Received: 8 July 2020 | Revised: 6 November 2020 | Accepted: 11 November 2020

DOI: 10.1002/edn3.168

ORIGINAL ARTICLE

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

eDNA metabarcoding reveals dietary niche overlap among herbivores in an Indian wildlife sanctuary

Anneke T.M. ter Schure[1] | Anusree A.S. Pillai[2] | Lisbeth Thorbek[2] | Maradani Bhavani Shankar[3] | Rajindra Puri[4] | Gudasalamani Ravikanth[3] | Hugo J. de Boer[2] | Sanne Boessenkool[1]

[1]Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway

[2]Natural History Museum, University of Oslo, Oslo, Norway

[3]Ashoka Trust for Research in Ecology and the Environment, Bangalore, India

[4]Centre for Biocultural Diversity, School of Anthropology and Conservation, University of Kent, Canterbury, UK

Correspondence
Anneke TM ter Schure and Sanne Boessenkool, Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway.
Emails: a.t.m.t.schure@ibv.uio.no and sanne.boessenkool@ibv.uio.no

Abstract

As many ecosystems are under increasing pressure from invasive species, habitat degradation, overgrazing and overharvesting, pollution, and climate change, dietary niche monitoring is gaining importance. The Malai Mahadeshwara Wildlife Sanctuary (MMH) in southern India is home to several long-standing ethnic and tribal groups and supports a considerable number of domestic herbivores (cattle, goats and water buffalo) as well as a range of wildlife (including several species of deer, bonnet macaque, and Asian elephant). We reconstructed dietary niche partitioning of the herbivores occurring in MMH using eDNA metabarcoding to quantify diet richness, composition, and overlap. In total, we distinguish 134 diet items (molecular operational taxonomic units), covering 31 plant families. Overall, our results indicate 35% overlap in domestic and wild herbivore diet items. The greatest overlap is found for the dietary niches of cattle and sambar deer (Pianka's niche overlap index: 0.68), and the dietary niche of cattle also overlaps considerably with those of Indian hare (0.65) and Asian elephant (0.46). This suggests that these herbivores may compete for these food plants in the case of limited availability, which could lead to exclusion of some herbivore species. Particular concern should go to bonnet macaque and Asian elephant as their below average dietary richness could make them vulnerable to changes in their environment. With increasing pressures on local wildlife from a range of different factors, DNA metabarcoding of fecal samples is a non-invasive method for monitoring changes in animal diets, providing valuable information for the management of biodiversity in mosaic natural and anthropogenic landscapes.

KEYWORDS

biological monitoring, ecological niche, ecology, environmental DNA, herbivory

1 | INTRODUCTION

In areas with species of similar ecology, the partitioning of ecological niches can reduce competition for resources, thus aiding species coexistence and biodiversity (Hutchinson, 1959; MacArthur & Levins, 1967; Pianka, 2011). Given that diet represents a fundamental aspect of a species' niche (Simberloff & Dayan, 1991), it is unsurprising that dietary niche analysis has been recognized as important

for understanding the mechanistic processes behind community ecology (Pompanon et al., 2012) and diversification (Cantalapiedra et al., 2014). More specifically, the dietary niche width of a species can provide information on the extent of dietary specialization (e.g., Sato et al., 2018), on the potential for competition between coexisting species (e.g., Lopes et al., 2015) as well as adaptive responses to environmental changes (Devictor et al., 2010; Pianka, 2011). Species with narrow niches are deemed more vulnerable (Carscadden et al., 2020; Clavel et al., 2011; Devictor et al., 2010) and thus should be monitored closely in light of climate change, invasive species and other anthropogenic pressures.

Many natural ecosystems are currently under pressure from invasive species, hunting, habitat degradation and destruction, threatening 26% of all mammal species (IUCN, 2020) and more than half of all large wild herbivore species with extinction (Ripple et al., 2015). Moreover, wild herbivores can be threatened by growing livestock populations (Food & Agriculture Organization of the United Nations, 2017). This is worrying as domesticated herbivores generally have competitive advantage over local wild herbivores owing to support they get from humans, for example, through supplemental feeding during periods of scarcity. This encroachment by domestic herbivores can potentially lead to competitive exclusion of wild herbivores if they occupy similar niches (Hardin, 1960; Pianka, 2011). Similarly, establishing nature reserves and wildlife sanctuaries in traditionally managed lands can disempower local communities as wild herbivores encroach on cattle grazing lands and raid croplands (Anand & Radhakrishna, 2017; Lamarque et al., 2009).

Traditional methods for studying dietary niche partitioning have provided insight into herbivore niche overlap, but can be time-consuming and dependent on the presence of undigested and identifiable plant remains as well as direct observation of foraging behavior. More recently, advances in eDNA metabarcoding have enabled broad application of this method in biodiversity monitoring (for reviews see, e.g., Bohmann et al., 2014; Cristescu & Hebert, 2018), and the application to fecal samples provides a valuable alternative approach for dietary reconstruction. This value is evidenced both by direct comparison of different approaches (e.g., Newmaster et al., 2013) and by the rapidly increasing number of studies using this method (for recent reviews see, e.g., Ando et al., 2020; de Sousa, Silva, & Xavier et al., 2019). So far, fecal DNA metabarcoding has been successfully applied to reconstruct the diets of a range of different herbivores, including birds, insects, molluscs (e.g., Valentini et al., 2009) and a wide range of mammalian herbivores such as small rodents (e.g., Lopes et al., 2015; Sato et al., 2018; Soininen et al., 2014, 2015), a number of deer species (e.g., Bison et al., 2015; Czernik et al., 2013; Fløjgaard, De Barba, Taberlet, & Ejrnæs, 2017; Rayé et al., 2011), tapirs (e.g., Hibert et al., 2013), several primate species (e.g., Bradley et al., 2007), the European bison (e.g., Kowalczyk et al., 2011, 2019), and large herbivore assemblages in Kenya (Kartzinel et al., 2015; Kartzinel & Pringle, 2020) and Mozambique (Pansu et al., 2019). From a different perspective, dietary niche analysis should also be able to triangulate traditional ecological knowledge on grazing of domestic and wild herbivores. All of the above studies analyzing herbivore diet used the P6 loop of the chloroplast *trn*L (UAA) intron (Taberlet et al., 2007), a universal plant marker specifically suited to environmental samples with degraded DNA. Most studies applying the *trn*L approach have focussed on the dietary reconstruction of a few species, providing valuable insight into the trophic ecology of these particular species. However, the approach has also been applied to analyze dietary niche partitioning of more complete herbivore assemblages (e.g., in African large herbivores; Kartzinel et al., 2015; Pansu et al., 2019).

Much of what is currently known about large mammalian herbivore diet comes from research in North America, Europe and Africa, while data from Asia are particularly scarce (Öllerer et al., 2019; Schieltz & Rubenstein, 2016). Furthermore, despite globally growing livestock numbers, there are relatively few studies specifically investigating impacts of domesticated herbivores on wild herbivores (see Schieltz & Rubenstein, 2016 for a review). Extrapolation from these other regions to Asia is typically not straightforward as effects of livestock on wildlife are highly context-dependent and species assemblages and biogeography differ greatly (Ahrestani & Sankaran, 2016). The potential competition from livestock is, however, of particular concern in Asia, and specifically in India. Here, the world's second largest livestock population is found (Food & Agriculture Organization of the United Nations, 2017) and many wildlife reserves are being grazed by livestock. Previous studies from India have shown that excessive livestock grazing seriously threatens elephant habitat contiguity (Silori & Mishra, 2001) and suggest livestock-mediated resource limitation as declining livestock numbers resulted in recovery of wild large herbivore densities (Madhusudan, 2004). Further insight into seasonal variation in diet and niche overlap among some of the most common large mammalian herbivores in India comes primarily from microhistological analyses (e.g., Ahrestani et al., 2012), and deer in particular are suggested to be impacted by livestock grazing (Bagchi et al., 2003). Overall, the limited current knowledge from this area is based on traditional methods that can be time-consuming and dependent on the presence of undigested and identifiable plant remains, as well as direct observation of foraging behavior that is extra challenging as most of the species-rich large herbivore assemblages are found in densely forested areas (Ahrestani et al., 2012; Ahrestani & Sankaran, 2016).

In the present study, we use eDNA metabarcoding of fecal samples to test for dietary niche partitioning by livestock and wild mammalian herbivores in the Malai Mahadeshwara Hills Wildlife Sanctuary (MMH) in southern India (Figure 1). The MMH is home to people from long-standing ethnic and tribal groups with their domestic animals (Harisha & Padmavathy, 2013; Kent & Dorward, 2015) as well as a wide range of wildlife. Although livestock rearing (forest grazing) has traditionally been part of the livelihoods of the local communities (Kent & Dorward, 2015), there is currently an effort to regulate forest access and livestock grazing in MMH (Thornton et al., 2019). At the same time, resource impacts from tens of thousands of pilgrims annually (Soumya et al., 2019a), invasive plant species and modernization, including developmental activities and tourism, are reported to reduce biodiversity in the area. This is evident from, among

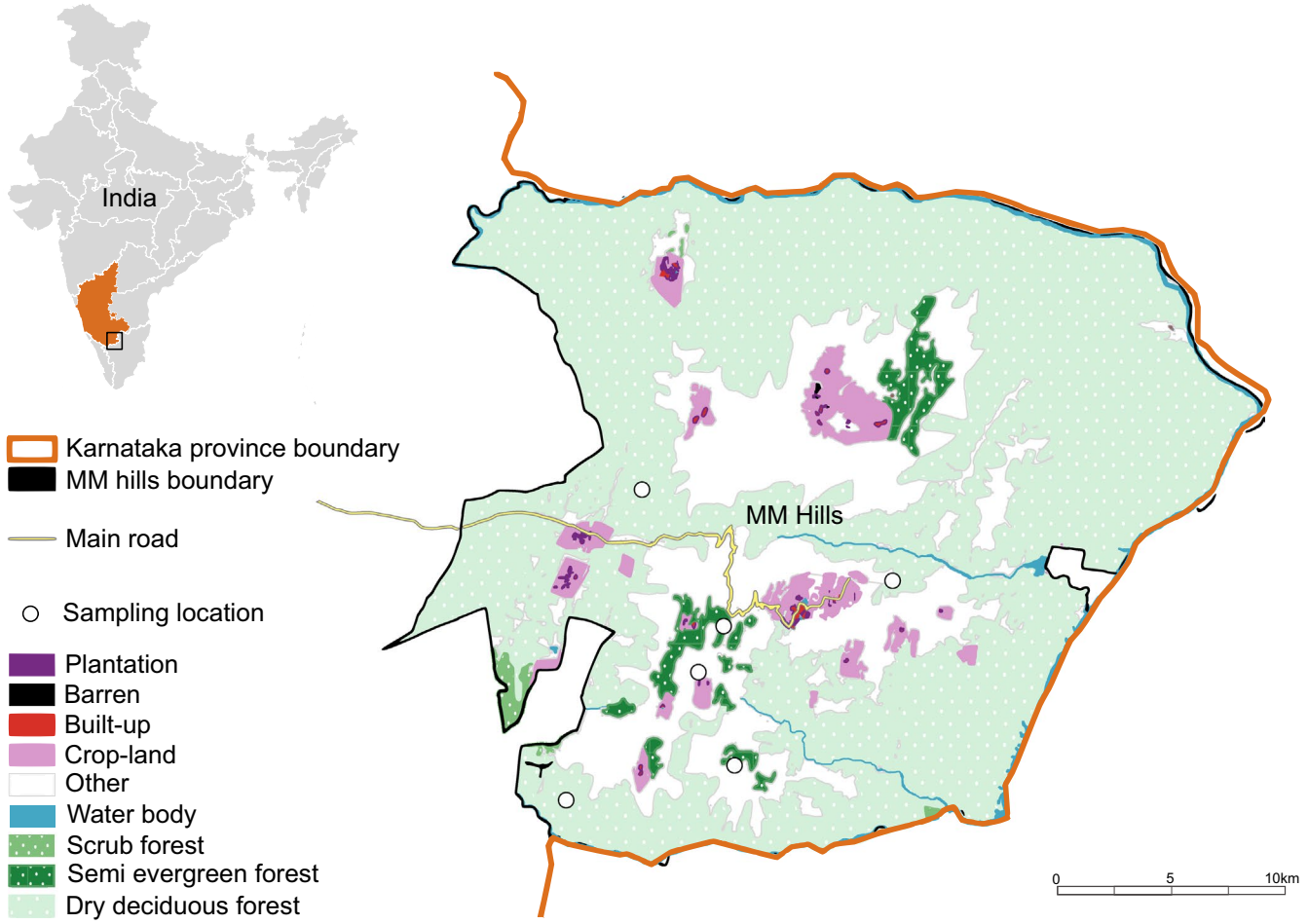

FIGURE 1 Map of Malai Mahadeshwara Hills Wildlife Sanctuary land use and sampling locations

others, interviews with local communities (Harisha et al., 2015) and research on the impacts of the invasive plant *Lantana camara* L. on vegetation (Soumya et al., 2019b; Varghese et al., 2015), bird assemblages (Aravind et al., 2010), and human adaptive responses (Kent & Dorward, 2015; Thornton et al., 2019). Our results provide a starting point for tracking the effects of the environmental changes in the area and urge the need to understand and monitor dietary niches of both local wildlife and livestock, especially in regions where their potential overlap is high and/or under-examined. The application of eDNA technology for such monitoring proves to be an efficient tool to address this need, allowing non-invasive analyses of fecal samples that provide valuable data for improving biodiversity management.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

Malai Mahadeshwara Hills Wildlife Sanctuary (MMH; 12.1.60N, 77.35.21E, 906 km^2) is a protected area of the Kollegala Range Forest in the state of Karnataka, southern India. The area is part of tiger habitat and acts as an important elephant corridor between two adjacent wildlife sanctuaries (i.e., Cauvery and Biligiriranganatha Swamy Temple Wildlife Sanctuary (BRT); Bawa, Joseph, & Setty, 2007; Gubbi et al., 2017). Most of the area is dry deciduous forest (64.3%) with scrub woodland (20.5%) and patches of moist deciduous and riparian forest (2.5%, Harisha & Padmavathy, 2013). MMH is home to approximately 12,000 people from several ethnic and tribal groups, but throughout the year the population is heavily elevated due to tens of thousands of religious pilgrims who visit the main temple and other shrines (Harisha & Padmavathy, 2013; Kent & Dorward, 2015; Soumya et al., 2019a). Despite its history of human interactions and anthropogenic character, the MMH forests host a wide range of wildlife.

We collected 116 fecal samples from 16 different mammal species in the winter and summer pre-monsoon seasons in 2015–2016 (December to April), 2017 (March and April) and 2018 (April). Seventy-seven samples were identified as from herbivorous animals, and 62 provided usable results after quality control filtering of the herbivore and plant DNA sequence data. These samples represent 10 herbivore species that can be subdivided into domestic herbivores, goat (*Capra hircus*), cattle (*Bos taurus*), water buffalo (*Bubalis bubalus*), and wild herbivores, sambar (*Rusa unicolor*) and barking deer (*Muntiacus muntjak*), Indian hare (*Lepus nigricollis*), Indian

crested porcupine (*Hystrix indica*), bonnet macaque (*Macaca radiata*), wild boar (*Sus scrofa*), and Asian elephant (*Elephas maximus*). Samples were dried or placed in ethanol and stored at −20°C.

2.2 | DNA analyses

We followed standard procedures for working with low copy DNA, such as the regular cleaning of work surfaces with bleach and changing gloves between the handling of each sample. Subsamples of fecal material were obtained by spreading the fecal sample on a Petri dish and randomly collecting 200 mg from the dish. Excess ethanol from storing the fecal samples was evaporated by briefly heating the sample to 50°C. We extracted the DNA using the PSP Spin Stool DNA Kit (Stractec Biomedical, Berlin, Germany) following manufacturer's instructions, using 100 μl elution buffer supplied with the kit and omitting the heating step (10 min at 95°C) to prevent further DNA degradation. Extraction blanks (6) were included in each extraction round, and these were pooled per two during the PCR step resulting in sequences from 3 sets of extraction blanks in the final dataset. DNA extraction, PCR preparation, and post-PCR work took place in separate dedicated rooms.

2.3 | Herbivore DNA amplification and sequencing

The identity of the herbivore fecal samples was confirmed using specifically designed primers for each target species (Table S1). Herbivore DNA amplifications were carried out in a final volume of 12.5 μl using 1 μl of DNA extract and 0.24 μM of each primer. The amplification mixture contained 0.5 U of AmpliTaq Gold DNA Polymerase with buffer II (Applied Biosystems, Foster City, CA), 1× Buffer II, 2.5 mM $MgCl_2$, 0.48 mM of each dNTP, and 0.048 μg/μl of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland). The mixture was denatured at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, 30 s at 48–55°C depending on the primer pair used (Table S1), 45 s at 72°C and a 3 min final elongation at 72°C.

The PCR products were visualized with agarose gel electrophoresis and cleaned for sequencing by adding 2 μl 1:10 dilution of Illustra ExoProStar (GE Healthcare, USA) to the PCR products and incubating them at 37°C for 45 min before enzyme inactivation at 80°C for 15 min. The cleaned PCR products were bidirectionally sequenced on an ABI 3730xl at Macrogen Europe BV (Amsterdam, the Netherlands).

2.4 | Plant DNA amplification and sequencing

Plant DNA metabarcoding was done using the *trn*L *g* and *h* primers (Taberlet et al., 2007). Both primers were tagged with a unique 8 or 9 bp barcode at the 5′ end to allow for multiplexing as described by Voldstad et al. (2020). We conducted three PCR replicates per sample, and both the extraction negative controls and PCR negative controls were included in the PCRs.

Plant DNA amplifications were carried out in a final volume of 25 μl containing 2 μl of DNA extract and 0.24 μM of each primer. The amplification mixture further contained 1 U of AmpliTaq Gold DNA Polymerase with Buffer II (Applied Biosystems), 1× Buffer II, 2.5 mM $MgCl_2$, 0.48 mM of each dNTP, and 0.048 μg/μl of bovine serum albumin (BSA, Roche Diagnostic). The mixture was denatured at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, and 30 s at 55°C, 45 s at 72°C, and a 2 min final elongation at 72°C.

Amplicons were quantified using a Bio-Rad Gel doc XR+ and the Image Lab v.6.0.0 software (Bio-Rad Laboratory, Inc.) and subsequently cleaned as described above. A Biomek 4000 liquid handling robot (Beckman Coulter) was used to pool amplicons equimolarly into three pools, whereby each pool contained one of the three replicate PCRs. For amplicons with concentrations lower than 1 ng/μl, the maximum amount of 15 μl was added to the pool. The resulting three pools were cleaned two times with Ampure XP (Beckman Coulter) according to the manufacturer's protocol using first a 1.4:1 and then a 2:1 ratio between Ampure XP beads and pool. Concentrations of the pools were measured on a Qubit 2.0 with the Qubit dsDNA HS kit (Thermo Fisher), and pools were visualized on a Fragment Analyzer using the DNF-488 kit (Advanced Analytical Technologies Inc.). Libraries were built from the pools with plant PCRs using the KAPA HyperPrep DNA kit (Roche) and pools were sequenced on the Illumina HiSeq 4000 at the Norwegian Sequencing Centre.

2.5 | Data processing and analyses

2.5.1 | Herbivore DNA identification

Sequence reads from the herbivore DNA were aligned and trimmed manually using Geneious Prime 2019.1.3 (https://www.geneious.com). The resulting consensus sequences were then checked against the NCBI nucleotide collection using megaBLAST (Morgulis et al., 2008). Sequences resulting in percentage ID < 95%, or poor quality reads (HQ% < 35) were excluded from further analyses.

2.5.2 | Plant DNA sequence analyses and filtering

Initial analyses and filtering of the plant DNA sequence data were performed using the OBITools package (http://metabarcoding.org/obitools/doc/index.html; Boyer et al., 2016). Assembling of the forward and corresponding reverse reads was done using *illuminapairedend*, followed by sample assignment with *ngsfilter*. We removed reads with a quality score < 40, <100% tag match, >3 mismatches with the primers, shorter lengths then expected (<8 bp), singletons, and those containing ambiguous nucleotides. Amplification and sequencing errors were identified using *obiclean*, with a threshold ratio of 5% for reclassification of sequences identified as "internal" to their corresponding "head" sequence. Finally, sequences were

compared to two taxonomic reference libraries using *ecotag*. These two reference libraries were prepared by performing an in-silico PCR with the *ecoPCR* software (Ficetola et al., 2010) and the NCBI Taxonomy database (https://www.ncbi.nlm.nih.gov/taxonomy). The first, local reference library contained 555 sequences of 134 plant taxa known to occur in MMH and the surrounding area from monitoring data (information provided by the Ashoka Trust for Research in Ecology and the Environment, ATREE) and published species lists (Appendix 1 of Harisha, Padmavathy, & Nagaraja, 2015). The majority of the sequences for this library were obtained from the EMBL database (release 137). An additional 35 species of locally occurring Poaceae were sequenced for the database (at ATREE, Bengaluru, see Appendix S2). As the plant taxa in the local reference library occur in MMH and neighboring areas, we prioritized matches against this library. To mitigate erroneous or missing taxonomic assignment due to lacking references in this library, we used a second reference library based on the global EMBL database (release 137), containing 111,146 sequences of 18,101 plant taxa.

In order to minimize any misidentifications, we filtered the identified sequences in R (version 3.5.2) to remove (a) sequences that were identified only as "internal" in the *obiclean* step, (b) sequences with higher occurrence (i.e., more reads) in negative controls than in samples, (c) sequences with a percentage identity < 95%, (d) 0.001% of each sequence read count per sample to correct for potential leakage, (e) unreliable PCR replicates, and (f) sequences that make up <1% of the sample (as advised during a workshop; see Appendix S3 for details). We identified unreliable PCR replicates by estimating Euclidian distances between all PCR replicates and their centroid based on square rooted rarefied read counts (similar to Kowalczyk et al., 2019). We estimated kernel densities for nonreplicates and replicates and compared them to identify the distance where the kernel density was higher for nonreplicates compared to replicates. Replicates were discarded when distances among replicates were the same or larger than this threshold distance. Remaining replicates were merged while averaging the read counts per molecular operational taxonomic unit (MOTU). In order to check and where possible narrow down some of the taxonomic identifications, the identified plant taxa were checked by a taxonomist with extensive knowledge of the locally occurring plants. Remaining unique plant sequences were designated as MOTUs. An overview of these steps and the remaining reads can be found in Appendix S3, and the resulting processed data in Tables S4–S7.

2.6 | Dietary niche analyses

All further data processing and statistical analyses were done in R (version 3.5.2). In order to quantify the diet composition, the obtained plant MOTU-by-samples matrix of the read counts was transformed using two distinct approaches: (a) the presence/absence of each plant MOTU in each fecal sample, and (b) the relative read abundance (RRA), that is, the proportional representation of each plant MOTU in each fecal sample. All further analyses were performed on both of the resulting transformed datasets. RRA data has been used in numerous other dietary metabarcoding studies (e.g., Kartzinel et al., 2015; Kartizinel & Pringle, 2020; Mychek-Londer et al., 2020; Pansu et al., 2019), as results based on RRA have been shown to be less sensitive to rare MOTUs (i.e., low-abundant reads that may for example result from PCR or sequencing errors or contamination; Deagle et al., 2019). We only present figures and analyses performed on the RRA data in the main text. Analyses on presence/absence data can be found in the Appendices S8–S12.

We assessed dietary niche width by calculating average MOTU richness and the Shannon diversity index for each sample using the *spaa* package (Zhang, 2016). We subsequently computed and visualized intersections of herbivore species' diets and calculated intersection sizes in number of shared MOTUs using *UpSetR* (Conway et al., 2017). Similar to Pansu et al. (2019), we used two complementary metrics to describe the (dis-)similarities between the different dietary niches: Bray–Curtis dissimilarity index and Pianka's niche overlap index (Pianka, 1974). Bray–Curtis dissimilarity was calculated between each pair of fecal samples in order to quantify dietary dissimilarity. We subsequently ordinated these values in two dimensions using nonmetric multidimensional scaling (NMDS) in the *vegan* package (Oksanen et al., 2019) to allow the visualization of the patterns of dietary dissimilarity among samples, and species (groups of samples). A stress level for the NMDS of <0.2 is considered acceptable (Clarke, 1993). We tested for dietary difference among species by doing a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the *adonis*-function in *vegan*. We tested the perMANOVA assumption of homogeneity of multivariate dispersions using the *permutest* function in *vegan* with 999 permutations. Pairwise calculations of Pianka's niche overlap index (Pianka, 1974) were performed with the *spaa* package (Zhang, 2016). We evaluated these results with reference to 1,000 permutations of a null model that retains the dietary niche width of each species while randomizing the values for the diet items using *EcoSimR* (Gotelli et al., 2015). See Appendix S12 for further details.

3 | RESULTS

3.1 | Description of the raw dataset

The 77 analyzed samples yielded 62 retained samples. Five samples were discarded due to poor quality sequences from the herbivore PCR, six samples were discarded due to percentage herbivore identity of < 95%, and four samples were discarded due to poor yield of plant DNA. After filtering and merging the replicates, the dataset contains a total of 12.5 M reads distributed over 134 plant MOTUs from 62 fecal samples, representing the diets of 10 herbivore species (Table S6). Read depth per sample ranged from 16 929 to 820 667 (average: 201 718 ± 16 615). 31% of the MOTUs are annotated with a plant species name, 13% with a genus name, and 40% with a plant family name. The remaining 16% are annotated to higher taxonomic ranks (Table S5). A total of 31 different plant families are

distinguished. The most abundant plant families in the dataset are Fabaceae and Poaceae, both in number of MOTUs (24 and 22) and in percentage read counts (30% and 35%, respectively; Table S4). These are followed by Malvaceae in terms of MOTUs (7), and by Anacardiaceae in terms of percentage of read counts (6%).

3.2 | Dietary niche width and composition

Average dietary niche width over all studied individuals is 8.23 ± 0.55 in MOTU richness and 1.24 ± 0.09 in Shannon diversity. MOTU richness is greatest for domestic goat, water buffalo and sambar deer (Table 1), with averages of 11.75 ± 1.53, 13.33 ± 2.73, and 12.40 ± 0.93, respectively. The Shannon diversity index also indicates the greatest dietary richness for these herbivore species, with index values: 1.78 ± 0.18, 1.88 ± 0.30, and 2.00 ± 0.18, respectively. The narrowest dietary niches are found for bonnet macaque and cattle samples (Table 1). Bonnet macaques have a dietary niche width of 4.83 ± 0.91 in MOTU richness and 0.78 ± 0.19 in Shannon diversity. The bulk of the bonnet macaque diet consists of Fabaceae (63%) of which 85% represents the *Senegalia* genus, followed by Malvaceae (13%) and Rhamnaceae (10%, Figure 2 and Table S7). Contrarily, the diet of cattle consists primarily of grass, as indicated by the RRA for Poaceae of 84%. Although the remaining 16% of the cattle diet is composed of 15 other plant families and at least 24 genera, the MOTU richness and Shannon diversity are still below average, with 6.22 ± 0.69, and 0.87 ± 0.13, respectively.

Comparing the dietary composition for the 10 studied herbivores, five species primarily consume Poaceae, whereas four species primarily consume Fabaceae. Members of the Poaceae family make up more than 50% of the diet of cattle, Asian elephant, wild boar, Indian hare, and more than 30% of the sambar deer diet. By contrast, the diets of domestic goat, bonnet macaque, Indian porcupine, and barking deer consist primarily of Fabaceae (43%–63%). The diet of water buffalo forms the exception with 31% of reads from the Anacardiaceae family (which consists for 84% of *Mangifera*), followed by 22% from the Moraceae family (which consists for 94% of *Ficus*) and 20% Poaceae reads (Figure 2 and Table S7).

3.3 | Dietary niche overlap

From the 134 plant MOTUs in the total dataset, 48 MOTUs are exclusive for domestic herbivore samples and 39 MOTUs are only found in wild herbivore samples (Figure 3 and Table S7). These sets make up 17% and 7% of the RRA dataset, respectively. The remaining 47 MOTUs (35% of all MOTUs) are shared between domestic and wild herbivores and represent the remaining 76% of RRAs in the total dataset. These 47 MOTUs are spread over 38 intersections each representing the number of plant MOTUs shared by a particular combination of herbivore species (Figure 3). The total number of shared plant MOTUs for a specific combination of herbivore species can be found by taking the sum of intersection sizes for all combinations that include the pair or group of herbivore species of interest. The largest number of shared MOTUs between two species is 17 and is found for two pairs of species: cattle and domestic goat, and domestic goat and sambar deer. This is followed by 14 shared MOTUs

TABLE 1 Overview of the sample size, a priori feeding guild assignment, and niche width described by average MOTU count and Shannon diversity index per herbivore species with standard errors (*SE*) for both measures

	Herbivore species	Scientific name	*N*	Feeding guild	MOTUs	(± *SE*)	Shannon	(± *SE*)
Domestic	Cattle	*Bos taurus indicus*	23	folivore (grazer)	6.22	(± 0.69)	0.87	(± 0.13)
	Domestic goat	*Capra hircus*	8	folivore (mixed feeder)	11.75	(± 1.53)	1.78	(± 0.18)
	Water buffalo	*Bubalus bubalis*	3	folivore (mixed feeder)	13.33	(± 2.73)	1.88	(± 0.30)
Wild	Asian elephant	*Elephas maximus*	5	folivore (mixed feeder)	8.40	(± 2.84)	1.09	(± 0.46)
	Barking deer	*Muntiacus muntjak*	2	folivore (browser)	7.50	(± 2.50)	1.22	(± 0.59)
	Bonnet macaque	*Macaca radiata*	6	frugivore	4.83	(± 0.91)	0.78	(± 0.19)
	Indian hare	*Lepus nigricollis*	3	folivore (grazer)	7.67	(± 1.76)	1.40	(± 0.19)
	Indian porcupine	*Hystrix indica*	4	frugivore	8.75	(± 1.97)	1.43	(± 0.43)
	Sambar deer	*Rusa unicolor*	5	folivore (mixed feeder)	12.40	(± 0.93)	2.00	(± 0.12)
	Wild boar	*Sus scrofa*	3	omnivore (browser)	9.33	(± 1.20)	1.51	(± 0.18)

Note:: Feeding guild assignments are based on Nowak and Walker (1999), Ahrestani et al. (2012), Ahrestani and Sankaran (2016), and IUCN (2020).

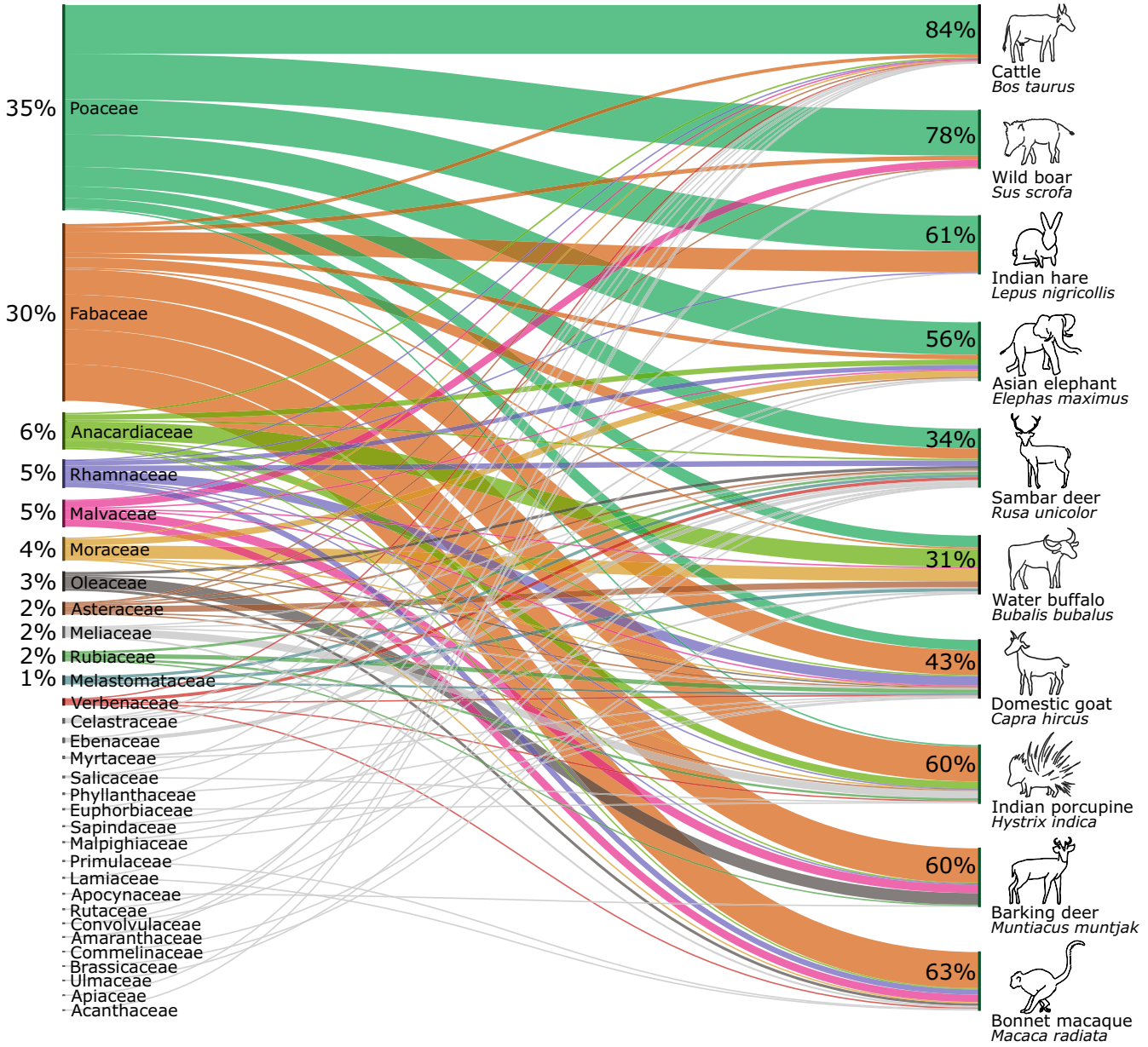

FIGURE 2 The relative read abundance (RRA) per plant family for the entire dataset (left; no percentage indication means RRA < 1%) and for each herbivore species (right), where percentages indicate the RRA of the most abundant plant family in the diet

for cattle and water buffalo, and 13 shared MOTUs for domestic goat and Asian elephant.

In order to quantify the degree of overlap in dietary niches between the different herbivores, we calculated Bray–Curtis dissimilarity (BC; 0: similar, 1: dissimilar) and complementary Pianka niche overlap indices (O; 0: no overlap, 1: full overlap) based on MOTU relative read abundances. Resulting Bray–Curtis dissimilarity index values are in the range of 0.61 to 1.00 with an average of 0.88 ± 0.02, whereas Pianka index values range from 0.00 to 0.68 with an average of 0.15 ± 0.04 (Table 2). 35 MOTUs, representing 79% of total RRAs are shared between wild herbivore species. Seven of these 35 MOTUs (13% of total RRAs) do not occur in the diets of the domestic herbivore species. The highest degree of dietary niche overlap for wild herbivores was observed between barking deer and porcupine (BC: 0.72, O: 0.63; Table 2) and between sambar deer and Indian hare (BC: 0.67, O: 0.60). Within the group of domestic herbivores, 30 MOTUs representing 53% of total RRAs are shared among species. Of these 30 MOTUs, nine (5% of total RRAs) do not occur in the diets of the wild herbivore species. The dietary niche of cattle overlaps with those of the other domestic herbivores (goat, BC: 0.75, O: 0.28; water buffalo, BC: 0.82, O: 0.23; Table 2), but comparison between goat and water buffalo reveals little overlap of their dietary niches (BC: 0.92, O: 0.05).

A perMANOVA on Bray–Curtis dissimilarities derived from the RRA data indicates significant dietary differences among species ($F_{9,52} = 3.79$, $r^2 = 0.40$, $p \leq .001$). The assumption of homogeneity

FIGURE 3 Upset plot visualizing intersecting sets of the two herbivore groups (domestic and wild; left) and herbivore species (right). Intersection size is presented in the number of shared plant MOTUs observed only for that particular combination of herbivore species indicated by the dots. Herbivore combinations and their respective intersection size bars are colored to indicate their composition of exclusively wild herbivore species (green), exclusively domestic herbivore species (purple), or both wild and domestic herbivore species (orange). Set size indicates the total number of MOTUs found per herbivore species. Scientific names for the herbivore species from top to bottom are as follows: *Lepus nigricollis*, *Sus scrofa*, *Muntiacus muntjak*, *Elephas maximus*, *Macaca radiate*, *Hystrix indica*, *Rusa unicolor*, *Bubalis bubalus*, *Capra hircus*, and *Bos taurus indicus*. The total number of shared plant MOTUs for a specific combination of herbivore species can be found by taking the sum of intersection sizes for all combinations that include the pair or group of herbivore species of interest. An example is shown for Asian elephant and cattle, where the total number of shared plant MOTUs is found by taking the sum of the bars indicated with "B." The bars specifying the number of MOTUs found for the Asian elephant but not for cattle are indicated with "A," and those found for cattle but not for Asian elephant are indicated with "C."

TABLE 2 Bray–Curtis dissimilarity (bottom left; 0: similar, 1: dissimilar) and Pianka's overlap index (top right; 0: no overlap, 1: full overlap) based on RRA data

	Cattle	Domestic goat	Water buffalo	Asian elephant	Barking deer	Bonnet macaque	Indian hare	Indian porcupine	Sambar deer	Wild boar
Cattle		0.28*	0.23*	0.49*	0.00	0.00	0.65*	0.01	0.68*	0.03
Domestic goat	0.75		0.05	0.52*	0.08*	0.06	0.04	0.11*	0.46*	0.00
Water buffalo	0.82	0.92		0.12*	0.00	0.02	0.20*	0.04	0.24*	0.12*
Asian elephant	0.70	0.76	0.84		0.02	0.05	0.00	0.03	0.10*	0.00
Barking deer	0.98	0.92	0.99	0.93		0.19*	0.00	0.63*	0.03	0.00
Bonnet macaque	0.99	0.87	0.97	0.94	0.79		0.00	0.02	0.04	0.00
Indian hare	0.61	0.93	0.85	0.97	1.00	1.00		0.10*	0.60*	0.24*
Indian porcupine	0.94	0.87	0.94	0.90	0.72	0.95	0.90		0.06	0.01
Sambar deer	0.68	0.65	0.77	0.87	0.96	0.89	0.67	0.90		0.08*
Wild boar	0.96	0.99	0.88	1.00	1.00	0.98	0.80	0.99	0.93	

Asterisks indicate statistically significant niche overlap (i.e., greater than expected by chance based on comparison with 1,000 null models, $\alpha = 0.05$, 95% CI [0.02, 0.07]).

of dispersion among species was supported by a nonsignificant *permutest* result ($p = .789$). In order to uncover which particular herbivore species drive these results, we performed a post hoc pairwise perMANOVA (using a Bonferroni correction of the *p*-values). Four of the 45 comparisons were statistically significant ($p < .05$): cattle versus domestic goat, cattle versus bonnet macaque, domestic goat versus bonnet macaque, and cattle versus wild boar (see Table S11). A perMANOVA on the presence/absence data also identifies these

pairs of herbivores to differ significantly in their dietary niches, but further indicates different niches for cattle compared to Asian elephant, Indian porcupine, and sambar deer (see Table S11).

Herbivore pairs with significantly different dietary niches according to the pairwise perMANOVA on RRA-based Bray–Curtis dissimilarities also score below average on Pianka's overlap index (O: 0.00–0.06), except for cattle versus domestic goat (O: 0.28). In contrast, the highest overlap in dietary niches of domestic and wild herbivore species is observed for cattle and sambar deer (BC: 0.68, O: 0.68), and cattle and Indian hare (BC: 0.61, O: 0.65). These are followed by domestic goat and Asian elephant (BC: 0.84, O: 0.52), cattle and Asian elephant (BC: 0.70, O: 0.49), and domestic goat and sambar deer (BC: 0.65, O: 0.46).

Overall, 20 out of 45 comparisons showed statistically significant niche overlap based on comparison with 1,000 null models (Table 2). Three out of seven wild herbivores have significant dietary overlap with cattle. In order of Pianka's niche overlap values from highest to lowest, these are sambar deer, Indian hare, and Asian elephant (O: 0.68, 0.65 and 0.49, respectively). Four wild herbivores have significant dietary overlap with domestic goat: Asian elephant, sambar deer, Indian porcupine and barking deer (O: 0.52, 0.46, 0.11 and 0.08). In the case of water buffalo, significant dietary overlap is also found for four wild herbivore diets: sambar deer, Indian hare, wild boar, and Asian elephant (O: 0.24, 0.20, 0.12, and 0.12).

4 | DISCUSSION

Environmental DNA metabarcoding of fecal samples has enabled us to reconstruct the dietary niche partitioning of 10 mammalian herbivore species present in the MMH in southern India. We specifically focused on dietary overlap that may arise by shared use of the forest and village habitats by domesticated animals and wildlife and we argue for monitoring of potential effects of environmental change as restrictions on grazing are enforced and impacts of invasive species change.

4.1 | Dietary niche reconstruction

The reconstructed diets represent a continuum of grazers, through mixed feeders and browsers to frugivorous mammals based on the RRAs of Poaceae compared to other plant families (Figure 2). Despite limited sample sizes for some herbivore species, these assignments are in agreement with the priori feeding guild assignments of the herbivores under study (Table 1) as based on Nowak and Walker (1999), Ahrestani et al. (2012), Ahrestani and Sankaran (2016), and IUCN (2020). One exception is observed: wild boar (*Sus scrofa*) is considered an omnivorous browser, but with 78% Poaceae in our study its diet is categorized together with the grazers (Figure 2). Wild boar are some of the most persistent crop raiders in the area, especially when local food staples finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.), and maize (*Zea mays* L.) ripen and are harvested (November to January). This interpretation also fits with other studies that show a variable diet for wild boar across geographic regions and habitats (Gray et al., 2016; Ickes, 2001; Robeson et al., 2018).

Considering the ability of mixed feeders to switch between grazing and browsing (Ahrestani & Sankaran, 2016), we expected these species to have a generalist diet and therefore a relatively large dietary niche width compared to more specialized feeders. In accordance with these expectations, the narrowest dietary niches were found for two specialized feeders: bonnet macaque and cattle with Shannon diversities of 0.78 ± 0.19 and 0.87 ± 0.13, respectively (Table 1). Bonnet macaques are conventionally described as frugivores and we primarily found diet items originating from the Fabaceae family (63% RRA, Figure 2) in their diet. Contrarily, cattle are grazers and primarily eat grass, as indicated by the high RRA for Poaceae of 84%. Although the remaining 16% of the cattle diet is composed of 15 other plant families, the average number of MOTUs and Shannon diversity are low (6.22 ± 0.69 and 0.87 ± 0.13, respectively), which include wild grasses that grow in the villages and in the forest, as well as bamboos and cereal and vegetable crops. The hare is also conventionally described as primarily grazing (Nowak & Walker, 1999), but the analyzed samples of the Indian hare contained a high proportion of Fabaceae (36%) as well as the expected Poaceae (61%). The high proportion of Fabaceae might be explained by crop raiding on various species of beans grown by farmers, and *Cajanus* sp. (pigeon pea) was indeed detected in the hare diet (Table S7). Species assigned to the feeding guild of mixed feeders scored above average in dietary richness in both average number of MOTUs and Shannon diversity (Table 1 and Table S8.1). The Asian elephant is the exception to this pattern, scoring below average on Shannon diversity for both datasets. We found the diet of elephants to consist mainly of grasses (56%), which is consistent with other reports from southern India, although their diets are suggested to shift toward less woody plants and more graminoids (Poaceae, Juncaceae and Cyperaceae) in the wet season (Ahrestani et al., 2012; Baskaran et al., 2010; Sukumar, 2006). The classification of mixed feeder is therefore only appropriate if one takes into account the complete diet, while it seems that within seasons they should be considered as either grazer or browser.

In the present study, samples were collected in the winter and summer pre-monsoon seasons in three subsequent years. Considering the seasonal availability of plants and the evidence for differences in herbivore feeding patterns over wet and dry seasons approximately 100 km from the study area (Ahrestani et al., 2012), it is likely that the dietary niches of the herbivores in MMH would shift over the seasons. Such shifts may also result in temporal variation in the dietary niche overlap between species pairs, though Ahrestani et al. (2012) found that the overlap in dietary niches of sambar deer and elephants remained constant across dry and wet seasons.

As species with narrow niches are deemed more vulnerable to environmental changes (Clavel et al., 2011; Devictor et al., 2010), it is especially important to closely monitor the dietary niches of bonnet macaque and Asian elephant. To obtain a more complete view of diet and dietary niche overlap, samples should be analyzed across

different seasons, and long-term monitoring of diet and its overlap should include temporally spaced sample collection ensuring both the wet and the dry season are covered.

4.2 | Dietary niche partitioning

Due to the limited number of samples for several herbivore species, an amount of uncertainty in their dietary reconstructions needs to be acknowledged, and dietary niche dissimilarity or overlap may be under- or overestimated as a result. Nonetheless, the dietary compositions of the samples within herbivore species are similar and clear clusters can be distinguished (Figure 4, Supplemental Information S9) indicating dietary niche partitioning between the studied species. Herbivore species pairs of diverse feeding guilds show high niche dissimilarity and low overlap, which is especially the case for cattle versus bonnet macaque, cattle versus wild boar, and domestic goat versus bonnet macaque (Table 2). Corresponding herbivore pairs also clearly segregate in the nMDS plot (Figure 4, Supplemental Information S9).

As expected based on previous indications of livestock-mediated resource limitation in India (Bagchi et al., 2003; Madhusudan, 2004), we found significant overlap in consumed MOTUs (35%) between domestic and wild herbivores in MMH representing 76% of RRAs in the dataset. The greatest overlap was found for the dietary niches of cattle and sambar deer (O: 0.68). The cattle diet also overlaps with the diets of Indian hare and Asian elephant and the diet of domestic goat overlaps with the diets of sambar deer and Asian elephant (Table 2). Bray–Curtis dissimilarities and perMANOVA results support these findings with below average dissimilarities and no significant differences in dietary niches for these herbivore pairs. Similar overlap between cattle diet and the diets of several wild herbivore species across dietary guilds such as elephant (mixed feeder), impala (mixed feeder), and Dik-Dik (browser) have previously been reported in Kenya (Kartzinel et al., 2015). Likewise, a study in the Greater Himalayas indicates high trophic niche overlap between livestock (sheep and goats) and wild ungulates, including sambar deer (Bhattacharya et al., 2012) further supporting our findings of dietary overlap between domesticates and wildlife.

Compared to RRA data, presence/absence data transformations result in a larger differentiation in some herbivore pairs (cattle paired with Asian elephant, Indian porcupine, and sambar deer) and a smaller differentiation in seven other pairs (see Table S10). This reflects the difference in the amount of rare, low-abundant MOTUs in the diet of the various animals, since results based on RRAs are less sensitive to the presence of rare MOTUs compared to results based on presence/absence alone (Deagle et al., 2019). Rare MOTUs also play a role in the seemingly contradictory results of dietary niche similarity indices of two domestic herbivores. Domestic goat and cattle have significantly different dietary niches according to the RRA-based perMANOVA test, but score significantly high on Pianka's niche overlap index. Comparison of quantitative metrics of niche overlap from species distributions by Rödder and Engler (2011) suggests that Bray–Curtis values more accurately reflect niche overlap than most other tested methods. Especially for species distributions made up of many grid cells with low occurrence, Pianka's niche

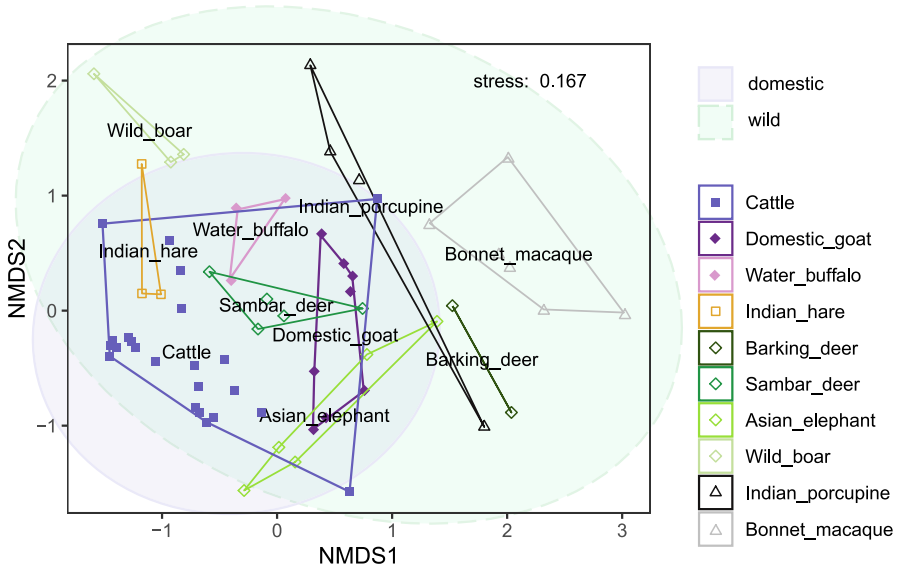

FIGURE 4 Dietary niche partitioning within and among domestic and wild herbivore species by nMDS of RRA-based Bray–Curtis dissimilarity of samples (adonis $F_{9,52} = 3.79$, $R^2 = 0.40$, $p \leq .001$). The positioning of the species label indicates the mean for that species. Depicted are as follows: cattle (*Bos taurus indicus*), domestic goat (*Capra hircus*), water buffalo (*Bubalis bubalus*), Indian hare (*Lepus nigricollis*), barking deer (*Muntiacus muntjak*), sambar deer (*Rusa unicolor*), Asian elephant (*Elephas maximus*), wild boar (*Sus scrofa*), Indian porcupine (*Hystrix indica*), and bonnet macaque (*Macaca radiata*). Samples from domestic herbivore species are indicated with filled symbols and the shaded ellipses indicate the standard deviation from the mean of domestic and wild herbivore groups. The shapes of the symbols refer to the different feeding guilds: grazer (square), mixed feeder (diamond), and frugivore (triangle). The stress level of 0.167 is under the cut-off value of 0.2 as posed by Clarke (1993) to indicate an interpretable ordination

overlap was shown to be prone to both under- as well as overestimation (Rödder & Engler, 2011), suggesting a potential bias when herbivore diet is made up of many MOTUs with low RRAs. As this is the case for the comparison of domestic goat and cattle in this study, we should conclude that despite the suggested overlap according to Pianka's niche overlap index, their dietary niches are different.

Although eDNA metabarcoding provides a relatively cost-effective and time-efficient alternative to microhistological analyses (Pompanon et al., 2012), a limitation of the use of eDNA metabarcoding for dietary niche partitioning studies is the lack of differentiation between plant tissues. In cases where some herbivores prefer to eat the fruits, while others eat the leaf material or the roots of a plant, the dietary niche overlap can be overestimated as partitioning does not take place on a taxonomic level, but is instead based on the consumption of different parts of the plants. For example, this could be the case for bonnet macaque and barking deer based on their feeding guild assignments (Table 2), although both are reported to prefer young, fresh leaves and fruits (Ahrestani & Sankaran, 2016; Krishnamani, 1994) and thus partitioning would more likely take place based on which parts of the plants the animals can reach. Otherwise, we found relatively low taxonomic overlap in diets between herbivore species that are known to feed on different plant tissues.

4.3 | Land use and invasive species

Apart from identifying dietary niches and quantifying dietary niche overlap, eDNA metabarcoding data from herbivore fecal samples also provide an opportunity to monitor the available plant taxa in the foraging areas of the herbivores under study. MMH is home to several ethnic groups that largely depend on the forest for their livelihoods (Harisha & Padmavathy, 2013; Kent & Dorward, 2015), and the area consists of forests, interspersed with anthropogenic lands for crops, plantations, or buildings (Figure 1). Human influences on dietary composition are therefore likely.

The traditional ecological knowledge in the MMH villages together with the locally occurring plant species and their use have been mapped in a previous study (Harisha et al., 2015). The local communities depend to a large degree on agriculture for their livelihood and grow both subsistence crops (e.g., the cereals and beans) as well as cash crops (e.g., maize and sunflower; Harisha et al., 2015). Not all known cultivated species could be identified to species level in our study; nevertheless, we detected several known crop species in the diets of the studied herbivores. For example, in the diet of the water buffalo, we observed *Brassica rapa* L. which is cultivated in the area for food and medicinal purposes (Harisha et al., 2015). In the cattle diet, *Achyranthes aspera* L. is present—a species used for food and cultural reasons (Harisha et al., 2015)—and *Sorghum bicolor* (L.) Moench—commonly cultivated as cash crop for use as fodder as well as human consumption (Harisha et al., 2015)—was found in the diet of both cattle and water buffalo. These domestic herbivores are likely to obtain these food sources by way of supplemental feeding.

We also found evidence for the consumption of agricultural crops by wild herbivores, for example, in the presence of *Amaranthus* spp. and Poaceae spp. in the diet of wild boar, and *Cajanus* spp. in the diet of Indian hare, all of which are cultivated for food (Harisha et al., 2015). Many other members from the Poaceae plant family are commonly used as fodder (e.g., *Apluda mutica* L., *Cynodon dactylon* (L.) Pers., *Melinis repens* (Willd.) Zizka, *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult., and *Themeda triandra* Forssk.), but a large proportion of Poaceae found could not be identified to genus or species level, thereby limiting inferences about the proportion of cultivated fodder versus wild Poaceae species. However, using the diet items that do have genus or species level identifications, some instances can be identified where typical fodder species are also eaten by wild herbivores. One example is *Cynodon dactylon*, which is present in the diets of water buffalo (4%) and cattle (< 1%), but also in the diets of sambar deer (1%), Indian hare (7%), and wild boar (37%). *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae) is also a known fodder species and is present in the diet of domestic goat and cattle at percentages below 1%, but at 17% represents a much larger proportion of the Indian porcupine diet. Wild boar are some of the most persistent crop raiders in the area, especially as local food staples ripen (finger millet, sorghum, and maize in November to January), and farmers stay up all night with smoky fires to scare away the boar, as well as deer, hare, and elephants. Reports from farmers in MMH indicate an increase in crop raiding with the increase of invasive *Lantana camara* L. (Verbenaceae) in the forest understory, while Mundoli et al. (2016) documented similar increases of crop raiding by boar in neighboring BRT Tiger Reserve over a 7-year period, leading to many farmers giving up growing food crops altogether in favor of commercial coffee.

Many plant species occur both in the wild as well as on agricultural lands, or are collected from the wild for use as food, medicine or cultural purposes. Harisha et al. (2015) identified 96 wild plant species that are used for food, 118 for medicine, 26 for cultural and 14 for economic purposes in the area. An example is the tamarind (*Tamarindus indica* L.) of which local communities use the fruits as food and as a source of income (Shaanker et al., 2004). We found sequence reads from *Tamarindus indica* in the diets of bonnet macaque, water buffalo and wild boar. Another example is *Semecarpus anacardium* L.f.; its fruits are used for food as well as medicine (Harisha et al., 2015) and sequence reads for this species were identified in the diets of Asian elephant, barking deer, cattle, domestic goat, Indian porcupine, sambar deer, and water buffalo. These herbivores are probably eating the leaves as the use of fruits is limited to the months of May to October (Harisha et al., 2015) and fecal samples were obtained between December and April. In addition to niche overlap between wild and domestic herbivores, there is a potential overlap between herbivores and humans in utilized plant species.

Finally, the introduction and spread of invasive species may influence the diet of herbivores in MMH. *L. camara* is a very abundant invasive plant species in the area and has been given academic attention (e.g., Aravind et al., 2010; Varghese et al., 2015) as well as in conservation management: Local communities are encouraged

to use it for the production of nontimber forest products (Kannan et al., 2016). The species makes up a small part of our dataset (0.78%) and is found in 13 of the 62 samples. Cattle and domestic goats eat it in small quantities, and also some of the wild herbivores, that is, porcupines and macaques that reportedly mostly eat the fruits. However, it seems to make up a more substantive part of the diet of particularly sambar deer (5%). Large herbivores are reported to avoid *L. camara* as its leaves and fruits contain toxins that cause cholestasis and hepatoxicity, which could ultimately lead to death (Sharma et al., 2007). Furthermore, the spread of *L. camara* in MMH is likely to reduce the availability of more suitable diet items, as its presence is associated to a decline in tree sapling densities and grass volume (Prasad, 2012; Varghese et al., 2015) and reduces access to the forests for wildlife, domesticates, and humans (Thornton et al., 2019). Widespread expansion of this invasive plant may therefore restrict resource availability and consequently change the foraging ecology of herbivores in invaded areas (Wilson et al., 2013). Continued monitoring of the presence of *L. camara* is therefore recommended.

4.4 | Wildlife management in MMH

MMH is known as an important elephant corridor and forms a large tiger habitat together with the adjacent BRT wildlife sanctuary (Bawa, Joseph, & Setty, 2007; Gubbi et al., 2017). Of the wild herbivores under study, the sambar deer and Asian elephant are, respectively, considered vulnerable and endangered (IUCN, 2020), while the other herbivores are considered of least concern under IUCN 3.1. A study of the food habits of tigers in northern India indicated that the sambar deer, together with wild boar and chital, constitutes a major part of the tiger's diet (Biswas & Sankar, 2002), which further indicates the importance of studying the wildlife in MMH.

The dietary niche overlap we identified between wild and domestic herbivores, combined with previous indications of livestock-mediated resource limitation in India (Madhusudan, 2004), suggests potential for competition between domestic and wild herbivores in the MMH area, especially in case of limited resource availability. Niche overlap does not necessarily equate to competition (Pianka, 2011), and assessment of the resource availability is needed to establish if there is direct competition. Previous authors have suggested that competitive exclusion of wild herbivores occupying similar niches may eventually occur if domestic herbivores are given an artificial competitive advantage (e.g., through extra feed in periods of scarcity) (Hardin, 1960; Pianka, 2011). For instance, an experimental study in Kenya showed a reduction of land use by wildlife (including zebra, oryx, buffalo, steenbok, gazelle, eland and elephant) with the presence of cattle (Kimuyu et al., 2017). The effect of livestock presence on wild herbivores will vary per species and geographic area, depending on the overlap in dietary niches, social intolerance, required forage quantity and quality, and several other factors (see Schieltz & Rubenstein, 2016 for a review). For example, Madhusudan (2004) described a muted effect for sambar deer, but a sharp decline in elephant population densities with increased livestock presence, followed by a clear increase after reduced livestock numbers in Bandipur national park, southern India. Furthermore, domestic herbivores can act as carrier of disease, such as foot-and-mouth disease, potentially spreading to wild herbivores as suggested for two wildlife sanctuaries close to MMH (Chandranaik et al., 2016; Silori & Mishra, 2001).

Since becoming a designated wildlife sanctuary in 2013, forest access has become more regulated and only daily livestock grazing is permitted as cowsheds have been forbidden in MMH (Thornton et al., 2019). Such measures are likely to reduce resource competition and interaction between wild and domestic species. Likewise, the encouraged use of the invasive plant *L. camara* by local communities may limit the negative impacts of this plant on the habitat and resource availability of herbivores in MMH. Continued monitoring could show if these particular conservation management strategies prove to be effective.

Based on our findings, particular concern should go to bonnet macaque and Asian elephant as their narrow dietary niches could make them vulnerable to changes in their environment (Clavel et al., 2011; Devictor et al., 2010), such as climate change, invasive species, and other anthropogenic pressures. Indeed, the range extension of rhesus macaque has already been suggested to threaten the declining bonnet macaque populations in southern India (Kumar et al., 2011) and Asian elephant habitats in India are under continuous threats of forest fragmentation and loss (Padalia et al., 2019). Moreover, our observations of dietary niche overlap suggest that especially the overlap of cattle and domestic goat with sambar deer and Asian elephants should be monitored closely, as these latter species are already considered vulnerable and endangered (IUCN, 2020).

Environmental DNA metabarcoding of fecal samples has provided a starting point for tracking the effects of the environmental changes in MMH on local wildlife. As environmental change continues to threaten biodiversity in the area, the need to continue monitoring both the wildlife species themselves and the interaction between wildlife and domestic livestock becomes more urgent. This is not only true for the wildlife sanctuary under study, but for many ecosystems across the world as they are under increasing pressure from globally increasing livestock population sizes, invasive species, habitat degradation, and other anthropogenic factors. DNA metabarcoding of fecal samples is an ideal, non-invasive method for such monitoring, providing a wide variety of valuable information for biodiversity management.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY

All read data are available at the European Nucleotide Archive (ENA) under study accession number PRJEB41139. All processed data are available in the supporting information of this article.

ORCID

Anneke T.M. ter Schure https://orcid.org/0000-0002-2177-5441

Hugo J. de Boer https://orcid.org/0000-0003-1985-7859
Sanne Boessenkool https://orcid.org/0000-0001-8033-1165

REFERENCES

Ahrestani, F. S., Heitkönig, I. M. A., & Prins, H. H. T. (2012). Diet and habitat-niche relationships within an assemblage of large herbivores in a seasonal tropical forest. *Journal of Tropical Ecology*, *28*(4), 385–394. https://doi.org/10.1017/S0266467412000302

Ahrestani, F. S., & Sankaran, M. (Eds.). (2016). *The Ecology of Large Herbivores in South and Southeast Asia* (Vol. *225*). Dordrecht: Springer. https://doi.org/10.1007/978-94-017-7570-0

Anand, S., & Radhakrishna, S. (2017). Investigating trends in human-wildlife conflict: Is conflict escalation real or imagined? *Journal of Asia-Pacific Biodiversity*, *10*(2), 154–161.

Ando, H., Mukai, H., Komura, T., Dewi, T., Ando, M., & Isagi, Y. (2020) Methodological trends and perspectives of animal dietary studies by noninvasive fecal DNA metabarcoding. *Environmental DNA*. *2*, 391–406 https://doi.org/10.1002/edn3.117

Aravind, N. A., Rao, D., Ganeshaiah, K. N., Shaanker, R. U., & Poulsen, J. G. (2010). Impact of the invasive plant, *Lantana camara*, on bird assemblages at Malé Mahadeshwara Reserve Forest, South India. *Tropical Ecology*, *51*(2), 325–338. https://doi.org/10.1111/aje.12698

Bagchi, S., Goyal, S. P., & Sankar, K. (2003). Niche relationships in an ungulate assemblage in a dry tropical forest. *Journal of Mammalogy*, *84*(3), 981–988. https://doi.org/10.1644/BBa-024

Baskaran, N., Balasubramanian, M., Swaminathan, S., & Desai, A. A. (2010). Feeding ecology of the Asian elephant *Elephas maximus* Linnaeus in the Nilgiri Biosphere Reserve, southern India. *Journal of the Bombay Natural History Society*, *107*(1), 3.

Bawa, K. S., Joseph, G., & Setty, S. (2007). Poverty, biodiversity and institutions in forest-agriculture ecotones in the Western Ghats and Eastern Himalaya ranges of India. *Agriculture, Ecosystems & Environment*, *121*(3), 287–295. https://doi.org/10.1016/j.agee.2006.12.023

Bhattacharya, T., Kittur, S., Sathyakumar, S., & Rawat, G. S. (2012). Diet overlap between wild ungulates and domestic livestock in the greater Himalaya: Implications for management of grazing practices. *Proceedings of the Zoological Society*, *65*(1), 11–21. https://doi.org/10.1007/s12595-012-0025-4

Bison, M., Ibanez, S., Redjadj, C., Boyer, F., Coissac, E., Miquel, C., Rioux, D., Said, S., Maillard, D., Taberlet, P., Yoccoz, N. G., & Loison, A. (2015). Upscaling the niche variation hypothesis from the intra- to the inter-specific level. *Oecologia*, *179*(3), 835–842. https://doi.org/10.1007/s00442-015-3390-7

Biswas, S., & Sankar, K. (2002). Prey abundance and food habit of tigers (*Panthera tigris tigris*) in Pench National Park, Madhya Pradesh. *India. Journal of Zoology*, *256*(3), 411–420. https://doi.org/10.1017/s0952836902000456

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). Obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*, 176–182. https://doi.org/10.1111/1755-0998.12428

Bradley, B. J., Stiller, M., Doran-Sheehy, D. M., Harris, T., Chapman, C. A., Vigilant, L., & Poinar, H. (2007). Plant DNA sequences from feces: Potential means for assessing diets of wild primates. *American Journal of Primatology*, *69*(6), 699–705. https://doi.org/10.1002/ajp.20384

Cantalapiedra, J. L., FitzJohn, R. G., Kuhn, T. S., Fernández, M. H., DeMiguel, D., Azanza, B., Morales, J., & Mooers, A. Ø. (2014). Dietary innovations spurred the diversification of ruminants during the Caenozoic. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1776), 20132746. https://doi.org/10.1098/rspb.2013.2746

Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., Livingstone, S. W., & Wiens, J. J. (2020). Niche breadth: Causes and consequences for ecology, evolution, and conservation. *The Quarterly Review of Biology*, *95*(3), 179–214. https://doi.org/10.1086/710388

Chandranaik, B. M., Shivashankar, B. P., Giridhar, P., & Nagaraju, D. N. (2016). Molecular characterisation and serotyping of *Pasteurella multocida* isolates from Asiatic elephants (*Elephas maximus*). *European Journal of Wildlife Research*, *62*(6), 681–685. https://doi.org/10.1007/s10344-016-1043-8

Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: Toward a global functional homogenization? *Frontiers in Ecology and the Environment*, *9*(4), 222–228. https://doi.org/10.1890/080216

Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: An R package for the visualization of intersecting sets and their properties. *Bioinformatics*, *33*(18), 2938–2940. https://doi.org/10.1093/bioinformatics/btx364

Cristescu, M. E., & Hebert, P. D. N. (2018). Uses and misuses of environmental DNA in biodiversity science and conservation. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 209–230. https://doi.org/10.1146/annurev-ecolsys-110617-062306

Czernik, M., Taberlet, P., Świsłocka, M., Czajkowska, M., Duda, N., & Ratkiewicz, M. (2013). Fast and efficient DNA-based method for winter diet analysis from stools of three cervids: Moose, red deer, and roe deer. *Acta Theriologica*, *58*(4), 379–386. https://doi.org/10.1007/s13364-013-0146-9

de Sousa, L. L., Silva, S. M., & Xavier, R. (2019). DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA*, *1*(3), 199–214. https://doi.org/10.1002/edn3.27

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., Kartzinel, T. R. & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, *28*(2), 391–406. https://doi.org/10.1111/mec.14734

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S., & Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, *47*(1), 15–25. https://doi.org/10.1111/j.1365-2664.2009.01744.x

Ficetola, G., Coissac, E., Zundel, S., Riaz, T., Shehzad, W., Bessière, J., Taberlet, P., & Pompanon, F. (2010). An In silico approach for the evaluation of DNA barcodes. *BMC Genomics*, *11*(1), 434. https://doi.org/10.1186/1471-2164-11-434

Fløjgaard, C., De Barba, M., Taberlet, P., & Ejrnæs, R. (2017). Body condition, diet and ecosystem function of red deer (*Cervus elaphus*) in a fenced nature reserve. *Global Ecology and Conservation*, *11*, 312–323. https://doi.org/10.1016/j.gecco.2017.07.003

Food and Agriculture Organization of the United Nations. (2017). *FAOSTAT statistical database* Retrieved from http://www.fao.org/faostat/en/#data

Gotelli, N., Hart, E., & Ellison, A. (2015). *EcoSimR: Null model analysis for ecological data*. R Package Version 0.1. 0. Available at Github. Com/Gotellilab/EcoSimR. Accessed August, 29, 2017. https://doi.org/10.1016/0304-3800(94)00117-z

Gray, T. N., Prum, S., & Phan, C. (2016). Density and activity patterns of the globally significant large herbivore populations of Cambodia's Eastern Plains landscape. In: F. Ahrestani & M. Sankaran (Eds.), *The Ecology of Large Herbivores in South and Southeast Asia* (pp. 207–222). Dordrecht: Springer. https://doi.org/10.1007/978-94-017-7570-0_9

Gubbi, S., Harish, N., Kolekar, A., Poornesha, H., Reddy, V., Mumtaz, J., & Madhusudan, M. (2017). From intent to action: A case study for the expansion of tiger conservation from southern India. *Global*

Ecology and Conservation, *9*, 11–20. https://doi.org/10.1016/j.gecco.2016.11.001

Hardin, G. (1960). The competitive exclusion principle. *Science*, *131*(3409), 1292–1297.

Harisha, R., & Padmavathy, S. (2013). Knowledge and use of wild edible plants in two communities in Malai Madeshwara Hills, Southern India. *International Journal of Botany*, *9*, 64–72. https://doi.org/10.3923/ijb.2013

Harisha, R. P., Padmavathy, S., & Nagaraja, B. C. (2015). Traditional Ecological Knowledge (TEK) and its importance in south India: Perspecive from local communities. *Applied Ecology and Environmental Research*, *14*(1), 311–326. https://doi.org/10.15666/aeer/1401_311326

Hibert, F., Taberlet, P., Chave, J., Scotti-Saintagne, C., Sabatier, D., & Richard-Hansen, C. (2013). Unveiling the diet of elusive rainforest herbivores in next generation sequencing Era? The tapir as a case study. *PLoS One*, *8*(4), e60799. https://doi.org/10.1371/journal.pone.0060799

Hutchinson, G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist*, *93*(870), 145–159. https://doi.org/10.1086/282070

Ickes,K.(2001).Hyper-abundanceofNativeWildPigs(*Susscrofa*)inaLowland Dipterocarp Rain Forest of Peninsular Malaysia 1. *Biotropica*, *33*(4), 682–690. 10.1646/0006-3606(2001)033[0682:haonwp]2.0.co;2

IUCN (2020). *The IUCN Red List of Threatened Species*. Version 2019–3. Retrieved from https://www.iucnredlist.org

Kannan, R., Shackleton, C. M., Krishnan, S., & Shaanker, R. U. (2016). Can local use assist in controlling invasive alien species in tropical forests? The case of Lantana camara in southern India. *Forest Ecology and Management*, *376*, 166–173. https://doi.org/10.1016/j.foreco.2016.06.016

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, *112*(26), 8019–8024. https://doi.org/10.1073/pnas.1503283112

Kartzinel, T. R., & Pringle, R. M. (2020). Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology 89*, 1482–1496 https://doi.org/10.1111/1365-2656.13206

Kent, R., & Dorward, A. (2015). Livelihood responses to *Lantana camara* invasion and biodiversity change in southern India: Application of an asset function framework. *Regional Environmental Change*, *15*(2), 353–364. https://doi.org/10.1007/s10113-014-0654-4

Kimuyu, D. M., Veblen, K. E., Riginos, C., Chira, R. M., Githaiga, J. M., & Young, T. P. (2017). Influence of cattle on browsing and grazing wildlife varies with rainfall and presence of megaherbivores. *Ecological Applications*, *27*(3), 786–798. https://doi.org/10.1002/eap.1482

Kowalczyk, R., Taberlet, P., Coissac, E., Valentini, A., Miquel, C., Kamiński, T., & Wójcik, J. M. (2011). Influence of management practices on large herbivore dietCase of European bison in Białowieża Primeval Forest (Poland). *Forest Ecology and Management*, *261*(4), 821–828. https://doi.org/10.1016/j.foreco.2010.11.026

Kowalczyk, R., Wójcik, J. M., Taberlet, P., Kamiński, T., Miquel, C., Valentini, A., Craine, J. M. Coissac, E. (2019). Foraging plasticity allows a large herbivore to persist in a sheltering forest habitat: DNA metabarcoding diet analysis of the European bison. *Forest Ecology and Management*, *449*, 117474. https://doi.org/10.1016/j.foreco.2019.117474

Krishnamani, R. (1994). Diet composition of the bonnet macaque (*Macaca radiata*) in a tropical dry evergreen forest of southern India. *Tropical Biodiversity*, *2*(2), 285–302.

Kumar, R., Radhakrishna, S., & Sinha, A. (2011). Of least concern? Range extension by rhesus macaques (*Macaca mulatta*) threatens long-term survival of bonnet macaques (M. radiata) in peninsular India. *International Journal of Primatology*, *32*(4), 945–959.

Lamarque, F., Anderson, J., Fergusson, R., Lagrange, M., Osei-Owusu, Y., & Bakker, L. (2009). *Human-wildlife conflict in Africa: Causes, consequences and management strategies (No. 157)*. Food and Agriculture Organization of the United Nations (FAO).

Lopes, C. M., De Barba, M., Boyer, F., Mercier, C., da Silva Filho, P. J. S., Heidtmann, L. M., Galiano, D., Kubiak, B. B., Langone, P., Garcias, F. M., Gielly, L., Coissac, E., de Freitas, T. R. O., & Taberlet, P. (2015). DNA metabarcoding diet analysis for species with parapatric vs sympatric distribution: A case study on subterranean rodents. *Heredity*, *114*(5), 525–536. https://doi.org/10.1038/hdy.2014.109

MacArthur, R., & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, *101*(921), 377–385. https://doi.org/10.1086/282505

Madhusudan, M. D. (2004). Recovery of wild large herbivores following livestock decline in a tropical Indian wildlife reserve: Livestock decline and wild herbivore recovery. *Journal of Applied Ecology*, *41*(5), 858–869. https://doi.org/10.1111/j.0021-8901.2004.00950.x

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schäffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics*, *24*(16), 1757–1764. https://doi.org/10.1093/bioinformatics/btn554

Mundoli, S., Joseph, G., & Setty, S. (2016). "Shifting agriculture": The changing dynamics of Adivasi farming in the forest-fringes of a tiger reserve in South India. *Journal of Agroecology and Sustainable Food Systems*, *40*(8), 759–782. https://doi.org/10.1080/21683565.2016.1189475

Mychek-Londer, J. G., Chaganti, S. R., & Heath, D. D. (2020). Metabarcoding of native and invasive species in stomach contents of Great Lakes fishes. *PLoS One*, *15*(8), e0236077. https://doi.org/10.1371/journal.pone.0236077

Newmaster, S. G., Thompson, I. D., Steeves, R. A., Rodgers, A. R., Fazekas, A. J., Maloles, J. R., McMullin, R. T., & Fryxell, J. M. (2013). Examination of two new technologies to assess the diet of woodland caribou: Video recorders attached to collars and DNA barcoding. *Canadian Journal of Forest Research*, *43*(10), 897–900. https://doi.org/10.1139/cjfr-2013-0108

Nowak, R. M., & Walker, E. P. (1999). *Walker's Mammals of the World*, Vol. *1*. JHU press.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). *Vegan: Community ecology package*.

Öllerer, K., Varga, A., Kirby, K., Demeter, L., Biró, M., Bölöni, J., & Molnár, Z. (2019). Beyond the obvious impact of domestic livestock grazing on temperate forest vegetation–A global review. *Biological Conservation*, *237*, 209–219.

Padalia, H., Ghosh, S., Reddy, C. S., Nandy, S., Singh, S., & Kumar, A. S. (2019). Assessment of historical forest cover loss and fragmentation in Asian elephant ranges in India. *Environmental Monitoring and Assessment*, *191*(3), 802.

Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R. & Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology*, *107*(3), 1355–1376. https://doi.org/10.1111/1365-2745.13113

Pianka, E. R. (1974). Niche overlap and diffuse competition. *Proceedings of the National Academy of Sciences*, *71*(5), 2141–2145. https://doi.org/10.1073/pnas.71.5.2141

Pianka, E. R. (2011). *Evolutionary ecology*, Eric R. Pianka.

Pompanon, F., Deagle, B. E., Symondson, W. O. C., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: Diet assessment using next generation sequencing: NGS DIET ANALYSIS. *Molecular Ecology*, *21*(8), 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

Prasad, A. E. (2012). Landscape-scale relationships between the exotic invasive shrub *Lantana camara* and native plants in a tropical

deciduous forest in southern India. *Journal of Tropical Ecology*, *28*(1), 55–64.

Rayé, G., Miquel, C., Coissac, E., Redjadj, C., Loison, A., & Taberlet, P. (2011). New insights on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: Chamois diet in autumn as a case study. *Ecological Research*, *26*(2), 265–276. https://doi.org/10.1007/s11284-010-0780-5

Ripple, W. J., Newsome, T. M., Wolf, C., Dirzo, R., Everatt, K. T., Galetti, M., Hayward, M. W., Kerley, G. I., Levi, T., Lindsey, P. A., Macdonald, D. W., Malhi, Y., Painter, L. E., Sandom, C. J., Terborgh, J., Van Valkenburgh, B. & Van Valkenburgh, B. (2015). Collapse of the world's largest herbivores. *Science Advances*, *1*(4), e1400103. https://doi.org/10.1126/sciadv.1400103

Robeson, M. S., Khanipov, K., Golovko, G., Wisely, S. M., White, M. D., Bodenchuck, M., Smyser, T. J., Fofanov, Y., Fierer, N. & Piaggio, A. J. (2018). Assessing the utility of metabarcoding for diet analyses of the omnivorous wild pig (Sus scrofa). *Ecology and Evolution*, *8*(1), 185–196. https://doi.org/10.1002/ece3.3638

Rödder, D., & Engler, J. O. (2011). Quantitative metrics of overlaps in Grinnellian niches: Advances and possible drawbacks. *Global Ecology and Biogeography*, *20*(6), 915–927. https://doi.org/10.1111/j.1466-8238.2011.00659.x

Sato, J. J., Shimada, T., Kyogoku, D., Komura, T., Uemura, S., Saitoh, T., & Isagi, Y. (2018). Dietary niche partitioning between sympatric wood mouse species (Muridae: Apodemus) revealed by DNA meta-barcoding analysis. *Journal of Mammalogy*, *99*(4), 952–964. https://doi.org/10.1093/jmammal/gyy063

Schieltz, J. M., & Rubenstein, D. I. (2016). Evidence based review: Positive versus negative effects of livestock grazing on wildlife. What do we really know? *Environmental Research Letters*, *11*(11), 113003. https://doi.org/10.1088/1748-9326/11/11/113003

Shaanker, R. U., Ganeshaiah, K. N., Krishnan, S., Ramya, R., Meera, C., Aravind, N. A., Kumar, A., Rao, D., Vanaraj, G., Ramachandra, J., Gauthier, R., Ghazoul, J., Poole, N., & Gauthier, R. (2004). Livelihood gains and ecological costs of non-timber forest product dependence: Assessing the roles of dependence, ecological knowledge and market structure in three contrasting human and ecological settings in south India. *Environmental Conservation*, *31*(3), 242–253. https://doi.org/10.1017/s0376892904001596

Sharma, O. P., Sharma, S., Pattabhi, V., Mahato, S. B., & Sharma, P. D. (2007). A review of the hepatotoxic plant *Lantana camara*. *Critical Reviews in Toxicology*, *37*(4), 313–352. https://doi.org/10.1080/10408440601177863

Silori, C. S., & Mishra, B. K. (2001). Assessment of livestock grazing pressure in and around the elephant corridors in Mudumalai Wildlife Sanctuary, south India. *Biodiversity & Conservation*, *10*(12), 2181–2195.

Simberloff, D., & Dayan, T. (1991). The guild concept and the structure of ecological communities. *Annual Review of Ecology and Systematics*, *22*(1), 115–143. https://doi.org/10.1146/annurev.es.22.110191.000555

Soininen, E. M., Ehrich, D., Lecomte, N., Yoccoz, N. G., Tarroux, A., Berteaux, D., Gauthier, G., Gielly, L., Brochmann, C., Gussarova, G., & Ims, R. A. (2014). Sources of variation in small rodent trophic niche: New insights from DNA metabarcoding and stable isotope analysis. *Isotopes in Environmental and Health Studies*, *50*(3), 361–381. https://doi.org/10.1080/10256016.2014.915824

Soininen, E. M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., Gussarova, G., Bellemain, E., Hassel, K., Stenøien, H. K., Epp, L., Schrøder-Nielsen, A., Brochmann, C., & Yoccoz, N. G. (2015). Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS One*, *10*(1), e0115335. https://doi.org/10.1371/journal.pone.0115335

Soumya, K. V., Shackleton, C. M., & Setty, S. R. (2019a). Harvesting and Local Knowledge of a Cultural Non-Timber Forest Product (NTFP): Gum-Resin from *Boswellia serrata* Roxb. in Three Protected Areas of the Western Ghats, India. *Forests*, *10*(10), 907. https://doi.org/10.3390/f10100907

Soumya, K. V., Shackleton, C. M., & Setty, S. R. (2019b). Impacts of gum-resin harvest and *Lantana camara* invasion on the population structure and dynamics of Boswellia serrata in the Western Ghats, India. *Forest Ecology and Management*, *453*, 117618

Sukumar, R. (2006). A brief review of the status, distribution and biology of wild Asian elephants *Elephas maximus*. *International Zoo Yearbook*, *40*(1), 1–8.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C. & Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*, *35*(3), e14. https://doi.org/10.1093/nar/gkl938

Thornton, T. F., Puri, R. K., Bhagwat, S., & Howard, P. (2019). Human adaptation to biodiversity change: An adaptation process approach applied to a case study from southern India. *Ambio*, *48*(12), 1431–1446.

Valentini, A., Miquel, C., Nawaz, M. A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J. E., & Taberlet, P. Taberlet, P. (2009). New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: The *Trn* L approach. *Molecular Ecology Resources*, *9*(1), 51–60. https://doi.org/10.1111/j.1755-0998.2008.02352.x

Varghese, A., Ticktin, T., Mandle, L., & Nath, S. (2015). Assessing the effects of multiple stressors on the recruitment of fruit harvested trees in a tropical dry forest, Western Ghats, India. *PLoS One*, *10*(3), e0119634.

Voldstad, L. H., Alsos, I. G., Farnsworth, W. R., Heintzmann, P. D., Håkansson, L., Kjellman, S. E., Rouillard, A., Schomacker, A., & Eidesen, P. B. (2020). A complete Holocene lake sediment ancient DNA record reveals long standing high-arctic plant diversity hotspot in northern Svalbard. *Quaternary Science Reviews*, https://doi.org/10.1016/j.quascirev.2020.106207

Wilson, G., Desai, A. A., Sim, D. A., & Linklater, W. L. (2013). The influence of the invasive weed Lantana camara on elephant habitat use in Mudumalai Tiger Reserve, southern India. *Journal of Tropical Ecology*, *29*(3), 199–207. https://doi.org/10.1017/s0266467413000205

Zhang, J. (2016). *Spaa: SPecies association analysis*.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: ter Schure ATM, Pillai AAS, Thorbek L, et al. eDNA metabarcoding reveals dietary niche overlap among herbivores in an Indian wildlife sanctuary. *Environmental DNA*. 2020;00:1–15. https://doi.org/10.1002/edn3.168