More than just meat: Carcass decomposition shapes trophic identities in a terrestrial vertebrate

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More than just meat: Carcass decomposition shapes trophic identities in a terrestrial vertebrate

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Abstract
1. Most food web models fail to account for the full complexity of interactions within a community, particularly where microbes are involved. Carcasses are microbe-rich resources and may represent a common nexus for the macrobiome and microbiome, effectively uniting autotrophs, consumers, predators and microbiota.

2. We evaluated the role of carcasses as multitrophic resources and explored dietary partitioning for a sexually dimorphic obligate scavenger known for its hierarchical social system. This study was set in a well-studied community of camelids Vicugna, Lama guanicoe, pumas Puma concolor and Andean condors Vultur gryphus in the Andes. We hypothesized that condors, by feeding on trophically distinct dietary substrates within any given carcass, would have highly variable trophic position (TP) values. Furthermore, we expected that the microbial consumers within the carcass would inflate TP values in both, the carrion and the condors. Thus, we expected that the trophic heterogeneity within a carcass could facilitate sex-based dietary partitioning in condors.

3. We used a multifaceted approach to assess the foraging of Andean condors, using regurgitated pellet and bulk isotopic analyses, and also quantified the TP of the entire community of graminoids, camelids, camelid carrion, pumas, and female and male condors employing compound-specific stable isotopes analysis of amino acids.

4. Our analysis of condor pellets and bulk isotopes revealed non-trivial plant consumption, close to 10% of condor diet. Isotope analysis of amino acids revealed that condors had highly variable TPs (2.9 ± 0.3) compared to pumas (3.0 ± 0.0) and camelids (2.0 ± 0.1), likely representing 'trophic omnivory', wherein the condors consume plants (TP = 1.0 ± 0.1) and microbe-colonized carrion (2.3 ± 0.1). Female condors exhibited a TP (2.8 ± 0.2) lower than strict carnivory, suggesting that they consume more plant biomass in a carcass, while males (TP = 3.1 ± 0.3) are likely consuming more of the microbe-rich animal tissue.
1 | INTRODUCTION

Predation is one of the most studied and captivating trophic interactions (Sergio et al., 2006; Wilson & Wolkovich, 2011). Apex carnivores exert disproportionate effects on prey both directly (‘consumptive’) and indirectly (‘non-consumptive’), sometimes with cascading consequences for lower trophic levels (Estes et al., 2011). Consequently, the regulation of food webs by apex carnivore has been widely explored not only in an ecological context, but also in terms of animal conservation and ecosystem restoration (Ritchie et al., 2012; Wallach et al., 2015). There has been, however, a growing interest in actors beyond the classic ‘green food web’ (i.e. living primary producers–consumers–predators) to include those in the ‘brown food web’ (i.e. detritus–microbiota–scavengers). It is becoming increasingly recognized that this traditional view of neatly compartmentalized green or brown food webs is not broadly representative of most food webs—indeed green and brown food chains are usually so intertwined that it becomes trivial to characterize a higher-order consumer as either ‘green’ or ‘brown’ (Steffan & Dharampal, 2019; Wilson & Wolkovich, 2011).

Carcasses are one example blurring the lines between green and brown food webs and uniting the macrobiome and microbiome (Barry et al., 2019; Burkepile et al., 2006; Shukla et al., 2018). Although vertebrate carcasses comprise a small fraction of total detritus in an ecosystem, they have a disproportionate local impact in nutrient deposition and cycling, especially of nitrogen, phosphorous and calcium, and represent nutritionally rich, yet ephemeral, resources (Carter et al., 2007; Parmenter & Macmahon, 2009). Consequently, carcasses are crucial for a diversity of scavengers and decomposers that compete for access to them (Burkepile et al., 2006). Although generally considered as a discrete and homogeneous dietary item, carcasses are a composite of organisms: animal tissue, plant matter and digesta held in the digestive tract, invertebrate scavengers and microbes that are consuming necrotic tissue. A single carcass can hold a diversity of organisms that span multiple trophic levels, from autotrophic biomass to secondary and tertiary consumers, including both macrobiota and microbiota. This so-called ‘necrobio­ome’ provides a framework to explore cross-kingdom interactions, where microbes can impact entire ecosystems (Benbow et al., 2019). Scavengers are likely consuming all components of a carcass (Pechal et al., 2019; Steffan & Dharampal, 2019)—not only the carrion (i.e. tissue of the dead animal), but also the innumerable microscopic consumers embedded within the carrion (Steffan et al., 2017; Steffan & Dharampal, 2019).

Obligate scavengers have evolved a suite of behavioural, physiological and anatomical adaptations to exploit ephemeral carrion resources (Ruxton & Houston, 2004).

Predators, by hunting prey regularly and often in a spatially predictable fashion, can provide some spatiotemporal consistency in carcass availability (Wilbers et al., 2003). Vultures occupy an important place in the food web by recycling nutrients (Moleón & Sánchez-Zapata, 2015), regulating disease outbreaks (Wilson & Wolkovich, 2011) and modulating predation rates due to competition–facilitation interactions with predators (Elbroch & Wittmer, 2013; Moleón et al., 2014). In the last century, the global abundance and distribution of vultures have declined in part due to decreased availability of safe carrion (Buecheley & Šekercioğlu, 2016; Pauli et al., 2018). These declines are likely disrupting important community interactions involving carcasses and the necrobio­ome (Benbow et al., 2019).

The Andean condor Vultur gryphus—the largest vulture (1.3 m height and 3 m wingspan) and one of the heaviest flying birds (13 kg body mass; Ferguson-Lees & Christie, 2001)—is considered carnivorous. Condors primarily consume carrion of large herbivores, generally provisioned by pumas Puma concolor in areas where native communities have been preserved, but they also can consume smaller-bodied vertebrates including exotic lagomorphs (Ballejo et al., 2018; Duclos et al., 2020; Perrig et al., 2017). Researchers have observed plant matter in the pellets of condors previously (Duclos et al., 2020; Pavez et al., 2019), although it is often considered to be incidental ingestion. Plant consumption among condors is a potential mechanism by which condors acquire pigments, notably carotenoids, which are associated with social dominance (Blanco et al., 2013; Marinero et al., 2018). Although male condors are up to 50% larger than females (Alarcón et al., 2017) and assume priority at carcasses (Alarcón et al., 2017; Donazar et al., 1999; Wallace & Temple, 1987), xanthophyll carotenoid concentrations in blood are highest in immature and female condors (Blanco et al., 2013). Possibly, then, dominant males are consuming the protein-rich components of the carcass and relegating subordinate individuals to plant material.

5. Our study highlights that carcasses represent a trophically heterogeneous resource and that vertebrate scavengers can feed across trophic groups within the carcass, from autotrophs to secondary consumers, and from both the macrobiome and microbiome. Thus, integration of microbes in macroecological contexts can help to resolve trophic identity, and better characterize the importance of microbes in detritivorous and omnivorous species.

KEYWORDS
carrion, detritivory, food webs, guanaco, microbe, necrobiome, omnivore, vulture
Research to-date has found little evidence of sex-based partitioning of diet (Perrig et al., 2021), although this could be overlooking fine-scale diet partitioning that happens within the carcass. Microbial or detrital impact on large vertebrate systems has been documented extensively (Hyodo et al., 2015; Stevens & Hume, 1998); however, the quantification of such microbivory has only recently been documented through trophic inflation of detrital complexes in invertebrates and fish (Dharampal et al., 2019; Steffan et al., 2017). Given that carcasses are a heterogeneous pool of resources, it is possible that sexes partition dietary resource at a carcass, possibly resulting in different trophic positions (TP). To date, the relative importance of carcass components to Andean condors has not been quantified.

To explore resource allocation from the carcass and to examine trophic identities of scavengers, we studied a camelid–puma–condor community in San Guillermo National Park (hereafter, San Guillermo NP; Figure 1). Located in northwestern Argentina, San Guillermo NP is one of the few places that maintained trophic interactions among native camels (vicuñas Vicugna vicugna and guanacos Lama guanicoe), pumas and Andean condors (Donadio et al., 2010, 2012; Perrig et al., 2017). Previous work in San Guillermo NP identified that puma predation is the primary cause of adult camelid mortality (accounting for >90% of carcasses; Donadio et al., 2012). Given that pumas are solitary hunters and leave large quantities of carcass materials behind (Elbroch et al., 2014), the majority (88%) of the condor diet in San Guillermo NP consists of camelids, 85% of which resulted from puma provisioning (Perrig et al., 2017).

We hypothesized that the trophic identity of a carcass would be an assemblage of multiple trophic positions—plant digesta (TP = 1), camelid biomass (TP = 2) and microbes (TP = 3)—and that obligate scavengers, by consuming them all, would have intermediate non-integer (2.5 < x < 3.5) trophic positions. Specifically, we predicted that camelids and pumas would have integer TPs corresponding to strict herbivory and carnivory (TP = 2 and TP = 3, respectively), and exhibit little variance. However, we predicted that cameld necrotic tissue, that is, carrion, would be troponically inflated due to microbial colonization (Steffan et al., 2017). Consequently, the Andean condors consuming the microbe-colonized carrion would develop a trophic position above pumas consuming only the camelid meat. In effect, microbes within a carcass are trophically analogous to pumas, thus when a condor consumes both the camelid and the embedded microbes, the condor feeds at a higher trophic position. We also predicted that condors were consuming non-trivial amounts of plant material and hence, the condor population would exhibit higher intra-populational variation in TP. Finally, we predicted that condors would exhibit sex-based dietary partitioning, in which dominant males would consume more carcass meat and females would consume more plant material. To test our hypothesis, we quantified the TP of the entire community using compound-specific stable isotopes analysis of amino acids (CSIA-AA). Additionally, we documented the relative contribution of individual food sources to scavengers via regurgitated pellets and the analysis of bulk isotopes (13C and 15N).

2 MATERIALS AND METHODS

San Guillermo NP (29°12′S–69°20′W) extends over 1,660 km2 in the Andean plateau in the province of San Juan, Argentina. This high-altitude region is characterized by a semi-arid climate (100–500 mm/year) with temperatures ranging between 27°C and −23°C (Salvioli, 2007), where vegetation is dominated by grass and shrub steppes. We reanalysed 177 regurgitated pellets from active Andean condors roosting sites collected by Perrig et al. (2017) in summer 2013 in the park to estimate the amount of plants consumed (plants were not accounted for in the original analysis). Pellets were oven-dried (55°C for 48 hr) and then microscopically identified dietary items to estimate, (a) occurrence, as the percentage of times an item occurred in the total number of pellets and (b) the relative volumetric content, in relation to the total volume of pellets, for each category of dietary items, grouped as camelids (vicuña and guanaco), plants, livestock (cow Bos taurus, horse Equus ferus, goat Capra aegagrus and sheep Ovis aries) and others (hares Lepus europaeus and southern mountain viscachas Lagidium viscacia).

We also used samples collected in summer 2013 for a complementary analysis of 13C and 15N bulk isotopes (Perrig et al., 2017), which estimate assimilated diet components avoiding biases associated with differential digestibility, as well as 15N CSIA-AA, to estimate the trophic position of all members of the food web. Past analyses have shown that the enrichment in 15N between the diet and the consumer is uneven across AAs but is highly consistent for particular AAs (Chikaraishi et al., 2009). Thus, by quantifying the ratio of 15N between an AA that reflects the source (e.g. phenylalanine), and one
that reflects the fractionation by the consumer (e.g. glutamic acid), one can robustly predict the TP of a consumer (Steffan et al., 2015).

For the $^{13}$C and $^{15}$N stable isotope analysis of bulk tissue, we used samples of condor feathers ($n = 47$, adult males = 14, adult females = 23, immature males = 2, immature females = 8) from feeding and roosting sites, hair from camelids (vicuña $n = 7$, guanaco $n = 7$) found on carcasses and hair from cows ($n = 10$) and goats ($n = 6$; Perrig et al., 2017). We additionally collected the dominant autotrophs and main dietary items of vicuñas (Cajal, 1989) in the park from living plants: rushes ($Juncus$ spp.; $n = 3$) and fescues ($Festuca$ spp.; $n = 3$). We molecularly identified the sex and individual for each condor feather and evaluated age class based on their colour (Perrig et al., 2019). We prepared hair and feather samples for bulk isotope analysis by washing and rinsing three times with a solution 2:1 trichloromethane–methanol to remove dirt and surface oil, then all samples were homogenized in small parts (<1 mm) with surgical scissors and dried for 72 hr at 60°C. Values for $^{13}$C and $^{15}$N in bulk tissue were calculated by weighing the samples (0.6 mg ± 0.005) and placing them in tin capsules, then measured with a Costech 4010 elemental analyzer attached to a Thermo Finnigan DeltaPLUS XP or V Flow Isotope Ratio Mass Spectrometer (University of Wyoming, Laramie, USA). Results are provided in per mil (%) notation relative to the international standards of PeeDee Belemnite (PDB) and atmospheric nitrogen (AIR) for carbon and nitrogen, respectively, calibrated against internal laboratory standards.

We estimated the proportional assimilation of isotopically distinct food sources by Andean condors using a Bayesian-based mixing model in MixSIAR (Stock & Semmens, 2016) with Markov chain Monte Carlo (MCMC: chain length = 300,000; burn = 200,000; thin = 100; chains = 3). Based on the pellet analysis, we identified five isotopically distinct and biologically meaningful dietary sources: large livestock (cows), small livestock (goats), camelids (vicuña and guanaco), fescues and rushes. We used raw isotopic compositions values of the dietary source and average and standard deviation for their concentration dependence (i.e. relative weight of N and C; Table S1). We used trophic discrimination factors of 3.1‰ for their concentration dependence (i.e. relative weight of N and values of the dietary source and average and standard deviation large livestock (cows), small livestock (goats), camelids (vicuña and

We estimated the trophic position for plants, vicuñas, guanacos, pumas, condor and camelid carrion based on the $^{15}$N values of glumatic acid ($\delta^{15}$N$_{\text{glu}}$) and phenylalanine ($\delta^{15}$N$_{\text{phe}}$) using the equation:

$$\text{TP} = \frac{\delta^{15}\text{N}_{\text{glu}} - \delta^{15}\text{N}_{\text{phe}} + \beta}{\Delta_{\text{glu}-\text{phe}}} + \lambda$$

(Chikaraishi et al., 2009),

where $\beta$ corrects for the difference in $^{15}$N values between glutamic acid and phenylalanine in $C_3$ plants; $\Delta_{\text{glu}-\text{phe}}$ represents the net trophic discrimination between glutamic acid and phenylalanine (7:2; Steffan et al., 2015, 2017); and $\lambda$ represents the basal trophic level (1). A TP estimate can be improved by using a $\beta$ value that has been empirically derived from the study system (Steffan et al., 2013). While most terrestrial

Research permits were issued by the Argentine National Park Administration (#DRC265 and DCM255 and subsequent renewals) and the Argentine Ministry of Environmental and Sustainable Development under CITES permit No. 15US94907A/9.
3 | RESULTS

Camelids were the most common dietary item found in Andean condor pellets, constituting 98.3% of occurrence and 86.9% (mean ± 21.9 SD) of volumetric content (Table 1). Plant matter was the second most important dietary item, found in 57.6% of the pellets analysed, which on average constituted 9.9% (±15.8) of the pellet volumetric content. Other sources of food, such as livestock, hares and mountain viscachas, were present only in 12.9% of the pellets and accounted for 3.1% (±4.2) of volumetric content.

Our dietary mixing model involving bulk δ¹³C and δ¹⁵N for the subset of condors that we also analysed TP (Figure 2), suggested that the diet of male condors was 65.2% (median, 95% credible interval [35–89]) camelids, whereas the diet of females was 53.1% [26–75] camelids. Additionally, livestock and plants represented 24.3% [3–58] and 7.8% [1–28] of male diet, respectively, while the diet of females were 32.6% [7–65] livestock and 13.0% [2–33] plants (Figure 2b). When we analysed the entire dataset of condors, mixing models revealed the same ranked importance in diet items at the population level (Table 1): camelids, followed by livestock and then plant matter, with sex and age-based differences in proportional intake (Figure 3). Bulk stable isotope analysis showed that the diet of females were 32.6% [7–65] livestock and 13.0% [2–33] plants (Figure 2). When we analysed the entire dataset of condors, mixing models revealed the same ranked importance in diet items at the population level (Table 1): camelids, followed by livestock and then plant matter, with sex and age-based differences in proportional diets overlapping (Table S2).

Our TP estimates for discrete trophic entities differed predictably (F_{1,21} = 284.9, p < 0.001), with plants [TP = 1.01 ± 0.11 (SD)] at the base of the food web, camelids (TP = 2.02 ± 0.05) registering as primary consumers, and pumas (TP = 3.00 ± 0.04) as strict consumers of herbivores. Camelid carrion was significantly elevated relative to living camelids (TP = 2.26 ± 0.1; t_{3.8} = −3.91, p = 0.02). TP estimates for male (TP = 3.09 ± 0.34) and female condors (TP = 2.83 ± 0.15) did not differ (t_{9.1} = −1.70, p = 0.13; condor population TP = 2.9 ± 0.29). However, the TP of pumas was significantly higher than the TP for female condors (t_{2.67} = 0.027), and marginally lower than male condors (t_{3.6} = −0.64, p = 0.054). Indeed, TP of female condors represent a non-integer value (t_{2.85} = 0.025), lower than strict carnivory (3.0), while male condor TP was indistinguishable from strict carnivory (t_{2.64} = 0.549). Among all the trophic groups in this Andean food web, Andean condors exhibited the widest range of TP values, from 2.6 to 3.8 (Figure 3), and had a significantly greater variance compared to that found in pumas (K² = 7.70, p = 0.006). TP variance was marginally higher in male than female condors (K² = 3.63, p = 0.057).

4 | DISCUSSION

Our results reveal that carcasses are not just meat to obligate scavengers but represent a heterogeneous pool of resources that span taxa and trophic positions. In the process, the consumption of a carcass and the attendant necrobiome effectively integrates the macrobiome and microbiome within scavenger populations. Indeed, we found that plant material was common within regurgitated pellets of condors and assimilated in relatively large amounts by these scavengers. Our estimates of TP suggested that carcasses also have non-trivial amounts of microbial matter and that there was a detectable, and important, consumption of microbes by condors. Finally, our non-integer estimates of TP for condors, as well as the high level of inter-individual variation in TP estimates, demonstrates that this highly specialized scavenger is, indeed, a ‘trrophic omnivore’ (Steffan & Dharampal, 2019) and consumes multiple trophic levels.

Andean condor diet partitioning among sexes and age classes has been widely debated. Strong sexual dimorphism and hierarchical dominance structures have been suggested as potential mechanisms that drive partitioning of space and time (Alarcón et al., 2017; Donazar et al., 1999), with eventual implications for the diet. However, evidence documenting sex-based dietary partitioning in condors is inconsistent between sites (Perrig et al., 2021). Our results show some support for dietary partitioning between the sexes. Similar to Perrig et al. (2017), but with the inclusion of plants, we did not detect difference in the assimilated diet between age-sex classes, nor did we detect TP differences between the sexes. On the other hand, adult male condors possessed TP estimates indistinguishable from strict carnivory (TP = 3.0), while females had lower TP estimates attributable to trophic omnivory. This aligns with studies showing that adult males can exert control and gain priority over high-quality sites (Alarcón et al., 2017; Mariner et al., 2018; Wallace & Temple, 1987) and, therefore the highest quality food, as well as with the observation that males have less pigments derived from plant material in the blood (Blanco et al., 2013). The lack of consistent evidence of partitioning between sexes may be due to high unpredictability in resource availability in the landscape (Mancini et al., 2013). That is to say, the cameld carcasses regularly provisioned by pumas contain varying amounts of residual meat (Elbroch et al., 2014), which could modulate the potential dietary differentiation. Hence, future research should evaluate the impact of carcass quality (i.e. amount of remaining meat) at a finer scale.

Diet estimates obtained from bulk isotopes and mixing model differed from those derived from pellets. Notably, bulk isotopes estimated greater reliance on livestock and less consumption of camelids. However, camelids remained the most important diet item regardless of the approach. This difference in point estimates is likely a result of the different integration times that these methods

**TABLE 1** Mean diet estimates of Andean condors based on isotopic mixing models (n = 47; ±95% credible interval) and the volumetric content in the pellets (n = 177; ±95% confidence interval) from San Guillermo National Park, Argentina, 2013. Diet components consisted of camelids (Vicugna vicugna, Lama guanicoe), plants (Juncus sp., Festuca spp.), livestock (Bos taurus, Capra aegagrus, Ovis aries, Equus ferus) and others (Lagidium viscacia, Lepus europaeus).

<table>
<thead>
<tr>
<th>Diet items</th>
<th>Bulk stable isotope (% diet)</th>
<th>Pellets (% volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camelids</td>
<td>68.8 (44.5–84.8)</td>
<td>86.9 (83.6–90.1)</td>
</tr>
<tr>
<td>Plants</td>
<td>8.3 (1.5–22.8)</td>
<td>9.9 (7.6–12.2)</td>
</tr>
<tr>
<td>Livestock</td>
<td>22.1 (3.9–47.3)</td>
<td>2.5 (1.1–03.9)</td>
</tr>
<tr>
<td>Others</td>
<td>—</td>
<td>0.7 (0.0–1.3)</td>
</tr>
</tbody>
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reflect: isotopes from feathers reveal assimilated diet over a wider spatiotemporal frame (~3 months), while the pellets reflect more immediate foraging events. Additionally, estimates of the mixing model could be affected by the wide range $^{15}$N found in the plant material, inflating error estimates. However, a previous study, that did not include plants as a potential diet item, found similar results in the relative contribution of camelids and livestock (Perrig et al., 2017). We found that condors consumed a considerable amount of plant biomass. The ingestion of plant material may be incidental (Duclos et al., 2020) and associated with the consumption of the guts of the carcass. However, its consistent presence in the diet of condors could link this feeding habit to other functions. It is possible that plant consumption allows the assimilation of carotenoids (Blanco et al., 2013), which can be important in social hierarchies for Andean condors (Marinero et al., 2018) and associated with their immune response (Plaza et al., 2020). Also, plant digesta and associated volatile fatty acids produced in the rumen of camelids may provide nutritional inputs that would otherwise be unavailable and limiting to condors, given that vultures do not possess the capacity for fermentation and the cecum is vestigial or absent (Clench & Mathias, 1995). Additionally, plant digesta can be a source of water,
as moisture is retained for longer periods in the internal tissues of the carcass (Carter et al., 2007; Schotsmans et al., 2011), which might be a relevant factor in arid and semi-arid environments. It is also possible that plant material is ingested not only for nutrition or digestion but also as an emetic, as suggested for other vultures (Thomson et al., 2013). Regardless of the ultimate function of plant ingesta, it showed as a non-trivial and consistent item across the condor population.

As predicted, cameldid carrion exhibited an inflated TP of 2.26, most likely produced by microbial consumption of the carrion (Steffan et al., 2017). As microbes consume carrion and propagate through the carcass, they stockpile 15N within certain amino acid pools, including glutamic acid (Steffan et al., 2013, 2015). This compound-specific enrichment is how microbes cause trophic inflation within a detrital mass (Steffan et al., 2017; Steffan & Dharampal, 2019). Autolytic processes during decomposition produce a general isotopic enrichment of bulk 15N in carrion as volatilized compounds such as ammonia are lost (Keenan & DeBruyn, 2019), although the role in 15N enrichment of glutamic acid over phenylalanine should be minor. Glutamic acid newly synthesized, and enriched in 15N, is unexpected to happen by itself in a dying tissue. Instead, enrichment of bulk 15N is only perceptible in tissues with a prolonged decomposition stage (Keenan & DeBruyn, 2019; Yurkowski et al., 2017) when microbes are dominant. Intra-trophic enrichment of glutamic acid 15N and elevation of TP by catabolic processes has been documented in living organisms, sometimes generating de novo tissues, that sustain these processes for prolonged periods like hibernation, starvation and egg production in animals (Whiteman et al., 2021) or flowers and bud development in plants (Takizawa et al., 2017). Because the primary cause of mortality of cameldids in San Guillermo NP is puma predation, and all carrion we sampled were the result of predation, trophic elevation of cameldid carrion due to prior starvation is highly unlikely; rather it is far more plausible that the trophic elevation of carrion was driven by microbial decomposition. While trophic inflation of decomposed tissues colonized by microbes has been described uniquely in detritus of vegetation and invertebrates (Steffan et al., 2017; Steffan & Dharampal, 2019) and bulk 15N enrichment has been reported in decaying vertebrate tissue (Keenan & DeBruyn, 2019); this study provides the first evidence for trophic position inflation of vertebrate tissue using CSIA.

Even though condors consumed and assimilated measurable amounts of plant material, their average TP was close to that of pumas (3.0), suggesting that condors are also eating non-trivial quantities of secondary consumer biomass (i.e. the microbes embedded in carcasses that are trophically analogous to pumas). Thus, it appears the consumption of the microbes likely elevates the condor TP, effectively neutralizing the depressive effects of the plant digesta. Given that condors unlikely scavenge on pumas due to their lower population density in relation to cameldids, and that cameldid carrion has an already inflated TP of 2.26, it is almost certain that condors are eating the heterotrophic microbes of the carrion as secondary consumers. This is one of the few studies to report trophic inflation (i.e. higher TP due to consumption of detritivores organisms) in a terrestrial vertebrate consumer (Pauli et al., 2019) and the first suggesting microbivory for a large vertebrate. These findings highlight the importance of CSIA in trophic positions and necromes where regular bulk isotopes analysis have shown variable differences (Burrows et al., 2014; Keenan & DeBruyn, 2019; Payo-Payo et al., 2013; Yurkowski et al., 2017). It is notable that estimated TPs of pumas showed little variability around 3.0, suggesting little-to-no ingestion of microbial-colonized tissues. Pumas in our study site abandon carcasses shortly after killing (on average 34 hr), which may be insufficient for microbial trophic inflation. Andean condors, in contrast, consume carcasses after pumas have given up and up to 3–7 days after death. It is possible that other organisms, notably scavenging insects, could have been also consumed by condors; however, the complete absence of insect remains or chitin in pellets, their infrequent occurrence on puma-killed carcasses and the fact that carrion is trophically inflated suggests that the TP of condors came primarily from microbes on the carrion.

Puma predation provides obligate scavengers not only with a regular supply of carrion (Barry et al., 2019; Elbroch & Wittmer, 2013), but also with other dietary components as plant digesta and high concentration of microbes. This is especially important given that vulture reliance on landfills or other artificial food sources is increasingly common (Duclos et al., 2020; Moreno-Opo et al., 2015; Plaza & Lambertucci, 2017) and these sources do not always provide all the components of naturally occurring carcasses. The existence of microbivory in vultures requires further study, as it could represent a link between scavenger gut microbiota and the microbiome of the carrion, as shown for other taxa (Weatherbee et al., 2017). Vulture gut microbiota similar to other scavenger, the American alligator, is composed mainly of Clostridia and Fusobacteria (Keenan & Elsey, 2015; Roggenbuck et al., 2014), generally considered pathogenic for other vertebrates and associated with flesh decomposition (Zepeda Mendoza et al., 2018). It could be that condors and other scavengers exploit microbes from carrion to inoculate gut flora and increase digestion efficiency, which is critical for birds that feed on patchy and ephemeral food resources (Grémillet et al., 2012). Our study represents an initial contribution to the understanding of the trophic heterogeneity found within a carcass and the role of scavengers in uniting the macrobiome and microbiome, emphasizing the utility of multifaceted approaches in reconstructing the diets of free-ranging animals within their natural habitat. Our work also highlights how the integration of microbes into food webs can help resolve the trophic identities and dietary differences for large vertebrates and that resolving interkingdom interactions can shed light on the necrobiome functioning.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHORS’ CONTRIBUTIONS
G.B., J.N.P. and S.A.S. designed the study; P.L.P. and E.D. collected the field data; G.B. and P.D. analysed the data. G.B. and J.N.P. lead the writing and all authors contributed critically and approved the final manuscript.

DATA AVAILABILITY STATEMENT
Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.vhmggnrj (Barceló et al., 2022).

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