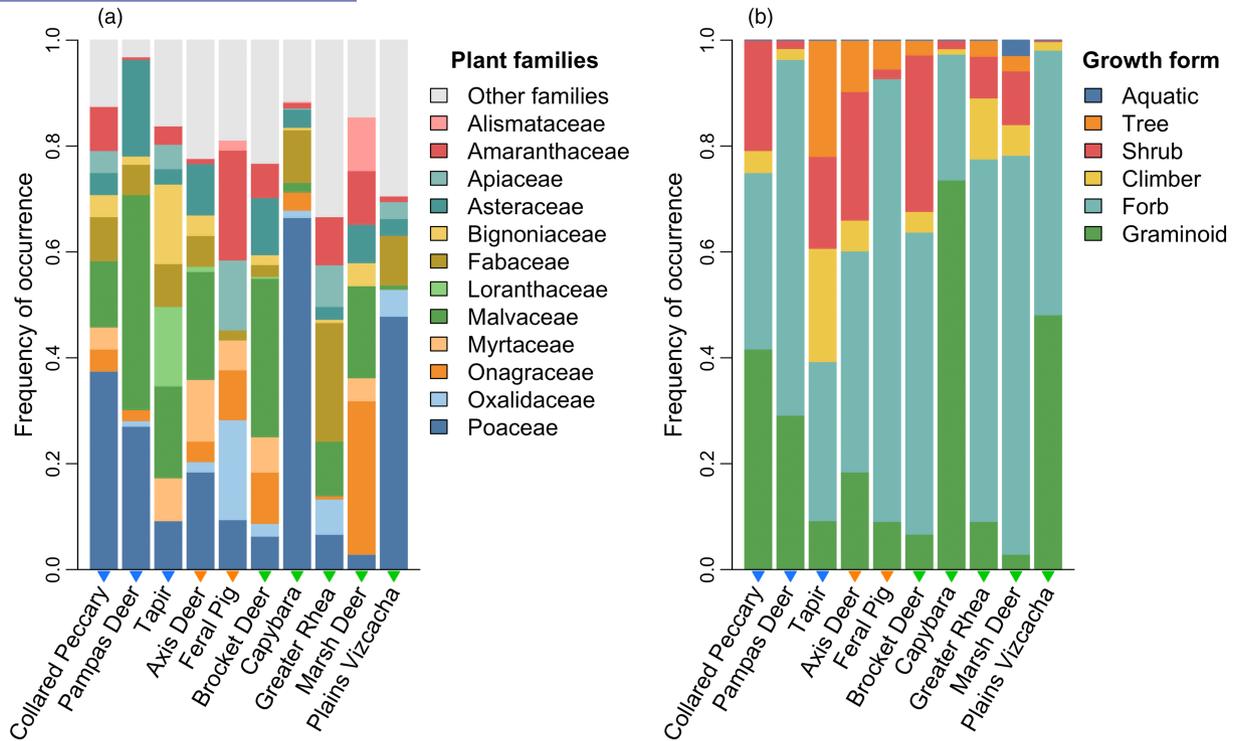


FIGURE 3 Herbivore diet composition in terms of the families (a) and growth forms (b) of plant species consumed. Frequency of occurrence is the number of times a plant species was detected, standardised to the total detections for each herbivore. Plant families with less than 10% occurrence for any animal species are grouped together as 'Other families'. The coloured triangles on the x-axis refer to the overarching groups: reintroduced (blue), non-native (orange) and native (green).

these two principal coordinates; they occupied the lower right corner, where Asteraceae prevalence was highest.

We found very strong evidence that diet composition, measured as Jaccard similarity, varied across herbivore species (PERMANOVA, $F_{(2, 448)} = 18.43$, $R^2 = 0.25$, $p < 0.001$); all pairwise comparisons were significant at $p < 0.05$ (Figure 5). All four deer species showed comparatively high levels of diet similarity, although the marsh deer was most different to the others (Figure 5a). Of the remaining herbivores, tapir diets were closest to the deer. Pampas deer had by far the narrowest dietary range; the mean similarity between faecal samples was 43%. The next highest intraspecific similarity was for the marsh deer and vizcacha (both 28%); meanwhile, the greater rhea had the broadest diet with only 9% mean similarity among samples. When comparing each species to all others in the assemblage (Figure 5b), we see that the deer species have less unique diets overall due to similarities with one another. Nonetheless, the marsh deer again stands out as the most unique deer, followed closely by the brocket deer. Feral pigs appeared to have the most compositionally unique diet.

We found very strong evidence for dietary differences between the three herbivore groups (PERMANOVA, $F_{(8, 442)} = 12.12$, $R^2 = 0.05$, $p < 0.001$; Figure S6); all pairwise comparisons were significant at $p < 0.05$. The highest diet similarity was between non-native species and reintroduced natives (7.1%). Never-extirpated native species had slightly more similar diets with reintroduced species (6.4%) than non-native species (5.1%). The highest mean within-group similarity

was for the reintroduced herbivores (21.2%), more than double the other two groups (both 8.3% intra-group similarity). We did not find strong correlations between the location of faecal samples and their similarity in plant species composition. While all correlations were positive, the only significant correlation was for samples from feral pigs, where closer samples were more similar, although the correlation was still weak ($r_M = 0.24$, $p = 0.013$; Figure S7, Table S2).

4 | DISCUSSION

Using DNA metabarcoding, we were able to conduct a detailed investigation into the dietary preferences of a novel herbivore assemblage in South America. We found differences in diet richness and composition that may facilitate herbivore coexistence in this anthropogenically modified assemblage. Almost all herbivore species exhibited high diet fidelity, with sample composition being more similar within than between species. The full grazer-to-browser continuum was represented, and some herbivores specialised on plant families that were otherwise rarely consumed. Overall, our results indicate that none of the 10 species appear to share herbivory strategies, with clear dietary differences separating even the most ecologically similar herbivores.

We found strong variation in diet composition, both taxonomically and in terms of plant growth forms. At one end of the spectrum, capybara stood out as a clear grazer, with their consumption

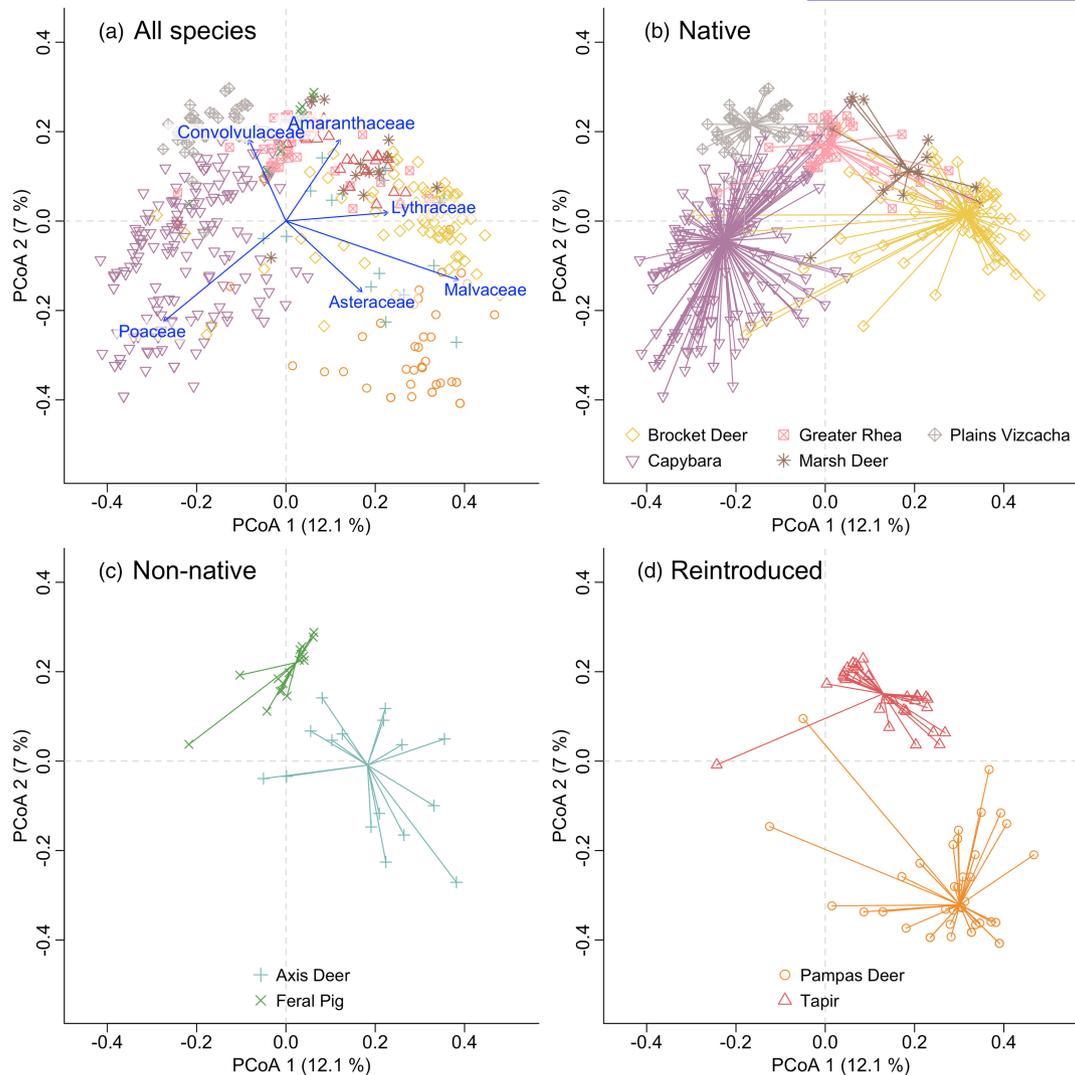


FIGURE 4 Ordination of herbivore diets. Principal coordinates analysis was applied to Jaccard distances between all samples. (a) Blue arrows show the direction of increase for the six plant species most correlated to the principal axes shown (scaled by fit; R^2). The centroid (spatial median) of all herbivores in ordination space (b–d) is shown with lines connecting to each sample. Some points are overlapping as they have identical plant composition.

of grasses and other graminoids (73%) in line with previous research (~70%–90%; Desbiez et al., 2011). The only other species that came close in its reliance on graminoids (excluding collared peccaries due to the low sample size) was the plains vizcacha, for which half of plant occurrences were graminoids. Previous research has shown that vizcacha diets are often dominated by grasses, especially in drier regions (Puig et al., 1998). It has been suggested that this high grass consumption may improve overall forage quality by facilitating the regeneration of certain forb species near vizcacha burrows (Branch et al., 1994). Indeed, the other half of the vizcacha diet almost exclusively constituted forb species.

We found that tapirs were closest to the other end of the herbivory spectrum, using a browse-dominated strategy with high proportions of tree, shrub and climbing plants. Tapirs are known frugivores, with 16%–50% of their diet comprising fruit at other South American sites (Chalukian et al., 2013). Being double the size of the second

largest species, tapirs may be the only potential disperser of seeds adapted to the now extinct megaherbivores of the region (O'Farrill et al., 2013). Evidence also suggests that the contribution of tapirs to local seed dispersal can persist, and even be augmented, in degraded landscapes, highlighting the potential value of their reintroduction for natural vegetation recovery (Paolucci et al., 2019). In the mostly open Rincón del Socorro reserve, we observed a low occurrence of fleshy fruits, and tapirs were mostly seen consuming leaves and stems. Researchers at another Argentinian site with low fleshy-fruit availability found that fruits still comprised 16% of tapirs' diet by dry weight (montane forest; Chalukian et al., 2013). All told, the importance of tapirs as seed dispersers in the reserve merits further investigation.

We found greater diet similarity among deer species than with other herbivores. Although three of the deer are typically classified as mixed feeders, there was still considerable variation among their

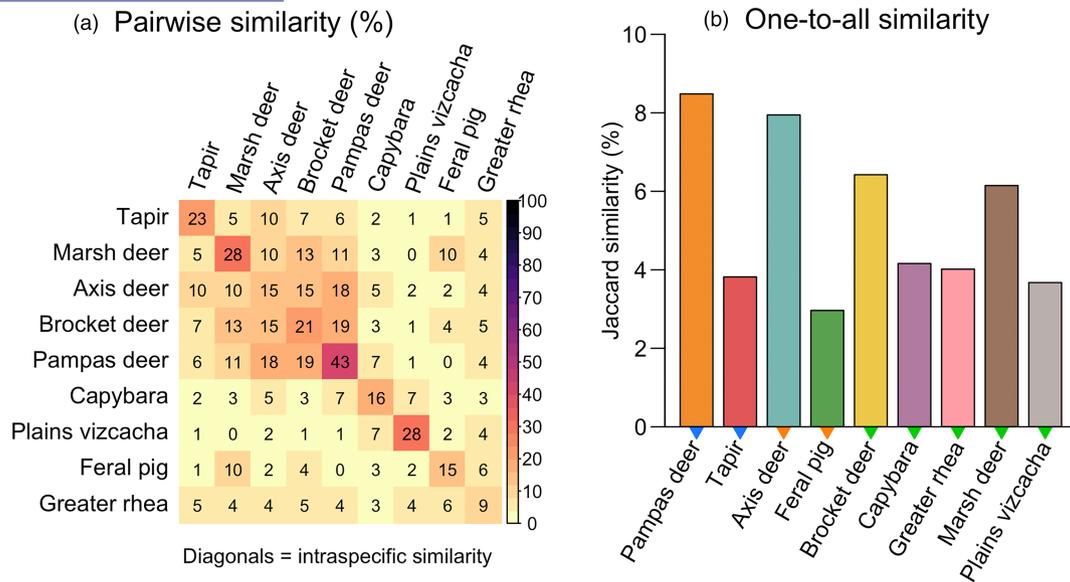


FIGURE 5 Comparison of diet across herbivores. Mean Jaccard similarity is shown for pairwise sample comparisons between herbivores (a) and for each herbivore compared to the remainder of the assemblage (b). Diagonal values in (a) are the similarity of samples within one species that is intraspecific similarity, which can be interpreted as dietary beta diversity (low similarity between samples indicates high diet variability and vice versa). The coloured triangles on the x-axis in (b) refer to the overarching groups: reintroduced (blue), non-native (orange) and native (green).

diets. Brocket deer consumed more shrubs than any other species, supporting their classification as browsers (Lundgren et al., 2021). Marsh deer consumed very few graminoids but a high proportion of marsh-associated families (Onagraceae, Alismataceae). Pampas deer had the highest intraspecific similarity of all herbivores and almost exclusively consumed forbs and graminoids from three families (>80%). A possible reason for the narrow diet of this endangered species could be the fact that Pampas deer at our study site were still in the process of being fully reintroduced and were being held in a large enclosure (30 ha; Zamboni et al., 2017), although it still contained savanna vegetation with a mixture of woody plants, grasses and forbs. Research on Pampas deer at other South American sites has shown a strong variation in their preference for grasses versus forbs that is probably driven by local vegetation differences (Desbiez et al., 2011; Jackson & Giulietti, 1988). Overall, they can be classified as opportunist, intermediate mixed feeders that avoid fibre when possible (González et al., 2010; Hofmann, 1989). Our results corroborate this designation and point towards the Pampas deer fulfilling the role of a grassland forb specialist in this system.

The greater rhea had the broadest diet, as evidenced by having the lowest intraspecific similarity and a high diversity of plant families. Despite this taxonomically generalist approach, however, the greater rhea had almost three times the occurrence of nitrogen-fixing plants than the next species. This result suggests a strong preference for highly nutritious nitrogen-fixing forbs that is consistent with the higher metabolic rates of birds (Clarke et al., 2010). Elsewhere in Argentina, Pereira et al. (2003) found that forbs accounted for approximately half of greater rhea's diets across the year, with the nutritious pods of just one leguminous tree (*Prosopis nigra*) providing over a third of their summer diet. Greater rhea in

our system had comparatively few plant species per faecal sample, which, paired with the broadness of their diet, suggests that they have high variability among foraging bouts.

We found that plant richness per faecal sample was negatively related to herbivore body mass. Yet, sample level richness does not necessarily correlate with total diet richness (i.e. gamma diversity) and therefore does not indicate a decrease in diet breadth with size. Although low taxonomic diet breadth has been observed elsewhere for very large mammals, they often consume a much greater range of plant parts instead (Jarman, 1974; Owen-Smith & Chafota, 2012). The differences in sample level richness that we observed could be related to differences in morphology and foraging strategy, such as differences in gut retention time (Demment & Van Soest, 1985). Even so, the negative relationship with body mass was largely influenced by the very high per-sample richness of the small plains vizcacha, which had one third the mass of the next smallest species. Due to their small size, higher nutrient and energy requirement per unit mass may drive vizcacha to selectively forage nutritious plant parts across many plant species (Demment & Van Soest, 1985). Vizcacha also engage in coprophagy, which may increase the mixing of feeding bouts and partly explain the high per-sample plant richness. However, this behaviour also occurs in Capybara (Herrera, 1985), which displayed average plant richness levels.

We found that feral pigs had the most unique diet composition compared to the rest of the assemblage. However, because Jaccard similarity accounts for both species turnover and nestedness (Baselga & Leprieur, 2015), part of the dietary uniqueness of feral pigs could be that they had comparatively few plant species per sample. This low per-sample richness may be explained by the omnivorous feral pigs targeting specific plants, such as those with

tubers. Alternatively, strong hunting pressure may constrain feral pigs to foraging in more sheltered forest areas, thereby restricting the diversity of their feeding bouts (Boitani et al., 1994; Tolon et al., 2009). Collared peccaries are ecologically similar to feral pigs; yet, the three samples we collected for collared peccary all had much higher plant richness. Desbiez et al. (2009) found that collared peccaries had greater diet diversity and breadth than feral pigs, with lower-than-expected dietary overlap due to key differences in the relative consumption of roots and leaves (higher in feral pigs) versus fibres (higher in collared peccaries). Feral pigs are powerful ecosystem engineers that modify soil and plant communities through disturbances related to foraging, rooting and nest building (Ickes et al., 2001). Most research on the impacts of feral pigs on vegetation has occurred in their introduced range and often finds that they reduce plant cover, impair regeneration and potentially aid the spread of invasive plants (Barrios-García & Ballari, 2012). However, these types of effects are generally expected from large herbivores, irrespective of nativeness, and there is contrasting evidence that feral pigs can enhance local plant diversity by suppressing dominant plant species (Cuevas et al., 2020; Hensel et al., 2022). Nonetheless, protected area managers in Argentina consider that feral pigs negatively impact the native plant community (Ballari et al., 2015), and there are studies showing that they may harm certain vulnerable species (Gürtler et al., 2023).

We were unable to directly compare the diets of the two suiform species due to the low number of samples found from collared peccaries; therefore, we cannot comment on the likelihood for diet overlap and resource competition at this site. This difficulty of finding samples also prevented us from calculating reliable estimates of total diet richness, an important metric relating to diet breadth. For many herbivores in our study, the plant species accumulation curves were still increasing above the number of samples that were needed to find a plateau in comparable studies based on microhistological analysis of faeces (e.g. Desbiez et al., 2011). This difference reflects the increased taxonomic resolution of DNA methods (Garnick et al., 2018), which may require more samples to achieve complete sampling because of rare species detections in the diet. Our analysis, nonetheless, captures the commonly consumed plant species that are important components of the herbivore's diets.

Although the Rincón del Socorro is strictly protected, domestic grazing animals such as cattle, horses and sheep do have access to other areas of the National Park and occur at great density across the region. Substantial changes to herbivore's diets and to the overall dietary niche space would likely occur in the presence of these domestic species. One potential cause of dietary shifts occurs when domestic animals affect the spatial and temporal activity patterns of native herbivores. This type of interference competition has been previously indicated, with cattle presence linked to behavioural changes in capybara, brocket deer and marsh deer (Di Bitetti et al., 2020). Cattle, the most abundant domestic species, along with the others mentioned, are all classified as grazers and are therefore likely to increase grazing-specific pressure on the ecosystem. We found that capybara, and to a lesser extent plains

vizcacha, also relied heavily on grasses, which suggests a potential for resource competition with domestic grazers (but see Desbiez et al., 2011). Alternatively, cattle as bulk grazers may facilitate smaller grazers by generating and maintaining open vegetation. Although likely involving a range of mechanisms, deforestation and cattle ranging have been linked to capybara expansion in the nearby Gran Chaco region (Campos-Krauer & Wisely, 2011). More generally, low-intensity cattle grazing in the broader region has been found to promote savanna structure and native herbaceous plant diversity (Durigan et al., 2022). This type of positive biodiversity response should be considered in the context of the region's rich diversity of very large mixed feeders and grazers that existed until recent prehistory (Varela et al., 2023).

The fundamental dietary niches of many herbivores in this study appeared to intersect as they often consumed the same plant species, and there was considerable overlap in the first two axes of dietary space. Yet, considering the plant species composition of samples, we find that there is in fact very low similarity between the herbivores' overall diets (mean = 5.6%). Comparably high levels of taxonomic diet partitioning have been recorded for diverse large-herbivore assemblages in African savannas (Kartzinel et al., 2015; Pansu et al., 2022). In Kenya, for example, Kartzinel et al. (2015) found that diets were consistent within each herbivore species; however, they were strongly divergent across species, even within the same feeding guild (e.g. grazers). The two species in our study with the most similar diets, the Pampas deer and brocket deer, still differed in composition by more than 80% (they also have distinct habitat preferences). Furthermore, every species bar one (the axis deer) showed the greatest sample similarity intraspecifically, corroborating the results from African assemblages. Interspecific competition for food, combined with the diversity of plant and herbivore functional traits, likely results in the high degree of taxonomic resource partitioning that is found among sympatric herbivores (Pansu et al., 2022). This fine-scale partitioning of plant species, which may be an important factor in species coexistence, can only be revealed thanks to the taxonomic resolution provided by DNA metabarcoding.

Considering the evidence, reintroduced and non-native species appear to be expanding dietary space in this assemblage by increasing the range of plant species consumed, and very likely the amount as well. Across South America, introduced herbivore species have helped restore some of the herbivore species richness (27%) and trait-space volume (47%) that was lost following Late Pleistocene–Early Holocene extinctions (2/3 herbivores ≥ 10 kg; Lundgren et al., 2020). The impacts of this trait-space expansion on local vegetation dynamics are still unclear. Rooting behaviour by feral pigs is often considered detrimental in its introduced range (Barrios-García & Ballari, 2012); however, it may functionally replace the rooting of extinct Tayassuidae species (Gasparini, 2013) and large armadillos (*Eutatus seguini*)—the feral pigs' closest functional analogue in South America (Lundgren et al., 2020). Reintroduced species also seem to be expanding the herbivore assemblage's functional dietary space. The tapir, for example, has a uniquely browsing-intensive strategy that increases predation on certain plant families (especially lianas)

and likely augments seed dispersal (Sica et al., 2014). The low diet similarity between never-extirpated native species and the two remaining groups is further evidence for an expansion of ecosystem-level herbivory following their (re)introduction. Depending on the absolute abundance of the individual species (Staver et al., 2021), these changes in the herbivore assemblage will have downstream consequences on landscape-scale vegetation biomass, composition and dynamics (Jia et al., 2018; Pringle et al., 2023).

The fine taxonomic resolution provided by DNA metabarcoding helped reveal important differences in herbivory strategy even among ecologically similar species. Based on diet composition, we find little evidence for resource competition between species and species groups. Instead, recently reintroduced native species and historically introduced non-natives are likely expanding the range of herbivory dynamics in the ecosystem. Further research will be needed to determine the full ecological effects of the (re)introduced herbivores, for example their influence on vegetation biomass and nutrient cycling, or their interactions with the recovering carnivore fauna. Camera trapping or other proximal sensing methods should be used in the future to detect spatial or temporal interactions among herbivore species, for example behaviours minimising competition. In conclusion, we provide detailed insights into the diets of herbivores in a novel assemblage and demonstrate that differences in diet breadth and composition are key to promoting resource partitioning and ultimately species coexistence.

AUTHOR CONTRIBUTIONS

Conceptualisation: CWD, JCM, J-CS, RB. Methodology: JCM, TGF, RB. Investigation: JCM, TGF, RB. Formal analysis: CWD. Visualisation: CWD. Writing—original draft: CWD. Writing—review & editing: JCM, J-CS, RB, TGF. Funding acquisition: J-CS. Supervision: J-CS, RB.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data supporting the analysis are available at [Zenodo.org](https://zenodo.org/doi/10.5281/zenodo.8370190): <https://doi.org/10.5281/zenodo.8370190> (Mata et al., 2023). A version of the inferred biodiversity data is available at [GBIF.org](https://doi.org/10.15468/yz5jxu): <https://doi.org/10.15468/yz5jxu> (Mata et al., 2024)—this version includes all

detections passing the bioinformatic quality filtering including non-plant, low specificity, low read-abundance detections and species outside of their known range.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Study animals and their characteristics.

Table S2. Mantel statistics (Pearson's *r*) for faecal samples belonging to each animal species.

Figure S1. Map of the study site and location in South America (inset). Points show sample collection coordinates for all animal species.

Figure S2. Relationship between animal body mass (log scale) and plant species richness per faecal sample.

Figure S3. Sample-based species accumulation curves with extrapolation to two times the sample size calculated using the Chao estimation (Chao et al., 2014; Hsieh et al., 2016).

Figure S4. Occurrence of plant families (A) and genera (B) across all faecal samples. Excluding taxa found in less than 5% of samples.

Figure S5. The composition nitrogen-fixing plants in herbivore diets. Frequency of occurrence is the number of times a plant species was detected, standardised to the total detections for each herbivore.

Figure S6. Comparison of diet across herbivore groups as mean Jaccard similarity.

Figure S7. Pairwise distances between samples for each herbivore species.

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