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The prospects of poop: a review of past achievements and future possibilities in faecal isotope analysis

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ABSTRACT

What can the stable isotope values of human and animal faeces tell us? This often under-appreciated waste product is gaining recognition across a variety of disciplines. Faecal isotopes provide a means of monitoring diet, resource partitioning, landscape use, tracking nutrient inputs and cycling, and reconstructing past climate and environment. Here, we review what faeces are composed of, their temporal resolution, and how these factors may be impacted by digestive physiology and efficiency. As faeces are often used to explore diet, we clarify how isotopic offsets between diet and faeces can be calculated, as well as some differences among commonly used calculations that can lead to confusion. Generally, faecal carbon isotope ($\delta^{13}C$) values are lower than those of the diet, while faecal nitrogen isotope values ($\delta^{15}N$) values are higher than in the diet. However, there is considerable variability both within and among species. We explore the role of study design and how limitations stemming from a variety of factors can affect both the reliability and interpretability of faecal isotope data sets. Finally, we summarise the various ways in which faecal isotopes have been applied to date and provide some suggestions for future research. Despite remaining challenges, faecal isotope data are poised to continue to contribute meaningfully to a variety of fields.

Key words: carbon, nitrogen, digestion, discrimination, turnover.

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I. INTRODUCTION

Faeces archive considerable information about both modern and ancient animals and humans, as well as the environments in which they live. Scientists from multiple disciplines have increasingly been using stable isotope values measured in faeces to investigate foraging behaviour, spatial ecology, and climate conditions in modern, ancient, and even palaeontological settings. Faeces are in many ways an ideal substrate for stable isotope research because they are readily locatable and identifiable, can be collected non-invasively (contact with the producing animal is not required), and record short-term dietary information at a resolution unlikely to be preserved in many other animal tissues. Under the right conditions, they can also preserve ecological information for decades to millennia. Nonetheless, the use of faecal material comes with several uncertainties. It is not yet clear, for example, how digestive physiology and dietary composition impact the composition of faeces. The period of time reflected in faecal isotope values also remains uncertain. Although a number of studies have now sought to determine the degree to which diet and faeces differ isotopically for a range of different organisms, their results vary tremendously.

Our goals in this paper are to: (*i*) synthesise current understanding of factors influencing faecal isotope values; (*ii*) summarise current applications in the fields of ecology, palaeoecology, palaeoclimatology, and archaeology; and (*iii*) make suggestions for future research, including recommendations for standardising experimental and field-based work. Although researchers are starting to work with non-traditional isotope systems like sulphur and strontium (Salvarina *et al.*, 2013; Lewis *et al.*, 2017; Crowley, Wultsch & Kelly, 2019; Crowley *et al.*, 2023; Weber *et al.*, 2020), the vast majority of research to date has focused on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values. We will therefore concentrate on these systems in this review.

Using stable isotopes to explore dietary ecology relies on the idea that 'you are what you eat plus a few per mil' (DeNiro & Epstein, 1976). The ratio of heavy to light stable isotopes of a given element in the tissues and waste products of animals reflects consumed food and water with a small offset, the size of which depends on the sampled material. While some materials, like bone collagen or muscle, integrate dietary information over months to years, others, like blood plasma, breath, and faeces, reflect much shorter periods of time, on the order of hours to days (Davis & Pineda-Munoz, 2016). Carbon isotope values are primarily driven by the photosynthetic pathway of plants (either consumed directly or at the base of the food web), with C_3 plants having much lower $\delta^{13}C$ values than C_4 plants and crassulacean acid metabolism (CAM) succulents exhibiting a large range of values depending on environmental conditions (e.g. O'Leary, 1988). Nitrogen isotope values primarily provide information about the amount and source of protein in an animals' diet (e.g. DeNiro & Epstein, 1981; Koch, 2007) but can also provide details about internal protein recycling, nutritional stress, and habitat aridity (Heaton, 1987; Sealy *et al.*, 1987; Ambrose, 1991; Sponheimer *et al.*, 2003*b*).

II. OVERVIEW OF C AND N ISOTOPES IN FAECES

In this section, we summarise our current knowledge of the factors that influence carbon and nitrogen isotopes in faeces. We discuss the composition of faeces; the degree to which diet and faeces differ isotopically (called discrimination, Δ) for different types of taxa; the period of time over which faeces integrate dietary information; how faecal isotope values may be influenced by digestive physiology, nutritional composition, and dietary variability; and finally, the impact of study design on discrimination values.

(1) What are faeces composed of?

Faecal material is not homogenous; it is composed of a combination of undigested food and endogenous debris, including sloughed epithelial cells, enzymes, mucous, and microbes (Robbins, 1993). The relative proportions of these materials are likely to vary not only among species, but also within individuals over time (e.g. ontogenetically, day-to-day, seasonally). The difficulty in apportioning these different materials within faecal samples has made faecal isotope data more challenging to interpret compared to data for animal tissues (Reid & Koch, 2017; R. E. B. Reid and B. E. Crowley, personal observations). The possibility for isotopic changes during digestion, and uncertainty in the amount of time represented by a given faecal sample, are additional complicating factors that we discuss in greater detail below.

Quantitatively partitioning faeces into components (i.e. undigested material *versus* bacterial or endogenous debris) has been of interest both from the perspective of nutrient cycling in agroecology and agronomy as well as digestive efficiency in animal physiology and nutrition. To our knowledge, studies of this nature have largely focused on ruminant ungulates with little to no attention paid to mammalian omnivores or carnivores, let alone other types of organisms.

Although limited, experiments with ruminants using ^{15}N-labelled food suggest that bacterial and endogenous debris make up a greater proportion of faeces than undigested foods. For example, undigested material accounted for just 11% of total nitrogen in faeces from a

cow (*Bos primigenius taurus*) fed ^{15}N-labelled hay, while 77% was derived from bacterial and endogenous debris (Langmeier *et al.*, 2002). Similarly, 33% of total faecal nitrogen was attributable to undigested material while 53% was derived from bacterial and endogenous debris in the faeces of a sheep (*Ovis aries*) fed ^{15}N-labelled hay (Bosshard *et al.*, 2011). Both these experiments included just a single individual animal, however, making it impossible to assess within-species variability.

Through the lens of animal physiology and nutrition, Schwarm *et al.* (2009) used a detergent analysis on faecal samples collected from 48 mammalian herbivore species to investigate the degree to which digestive physiology influences the partitioning of total faecal nitrogen among components. Again, most of the species investigated in this study were represented by just one individual and one faecal sample. Nevertheless, the authors found that, with one exception, metabolic faecal nitrogen (MFN; a combination of bacterial and endogenous nitrogen) accounted for 65–91% of total faecal nitrogen and there were no significant differences in the proportion of MFN among species with different digestive physiologies. In the only outlier, a red panda (*Ailurus fulgens*), MFN made up just 45% of total faecal nitrogen, which may be due to this species being a secondarily herbivorous (sometimes termed hypocarnivorous) carnivoran (Schwarm *et al.*, 2009). A general trend in the evolution of various vertebrate digestive systems is that increased consumption of animal protein leads to simplification of gastrointestinal length and complexity (Stevens & Hume, 2004; Fig. 1). Sometimes clades that have dental and digestive physiological adaptations for animal protein consumption will 'reverse' their trajectory. There are multiple instances of physiologically faunivorous animals, including the red panda, giant panda

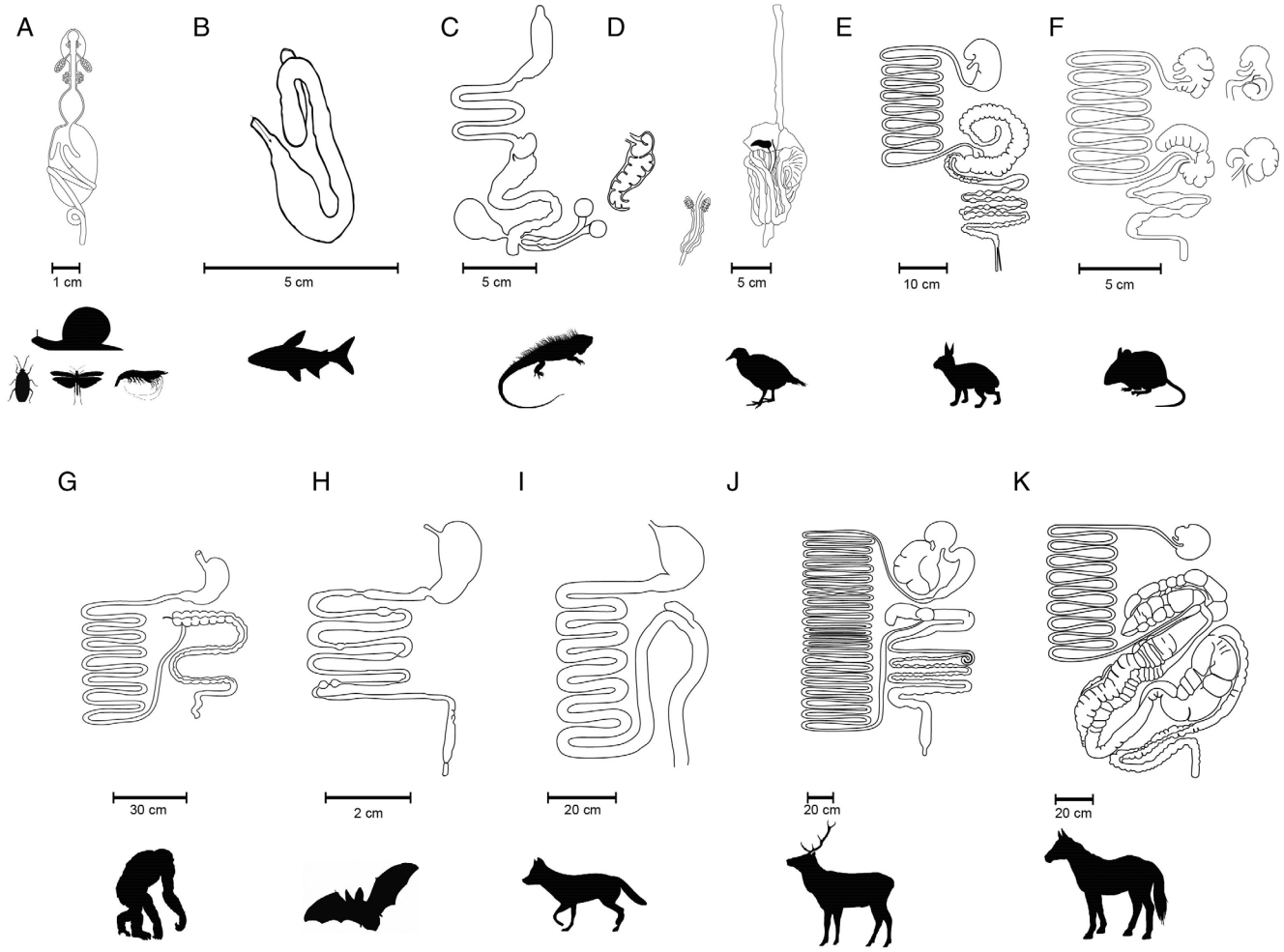

Fig. 1. Comparative digestive physiology for the various groups discussed in this review: (A) generalised invertebrate; (B) fish; (C) herbivorous reptile; (D) omnivorous bird; (E) coprophagous lagomorph; (F) generalised rodent; (G) primate; (H) bat; (I) generalised carnivorous mammal; (J) foregut fermenting herbivorous artiodactyl; and (K) hindgut fermenting herbivorous perissodactyl. Insets in (C) and (D) highlight the cecum within the interior of the anterior part of the large intestine or within the folds of the rest of the intestinal track, respectively, while the insets in (E) represent a relatively empty stomach (top) and cecum (bottom). Illustrations modified from Stevens & Hume (1998), Cardoso *et al.* (2012), Vieira-Lopes *et al.* (2013), and Crompton & Nesheim (2016).

Table 1. Details of controlled-feeding studies, including information about the organism studied, the length of the experiment, the type of food provided to the study organism, the number of individuals in the study as well as the number of faecal samples analysed, and the calculated diet–faeces discrimination factors. In some studies, different discrimination factors were determined for individuals consuming different diets; these have been listed separately. Standard deviations are provided when possible.

Taxon	Taxon type	Consumer type	Study length	Diet	# Individuals/ # samples	$\Delta^{13}C_{diet-faeces}$ (‰)	$\Delta^{15}N_{diet-faeces}$ (‰)	Notes	Source
Snail (*Cornu aspersum*)	Cephalopod, mollusc	Herbivore/ mixed	Not specified. Animals fed diet for multiple generations	Romaine lettuce leaves	/4	−0.5 ± 0.7		1	1
Opossum shrimp (*Mysis mixta*)	Arthropod, crustacean	Faunivore	12–13 weeks	Brine shrimp	/5	−1.4 ± 1.2	−3.5 ± 1.3	2	2
Milkweed bug (*Oncopeltus fasciatus*)	Arthropod, insect	Herbivore/ mixed	Not specified. Animals fed diet for multiple generations	Milkweed seeds	/1	−0.9		1	1
Grasshopper (*Melanoplus sanguinipes*)	Arthropod, insect	Herbivore/ mixed	Not specified. Animals fed diet *ad libitum* since newly hatched nymphs	Corn seedlings; wheat seedlings	/2; /6	−1.7 ± 0.7; −0.8 ± 0.9		1	1
Rock iguana (*Cyclura lewisi*)	Reptile, lizard	Herbivore/ mixed	12 months (faeces collected once at 6 months and once at 12 months)	15 types of plant foods (leafy greens, root vegetables and fruit)	14/28	0.6 ± 1.2	−2.8 ± 1.4	2, 3	3
Rock iguana (*Cyclura collei*)	Reptile, lizard	Herbivore/ mixed	12 months (faeces collected once at 6 months and once at 12 months)	15 types of plant foods (leafy greens, root vegetables and fruit)	4/8	2.0 ± 0.6	−3.9 ± 0.7	2	3
Rock iguana (*Cyclura pinguis*)	Reptile, lizard	Herbivore/ mixed	12 months (faeces collected once at 6 months and once at 12 months)	15 types of plant foods (leafy greens, root vegetables and fruit)	3/6	1.1 ± 1.3	−2.2 ± 2.4	2	3
Common coot (*Fulica atra*)	Bird, gruiform	Trophic omnivore	2 consecutive days in Autumn; 2 days in Spring	Commercial bird food for 2 days during autumn and spring; pondweed (*Potamogeton pectinatus*) for 2 days during autumn and spring	16/	0.7 ± 0.9; 1.0 ± 0.9; 0.7 ± 1.1; 0.0 ± 0.3	−2.6 ± 1.7; −2.4 ± 0.9; −0.4 ± 0.3; 0.0 ± 1.0	2, 4	4
Red knobbed coot (*Fulica cristata*)	Bird, gruiform	Trophic omnivore	2 consecutive days in Autumn; 2 days in Spring	Commercial bird food for 2 days during autumn and spring; pondweed (*Potamogeton pectinatus*) for 2 days during autumn and spring	16/	0.4 ± 0.8; 1.1 ± 0.9; 0.2 ± 0.5; 0.0 ± 0.2	−3.3 ± 1.3; −3.4 ± 1.1; −1.2 ± 0.4; −4.5 ± 4.2	2, 4	4
Red-necked stint (*Calidris ruficollis*)	Bird, charadriiform	Trophic omnivore	7 days; faecal sampling beginning 2 days after isolation	Cereal-based pellet or fish-based pellet	3/77; 3/20	0.2 ± 0.5; −0.1 ± 0.3	0.6 ± 0.6; 0.5 ± 0.5	5, 6	5
Yellow-vented bulbul (*Pycnonotus xanthopygos*)	Bird, passeriform	Trophic omnivore	75 days on first diet; 95 days on experimental diet	Low casein protein, medium protein, and high protein	5/; 4/; 4/		−0.9 ± 0.2; −0.8 ± 0.3; −0.6 ± 0.3	1, 7	6
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	12–22 days on each diet	Pure C_3 legume (*Stylosanthes humilis*); pure C_4 (*Heteropogon contortus* and *Borhiochloa decipiens*)	2/; 6/	−2.4 ± 0.17; 0.9 ± 0.21		2, 7	7
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	123 days; 115 days. Faeces collected daily after diet change for 10 days, and once a week subsequently	C_3 cow pea hay (*Vigna sinensis*); C_4 pangola grass (*Digiaria decumbens*)	2/	1.8 ± 0.09; 2.4 ± 0.12		1, 7	7
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Browser	3 weeks	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/	1.0 ± 0.2; 0.9 ± 0.2		2, 8	8
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	60; 85; 39 days	Italian ryegrass wafer and concentrate mixture; ryegrass silage, concentrate mixture, and alfalfa haycube; concentrate mixture, corn silage, and alfalfa haycube	4/4; 6/6; 4/4		−0.6 ± 0.3; −0.4 ± 0.2; −1.4 ± 0.3	2, 9	9

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Table 1. *(Cont.)*

Taxon	Taxon type	Consumer type	Study length	Diet	# Individuals/ # samples	$\Delta^{13}C_{diet-faeces}$ (‰)	$\Delta^{15}N_{diet-faeces}$ (‰)	Notes	Source
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	3 weeks (samples collected for last 7 days)	Ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture; hay of this pasture; silage of pasture; fibrous cake made from macerated pasture with juice removed	2/;2/;2/;2/		−2 ± 0.0; −2 ± 0.6; −2 ± 0.1; −1.7 ± 0.3	10	10
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	27 days on each diet; one sample collected from each individual at the end of each period	Four different formulated isoenergetic diets with varying crude protein, starch, and fibre content	5/5; 5/5; 5/5; 5/5		−1.2; −1.8; −2.0; −2.5	4, 5, 6	11
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	4 weeks initial diet; 4 weeks second diet (faeces collected during last 3 weeks of each period)	Groups initially fed pasture *ad libitum* and corn or provisioned with freshly clipped pasture and corn. Then switched to other diet.	8/256	1.7 ± 0.7		5, 11	12
Domestic cow (*Bos primigenius taurus*)	Mammal, artiodactyl	Herbivore/ grazer	10–14 days on C_3 diet and fasted for 24 h; 8–9 days on C_4 diet (samples collected during last 2 days on each feed)	C_3 ryegrass (*Lolium* spp.), white clover (*Trifolium repens*), and pelleted barley meal (*Hordeum vulgare*); C_4 pelleted corn meal, corn silage, and hay (*Paspalum dilatatum*)	4/; 4/	1.7 ± 0.1; 2.2 ± 0.3		10	13
Domestic sheep (*Ovis aries*)	Mammal, artiodactyl	Herbivore/ grazer	12–22 days on each diet	Pure C_3 legume (*Stylosanthes humilis*); C_4 (*Heteropogon contortus* and *Borhiochloa decipiens*)	5/; 10/	−2.3 ± 0.4; 0.4 ± 0.3		2, 4, 7	7
Domestic sheep (*Ovis aries*)	Mammal, artiodactyl	Herbivore/ grazer	45 days on mixed diet (samples collected at end of this 'acclimation period'); 104 days on pure diet (samples collected every 1–7 days, $N = 24$ total)	50% C_3/50% C_4 diet; pure C_4 corn silage; pure C_3 alfafa (*Medicago sativa*)	8/;4/;4/	0.3 ± 0.1; 0.5 ± 0.4; 0.9 ± 0.2		2	14
Domestic sheep (*Ovis aries*)	Mammal, artiodactyl	Herbivore/ grazer	2 weeks; 2 weeks (samples collected during last 3 days of each trial)	Alfalfa hay cubes; alfalfa hay cubes with sucrose	4/; 4/		−2.8; −3.1	5, 12	10
Domestic goat (*Capra hircus*)	Mammal, artiodactyl	Herbivore/ browser	141 days	100% C_3 alfalfa (*Medicago sativa* L.); 25% C_4; 50% C_4; 75% C_4 (*Themeda triandra*)	6/;5/;5/;5/ ;3/	0.5 (0.3–0.7); 1.5 (1.0–2.3); 2.3 (1.3–2.9); 3.9 (3.2–4.4); 0.6 (0.4–0.9)		13, 8	15
Domestic goat (*Capra hircus*)	Mammal, artiodactyl	Herbivore/ browser	60 days (following a diet switch)	100% C_3 alfalfa (*Medicago sativa* L.); 25% C_4; 50% C_4; 75% C_4 (*Themeda triandra*)	5/;5/;5/;3/ ;5/	0. (−0.3 to 0.3); 2.0 (1.1–3.1); 2.6 (1.7–4.1); 2.3 (1.9–2.8); 0.7 (0.5–0.9)		13, 8	15
Domestic goat (*Capra aegagrus hircus*)	Mammal, artiodactyl	Herbivore/ browser	20 days on each diet	C_3 grass hay (species not specified); C_3 browse (poplar, raspberry, and chestnut)	5/5; 5/5	0.5 ± 0.9; 2.3 ± 0.3	−2.1 ± 0.7; −0.5 ± 0.6	1	16
Domestic goat (*Capra aegagrus hircus*)	Mammal, artiodactyl	Herbivore/ browser	3 weeks	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/	0.8 ± 0.1; 1.0 ± 0.4		2, 8	8
Domestic goat (*Capra aegagrus hircus*)	Mammal, artiodactyl	Herbivore/ browser	60 days	Italian rye grass wafer and concentrate mixture	3/3		−3.5 ± 2.6	1, 9	9

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Table 1. *(Cont.)*

Taxon	Taxon type	Consumer type	Study length	Diet	# Individuals/ # samples	$\Delta^{13}C_{diet-faeces}$ (‰)	$\Delta^{15}N_{diet-faeces}$ (‰)	Notes	Source
Rheem gazelle (*Gazella leptoceros*)	Mammal, artiodactyl	Herbivore/ mixed	20 days	Mix of C_4 hay (species not specified) and C_3 alfalfa (*Medicago sativa*)	7/7	−0.6 ± 1.0	−1.7 ± 0.6	1	16
Alpaca (*Vicugna pacos*)	Mammal, artiodactyl	Herbivore/ grazer	3 weeks	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/	0.4 ± 0.4; 1.3 ± 0.2		2, 8	8
Llama (*Llama llama*)	Mammal, artiodactyl	Herbivore/ mixed	3 weeks	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/	0.4 ± 0.5; 1.2 ± 0.4		2, 8	8
Llama (*Llama llama*)	Mammal, artiodactyl	Herbivore/ mixed	5 days (following a 3-week acclimation period)	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/		−2.9 ± 0.3; −3.0 ± 0.4	2, 8	17
Horse (*Equus caballus*)	Mammal, perissodactyl	Herbivore/ grazer	3 weeks	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	4/	0.5 ± 0.4; 0.7 ± 0.2		2, 8	8
Horse (*Equus caballus*)	Mammal, perissodactyl	Herbivore/ grazer	5 days (following a 3-week acclimation period)	C_3 alfalfa (*Medicago sativa*); C_4 Bermuda grass (*Cynodon dactylon*)	1/		−2.6; −3.3	2, 8	17
Wild boar (*Sus scrofa*)	Mammal, artiodactyl	Trophic omnivore	Not specified	Not specified	3/3		−1.2 ± 0.4	2	18
European rabbit (*Oryctolagus cuniculus*)	Mammal, lagomorph	Herbivore/ mixed	3 weeks	C_3 alfalfa (*Medicago sativa*)	4/	0.3 ± 0.1		2, 8	8
Wistar lab rat (*Rattus norvegicus*)	Mammal, rodent	Trophic omnivore	Faeces collected after 24 h of fasting	Rat chow	Not specified	2.1	−1.1	2, 12	19
Western jumping mouse (*Zapus princeps*)	Mammal, rodent	Herbivore/ mixed	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	5.9	−2.2	5, 12, 14	20
Yellow-pine chipmunk (*Tamias amoenus*)	Mammal, rodent	Trophic omnivore	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	3.0	−1.4	5, 12, 14	20
Deer mouse (*Peromyscus maniculatus*)	Mammal, rodent	Trophic omnivore	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	3.2	−2.1	5, 12, 14	20
Long-tailed vole (*Microtus longicaudus*)	Mammal, rodent	Herbivore/ mixed	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	2.7	−2.2	5, 12, 14	20
Meadow vole (*Microtus pennsylvanicus*)	Mammal, rodent	Trophic omnivore	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	3.6	−2.5	5, 12, 14	20
Red-backed vole (*Myodes gapperi*)	Mammal, rodent	Trophic omnivore	9 days total: 2 days (initial diet), 7 days (subsequent diet). Faeces collected once at end	Sunflower seeds, oats, and apples (initial); rodent chow (subsequent)	5/5	4.2	−2.2	5, 12, 14	20
Red-backed vole (*Myodes gapperi*)	Mammal, Rodent	Trophic omnivore	61 ± 12 days (samples collected at the end of this period)	High protein diet (26%) of mixed greens and sunflower seeds; medium protein diet (17%) of mixed greens and oats; low protein diet (14%) of mixed greens	11/;10/;10/	−0.2 ± 0.4; 1.2 ± 0.3; 0.5 ± 0.4	−1.3 ± 0.7; −1.2 ± 0.5; −1.8 ± 0.4	12, 15	21
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 1; all C_3; 23.5% soybean meal, 4% soy oil, 58% ground wheat, 10% oat hulls, 4.5% vitamins/ minerals	4/4	0.2 ± 0.08		7	22

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Table 1. *(Cont.)*

Taxon	Taxon type	Consumer type	Study length	Diet	# Individuals/ # samples	$\Delta^{13}C_{diet-faeces}$ (‰)	$\Delta^{15}N_{diet-faeces}$ (‰)	Notes	Source
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 2; C_4 cellulose; 49.3% soybean meal, 3.7% soy oil, 23.5% ground wheat, 19% corn bran, 4.6% vitamins/minerals	4/4	−4.5 ± 0.08		7	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 3; C_4 starch; 26.5% soybean meal, 2.7% soy oil, 10% oat hulls 56.3% ground corn, 4.5% vitamins/ minerals	4/4	1.7 ± 0.07		7	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 4; C_4 lipid; 24% soybean meal, 57.7% ground wheat, 10% oat hulls, 3.8% corn oil, 4.5% vitamins/ minerals	4/4	0.6 ± 0.04		7	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 5; C_4 protein; 3.8% soy oil, 61.5% ground wheat, 10% oat hulls, 18.5% corn gluten, 6.2% vitamins/ minerals	4/4	0.9 ± 0.04		7	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 6; 2× C_4 protein; 2% soy oil, 28.3% ground wheat, 10% oat hulls, 54.3% corn gluten, 5.4% vitamins/minerals	4/4	2.0 ± 0.03		7, 16	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	4 months	Diet 7; 0.25× C_4 protein; 5.3% soy oil, 62.4% ground wheat, 17% oat hulls, 6.5% corn gluten, 8.8% vitamins/minerals	4/4	1.9 ± 0.05		7, 16	22
Mouse (*Mus musculus*)	Mammal, rodent	Trophic omnivore	2 months	Diet 8; 92% C_4; 1.5% soybean meal, 0.2% soy oil, 1.5% ground wheat, 92% ground corn, 4.8% vitamins/ minerals	4/4	1.6 ± 0.04		7	22
Greater mouse-eared bat (*Myotis myotis*)	Mammal, chiropteran	Insectivore	12 days total; diet switched after 2 days and 9 days	Isotopically spiked mealworms: ^{13}C-depleted (2 days), ^{13}C-enriched (7 days), ^{13}C-depleted (3 days)	3/15; 3/21	−0.2 ± 1.1; −0.3 ± 0.8	−1.8 ± 1.3; −2.3 ± 2.2	5, 10, 17	23
Greater horseshoe bat (*Rhinolophus ferrumequinum*)	Mammal, chiropteran	Insectivore	12 days total; diet switched after 2 days and 9 days	Isotopically spiked mealworms: ^{13}C-depleted (2 days), ^{13}C-enriched (7 days), ^{13}C-depleted (3 days)	3/14; 3/21	−0.2 ± 1.0; −0.1 ± 0.4	−0.5 ± 0.5; −1.0 ± 0.5	5, 10, 17	23
Common chimpanzee (*Pan troglodytes*)	Mammal, primate	Trophic omnivore	Diet similar for several months. Faeces collected *ad libitum* for 7 days	Commercial primate pellets and a variety of fruits and vegetables	5/	1.0 ± 0.3; 2.2 ± 0.3	−1.9 ± 0.3; −1.8 ± 0.3	5, 10, 6, 18	24
Human (*Homo sapiens*)	Mammal, primate	Trophic omnivore	8 days on each diet	Fish; meat; 'vegetarian'	14/; 14;4/	1.1 (0.8–1.5); 0.4 (0.2–0.6); 1.5 (1.3–1.7)	0.4 (0.2–1.0); −0.4 (−0.6 to −0.1); −1.2 (−2.1 to −1.8)	19	25
François' langur (*Trachypithecus francoisi*)	Mammal, primate	Herbivore/ mixed	13 months	45% vegetables, 45% leafy greens, and 10% primate 'leafeater' biscuit	1/75	−1.2 ± 1.8	−1.2 ± 3.8	20	26
Capuchin (*Sapajus libidinosus*)	Mammal, primate	Trophic omnivore	Not specified	Mazuri NW Primate Biscuit, fresh and dried fruit, commercial cereal, vegetables, assorted fruits	7/	−0.8 ± 0.7	−0.2 ± 0.4	5	27

(Continues on next page)

Table 1. *(Cont.)*

Taxon	Taxon type	Consumer type	Study length	Diet	# Individuals/ # samples	$\Delta^{13}C_{diet–faeces}$ (‰)	$\Delta^{15}N_{diet–faeces}$ (‰)	Notes	Source
Orangutan (*Pongo pygmaeus*)	Mammal, primate	Trophic omnivore	7 days	Fruit, vegetables, dried sardine, egg yolk, skim milk, grains, tree leaves	4/	1.8 ± 0.7; 3.5 ± 0.8	−1.6 ± 0.7; −1.6 ± 0.7	4, 5, 6, 18	28
Meerkat (*Suricata suricatta*)	Mammal, carnivoran	Trophic omnivore	1 month	Carrots, apples, horsemeat, dog biscuits, mice, and chicks	7/24	−0.1 ± 1.5	−1.5 ± 1.1	2, 6, 21	29
Tiger (*Panthera tigris*)	Mammal, carnivoran	Carnivore	3 days (samples collected once)	Feline diet (blend of meat, meat by-products, fishmeal, vitamins, and trace minerals)	7/7	−1.3 ± 3.3	−1.6 ± 2.1	10	30
Snow leopard (*Uncia uncia*)	Mammal, carnivoran	Carnivore	3 days (samples collected once)	Feline diet (blend of meat, meat by-products, fishmeal, vitamins, and trace minerals)	10/10	−2.3 ± 3.6	−2.5 ± 1.5	10	30
House cat (*Felis catus*)	Mammal, carnivoran	Carnivore	3 days (samples collected every day)	Dry kibble	4/12	1.4 ± 0.7	−1.7 ± 0.6	4	31

Notes:
1. $\Delta_{diet–faeces}$ estimated using faecal data extracted with https://apps.automeris.io/wpd/.
2. $\Delta_{diet–faeces}$ calculated using summary data for diet and faecal samples provided by the authors and propagating error.
3. Data combined for both juveniles and adults.
4. Values rounded to nearest 0.1‰.
5. Summary $\Delta_{diet–faeces}$ provided by authors.
6. Values inverted (to reflect $\Delta_{diet–faeces}$ rather than $\Delta_{faeces–diet}$).
7. No error for diet data available. Standard deviations based solely on faecal $\delta^{13}C$ data.
8. Values reported are apparent enrichment (ε^*) rather than simple offset (Δ), where $\varepsilon^* = [(10^3 + \delta^{13}C_{faeces})/(10^3 + \delta^{13}C_{diet}) - 1]*10^3$. The equations yield comparable results.
9. The concentrate mixture was 30% milo, 20% corn, 20% bary (we speculate the authors may have meant barley), 10% wheat bran, 10% alfalfa meal, 5.5% sugar molasses, 1% urea, and added minerals and vitamins.
10. Calculated using raw data provided by authors.
11. Reported values include data from both groups of cows. Cows equilibrated on pasture diet for 6 weeks prior to study.
12. No error values provided.
13. Raw data not provided. Values reported are means and ranges of diet–tissue discrimination values for each group.
14. Animals were wild-caught so diet is unknown prior to beginning of the study period.
15. Error values are standard error.
16. Mice fed the 2× C_4 protein diet received twice the amount of protein in the standard diets while those fed the 0.25× C_4 protein diet received one-quarter the amount of protein in the standard diets.
17. Separate $\Delta_{diet–faeces}$ reported by authors for bats consuming ^{13}C-depleted and ^{13}C-enriched worms.
18. $\Delta_{diet–faeces}$ are for whole 'atomic' diet as well as just dietary protein.
19. Raw data not provided; median and interquartile ranges are reported.
20. Study included a mother–infant pair; only data for the adult female included here. Summary $\Delta_{diet–faeces}$ calculated using monthly data reported by author.
21. Samples collected opportunistically from an enclosure containing all individuals.
Sources: 1 = DeNiro & Epstein (1978); 2 = Gorokhova & Hansson (1999); 3 = Steinitz *et al.* (2016); 4 = Varo & Amat (2008); 5 = Kuwae *et al.* (2022); 6 = Tsahar *et al.* (2008); 7 = Jones *et al.* (1979); 8 = Sponheimer *et al.* (2003*a*); 9 = Sutoh *et al.* (1987); 10 = Steele & Daniel (1978); 11 = Cantalapiedra-Hijar *et al.* (2015); 12 = Schneider *et al.* (2015); 13 = Wilson *et al.* (1988); 14 = Martins *et al.* (2012); 15 = Codron *et al.* (2011*a*); 16 = Codron *et al.* (2012); 17 = Sponheimer *et al.* (2003*b*); 18 = Sutoh *et al.*, 1993); 19 = Nakagawa *et al.* (1985); 20 = Hwang *et al.* (2007); 21 = Sare *et al.* (2005); 22 = Tieszen & Fagre (1993); 23 = Salvarina *et al.* (2013); 24 = Tsutaya *et al.* (2017); 25 = Kuhnle *et al.* (2013); 26 = Reitsema (2012); 27 = Reitsema *et al.* (2020); 28 = Tsutaya *et al.* (2021); 29 = Montanari (2017); 30 = Montanari & Amato (2015); 31 = this study.

(*Ailuropoda melanoleuca*), kinkajou (*Potos flavus*), binturong (*Arctictis binturong*), and iguana (Iguanidae), that have 'reverted' to herbivory (Troyer, 1983; Nie *et al.*, 2015). Such shifts require substantial dental, morphological, and metabolic adaptations to accommodate the digestion of plant materials within a gastrointestinal tract previously adapted to a high-protein and low-fibre diet (Sues, 2000). The red panda, which primarily consumes bamboo shoots and leaves (Yonzon & Hunter Jr., 1991), is likely not as efficient at digesting plant material as herbivores with more specialised gut morphologies. This would explain why red panda faeces contain a greater proportion of undigested material than those produced by other herbivores.

While the results from the above studies vary, they collectively suggest that undigested material accounts for a relatively small proportion of mammalian herbivore faecal nitrogen and that the bulk of faecal nitrogen is instead derived from bacterial and endogenous debris. This has two important ramifications. First, it means that faecal isotope values will reflect more than just an animal's most recent meal. For animals that eat a relatively unvaried diet (e.g. grazers), this should not significantly impact dietary inferences made from faecal isotope values. However, it will be difficult to estimate short-term dietary intake confidently using faecal isotope values for animals that have more variable diets. Second, one will have to account for isotopic offset, called apparent fractionation, during digestion and tissue formation when using faecal isotope values to estimate diet.

Given that faeces are a mixture of components, sample processing becomes an important consideration, as different processing techniques may preferentially remove different faecal components. In a recent study, Oelze *et al.* (2022) found significant isotopic differences among different size fractions of primate faeces. They advocated for the adoption of a standardised faecal sample preparation technique in which large particles of undigested materials are removed. Other authors have additionally used chemical pretreatments, such as acidification to remove carbonates (e.g. Reid & Koch, 2017; Kuwae *et al.*, 2022), and/or treatment with solvents such as chloroform: methanol or sodium hydroxide (NaOH) to remove isotopically fractionated metabolites like urea and ammonium (e.g. Wurster *et al.*, 2017; Kuwae *et al.*, 2022). In bat and seabird guano accumulations, ammonia volatilisation has been shown to increase guano $\delta^{15}N$ values significantly (e.g. Mizutani & Wada, 1988; McFarlane, Keeler & Mizutani, 1995), making solvent-extraction important for removing this secondary signal. Lack of standardisation in sample preparation across studies is another likely driver for variation in faecal isotope results, discussed in more detail below.

(2) How do diet and faeces differ isotopically?

The $\delta^{13}C$ and $\delta^{15}N$ values of consumed foods are passed on to faecal material, but with some offset. The most rigorous way to determine the degree to which faecal isotope values reflect diet is to conduct a controlled feeding study to establish the isotopic difference, or discrimination (Δ), between diet and faecal matter (where $\Delta^{13}C_{diet–faeces} = \delta^{13}C_{diet} - \delta^{13}C_{faeces}$, and $\Delta^{15}N_{diet–faeces} = \delta^{15}N_{diet} - \delta^{15}N_{faeces}$). We note that some researchers alternatively report faeces–diet discrimination factors, or apparent enrichment (ε*). Apparent enrichment and discrimination yield comparable results. However, calculating faeces–diet discrimination will produce opposite values from diet–faeces discrimination (the sign of the equation is different). It is therefore critical that researchers are clear as to how discrimination is calculated.

Researchers have used semi-controlled approaches to quantify $\Delta^{13}C_{diet–faeces}$ and $\Delta^{15}N_{diet–faeces}$ values for a variety of animals (summarised in Table 1). Estimating diet–faeces discrimination in the field is also possible, but this is not as precise and the data are more challenging to interpret,

Table 2. Details for published field-based studies, including information about the organism studied, both the number of individuals included in the study and the number of faecal samples analysed, and the calculated diet–faeces discrimination factors.

Taxon	Taxon type	Consumer type	# Individuals/ # samples	$\Delta^{13}C_{diet–faeces}$ (‰)	$\Delta^{15}N_{diet–faeces}$ (‰)	Notes	Source
Mountain gorilla (*Gorilla beringei*)	Mammal, primate	Herbivore/ folivore	4/121	−0.3	−0.6	1	1
Common chimpanzee (*Pan troglodytes*)	Mammal, primate	Trophic omnivore	10/115	−1.3 ± 0.7	−0.5 ± 0.7	2	2
Coyote (*Canis latrans*)	Mammal, carnivoran	Trophic omnivore	4/4	1.5 ± 1.6	−2.3 ± 1.3	3	3
Jaguar (*Panthera onca*)	Mammal, carnivoran	Carnivore	10/10	4.0 ± 3.3	−2.8 ± 2.5	4	4

Notes:
1. The authors provided weighted mean apparent enrichment (ε*) values (which are comparable to Δ values). Here we have inverted their values to $\Delta^{13}C_{diet–faeces}$ (rather than $\Delta_{faeces–diet}$).
2. Summary data estimated using mean data provided for each individual chimpanzee.
3. Diet was estimated using $\Delta_{scat–fur}$ for coyotes combined with $\Delta_{fur–diet}$ estimates for red foxes (*Vulpes vulpes*) and wolves (*Canis lupus*).
4. Diet was estimated using bone collagen from digested prey.
Sources: 1 = Blumenthal *et al.* (2012); 2 = Phillips & O'Connell (2016); 3 = Reid & Koch (2017); 4 = Crowley *et al.* (2019).

particularly when dealing with animals that have variable diets. Consequently, there have been fewer field-based studies (Table 2). In general, faecal $\delta^{13}C$ values tend to be lower than diet (positive $\Delta^{13}C_{diet–faeces}$), while faecal $\delta^{15}N$ values tend to be higher than diet (negative $\Delta^{15}N_{diet–faeces}$). There is, however, considerable variability both within and among species. As we noted above, and as the tables demonstrate, research to date has focused nearly entirely on mammals, particularly ungulates. Very little work has been conducted on non-mammalian taxa and there is still much to learn about $\Delta_{diet–faeces}$ in these groups. Below, we review the findings from studies focused on invertebrates, reptiles, birds, and mammals (to the best of our knowledge, there has been no work on this topic for fish). We subsequently discuss the potential drivers for variability in $\Delta_{diet–faeces}$ both within and among taxa, including digestive physiology, diet composition, diet digestibility, and study design.

(a) *Discrimination between diet and faeces in non-mammalian taxa*

Although discrimination studies centred on invertebrates are limited, existing data suggest that arthropods and molluscs have negative $\Delta^{13}C_{diet–faeces}$ values (i.e. faecal samples have higher $\delta^{13}C$ values than diet), which is opposite to the relationship seen in most vertebrates (Table 1, Fig. 2A). To date, two species of insect (milkweed bug, *Oncopeltus fasciatus*; and grasshopper, *Melanoplus sanguinipes*), one mollusc (the garden snail, *Cornu aspersum*), and one small-bodied marine crustacean (opossum shrimp, *Mysis mixta*) have been studied (DeNiro & Epstein, 1978; Gorokhova & Hansson, 1999). Insect exoskeletons tend to have much lower $\delta^{13}C$ values than soft tissues (Perkins *et al.*, 2013); it may be that these organisms preferentially incorporate light ^{12}C into their exoskeletons, leaving their waste relatively enriched in ^{13}C. This is not necessarily the case for crustaceans, however, as carapace $\delta^{13}C$ values are higher than those recorded for muscle tissue in freshwater crayfish (*Cherax destructor*; Mazumder *et al.*, 2018).

Invertebrate $\Delta^{15}N_{diet–faeces}$ remains more ambiguous because so few data exist. The $\Delta^{15}N_{diet–faeces}$ value for the crustacean *Mysis mixta* (-3.48 ± 1.34‰) is similar in direction (faeces had higher $\delta^{15}N$ values than diet), but greater in magnitude than has been reported for most other organisms (Fig. 2B; Gorokhova & Hansson, 1999). To the best of our knowledge, these are the only conclusive $\Delta^{15}N_{diet–faeces}$ data published for invertebrates. Overmyer, MacNeil & Fisk (2008) examined $\Delta^{15}N_{diet–tissue}$ for black fly larvae (*Simulium vittatum*) and included a single faecal sample in their experiment. However, because the authors were working with ^{15}N-labelled food, the observed $\Delta^{15}N_{diet–faeces}$ value (-170‰) is not useful beyond suggesting that fly faeces may be ^{15}N-depleted relative to diet. One additional study estimated $\Delta^{15}N_{diet–faeces}$ for zooplankton (Altabet & Small, 1990), but these authors did not directly measure diet, and they assumed trophic enrichment from previously published observations for other organisms. As noted by DeNiro & Epstein (1978), arthropods excrete all of their waste in faeces rather than in separate faecal and urine pools. Because urine tends to have much lower $\delta^{15}N$ values than diet (e.g. Sponheimer *et al.*, 2003*b*), we might expect the combination of these two waste streams to increase $\Delta^{15}N_{diet–faeces}$ values, but the opposite is evident here. As seen for carbon, arthropod exoskeletons tend to have low $\delta^{15}N$ values relative to both soft tissues (Perkins *et al.*, 2013) and diet (Gorokhova & Hansson, 1999). If ^{14}N is preferentially incorporated into chitin, then waste would be relatively enriched in ^{15}N.

We are aware of just one study on reptile diet–faeces discrimination. Steinitz *et al.* (2016) determined discrimination factors between diet and skin, blood, and faeces for three species of rock iguana (*Cyclura* spp.). In their study, 34 captive and individually housed iguanas were fed a consistent diet composed of 15 different plant types for over a year. Of the tissues considered (blood, skin, and faeces), faeces had the most variable discrimination factors. The authors observed positive $\Delta^{13}C_{diet–faeces}$ values for all three species, and also found that $\Delta^{13}C_{diet–faeces}$ was correlated with iguana age, size (assessed using snout–vent length), and mass (Fig. 2A). Conversely, $\Delta^{15}N_{diet–faeces}$ values were consistently negative and there was no discernible relationship with age, size, or mass (Fig. 2B).

Notably, iguanas, which are herbivorous lizards, are an exception among Reptilia. The majority of reptiles are carnivores or omnivores, and even herbivorous reptiles are carnivorous or omnivorous as juveniles until their gut capacity reaches a size where plants can be effectively digested (Pough, 1973; Troyer, 1984). Similar to the hypocarnivorous red panda discussed above, it is likely that rock iguana faeces also contain a large amount of undigested food, and their discrimination values may therefore be smaller than other reptiles.

Useful estimates of $\Delta_{diet–faeces}$ are also limited for birds. Varo & Amat (2008) estimated $\Delta_{diet–faeces}$ for two species of coot (*Fulica* spp.), which are omnivores, by feeding 16 individuals of each species commercial bird feed for 6 days, then feeding the birds either commercial feed or pondweed (*Potamogeton pectinatus*) for 2 days, and finally switching individuals to the alternative diet for 2 days. The authors recognised that 2 days would likely be insufficient for ensuring that the birds were equilibrated on their diets, and to help account for this, they only collected faeces during the second day of each feeding experiment. Overall, they found that faeces had lower $\delta^{13}C$ values and higher $\delta^{15}N$ values than diet for both species (as seen in most vertebrates, Table 1), but the results were highly variable, which is likely in part a reflection of the study design (Fig. 2). Kuwae *et al.* (2022) used a similar sample design for red-necked stints (*Calidris ruficollis*). Faecal samples were collected from three captive individuals after they were fed either a cereal-based pellet diet for 2 days, or a fish-based pellet diet for 2 days. The authors found $\Delta_{diet–faeces}$ was small for both carbon and nitrogen and concluded that faecal isotopes closely match those in the diet, at least for shorebirds that have high metabolisms and frequently excrete their waste. Lastly, Tsahar *et al.* (2008) found that omnivorous yellow-vented bulbuls (*Pycnonotus xanthopygos*)

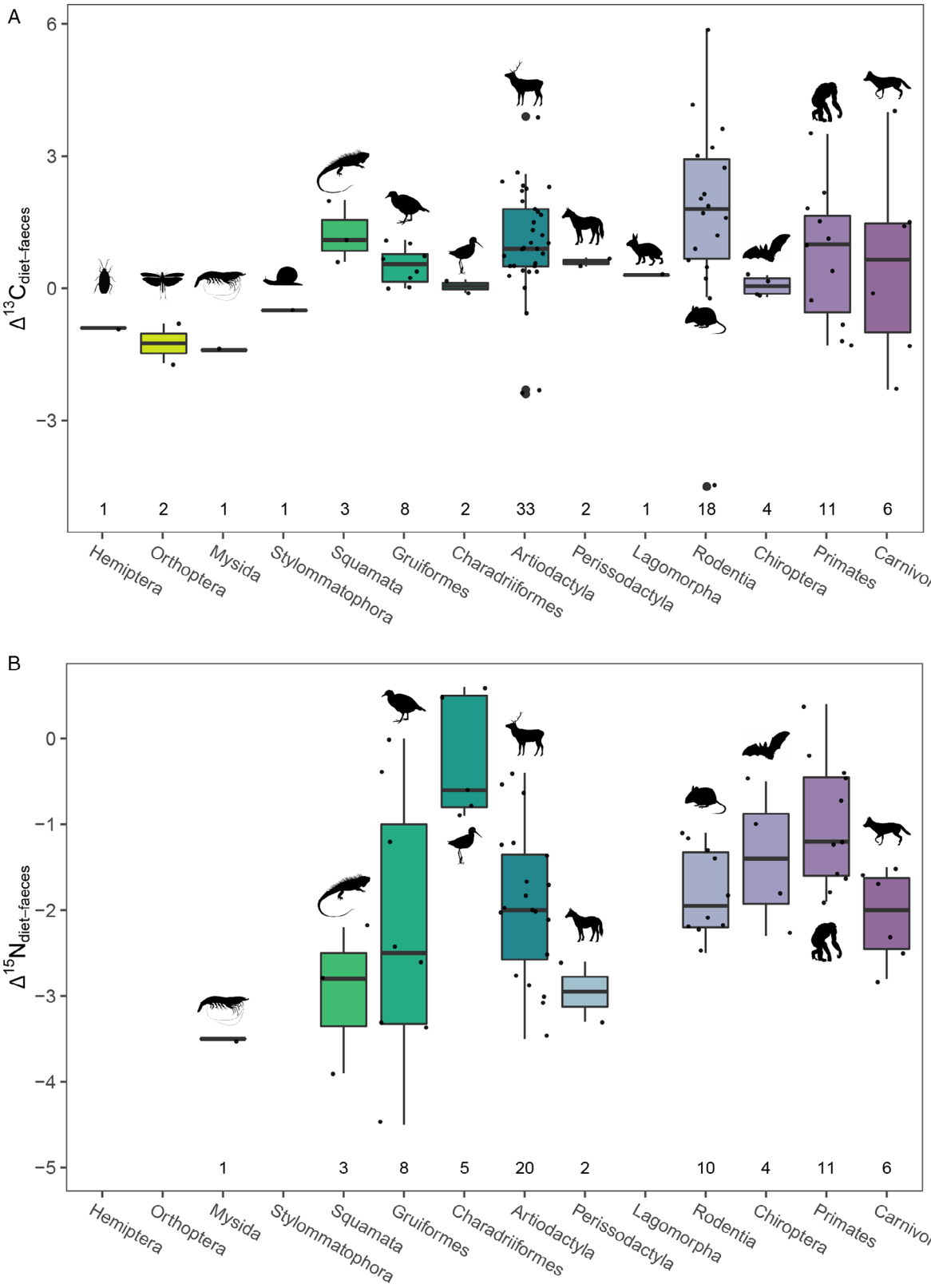

Fig. 2. Calculated isotopic discrimination between diet and faeces in the published literature organised by order for (A) $\Delta^{13}C_{diet-faeces}$ and (B) $\Delta^{15}N_{diet-faeces}$. Data sources are listed in Table 1. The number of discrimination factors included within each group is included below each box. Within each box, the median value is illustrated by a thick horizontal line, the boxes depict the interquartile range (IQR) from the 25th to 75th percentile, the upper and lower whiskers represent data falling within 1.5 × IQR, and the large dots denote outliers.

had excreta enriched in ^{15}N relative to diet (negative $\Delta^{15}N_{diet–faeces}$), and the magnitude of the difference was dependent on dietary protein content (smaller in birds fed higher protein diets). Two additional controlled feeding studies on passerine songbirds included faecal isotope data (Podlesak, McWilliams & Hatch, 2005; Carleton *et al.*, 2008), but the study designs preclude our ability to estimate discrimination factors.

(*b*) *Discrimination between diet and faeces in mammals*

Like reptiles and birds, mammals, which are by far the most studied group, tend to have positive $\Delta^{13}C_{diet–faeces}$ values and negative $\Delta^{15}N_{diet–faeces}$ values. However, there is considerable variability among taxa (Tables 1 and 2, Fig. 2). With the exception of several rodent species, especially when they are fed highly contrasting diets (Tieszen & Fagre, 1993; Hwang, Millar & Longstaffe, 2007), isotopic offsets between diet and faeces in mammals tend to be relatively small. Faecal $\delta^{13}C$ values are often not statistically distinguishable from diet, leading many researchers to conclude that faecal $\delta^{13}C$ values are faithful recorders of diet $\delta^{13}C$ values (e.g. Sponheimer *et al.*, 2003*a*; Codron *et al.*, 2012; Salvarina *et al.*, 2013; Montanari, 2017). For those mammals with positive $\Delta^{13}C_{diet–faeces}$ values, it has been suggested that small amounts of lipids, which tend to have very low $\delta^{13}C$ values, could be responsible for lowering faecal $\delta^{13}C$ values (and therefore increasing $\Delta^{13}C_{diet–faeces}$ values; Reid & Koch, 2017). Alternatively, the microfloral component of mammal faeces could be depleted in ^{13}C, but Sponheimer *et al.* (2003*a*) did not observe a change in faecal $\delta^{13}C$ values after removing microbes using acid-detergent. Faecal $\delta^{15}N$ values, on the other hand, are consistently elevated relative to diet in mammals. This could be driven by the fact that a significant proportion of the nitrogen found in mammal faeces is sourced from bacterial and endogenous debris, as discussed in Section II.1. Perhaps faecal samples also contain isotopically fractionated nitrogenous metabolites that could be removed using a pretreatment procedure, as advocated by Kuwae *et al.* (2022).

(3) What is the impact of digestive physiology on diet–faeces discrimination?

The digestive physiologies of non-mammalian taxa tend to be relatively simple (Fig. 1A–D). Invertebrates have the simplest gastrointestinal tracts (Fig. 1A; Cardoso *et al.*, 2012). Indeed, differentiation in the development of the digestive system is one of the major evolutionary distinctions between protostomes (most invertebrates) and deuterostomes (echinoderms, hemichordates, and chordates). While some invertebrates digest their food intracellularly, the taxa for which diet–faeces discrimination has been estimated digest their food extracellularly within an alimentary canal. Although data are limited, arthropods and molluscs are the only groups considered in this review to exhibit consistently negative $\Delta^{13}C_{diet–faeces}$ values, and their relatively simple digestive systems could be part of the explanation for that anomaly. The generalised gastrointestinal tract for a carnivorous or omnivorous reptile is characterised by a relatively long midgut and a short hindgut (Stevens & Hume, 1998), but herbivorous reptiles have a shortened midgut and a longer hindgut optimised for fermentation (Fig. 1C, Bjorndal & Bolten, 1990). Waste is excreted *via* a cloaca and there is no differentiation between urine and faeces (Thomas & Skadhauge, 1988). Birds, although technically reptiles, are highly derived bipedal toothless endotherms and many species fly, which places mass-specific limitations on their total gut capacity (Stevens & Hume, 1998). While there is a wide diversity of beak morphology, bird jaws are inefficient for grinding food, so many birds possess a specialised digestive organ called a gizzard for this purpose. Bird digestive systems vary with diet, but the majority of birds are omnivores and possess a relatively short midgut and a hindgut with a short, straight colon and paired ceca (Fig. 1D; Poppema, 1990; Stevens & Hume, 1998). This shortened gastrointestinal tract leads to shorter gut retention times; because birds pass food quickly and frequently, it has further been suggested that their faeces (and faecal isotopes) should be excellent recorders of short-term dietary trends (Kuwae *et al.*, 2022).

The impact of digestion and gastrointestinal complexity on isotopic discrimination has been specifically investigated for rodents and ruminant ungulates (Sutoh, Koyama & Yoneyama, 1987; Hwang *et al.*, 2007; Codron *et al.*, 2012). Ruminant ungulates (suborder Ruminantia) have a relatively complicated digestive tract (Fig. 1J, K) that generally includes a four-chambered stomach where food is sequentially fermented with the aid of microbes, rechewed, filtered, and digested (Stevens & Hume, 1998, 2004). In comparison, mammalian omnivores (e.g. Fig. 1G) have relatively simple stomachs with intestinal lengths that vary primarily as a function of body length. Gastrointestinal tracts are further shortened and simplified in mammalian faunivores (which consume both invertebrate and vertebrate prey), with some entirely lacking a hindgut, the distal portion of the digestive tract (Fig. 1I).

Trends in $\delta^{15}N$ values across the gastrointestinal (GI) tract are reasonably similar among both ruminants and largely omnivorous rodents (see Fig. 3). Digesta in the stomach or rumen tend to have relatively low $\delta^{15}N$ values, the proximal midgut (i.e. omasum and abomasum in ruminants, where food is respectively fermented and enzymatically digested) have slightly higher $\delta^{15}N$ values, the small intestine and cecum have the highest values, and digesta in the colon are isotopically comparable to the foregut (oesophagus and/or stomach) or midgut for ruminants and more similar to the intestine for rodents (Sutoh *et al.*, 1987; Codron *et al.*, 2012). By contrast, trends in $\delta^{13}C$ values across the GI tract are remarkably different for ruminants and rodents (Fig. 3). For ruminants, digesta tend to be reasonably isotopically similar along the GI tract with the exception of elevated values in the small intestine, where significant nutrient absorption occurs (Codron *et al.*, 2012). Rodent stomach contents, on the other hand, tend to have slightly elevated $\delta^{13}C$ values relative to the rest of the

GI tract (Hwang *et al.*, 2007). It would be useful to investigate isotopic variability along the GI tract for additional taxa, including hindgut fermenting ruminants, mammalian carnivores, and non-mammalian taxa.

(4) What is the temporal resolution of a faecal sample?

Faecal isotope turnover (the time it takes for an animal's faeces to reflect the isotopic composition of its diet) likely depends on both diet and faecal composition (i.e. undigested material *versus* bacterial and endogenous debris), which in turn depend on digestive physiology. While there have been numerous studies that use isotopes to investigate gut passage rates in different types of organisms (e.g. Südekum *et al.*, 1995; Warner *et al.*, 2014; de Sandre *et al.*, 2016; Botteon *et al.*, 2019; Gilannejad *et al.*, 2019), because faeces also contain tissues that take time to form (e.g. epithelial cells), gut passage rate and faecal isotope turnover may not operate on the same timescale.

For endotherms (organisms not dependent on an external heat source for body heat), gut retention time is largely a function of body mass, metabolic rate, gut length, and gut complexity, which are themselves autocorrelated [Demment & Van Soest (1985) and references therein]. In mammals, which exhibit a considerable range in body mass, there is a consistent trend towards larger-bodied mammals having a slower basal metabolic rate by a factor of ∼0.75 on average (Kleiber, 1932, 1947). Larger endotherms can persist on lower quality food than smaller-bodied endotherms, due in part to a longer gut retention time allowing for more fermentation and absorption of nutrients from higher fibre forage (also known as the Jarman–Bell Principle; Geist, 1974). The higher metabolic rates of small endotherms also place an effective upper limit on digesta passage rate, so nutrients must be absorbed as quickly as possible. This drives them to pursue more nutrient-dense and easily digestible foods (Geist, 1974). Regardless of body size and metabolic rate, gut passage rates tend to be quite short, on the order of hours to days. For example, an experiment examining gut passage rate in sheep found that faecal $\delta^{13}C$ values peaked between 12 and 40 h after sheep consumed a ^{13}C-labelled meal (Svejcar, Judkins & Boutton, 1993). It was not possible, however, to determine when the labelled food no longer contributed to faecal $\delta^{13}C$ values because the study was stopped after 100 h (Svejcar *et al.*, 1993).

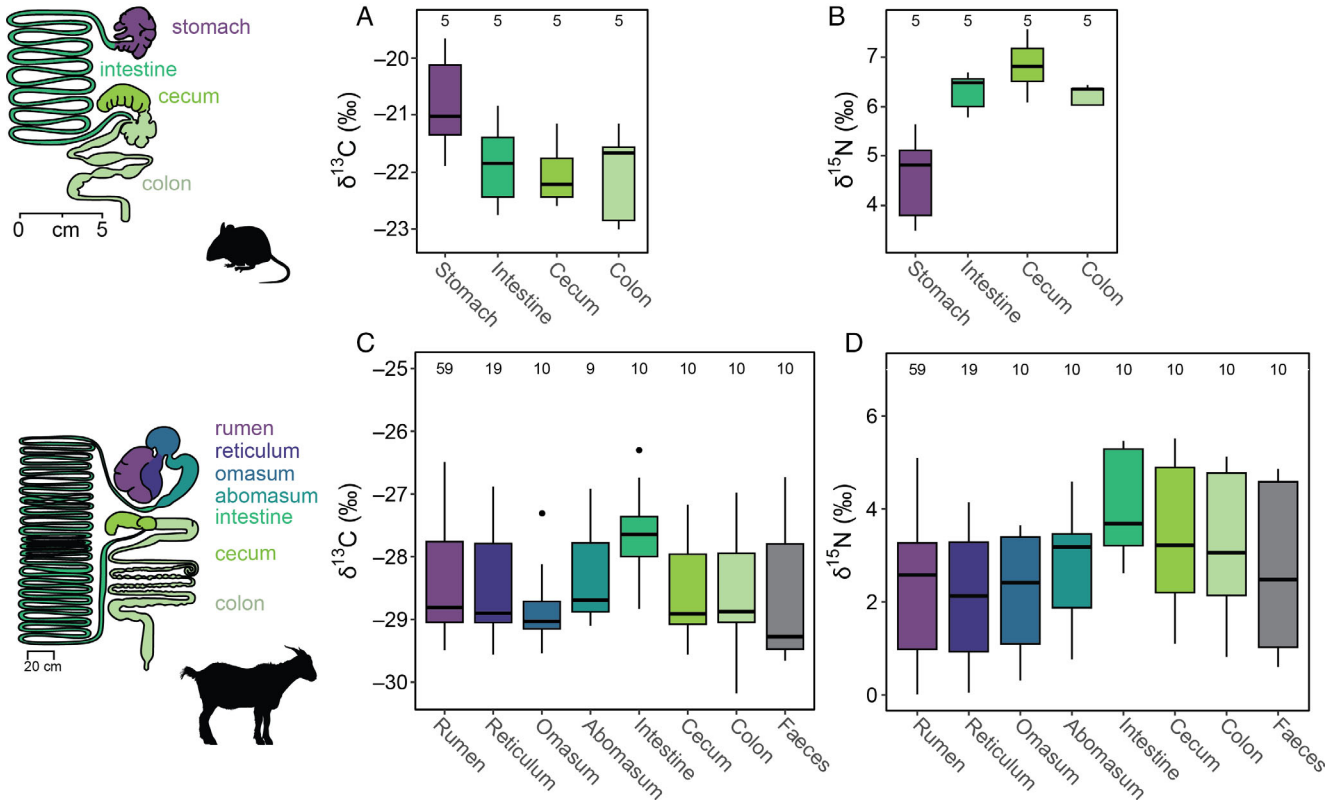

Fig. 3. Variation in carbon and nitrogen isotope values of digesta along the gastrointestinal tract of (A, B) deer mice (*Peromyscus maniculatus*; data from Hwang *et al.*, 2007), and (C, D) goats (*Capra hircus*; data from Codron *et al.*, 2012). The number of samples analysed is included above each boxplot. We combined data from goats fed either a C_3 or C_4 diet because the patterns were consistent between these two groups. For description of box plots see legend to Fig. 2.

For ectotherms (animals dependent on external heat sources for body heat) digestive efficiency is primarily a function of temperature, as many of the reactions necessary for the digestive process are temperature dependent (Naulleau, 1983; Zimmerman & Tracy, 1989). Such a relationship can be expressed using a Q_{10} value, which is a quantification of temperature sensitivity across a span of 10 °C of a given reaction or biological function (Skoczylas, 1978; Fänge & Grove, 1979; Waldschmidt, Jones & Porter, 1986). In both ectotherms and heterothermic endotherms, which can vary between self-regulation of body temperature and reliance on the external environment (e.g. sloths), reduced body temperatures correlate with reduced food intake and increased gut retention time (Zimmerman & Tracy, 1989; Cliffe *et al.*, 2015). For example, following a diet switch, it took between 6 and 15 h (shorter at higher temperatures) for the isotopic signal in the new food to be detectable in the faeces of juvenile pacu fish (*Piaractus mesopotamicus*; de Sandre *et al.*, 2016). But, as with the above example with sheep, because the authors' goal was to determine gut passage rates, they did not extend their monitoring beyond 30 h, which means we cannot assess when full isotopic turnover occurred. It is important to note that slower digestion at lower temperatures does not affect the fundamental digestibility of consumed food, but instead enables more microbial fermentation and better nutrient absorption, which also occur more slowly at lower temperatures (Zimmerman & Tracy, 1989; Stevens & Hume, 1998).

Studies that extend for longer periods and allow for animal faeces to reach isotopic equilibrium with diet suggest that isotopic turnover can take much longer than gut passage rates (days to months). Researchers typically monitor both the amount of time it takes for faeces (or animal tissues) to reflect a new diet after a dietary switch experiment using a half-life system (the time it takes for half of the ^{13}C or ^{15}N isotopes in the faecal matter to reflect the isotopic composition of the new diet) and the amount of time it takes for the faecal isotope value to stabilise and completely reflect the new diet. Work with sheep has suggested that the ^{13}C half-life of faecal matter is 24–29 h, but the time needed for the isotopic signal to stabilise was 6–8 days (Norman *et al.*, 2009; Martins *et al.*, 2012). Equilibration of the carbon isotope values in faeces and diet took 7 days for alpaca (*Vicugna pacos*), but only 3 days for horses (*Equus caballus*), which are a hindgut fermenter, meaning that microbial fermentation occurs in the large intestine and cecum, as opposed to the stomach chambers found in ruminants (Sponheimer *et al.*, 2003*a*; Fig. 1J, K). Goat (*Capra aegagrus hircus*) faecal isotope values also began to change within 2–3 days after a diet switch, but it took at least 60 days for faeces to reach isotopic equilibrium with the goats' new diet (Codron *et al.*, 2011*a*). Collectively, these studies suggest that isotopic turnover operates on a much longer timescale than gut passage rate, and that it can vary tremendously across taxa. Thus, the period of time represented in a faecal sample can range from potentially just hours to months depending on species.

In addition to variability among taxa, the period of time represented by a faecal sample may vary further within taxa, as different dietary items may be more or less digestible. For example, fruits are often more easily and quickly digestible than leaves (e.g. Wenninger & Shipley, 2000) and may therefore have quicker gut passage rates. Should certain dietary items, such as masting fruits, gain importance seasonally, then the amount of time represented by a faecal sample may also vary seasonally. Furthermore, faeces that are passed quickly may contain a larger proportion of undigested materials (and therefore a smaller proportion of gut tissue and epithelial cells), thereby reducing the magnitude of $\Delta_{diet–faeces}$ values, as suggested by Kuwae *et al.* (2022) for shorebirds. This potential variability in diet–faeces discrimination with diet is important for researchers to keep in mind when comparing faecal isotope data across seasons.

(5) How do nutritional composition and diet variability impact diet–faeces discrimination?

Given that faecal matter is a mixture of undigested food (perhaps incorporating multiple different feeding events), sloughed gut lining and microbes, $\Delta_{diet–faeces}$ values might be expected to vary with the nutritional composition and digestibility of food, in addition to the digestive physiology of the study organism. For example, animals that consume low-fibre diets (e.g. carnivores or insectivores) should have less undigested food in their faecal matter than herbivores (although hair, nail, and bone may be present). This would be especially true for hindgut fermenters, like horses. More than 40 years ago, Jones *et al.* (1979) determined that varying the amounts of C_3 legumes *versus* C_4 grass in herbivore diets affected both faecal $\delta^{13}C$ values and $\Delta^{13}C_{diet–faeces}$ values for cows, sheep, goats, and rabbits. Varying the relative proportion of C_3 and C_4 feeds resulted in a reasonably predictable linear, and even better quadratic, relationship between average diet and faecal $\delta^{13}C$ values. However, both the magnitude and direction of $\Delta^{13}C_{diet–faeces}$ varied: sheep, goats, and cows fed a pure C_3 legume diet had faecal $\delta^{13}C$ values that were higher than diet values (and a larger difference in $\delta^{13}C$ values between diet and faeces), while those fed pure C_4 grass had smaller $\Delta^{13}C_{diet–faeces}$ values and faecal $\delta^{13}C$ values were both slightly lower and higher than diet (Table 1). In turn, animals fed mixed diets had variable $\Delta^{13}C_{diet–faeces}$ values, depending on the composition of the consumed food (Table 1). The authors speculated that there may be some influence (or in their words, 'contamination') of previously consumed food on faecal $\delta^{13}C$ values, that activity of gut microbes might differ among diets, and most importantly, that differences in the digestibility of dietary components results in more variable results (which could lead to over- or under-estimation of dietary components). Diet–faeces discrimination also varied considerably among mice (*Mus musculus*) that were fully equilibrated on eight different diets comprised of alternately C_4-labelled cellulose, starch, lipids or protein (Tieszen & Fagre, 1993). The authors found

that dietary cellulose and starch accounted for the majority of faecal $\delta^{13}C$ values, suggesting that these macronutrients were the least digested.

More recently, Codron *et al.* (2011*a*) found that goats fed varying mixtures of C_3 and C_4 hay had larger and more variable $\Delta^{13}C_{diet–faeces}$ values than goats fed pure C_3 or C_4 diets (Table 1). These researchers observed some shifts in discrimination when animals were still equilibrating to their new feed after a diet switch. Following equilibration, however, individuals fed mixed C_3–C_4 diets once again had larger and more variable discrimination values than those fed pure C_3 or C_4 diets. Similar to Jones *et al.* (1979), the authors reasoned that C_3 hay, which has a lower fibre digestibility, had more rapid turnover rates and also contributed more to faecal $\delta^{13}C$ values than C_4 hay when the two were fed in combination. Comparable results were observed for gazelles (*Gazella leptoceros*) fed a similar mixed diet (Codron *et al.*, 2012). Conversely, sheep fed a 50:50 mix of C_3 alfalfa hay and C_4 silage had small and quite homogenous $\Delta^{13}C_{diet–faeces}$ (Table 1; Martins *et al.*, 2012).

Variable $\Delta_{diet–faeces}$ resulting from different diets is expected, given the effect of diet on discrimination between diet and animal body tissues, like blood and muscle (e.g. Pearson *et al.*, 2003; Mirón *et al.*, 2006; Lecomte *et al.*, 2011). In particular, the amount of protein consumed has been shown to impact both $\Delta^{13}C_{diet–tissue}$ and $\Delta^{15}N_{diet–tissue}$ values, although the influence of diet on diet–tissue discrimination is not uniform across taxa (e.g. Hobson & Clark, 1992; Pearson *et al.*, 2003; Mirón *et al.*, 2006). Both digestive physiology and the nutritional makeup of the foods are likely responsible for these differences (e.g. Vanderklift & Ponsard, 2003; Caut, Angulo & Courchamp, 2009). For example, the specific amino acid profiles of different consumed foods can affect $\Delta^{15}N_{diet–tissue}$ values; if foods with complementary amino acids are consumed at the same time, this can effectively increase the quality of the foods, which in turn increases efficiency in nitrogen uptake and thus decreases tissue $\delta^{15}N$ values (Robbins, Felicetti & Florin, 2010). Recent work further suggests that intestinal microbes might play an important role in supplying essential amino acids to their hosts (Newsome *et al.*, 2020). Mice fed low-protein diets had a higher relative abundance of Firmicutes in their gut microbiome and used a greater proportion of microbially derived essential amino acids in their tissues (up to 60%; Newsome *et al.*, 2020). Another consideration is variability in routing of proteins and/or lipids to consumer tissues, which has also been shown to impact diet–tissue isotopic discrimination (e.g. Wolf *et al.*, 2015).

As has been observed for diet–tissue discrimination, variation in the amount of dietary protein does not uniformly impact diet–faeces discrimination across taxa. Looking at Table 1, we see that larger $\Delta^{13}C_{diet–faeces}$ values have been observed for humans, llamas (*Llama llama*), alpacas, and grasshoppers fed lower protein diets (DeNiro & Epstein, 1978; Sponheimer *et al.*, 2003*a*; Kuhnle *et al.*, 2013), while the opposite was seen for cows, goats, and sheep (Jones *et al.*, 1979). However, ungulates in general have highly variable results (e.g. Wilson *et al.*, 1988; Sponheimer *et al.*, 2003*a*). Red-backed voles (*Myodes gapperi*) also have quite variable $\Delta^{13}C_{diet–faeces}$ values (Sare, Millar & Longstaffe, 2005; Hwang *et al.*, 2007); individuals fed high- and low-protein diets had indistinguishable $\Delta^{13}C_{diet–faeces}$ values, while individuals fed medium-protein diets had considerably larger $\Delta^{13}C_{diet–faeces}$ values and individuals fed manufactured pellets had the largest $\Delta^{13}C_{diet–faeces}$ values. By contrast, mice fed high- and low-protein diets also had indistinguishable $\Delta^{13}C_{diet–faeces}$ values, but individuals fed medium-protein diets had smaller $\Delta^{13}C_{diet–faeces}$ values (Tieszen & Fagre, 1993). Large $\Delta^{15}N_{diet–faeces}$ values have been observed for humans, horses, red-backed voles, and yellow-vented bulbuls consuming lower protein diets (Sponheimer *et al.*, 2003*b*; Sare *et al.*, 2005; Tsahar *et al.*, 2008; Kuhnle *et al.*, 2013). Conversely, dairy cows fed higher protein diets had larger $\Delta^{15}N_{diet–faeces}$ values than those fed lower protein diets, and llamas fed diets with differing protein content had indistinguishable $\Delta^{15}N_{diet–faeces}$ (Sponheimer *et al.*, 2003*b*; Cantalapiedra-Hijar *et al.*, 2015). Thus, diet composition can clearly have a significant impact on diet–faeces discrimination, but the available data do not allow for generalisation; this is an area that would benefit from further research.

(6) How does study design impact diet–faeces discrimination?

Many of the discrepancies in $\Delta_{diet–faeces}$ values among studies may reflect differences in how controlled feeding studies are designed and executed. Indeed, much of the considerable variability in $\Delta_{diet–faeces}$ values evident in ungulate taxa that have been the subject of multiple studies reflects variability in both study length and provisioned diet (Fig. 4). For example, cattle held on C_3 diets for 123 days *versus* just 22 days had opposite $\Delta^{13}C_{diet–faeces}$ values (1.8‰ *versus* −2.4‰; Table 1; Jones *et al.*, 1979). As noted above, despite the fact that gut passage rates may be short and the most recently consumed meal may be visible in faeces in as little as 2–3 h for small-bodied mammals and birds with simple digestive tracts, previously consumed foods can still be detectible in faeces for >6 days for animals with complex digestive tracts (Jones *et al.*, 1979; Sponheimer *et al.*, 2003*b*; Martins *et al.*, 2012), and equilibrium between diet and faecal isotope values may not be reached for months (Codron *et al.*, 2011*a*). This is again because faeces are more than just undigested food. True steady state will only be reached when sloughed microbes and gut lining are also in equilibrium with diet. Thus, if an animal was only fed a controlled diet for a short period of time, which has been the case with many of the captive feeding studies to date (see Table 1), faecal isotope values may still be affected by foods consumed prior to the dietary switch.

Differences in diet–faeces discrimination values among studies may also have to do with how diet is calculated. Perhaps unsurprisingly, $\Delta_{diet–faeces}$ values (particularly $\Delta^{13}C_{diet–faeces}$) can vary greatly depending on whether whole diet, or just dietary protein, is used in the calculation (Wolf *et al.*, 2015). Equally importantly, researchers should not assume that the

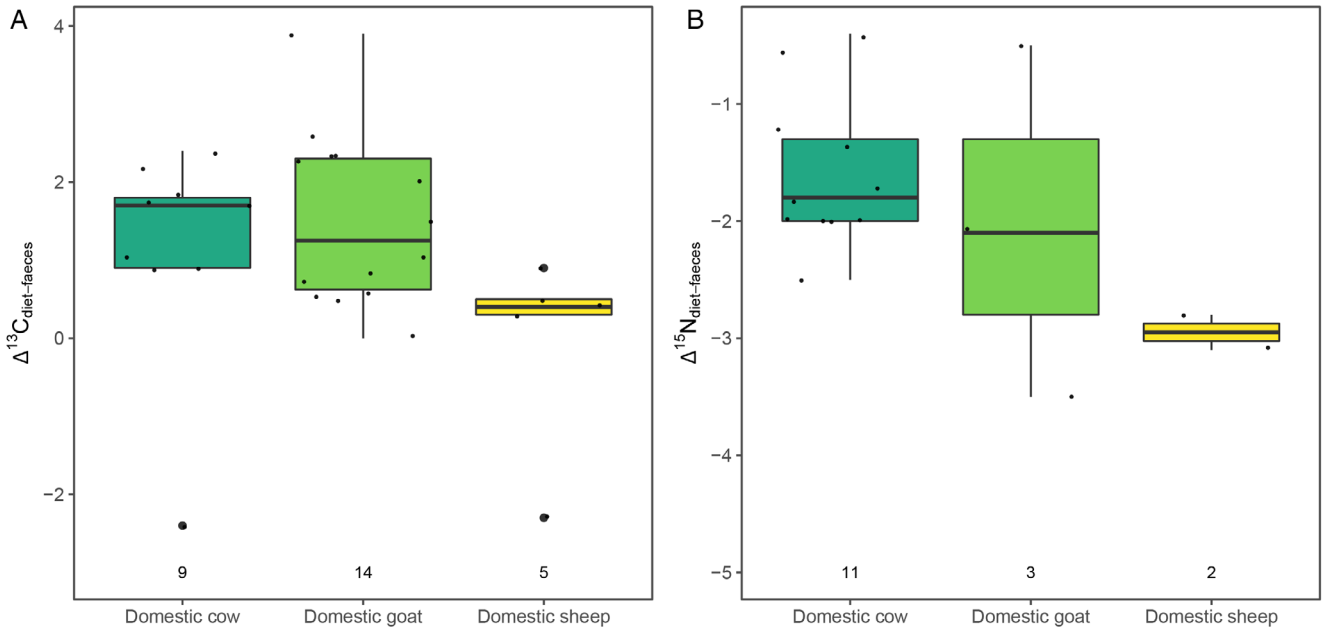

Fig. 4. Variation in calculated diet–faeces isotope discrimination values for the three most commonly studied taxa in the published literature: (A) $\Delta^{13}C_{diet-faeces}$ and (B) $\Delta^{15}N_{diet-faeces}$. The number of studies incorporated are included below each boxplot. For description of box plots see legend to Fig. 2.

isotopic composition of food is constant across space or over time. Even simple foods, such as monospecific hay and manufactured chow, can have variable isotope values (e.g. Schneider *et al.*, 2015; Montanari & Amato, 2015; B.E. Crowley, personal observations). Moreover, because multiple meals may be combined into a single faecal sample, or alternatively, undigested portions of the same meal may be spread across multiple faeces produced at different times, there may be considerable variability in estimated $\Delta_{diet-faeces}$ for animals that eat diverse diets. This potential issue likely explains the large variance in $\Delta_{diet-faeces}$ for captive meerkats (*Suricata suricatta*) and langurs (*Trachypithecus francois*; Table 1), as well as wild coyotes (*Canis latrans*) and jaguars (*Panthera onca*; Table 2). Such pronounced isotopic variance, both among individuals and among faeces produced by the same individual, can complicate the ecological information one can glean from faecal isotope data (Codron *et al.*, 2012; Crowley *et al.*, 2019, 2023; Reitsema *et al.*, 2020).

It is imperative to consider dietary variability within and among individuals as well as across multiple timescales when calculating $\Delta_{diet-faeces}$ values. If individuals eat variable diets on a daily basis, then averaging dietary $\delta^{13}C$ or $\delta^{15}N$ values over several days may be appropriate. Similarly, if individuals eat seasonally variable diets, but those shifts are consistent and predictable, then averaging dietary $\delta^{13}C$ or $\delta^{15}N$ values within a season may be appropriate. However, if an individual consumes different foods on different days, or if different individuals within a study have different dietary preferences, averaging diet may result in inaccurate discrimination estimates. The importance of this point is illustrated in a study on omnivorous captive meerkats (Montanari, 2017). The author concluded that there is no discrimination in carbon isotope values between diet and faeces ($\Delta^{13}C_{diet-faeces} = -0.1 \pm 1.5‰$), but those calculations were based on data averaged across days as well as individuals within a common enclosure. The meerkats were fed isotopically and compositionally variable foods (including fruits, dog food, horsemeat, and whole chicks and mice), but dietary intake was not monitored for specific individuals, nor was the author able to track which individuals produced the faecal samples analysed. As discussed in Section II.5, given that offsets may vary depending on diet composition and protein quality (e.g. Robbins *et al.*, 2010), definitively concluding a lack of offset between diet and faeces based on these data seems unwise.

There are several steps researchers can take to minimise the impact of study design on $\Delta_{diet-faeces}$. Experiments should be long enough to ensure that (*i*) the study subjects have reached equilibrium with their diets, and (*ii*) the isotopic composition of previous, non-experimental diets does not introduce variability into calculated $\Delta_{diet-faeces}$ values. Experimental diets can be simplified or homogenised over the length of the experiment. Measuring and reporting both the isotopic and elemental composition (wt% C and N) of foods as well as the nutritional composition (including fibre, protein, carbohydrate, and lipids), would also improve interpretation of experimental results. Finally, if animals are provisioned with more than one food item, then dietary intake and faecal output should be monitored for specific individuals (rather than groups of animals in a shared enclosure) to ensure that the dietary preferences of specific individuals do not cloud study results. Accounting for sex, age, health, and individual behavioural differences may also be informative.

III. APPLICATIONS OF C AND N ISOTOPES IN FAECES

Despite the challenges associated with faecal isotope work, faecal isotope analysis has proved incredibly useful in a number of different fields, contributing to valuable discoveries in dietary ecology, spatial ecology, biogeochemical research on nutrient inputs and cycling, and palaeoclimate and palaeoenvironmental reconstructions. Below we provide an overview of each of these broad applications.

(1) Dietary analysis

One of the earliest and most frequently used faecal isotope applications is in interpreting diet. Studies range from characterising diet generally (e.g. Codron *et al.*, 2005; Painter *et al.*, 2009) or seasonally (e.g. Botha & Stock, 2005; Djagoun *et al.*, 2013), to evaluating dietary niche partitioning (e.g. Stewart *et al.*, 2003; Codron *et al.*, 2016), and monitoring ontogenetic dietary shifts (e.g. Trakimas *et al.*, 2011), including nursing and weaning (e.g. Reitsema, 2012; Bădescu *et al.*, 2016, 2017). Researchers have also used faecal isotopes to detect the consumption of marine or anthropogenic foods by wild terrestrial animals (e.g. Christie, Hocking & Reimchen, 2008; Reid, Gifford-Gonzalez & Koch, 2018).

Early research using faecal isotopes focused on establishing the degree to which faecal isotope ratios could serve as proxies for the diets of African herbivores (Codron *et al.*, 2005; Codron & Codron, 2009). These studies established that browsers (C_3 feeding) and grazers (C_4 feeding) were easily and accurately distinguishable using faecal $\delta^{13}C$ values and that faecal $\delta^{15}N$ values tracked both dietary protein intake (as reflected by faecal %N) and spatial trends in plant $\delta^{15}N$ values. The authors concluded that, for ungulate herbivores at least, faecal isotope values are reliable proxies for diet.

To determine the degree to which faecal isotope values can be used to reconstruct the diets of trophic omnivores, which have more varied diets than herbivores, researchers have compared dietary information from faecal isotope data with data obtained using other methods, such as macroscopic faecal analysis or feeding observations. For example, Hatch *et al.* (2011) compared estimates for diet of wild black bears (*Ursus americanus*) derived from faecal carbon and nitrogen isotope data and from macroscopic scat dissection, also known as gross faecal analysis. Using a similar approach, Reid & Koch (2017) compared dietary estimates from coyote faecal stable isotope data and scat dissection while Phillips & O'Connell (2016) compared carbon and nitrogen isotope ratios for wild chimpanzee (*Pan troglodytes schweinfurthii*) faeces with the proportion of macroscopically identified food items in the faeces as well as observed food intake. Together, these studies suggest that dietary reconstructions drawn from faecal stable isotope data are robust for herbivores and omnivores so long as there is sufficient isotopic variability within the food web.

Because faeces are thought to integrate diet over a relatively short period of time, they can help elucidate seasonal dietary shifts (e.g. Botha & Stock, 2005; Blumenthal *et al.*, 2012; Tsutaya *et al.*, 2022). Significant intra-annual variability in faecal isotope values for wild mountain gorilla (*Gorilla beringei beringei*), for example, has been documented with faecal isotope ratios; even within a purely C_3 ecosystem, different plant foods were isotopically distinguishable and faecal isotope values reflected seasonal reliance on fruit (higher $\delta^{13}C$ values during periods of increased frugivory; Blumenthal *et al.*, 2012).

Faecal isotope values can be robust indicators of dietary resource subsidies (the consumption of resources that are externally sourced, such as marine or anthropogenic foods like crops or human refuse) by terrestrial animals (e.g. Codron *et al.*, 2006; Reid *et al.*, 2018; Larson *et al.*, 2020; Reitsema *et al.*, 2020). This is possible because these foods tend to be isotopically distinguishable from naturally available terrestrial foods (Newsome, Clementz & Koch, 2010; Hopkins *et al.*, 2014).

Faecal isotope analyses have also proved useful for exploring dietary niche partitioning, that is, the division of food resources to reduce competition among species within a shared ecosystem (e.g. Stewart *et al.*, 2003; Codron & Brink, 2007; Codron *et al.*, 2011*b*, 2018; Sawyer, 2020). For example, Stewart *et al.* (2003) evaluated dietary overlap among three free-ranging ungulates with different body sizes (mule deer, *Odocoileus hemionus*; elk, *Cervus elaphus*; and domestic cattle, *Bos taurus*) in northeastern Oregon, USA. Using faecal $\delta^{13}C$ and $\delta^{15}N$ values in conjunction with microhistological analysis (the identification under the microscope of plant fragments found in faecal samples) the authors found dietary separation among the species.

Primatologists have used faecal isotope values to monitor when juveniles stop nursing. These data complement observational data, which may miss portions of the diurnal cycle (e.g. night suckling), and cannot clearly distinguish when infants obtain nutrition from suckling *versus* simply suckle for comfort. If an infant regularly consumes breast milk, it will have a distinct diet (a by-product produced by its mother), and this should be isotopically detectable (e.g. Fuller *et al.*, 2006). If the infant obtains its nutrition from its mother, then the two should be isotopically distinct; if they consume the same foods, then they should be isotopically comparable (e.g. Reitsema, 2012; Bădescu *et al.*, 2017).

(2) Spatial ecology

While not as established as some of the other faecal isotope applications, the potential to non-invasively monitor habitat and landscape use, spatial niche partitioning, and individual mobility using faecal isotope data has also been recognised by several researchers in the past decade. Although scant, this existing work paves the way for future research. We summarise several applications below to highlight the types of spatial questions that can be addressed.

Walter *et al.* (2010) found that by combining faecal $\delta^{13}C$, $\delta^{15}N$ and %N values with faecal microhistology, radiotelemetry, and observational data it was possible to track differences in habitat and resource use (including consumption of cultivated crops) by North American elk. Conversely,

Gustine *et al.* (2012) found that faecal $\delta^{15}N$ values were not particularly well suited for distinguishing populations of caribou (*Rangifer tarandus*) in Alaska. The authors hypothesised that this was likely due to both the highly generalist diet of any given caribou individual (including lichens, mosses, forbs, graminoids, as well as woody vascular evergreen and deciduous shrubs), and the highly mobile nature of caribou (they can rapidly traverse quite variable landscapes). More recently, Hixon *et al.* (2022) used faecal isotope data to determine that introduced domesticated dogs (*Canis familiaris*) consume animals in protected forests in eastern Madagascar. Lastly, Crowley *et al.* (2019) compared isotopic data for faecal matter and bone from consumed and digested prey for wild jaguars from a protected area in Central Belize. The authors determined that although isotope data for jaguar faecal matter are messier than those for bones, they can be used to evaluate landscape use. The authors subsequently combined non-invasive molecular scatology and faecal isotope data to determine areas of use for individual male jaguars (Crowley *et al.*, 2023).

(3) Tracking nutrient inputs and cycling

As discussed above, faecal isotope values have proved useful for detecting the consumption of allochthonous food resources by individuals, but faeces themselves can also be considered nutrient subsidies – that is, they can add nutrients to ecosystems. Faeces from large herbivores in savanna and temperate grassland ecosystems increase net primary productivity and improve nutrient availability (e.g. Frank & Evans, 1997; McNaughton, Banyikwa & McNaughton, 1997; Augustine, McNaughton & Frank, 2003; Craine *et al.*, 2009), and humans have intentionally added manure to agricultural fields as fertiliser for thousands of years (e.g. Jones, 2013). Even incidental exposure to manure *via* temporary herbivore enclosures can have long-term impacts on the primary productivity of an area (Fox-Dobbs *et al.*, 2010). Because nitrogen is both a significant component of faeces and often a limiting resource, a number of researchers have used nitrogen isotopes to establish the existence of a faecal nutrient subsidy and subsequently trace the fate of that subsidy through food webs (e.g. Fujita & Koike, 2007; McCauley *et al.*, 2015; Schrama *et al.*, 2013). Other researchers operate under the assumption that elevated nitrogen isotope values in soil, plants, or animal tissues reflect faecal inputs (e.g. Frank & Evans, 1997; Hawke & Newman, 2007). For example, Hawke & Newman (2007) evaluated the degree to which breeding seabirds provide nutrient enrichments to coastal forest communities on small islands around New Zealand by comparing $\delta^{13}C$ and $\delta^{15}N$ values in the leaves of three different tree species sampled on islands with and without seabirds. They found elevated $\delta^{15}N$ values in forest vegetation on islands with birds, and concluded that this reflected nutrient inputs from seabird faeces.

Because it is often not possible to measure isotope values in faecal material directly when studying the past, researchers frequently rely on sediments or palaeobotanical samples, such as charred seeds to interpret historical and ancient nutrient subsidies and manuring practices (Bogaard *et al.*, 2007; Fraser *et al.*, 2013; Kanstrup *et al.*, 2014; Styring *et al.*, 2017). For example, Marshall *et al.* (2018) compared $\delta^{15}N$ values of sediments collected both within and outside of ancient herbivore corral sites in East Africa. They found that these 2000–3000-year-old sites, despite being long abandoned, have remained nutrient-enriched (and have elevated $\delta^{15}N$ values) into the present. This suggests that early pastoralists had a much more enduring influence on this landscape than previously thought, creating nutrient-rich grassy patches in wooded grassland savanna that continue to be important to both wildlife and humans today.

(4) Reconstructing palaeoclimate/palaeoenvironment

Most of the existing research using faecal stable isotopes to investigate past climate and environment has relied on large accumulations of faeces, such as guano piles, middens, and protected accumulations of domesticated herbivore dung. Guano deposits, which accrue from the detritus of bats and birds that roost in large numbers in caves, are comprised of a mixture of both unconsolidated organic material and inorganic material, including phosphate and carbonate minerals, silts, and clays (Mizutani, McFarlane & Kabaya, 1992*a*,*b*). Protected from surface weathering conditions, these deposits can accumulate undisturbed over millennia, potentially providing a high-resolution archive of past climate and environment. Such records are particularly valuable for the low latitudes, where other more typical archives, such as tree rings, are less reliable. Des Marais *et al.* (1980) were the first to posit a connection between carbon isotope values in bat guano and local climate and environment. They reasoned, and ultimately confirmed, that the $\delta^{13}C$ values of arthropods consumed by insectivorous bats reflect the local vegetation in and around Carlsbad Caverns, New Mexico, and that this in turn, could vary over time in response to changes in the abundance and distribution of C_3 *versus* C_4 vegetation driven by regional climate or human activity. Although this pioneering work was conducted 40 years ago, additional research in this arena was nearly non-existent until after the year 2000 and has really only taken off in the past 10 years (e.g. Onac *et al.*, 2014, 2015; Widga & Colburn, 2015; Forray *et al.*, 2015; Royer *et al.*, 2015; Choa *et al.*, 2016; Wurster *et al.*, 2017, 2019; Cleary *et al.*, 2018, 2019; Cleary & Onac, 2021). Some authors have also argued that guano $\delta^{15}N$ values can serve as a proxy for water availability in the past, although interpretations of these data have been hampered by the complexity of the nitrogen cycle and the possibility for diagenetic alteration (Cleary *et al.*, 2016; Reid *et al.*, 2022).

Packrat (*Neotoma* spp.) and rock hyrax (*Procavia capensis*) middens also have the potential to be highly informative palaeoenvironmental archives. Both species live in arid environments and their middens, which tend to be built in sheltered places such as caves, rock ledges, or rock crevices, can be used by generations of animals in the same location for

millennia. These factors all assist in the long-term preservation of the midden materials. Scott & Vogel (2000) introduced the idea of using hyrax faecal $\delta^{13}C$ values to study past changes in climate and environment. They argued that, because hyraxes are herbivores that eat a wide range of plant tissues and species, changes in their dung $\delta^{13}C$ values are likely to be driven by vegetation and climate change. Hyrax midden $\delta^{15}N$ values are thought primarily to reflect water availability. In a study evaluating Holocene palaeoenvironmental change in Namibia, Chase *et al.* (2009) observed that $\delta^{15}N$ values from hyrax middens were negatively correlated with other indicators of past precipitation, as has been observed in modern plants, soils, and vertebrate tissues (e.g. Cormie, Luz & Schwarcz, 1994; Robinson, 2001; Amundson *et al.*, 2003; Crowley *et al.*, 2011). Carr *et al.* (2016) later observed that modern faecal $\delta^{15}N$ values were positively correlated with both plant and midden $\delta^{15}N$ values, a relationship later used by Chase *et al.* (2019) to argue that hydroclimate variability in the Namib desert region was orbitally paced. Palaeoenvironmental work using rock hyrax middens was reviewed most recently by Chase *et al.* (2012).

While most palaeoenvironmental work has focused on natural faecal deposits in caves and other protected sites like rock ledges, it is also possible to track historical vegetation changes using accumulations of faeces from domesticated taxa. For example, a series of studies (Witt, Moll & Beeton, 1997; Witt *et al.*, 2000; Witt, Luly & Fairfax, 2006) examined historic vegetation change in Australian rangelands since the 1930s using sheep faecal accumulations beneath shearing sheds. Elevated shearing sheds are common in the semi-arid rangelands of Australia and provide a protected area where sheep faeces can accrue over decades. These accumulations preserve a record of sheep diet in the days to weeks leading up to shearing, which occurs at roughly the same time each year.

IV. FUTURE RESEARCH OPPORTUNITIES

Despite decades of research on faecal isotopes, there is still ample room for improving our understanding of $\Delta_{diet–faeces}$, especially regarding the impact of digestion, gut passage rates, and diet composition. Most studies working with faecal isotopes have been done using captive animals, but not necessarily in a way that strictly controlled the diet of those animals, nor were captive individuals provisioned with the exact types and proportions of foods that may be available in natural settings. Nonetheless, captive feeding studies are still likely the best approach for constraining $\Delta_{diet–faeces}$, as they allow for many factors to be controlled, or at least accounted for. As we suggested in Section II.6, some important considerations for future controlled feeding experiments include ensuring that studies are of sufficient duration for subjects to reach equilibrium with their experimental diets, simplifying and homogenising experimental diets, and measuring and reporting the isotopic, elemental, and nutritional composition of dietary items. While some progress has been made in the investigation of the relationship between diet composition and $\Delta_{diet–faeces}$, ample opportunities remain for conducting controlled feeding experiments that explicitly test the effect of diet composition on $\Delta_{diet–faeces}$.

Additional factors that influence the nutritional balance of an individual and impact $\Delta_{diet–tissue}$ [e.g. age, health, or reproductive status (Hobson & Clark, 1992; Vanderklift & Ponsard, 2003; Lecomte *et al.*, 2011)], could in theory also affect $\Delta_{diet–faeces}$. Yet, the few studies that have explicitly looked at one or more of these factors have not detected compelling patterns. For example, Wilson *et al.* (1988) found negligible (<0.5‰) differences in $\Delta_{diet–faeces}$ between 'early' and 'late' lactating cows, as well as between cows with different genetic potential for milk production, and Reitsema *et al.* (2020) found no differences in $\Delta_{diet–faeces}$ between infant and adult capuchin monkeys (*Sapajus libidinosus*). As these factors have only been cursorily investigated in faecal isotope research, further work would be welcome.

Lastly, comparability among studies would be significantly improved by the adoption of standardised faecal sample preparation techniques. To our knowledge, most authors analyse bulk, homogenised faecal samples. However, several authors have suggested that faeces should be physically or chemically processed prior to analysis (e.g. Wurster *et al.*, 2017; Kuwae *et al.*, 2022; Oelze *et al.*, 2022). This deserves further attention.

V. CONCLUSIONS

(1) Faeces are not homogenous. They contain a mixture of bacterial and endogenous debris in addition to undigested foods.
(2) Isotopic turnover likely operates on a longer timescale than gut passage rate. Faecal isotope values could record dietary intake over several hours to several months.
(3) Faecal $\delta^{13}C$ and $\delta^{15}N$ values broadly reflect diet. However, $\Delta_{diet–faeces}$ is also highly variable both within and among taxa. This is likely the result of a combination of factors, including physiology, the nutritional composition of food, dietary variability, and study design.
(4) Much of our current knowledge is heavily taxonomically biased towards ruminant ungulates. Exploring variability in $\Delta_{diet–faeces}$ for other taxa, particularly non-mammalian taxa, should be a priority.
(5) Faecal isotope research can be improved through careful study design and the adoption of standardised sample preparation procedures.
(6) Despite uncertainties in the drivers of variability in $\Delta_{diet–faeces}$ within and among taxa, much can still be learned from faecal isotope values. Faecal $\delta^{13}C$ and $\delta^{15}N$ values have been successfully used to make important contributions to both dietary and spatial ecology, biogeochemical research on nutrient inputs and cycling, and palaeoclimate and palaeoenvironmental reconstructions.

(7) Greater awareness of the unknowns and assumptions involved in using faecal isotope data will serve both to refine the research questions we can currently address as well as to direct avenues for future research.

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