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Revealing cryptic interactions between large mammalian herbivores and plant-dwelling arthropods via DNA metabarcoding

Tali S. Berman and Moshe Inbar[1]

Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 3498838 Israel

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Abstract. In the past decade, it has become clear that omnivory, feeding on more than one trophic level, is important in natural and agricultural systems. Large mammalian herbivores (LMH) frequently encounter plant-dwelling arthropods (PDA) on their food plants. Yet, ingestion of PDA by LMH is only rarely addressed and the extent of this direct trophic interaction, especially at the PDA community level, remains unknown. Using a DNA-metabarcoding analysis on feces of free-ranging cattle from a replicated field experiment of heavily and moderately grazed paddocks, we reveal that feeding cattle (incidentally) ingest an entire food chain of PDA including herbivores, predators and parasites. Overall, 25 families of insects and four families of arachnids were ingested, a pattern that varied over the season, but not with grazing intensity. We identified the functional groups of PDA vulnerable to ingestion, such as sessile species and immature life stages. Most of the fecal samples (76%) contained sequences belonging to PDA, indicating that direct interactions are frequent. This study highlights the complex trophic connections between LMH and PDA. It may even be appropriate to consider LMH as omnivorous enemies of PDA.

Key words: arthropods; DNA metabarcoding; grazing; incidental ingestion; large mammalian herbivores; omnivory; trophic cascades.

Introduction

Understanding natural food webs is challenging, especially when consumers feed on more than one trophic level (i.e., omnivory). Large mammalian herbivores (LMH) widely affect the function of terrestrial ecosystems by altering the structure, diversity and distribution of vegetation (McNaughton et al. 1989, Gordon and Prins 2007). These effects can indirectly influence plant-dwelling arthropods (PDA) that depend on the plants for food and shelter. LMH may also directly affect PDA by ingesting them when feeding. This direct interaction, especially the ingestion of herbivorous arthropods, is a classic case of intraguild predation; a given species feeding on another species that shares the same food resource (Polis and Holt 1992). While plant-mediated indirect effects of LMH on PDA are well documented (van Klink et al. 2015), direct trophic effects have hardly been studied (Gish and Ben-Ari 2017).

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[1] Corresponding Author. E-mail: minbar@research.haifa.ac.il

Large mammalian herbivores consume large amounts of diverse plants, which are often inhabited by PDA. Therefore, PDA are in danger of being ingested. The importance of this direct trophic interaction can be inferred from studies that examined its impact on PDA that feed inside fruits and seeds; a fact that also makes them relatively easy to study and quantify (Gish et al. 2017). Yet, most PDA feed inside leaves, stems and shoots or feed freely on the surface of plants. Only rare observations documented incidental ingestion of PDA by LMH (Gish and Dafni 2010, van Noordwijk et al. 2012). While LMH may ingest small, harmless, and relatively immobile PDA, they can efficiently avoid ingesting noxious arthropods (Berman et al. 2017, 2018, Berman et al. 2019*a*, *b*) that may harm them (Webb et al. 2004, Ferrer et al. 2007). The lack of data on the extent and impact of these direct trophic interactions, especially at the PDA community level, can be attributed to the difficulty of observing and quantifying their ingestion by LMH. Currently it is unknown which PDA are vulnerable (or not) to ingestion or how this interaction may influence the overall functioning of terrestrial food webs.

DNA metabarcoding of environmental DNA has opened up new ways for studying food webs. It involves high-throughput sequencing of target DNA from bulk samples that are compared with a database to identify taxonomic origin. This method has characterized animal diets (Clare 2014), detected hidden (i.e., cryptic) trophic interactions (Pringle and Hutchinson 2020) and revealed unknown diet components, such as niche partitioning and specialization in a variety of animals (Sousa and Silva 2019), including LMH (Kartzinel et al. 2015, Garnick and Barboza 2018). Despite the potential of DNA metabarcoding to provide new insights into poorly understood trophic interactions, it has never been used to examine direct trophic impacts of LMH on PDA.

Using a replicated field experiment of moderately and heavily grazed paddocks, we assessed PDA ingestion over time in feces of free-ranging cattle using primers targeting mitochondrial markers (COI) of arthropods. In addition, we sampled PDA from the pasture as a reference to the molecular analysis. We addressed the following questions: (1) Which functional feeding groups of PDA (herbivores, predators, parasites, etc.) are ingested by cattle? (2) Does the composition of ingested PDA change over time and grazing intensity? (3) Does the composition of ingested PDA reflect that observed in the pasture? (4) How frequent are these interactions?

Methods

Experimental setup and sampling protocols

The study was conducted in 2019 at the 'Karei Deshe' research farm, located in the eastern Galilee of Israel (Mediterranean climate grassland, see Appendix S1). The farm (1,450 ha) has been divided since 1994 into paddocks that are subjected to moderate and heavy cattle grazing throughout the year (0.55 and 1.1 cows ha^{-1}, respectively). Arthropod abundance and diversity may vary due to grazing intensity (Takagi and Miyashita 2014, van Klink et al. 2015), therefore our experiment included two moderately and two heavily grazed paddocks (~27 ha each). Cattle feces and arthropods were collected from the paddocks once a month, from March to May (beginning until the end of spring, Data S1). During this period, arthropod abundance is high due to warm temperatures and increased vegetation growth. Samples were collected under sterile conditions.

Cattle fecal samples.—We walked across each paddock in search of fresh (hours old) dung piles, which were at least 20 m apart (to reduce the likelihood of collecting feces from the same cow). Once located, a sample of 50 mL was collected with a spatula from several parts within the center of the dung pile (avoiding the outer crust and any visible coprophagous arthropods) and stored in a cooler box with ice packs. In total, we obtained 120 samples (10 samples from four paddocks, over three months). Upon return to the laboratory (within 6 h) the samples were homogenized (thoroughly mixed) and kept at −80°C until DNA extraction.

Arthropod samples.—Arthropods were collected with a Vortis insect suction sampler (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) from six 3 m-long transects located randomly within each paddock (minimum of 20 m apart). Each suction was performed on the vegetation at a height of 30–50 cm from the ground, for 15 s. After each sampling (transect), the contents of the suction sampler were emptied into 50 mL tubes containing 75% ethanol and stored in a cooler box with ice packs. Samples were refrigerated until identification.

Identifying arthropods using DNA metabarcoding

Arthropod DNA was amplified using the Zeale et al. (2011) COI mitochondrial markers (~157 bp amplicon) and sequenced on an Illumina MiniSeq system (Illumina, San Diego, CA, USA) at the DNA Services Facility, University of Illinois, Chicago, USA (87 fecal samples were sequenced based on gel verification, Data S1; see Appendix S1). COI markers were chosen because they are supported by a large and well curated reference database (Ratnasingham and Hebert 2013) and enable species-level discrimination (Deagle et al. 2014). The Zeale et al. (2011) primers have been specifically applied in a variety of dietary studies (Alberdi et al. 2018).

Sequence analysis and taxonomic identification.—Sequences were processed using the DADA2 pipeline (Callahan et al. 2016) in R. This pipeline turns amplicon data into denoised, merged, chimera-free, inferred sequences by correcting errors present after Illumina sequencing (Appendix S1). The process generated an amplicon sequence variants (ASVs) table containing 1,641,797 high-quality reads. ASVs with sequences longer or shorter than the expected barcode length (>170 bp or <150 bp) were eliminated, retaining 695,007 reads binned in 417 ASVs.

The ASV sequences were aligned to the NCBI GenBank nt-database and the BLAST output files were imported into MEGAN v.6 (Huson et al. 2016) for taxonomic analysis (Appendix S1). Unassigned sequences were further examined against the BOLD database (Ratnasingham and Hebert 2007). Only sequences assigned to insects and arachnids were kept. To provide a more accurate and conservative community estimate, we removed any taxa found in a single sample alone (listed in Data S2), retaining 526,892 reads and 101 ASVs (Data S3). The raw sequence data are available at the NCBI database under BioProject accession number PRJNA579572.

Data analysis and statistics

DNA metabarcoding data.—To begin, the data were rarefied to 925 reads per sample (Appendix S2: Fig. S1) to avoid sequencing depth-related bias using the

"vegan" R package (Oksanen et al. 2007). As a result, 51 of 87 fecal samples were retained and binned in 77 ASVs (Data S4).

Two approaches are commonly used to interpret sequence data—occurrence of taxa (presence/absence) and relative read abundance (RRA). While occurrence is considered to be a more conservative method, RRA may actually provide a more accurate view of population-level estimates (Deagle et al. 2018). We therefore chose to use RRA as the primary basis for inference in this study (calculated as the proportion of reads of each ASV in each fecal sample), as has also been done in previous DNA-metabarcoding studies of insectivore diets (Pringle 2019). As a sensitivity check against the possible biases in RRA (which can arise from differential digestibility, amplification biases, etc.), we also conducted a supporting analysis of presence/absence data using percentage of occurrence (POO, calculated as the number of samples containing a given food item, rescaled to 100% across all food items; see Deagle et al. 2018; Appendix S1, Data S5). In both datasets we set a "true presence" threshold—any sequences with an abundance of <1% within samples were removed (Deagle et al. 2018; Data S6). No assumptions were made regarding the actual proportions or biomass of arthropods ingested.

To investigate how similar the overall arthropod community (ASVs) was among sampling months (March–May) and between grazing intensities (moderate vs. heavy), a non-metric multidimensional scaling analysis (NMDS) was performed for each treatment using Primer v7 software (RRA: Bray–Curtis similarity matrix; POO: Jaccard similarity matrix). To test whether the community differed among sampling months and between grazing intensities, a permutational multivariate analysis of variance (PERMANOVA) was run with 999 permutations using the R "vegan" function *adonis* (RRA: Bray–Curtis; POO: Jaccard). As no significant difference was found between grazing intensities for RRA or POO (*adonis*: RRA, $R = 0.014$, $P = 0.629$; POO, $R = 0.021$, $P = 0.255$; Appendix S3: Fig. S2c, d, Table S1), and since the interaction between treatments was non-significant (*adonis*: RRA, $R = 0.037$, $P = 0.442$; POO: $R = 0.044$, $P = 0.205$), grazing intensity was removed from the analysis, resulting in four replicate paddocks per month.

Diversity (Shannon H') and richness (Fisher's alpha) indices were calculated for RRA with PAST (Hammer and Harper 2001) for the total arthropod community (ASVs) detected in the feces and separately for arthropods presumed to have been ingested (i.e., excluding dung-associated arthropods, which are likely to have colonized the feces prior to collection). The indices were compared among sampling months using a Kruskal–Wallis test (Dunn–Bonferroni post hoc) in SPSS software v.25 (IBM, Armonk, NY, USA).

Arthropod taxa were assigned to functional feeding groups based on their biology (Data S7, see Appendix S1): herbivores, predators, parasites, dung-associated arthropods (coprophages), aquatic arthropods or unknown. Herbivores were further classified by feeding niche: (1) exophages: feed on the surface of the plant; (2) endophages: feed within plant tissue; (3) unknown. The mean RRA of the functional feeding groups were compared among sampling months using a Kruskal–Wallis test (Dunn–Bonferroni post hoc). The number of genera per functional feeding group was compared among sampling months using the Pearson chi-squared test. Both tests were performed using SPSS software v.25.

Arthropod suction sampler data.—Arthropods collected using the suction sampler were sorted under a dissecting microscope and identified at least to the family level. To estimate the number of arthropods collected in each sample (transect), we ranked them based on categories (intervals of 25): ≤10, ≤25, ≤50, ≤75 and so forth. Categories were averaged per month (Data S8).

Results

We uncovered DNA sequences from a variety of taxonomic and functional groups, including PDA ingested while grazing, aquatic arthropods ingested while drinking and dung-associated arthropods (coprophagous) that infested the feces after defecation (Fig. 1a). Altogether, 70 ASVs of arthropods were identified from 51 fecal samples (5.5 ASVs per sample on average). The final reads represented a total of 39 genera in 33 families and eight orders of insects (94% RRA; 93% POO; for full occurrence results; see Appendix S3), as well as seven genera in six families and four orders of arachnids (6% RRA; 7% POO).

As noted above, fecal samples collected under field conditions may be contaminated with DNA of coprophages. Indeed, a large proportion of RRA belonged to dung-associated arthropods (60% RRA, Fig. 1b; 35% POO; Appendix S3: Fig. S1b), mostly Diptera (house flies, Muscidae, and black scavenger flies, Sepsidae). Approximately 6% of reads (7% POO; Appendix S3: Fig. S1b) belonged to arthropods whose functional group could not be determined (Fig. 1b; Data S7).

PDA ingested by cattle

The cattle ingested an entire food chain of PDA while grazing, including a large variety of herbivores together with their predators and parasites (34% RRA, Fig. 1b; 57% POO; Appendix S3: Fig. S1b). Overall, the cattle ingested 22 families of herbivores (87% of both RRA and POO of PDA) mainly Lepidoptera, Diptera, Hemiptera, Coleoptera and Trombidiformes. They also ingested four families of predatory Araneae (spiders) and Neuroptera (8% RRA and 9% POO of PDA); and two families of parasitic wasps (Hymenoptera, 5% RRA of PDA; Fig. 1b, c; 4% of POO of PDA; Appendix S3: Fig. S1b, c). Half of the ingested herbivores were exophages (54%, 18 genera; 58% POO), and one-quarter

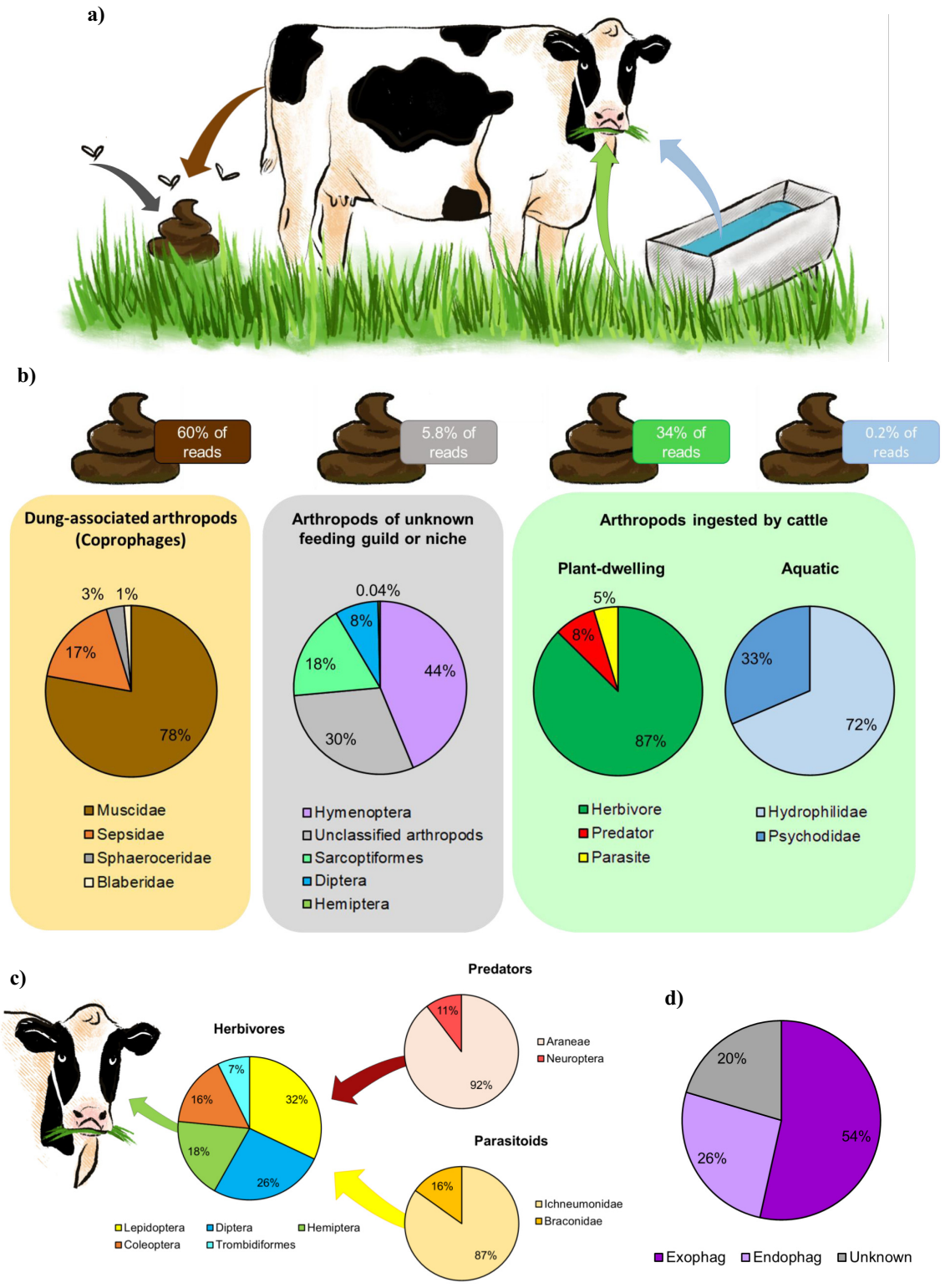

FIG. 1. Diversity of arthropods ingested by cattle. The pie charts show the mean proportion of reads (RRA) averaged across March–May. (a) The different sources of arthropods detected in the cattle feces. Plant-dwelling arthropods (PDA) ingested while grazing, aquatic arthropods ingested while drinking and dung-associated arthropods (b) Assembly of the main functional feeding groups of arthropods detected in cattle feces. (c) Assembly of PDA detected in cattle feces. (d) Feeding niches of the ingested herbivorous arthropods (exophages: feed on the plant surface; endophages: feed within plant tissue). Cow illustration by Lina Gurevich.

were endophages (26% of both RRA and POO, 10 genera; Fig. 1d; Appendix S3: Fig. S1d). Interestingly, the cattle also ingested aquatic arthropods while drinking (0.2% RRA; Fig. 1b; 2% POO; Appendix S3: Fig. S1b), mostly larvae of water beetles (Hydrophilidae; Fig. 1b), that were observed in the watering troughs throughout the farm. The frequency of direct consumptive interactions between cattle and PDA was high; 76% of samples (39/51) that passed quality control contained arthropods ingested by the cattle.

Seasonal variation of PDA ingested by cattle.—Arthropod abundance and composition in Mediterranean habitats change over the season. This also reflected in the cattle feces, as the overall arthropod community varied significantly among sampling months for both RRA and POO (*adonis*: RRA, $R = 0.077$, $P = 0.021$; POO, $R = 0.083$, $P = 0.002$; Appendix S3: Fig. S2a, b), even when analyzing arthropods ingested by cattle alone (ASVs of coprophages were removed from RRA and POO analysis, retaining 57/52 ASVs from 47/42 samples respectively, *adonis*: RRA, $R = 0.102$, $P = 0.001$; POO, $R = 0.111$, $P = 0.001$).

Diversity (Shannon H'), yet not richness (Fisher's alpha), was significantly higher in May (end of spring) compared with April (spring) for the overall arthropod community (Kruskal–Wallis test: Shannon H', $\chi^2_2 = 7.496$, $P = 0.024$; Fisher's alpha: $\chi^2_2 = 5.466$, $P = 0.065$; Data S9a). Diversity estimates of arthropods ingested by cattle alone did not differ among sampling months (Kruskal–Wallis test: Shannon H', $\chi^2_2 = 4.503$, $P = 0.105$; Fisher's alpha: $\chi^2_2 = 3.042$, $P = 0.219$; Data S9b).

To investigate which PDA families might be more prone to ingestion over the season, those with the highest relative abundances were examined (for order abundance see Appendix S3: Fig. S3). In total, 15 families displayed a mean relative abundance of over 2% across all sampling months, which changed over the season (Fig. 2a). Most PDA ingested across the season were herbivores (mostly exophages; Appendix S4: Fig. S1). Parasitoids and endophages were abundant in May, while the abundance of predators remained steady over time. No seasonal trend of ingestion was evident at the genus level (Appendix S4: Fig. S1).

Arthropods that were not ingested by cattle

Half of the arthropod taxa collected by the suction sampler were also detected in the cattle feces, including Diptera, Hymenoptera, Lepidoptera, Hemiptera, Coleoptera, Araneae and Sarcoptiformes (Fig. 2b). As seen in the feces, Diptera was one of the most abundant orders collected by the suction sampler. The other half consisted of arthropods that were present in the suction sampler but were absent in the feces. Spittlebugs (Cercopidae, Hemiptera) and grasshoppers (Orthoptera) that were common in the field; bees and ants (both Hymenoptera) were not detected in any sample (Fig. 2b). Endophages cannot be collected using the suction sampler, therefore they only appeared in the cattle feces.

Discussion

Ingestion of PDA by LMH is only rarely addressed and the extent of this direct trophic interaction is currently unknown (van Klink et al. 2015, Gish et al. 2017). Our research reveals for the first time that free-ranging cattle incidentally ingest an entire food chain of arthropods from multiple trophic levels. Most of the cattle feces (76%) contained PDA, implying that this interaction is prevalent and may be a significant form of top-down control on PDA. The composition and diversity of PDA ingested by cattle, and LMH in general, is likely to be influenced by: the availability and palatability of the plants in the habitat, the foraging behavior of the specific LMH and the seasonal shifts in both plant and PDA communities. In this study we identified the key factors that may influence the intensity of this direct trophic interaction: feeding niche and the mobility of PDA, grazing intensity and seasonality.

Although cattle incidentally prey on a variety of PDA (Fig. 1b), the most vulnerable groups were characterized by reduced mobility.

Endophagous PDA

One-quarter of the ingested PDA were endophages (Fig. 1d). These PDA are more susceptible to ingestion as they feed within plant parts from which they are unable to escape. Consequentially, they were not collected with the suction sampler. Ingestion of endophages by LMH has been documented in species feeding within fruits and seeds (Gish et al. 2017). We revealed additional endophagous species that are prone to ingestion, including leaf and grass miners (Agromyzidae and Elachistidae), plant parasitic mites (Eriophyidae), gall midges (Cecidomyiidae), and boring caterpillars.

Immature life stages of PDA

Part of the PDA ingested by the cattle have highly mobile adult stages. These included herbivorous moths (Noctuidae) and fruit flies (Drosophilidae); predatory lacewings (Chrysopidae) and parasitic wasps, which were probably ingested with their hosts (secondary ingestion). Although DNA metabarcoding cannot discriminate between larvae and adults, we can assume that these PDA were ingested during their immobile, immature phases (i.e., eggs, pupae, larvae) as winged adults can more readily escape danger.

Some PDA groups were absent from the cattle feces. These included grasshoppers, bees, ants and spittlebugs; which were collected by the suction sampler (Fig. 2b). Mobility (flying, jumping walking) and avoidance

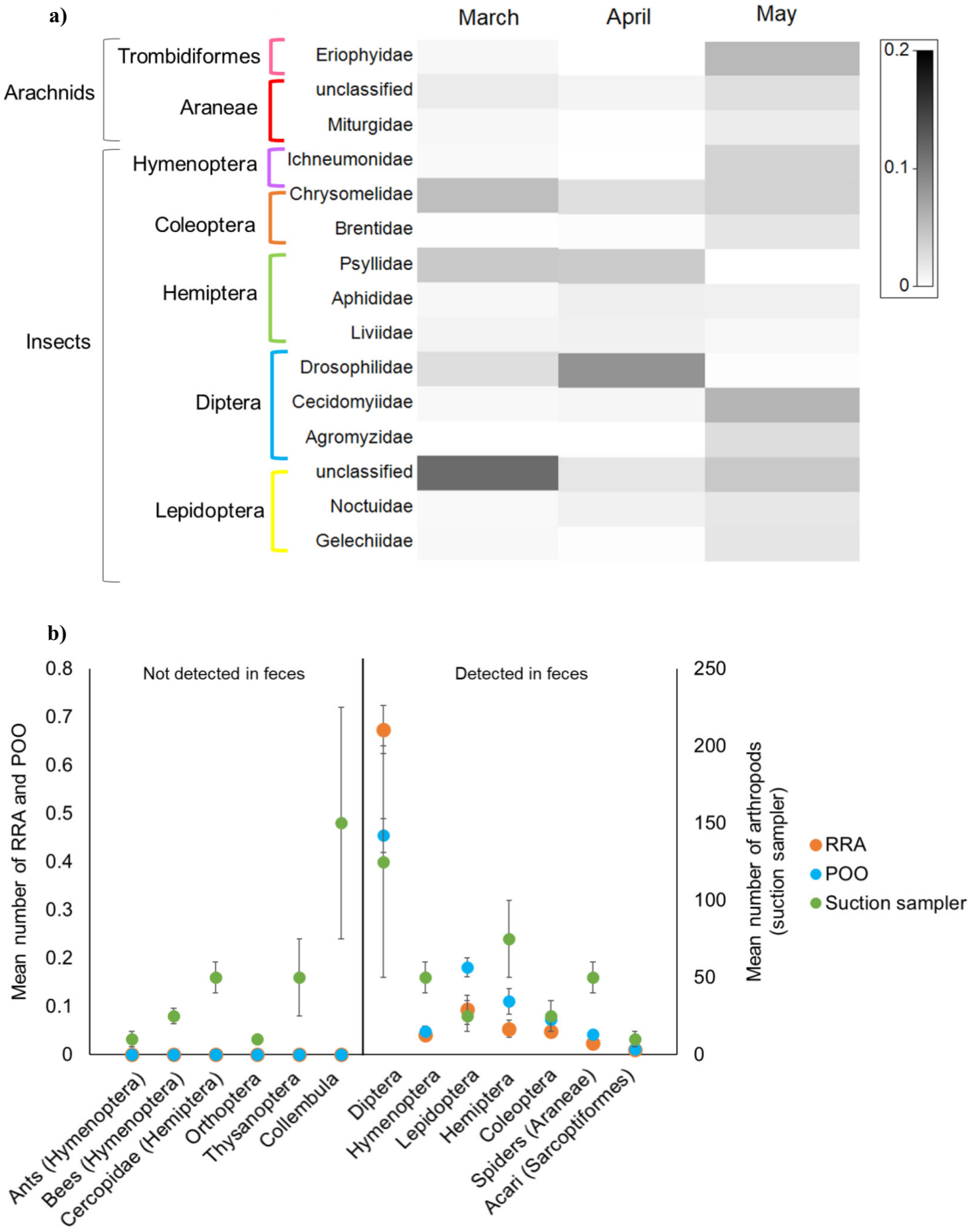

FIG. 2. (a) Heat map of the most abundant plant-dwelling arthropod families ingested by cattle and their taxonomic classification (rows) across three sampling months (columns). The 15 most abundant families (>2% of reads) across sampling months are presented. The gray scale represents the mean relative abundance of each family (white squares indicate an absence of reads). (b) Arthropods ingested by cattle (DNA metabarcoding) and arthropods present in the paddocks (suction sampler). The mean relative abundance (RRA), percentage of occurrence (POO) and the mean number of arthropods collected using a suction sampler were averaged across March–May. Points indicate means ± SE. The left-hand side shows arthropods that were present in the suction sampler but were hardly detected or absent in the feces. The right-hand side shows arthropods that were present both in the suction sampler and the feces. Endophages (feed within plant tissue) could not be collected with the suction sampler and therefore do not appear in this illustration.

behaviors, both of PDA and cattle, probably explain why certain PDA were not ingested.

Mobile PDA

Grasshoppers and spittlebugs, common PDA in the farm, are highly mobile and therefore able to escape the plant when approached by a cow. Grasshoppers may even be common in grazed habitats (Zhong et al. 2014), primarily due to plant-mediated mechanisms. But the fact that they can escape ingestion may contribute to their success in grazed areas. Not surprisingly, these PDA were never detected in the feces.

Exophagous PDA

Mobile exophages feed freely on the plant surface and may be able to detect and avoid ingestion. Even less immobile exophages may actively escape ingestion by dropping or rolling off the plant (Brackenbury 1997, Gish et al. 2010). By moving the plant while feeding, LMH may also cause exophages to passively fall of the plant. Certain PDA have even developed specific adaptations to minimize the risk of being ingested by LMH (Bennett et al. 2015, Ben-Ari et al. 2019). These abilities suggests that direct interactions are frequent and important.

Noxious PDA

Some PDA (e.g., ants and bees) can deter LMH with aggressive behavior (King and Douglas-Hamilton 2007, Martins 2010). Moreover, LMH may actively avoid ingesting noxious PDA. We recently showed that grazing cattle and goats are able to avoid ingesting webworms (Berman et al. 2017, 2018), which may harm them (Webb et al. 2004). Not surprisingly, webworms were never detected in the feces despite their abundance in the study area (Berman et al. 2018).

The composition and diversity of PDA ingested by cattle changed significantly over the season (Fig. 2; Appendix S3: Fig. S2a, b, Data S9b). This might occur as the abundance and composition of PDA and their food plants change over time in Mediterranean habitats. Grazing itself may also indirectly affect PDA abundance over the season (Lazaro et al. 2016, Zhu et al. 2020). Higher trophic levels (parasitoids and some predators) seemed to be ingested later in the season (Fig. 2a; Appendix S3: Fig. S4), probably as they follow the development of their hosts/prey (herbivores). Similarly, endophages were more prevalent in May as they develop within plant tissue that protects them from the hot Mediterranean summer. As LMH graze all year round, they are likely to consume different PDA species during different seasons of the year.

Moderate grazing, as opposed to heavy grazing, may positively affect PDA diversity and composition through plant-mediated mechanisms (van Klink et al. 2015). In our study however, PDA communities ingested by cattle were similar between moderately and heavily grazed paddocks (Appendix S3: Fig. S2c,d). A long-term experiment conducted in "Karei Deshe" farm showed that despite significant differences between grazing intensities, the plant community remained relatively steady in high stocking densities (Sternberg et al. 2015). This might explain why the diversity of PDA in the feces was similar between paddocks. Variation in ingested PDA is expected to show when grazing intensity significantly alters the plant community.

DNA metabarcoding has made it possible to detect and resolve previously cryptic interactions within food webs (Pringle and Hutchinson 2020). Such interactions between LMH and PDA at the community level, as uncovered in this study, would be nearly impossible to detect using conventional techniques. This method can enable us to identify vulnerable PDA and species adapted to avoid incidental ingestion by LMH. Yet, DNA metabarcoding has its limitations (Deagle et al. 2018, Pringle and Hutchinson 2020). Wide range primers used in these studies may be less specific, may amplify non-target taxa and provide less data at the species level. The Zeale et al. (2011) primers have been frequently used to analyze arthropods in animal diets (Alberdi et al. 2018). Yet, they have been claimed to overestimate Lepidoptera and Diptera (Clarke et al. 2014). These orders were dominant in the cattle feces (~50% of RRA), however they are also abundant in Mediterranean grasslands. Future studies should include an additional set of primers or barcodes, such as ribosomal markers, to reduce potential biases (Deagle et al. 2014, Alberdi et al. 2018). Overall, the taxonomic resolution of these primers was sufficient to assign most arthropods to their functional feeding groups.

The main drawback of DNA metabarcoding is its limited ability to provide quantitative data (Deagle et al. 2018, Pringle and Hutchinson 2020), whether using occurrence or RRA data. Despite some differences in the proportion of arthropods detected using RRA and occurrence data, both methods strongly correlated and presented qualitatively similar outcomes (Appendix S3).

Most of the cattle fecal samples (76%) contained DNA of PDA. In reality, cattle herds, in which a single cow can produce manure equivalent to 5–6% of its body weight each day (Font-Palma 2019), may prey on numerous PDA a day. The large amounts of plants eaten by cattle and the frequency of arthropod DNA detected in their feces suggest that this direct interaction between them is strong and common. Intraguild predation of PDA by LMH may shape the entire community structure of grazing ecosystems.

The ingestion of PDA by LMH may initiate cascading effects down the food chain. Trophic cascades are indirect species interactions that originate with a predator and spread down the food web. Most studies of trophic cascades including LMH have focused on the indirect effect that large predators exert on plant communities through predation of LMH (Ford et al. 2014, Ripple

et al. 2014). We suggest that large predators may have deeper cascading effects on PDA communities. By removing LMH from the ecosystem, large predators might benefit certain PDA species that are vulnerable to ingestion. Ingestion of PDA by LMH may also have knock-on effects, effects that spin-off from the main interaction chain, on other members of the food web. For instance, by reducing the population of PDA in a habitat, LMH may negatively impact insectivorous birds and reptiles who prey on them (Gill and Fuller 2007, Mohanty et al. 2016). Therefore, by ingesting a large variety of PDA, LMH may considerably impact other insectivorous animals in the shared habitat. Future studies in the field should focus on understanding the role of direct vs. plant-mediated effects of LMH on PDA communities.

Our previous findings (Gish et al. 2017) suggested that direct trophic interactions are common and more complex than previously considered, having profound consequences for both LMH and PDA. Direct ingestion by LMH can locally remove vulnerable PDA from the habitat especially in chronically grazed systems. LMH may select for escape strategies in PDA to minimize the risk of incidental ingestion. LMH and herbivores in general, may benefit from ingesting innocuous arthropods by supplementing their plant diet with important (but scarce) nutrients and minerals (e.g., nitrogen, sodium and magnesium). Direct ingestion of PDA probably occurs in other grazing animals, such as manatees, geese and phytophagous reptiles. It may therefore be appropriate to consider these obligate herbivores as omnivores.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3548/suppinfo

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All data supporting the results is available at the NCBI database under BioProject accession number PRJNA579572 at: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA579572. Data (Inbar and Berman 2021) are also available on Dryad: https://doi.org/10.5061/dryad.ns1rn8pt7.