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

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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), taupirs (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 trnL (UAA) intron (Taberlet et al., 2007), a universal plant marker specifically suited to environmental samples with degraded DNA. Most studies applying the trnL 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; Schiltz & Rubenstein, 2016). Furthermore, despite globally growing livestock numbers, there are relatively few studies specifically investigating impacts of domesticated herbivores on wild herbivores (see Schiltz & 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
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 km²) 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 hare (*Lepus nigricollis*),

![Figure 1](image.png)

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

Karnataka province boundary
MM hills boundary
Main road
Sampling location
Plantation
Barren
Built-up
Crop-land
Other
Water body
Scrub forest
Semi evergreen forest
Dry deciduous forest

*Environmental DNA: Optimization of the study and use of environmental DNA for invasive species management.*
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 (Stratec 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), 1x Buffer II, 2.5 mM MgCl₂, 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 v6.0.0 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
MOTU in each fecal sample. All further analyses were 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 spa 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 permustest function in vegan with 999 permutations. Pairwise calculations of Pianka’s niche overlap index (Pianka, 1974) were performed with the spa 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

comparing 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.
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 $8.23 \pm 0.55$ in MOTU richness and $1.24 \pm 0.09$ in Shannon diversity. MOTU richness is greatest for domestic goat, water buffalo and sambar deer (Table 1), with averages of $11.75 \pm 1.53$, $13.33 \pm 2.73$, and $12.40 \pm 0.93$, respectively. The Shannon diversity index also indicates the greatest dietary richness for these herbivore species, with index values: $1.78 \pm 0.18$, $1.88 \pm 0.30$, and $2.00 \pm 0.18$, respectively. The narrowest dietary niches are found for bonnet macaque and cattle samples (Table 1). Bonnet macaques have a dietary niche width of $4.83 \pm 0.91$ in MOTU richness and $0.78 \pm 0.19$ 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 diet of cattle is composed of 15 other plant families and at least 24 genera, the MOTU richness and Shannon diversity are still below average, with $6.22 \pm 0.69$, and $0.87 \pm 0.13$, 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 94% of *Ficus*) and 20% Poaceae reads (Figure 2 and Table S7).

### 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

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

Note: Feeding guild assignments are based on Nowak and Walker (1999), Ahrestani et al. (2012), Ahrestani and Sankaran (2016), and IUCN (2020).
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
of dispersion among species was supported by a nonsignificant permutation test ($p = .789$). 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 bamboo 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 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 $F_{9,52} = 3.79, R^2 = 0.40, p ≤ .001$). 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 *Sesamum indicum* L.; 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 cholesta-
sis 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 for-
estrs 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 herbivo-

res under study, the sambar deer and Asian elephant are, respec-
tively, 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 do-
mestic herbivores, combined with previous indications of live-
stock-mediated resource limitation in India (Madhusudan, 2004), suggests potential for competition between domestic and wild her-

bivores in the MMH area, especially in case of limited resource avail-

ability. 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 sug-
gested that competitive exclusion of wild herbivores occupying sim-
ilar 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 experi-

mental study in Kenya showed a reduction of land use by wildlife (includ-
ing 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 her-

bivores can act as carrier of disease, such as foot-and-mouth dis-

ease, potentially spreading to wild herbivores as suggested for two wildlife sanctuaries close to MMH (Chandranai 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 competi-
tion and interaction between wild and domestic species. Likewise, the encouraged use of the invasive plant L. camara by local commu-
nities 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 ex-
tension 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 contin-
uous 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 pro-
vided a starting point for tracking the effects of the environmental changes in MMH on local wildlife. As environmental change con-
tinues 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.

ACKNOWLEDGEMENT

We thank Dr. Harisha and Dr. Siddappa Setty for their botanical ex-
pertise of the MMH area and help with the taxonomical identifica-
tions and Dr. Ravichandran for his identification of fodder grasses. We gratefully acknowledge the help from Mr. Madesha and Mr. Narayanan during sample collections. We are further grateful to the Ecoinformatics lab at ATREE for use of their map and The Norwegian Sequencing Centre for their advice and plant DNA sequencing. The analyses were performed on the Abel computer cluster, owned by
the University of Oslo (UiO) and Uninet/Sigma2, and operated by the Department for Research Computing at USIT (UiO). GR acknowledges the support from Norwegian embassy for the travel grant to the UiO. Our work was conducted under Malai Mahadeshwara Hills Wildlife Sanctuary Permit -PCCF/[WF]/E2/CR/22/2014-15.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION
GR, RP, HdB and SB designed the study. GR, RP and BS collected the samples. AP and LT performed the laboratory work. BS performed the laboratory work for the reference library. AtS did the bioinformatic and statistical analyses. AtS made the figures and wrote the paper with input from RP, HdB and SB. All authors edited and made suggestions on the final text.

DATA AVAILABILITY STATEMENT
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.

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**SUPPORTING INFORMATION**

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