

**ASPECTOS MICROBIOLÓGICOS E NUTRICIONAIS RELACIONADOS À
DIETA DE LOBOS-GUARÁ (*Chrysocyon brachyurus* – CARNIVORA:
CANIDAE) NO CERRADO, OBTIDOS PELO MÉTODO DNA
METABARCODING.**

ANA RAQUEL GOMES FARIA

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO –
UENF

CAMPOS DOS GOYTACAZES – RJ

MARÇO/2026

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Fluminense Darcy Ribeiro como
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obtenção do título de Doutor em
Ecologia e Recursos Naturais

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE – UENF

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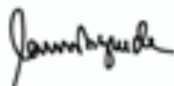
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Resumo

O lobo-guará (*Chrysocyon brachyurus*), maior canídeo da América do Sul e endêmico do Cerrado, enfrenta pressões crescentes decorrentes da fragmentação e transformação das paisagens naturais. Embora apresente elevada plasticidade dietética espacial e sazonal, característica associada à sua persistência em ambientes heterogêneos, as implicações dessa flexibilidade para a saúde e a conservação da espécie ainda são pouco compreendidas. A microbiota intestinal, componente central da fisiologia do hospedeiro, constitui uma interface sensível entre a dieta, o ambiente e a condição sanitária, oferecendo um arcabouço promissor para avaliar respostas ecológicas a distúrbios ambientais. Esta tese apresenta a primeira caracterização integrada da dieta e da microbiota intestinal de populações de vida livre de lobo-guará no bioma Cerrado, avaliando variações sazonais, espaciais e associadas a diferentes impactos humano. O Capítulo 1 sintetiza padrões gerais da microbiota em canídeos selvagens por meio de uma revisão de 21 estudos publicados entre 2012 e 2023, abrangendo 15 espécies. Os resultados indicam maior variabilidade microbiana em canídeos generalistas em comparação a carnívoros estritos e apontam que ambientes antropizados tendem a favorecer perfis compatíveis com disbiose, caracterizados por redução de Bacillota e aumento de Pseudomonadota. No Capítulo 2, foi aplicado *metabarcoding* dietético integrado (ITS2 e COI) a 49 amostras fecais coletadas em três localidades com diferentes contextos de paisagem: BA1 (vegetação nativa preservada), BA2 (matriz agrícola intensiva) e DF (unidades de conservação com pressão periférica). Foram identificadas 57 famílias vegetais (117 espécies) e 42 famílias animais (54 espécies), superando a resolução de métodos morfológicos. A composição vegetal diferiu significativamente entre localidades ($p = 0,001$), com forte dominância de Poaceae em BA2, enquanto a dieta animal revelou ampla participação de invertebrados, especialmente cupins (Termitidae), além de vertebrados silvestres e domésticos. O Capítulo 3 caracteriza a microbiota intestinal por *metabarcoding* do gene 16S rRNA, identificando 31 filos, 282 famílias e 789 gêneros bacterianos. A diversidade alfa não variou entre estações ($p > 0,05$), indicando estabilidade funcional sazonal, enquanto a diversidade beta revelou diferenças significativas entre localidades ($R^2 = 0,126$; $p = 0,001$), com BA2 apresentando o perfil mais distinto. Táxons indicadores refletiram associações com a dieta e o contexto ambiental, incluindo *Streptococcus gallolyticus* em áreas mais preservadas, *Clostridium perfringens* em paisagens agrícolas e *Escherichia coli* em Unidades de Conservação sob pressão periférica. Em conjunto, os resultados demonstram que a localização geográfica e o uso da paisagem exercem influência mais pronunciada sobre a microbiota intestinal do lobo-guará do que a sazonalidade,

evidenciando resiliência funcional associada à plasticidade dietética, mas sensibilidade microbiana a perturbações espaciais. Esses achados fornecem subsídios relevantes para estratégias de conservação que integrem a dieta, a saúde e a integridade ecológica em diferentes paisagens do Cerrado.

Palavras-chave: ecologia alimentar; canídeos selvagens; diversidade microbiana; uso da paisagem.

Abstract

The maned wolf (*Chrysocyon brachyurus*), the largest canid in South America and endemic to the Cerrado biome, faces increasing pressures resulting from the fragmentation and transformation of natural landscapes. Although it exhibits high spatial and seasonal dietary plasticity, a trait associated with its persistence in heterogeneous environments, the implications of this flexibility for species health and conservation remain poorly understood. The intestinal microbiota, a central component of host physiology, represents a sensitive interface among diet, environment, and sanitary condition, offering a promising framework for evaluating ecological responses to environmental disturbances. This thesis presents the first integrated characterization of diet and intestinal microbiota in free-ranging maned wolf populations within the Cerrado biome, assessing seasonal, spatial, and human-impact-associated variation. Chapter 1 synthesizes general microbiota patterns in wild canids through a review of 21 studies published between 2012 and 2023, encompassing 15 species. The findings indicate greater microbial variability in generalist canids compared to strict carnivores and suggest that anthropogenic environments tend to favor profiles compatible with dysbiosis, characterized by reduced Bacillota and increased Pseudomonadota. In Chapter 2, integrated dietary *metabarcoding* (ITS2 and COI) was applied to 49 fecal samples collected from three localities with distinct landscape contexts: BA1 (preserved native vegetation), BA2 (intensive agricultural matrix), and DF (protected areas under peripheral pressure). A total of 57 plant families (117 species) and 42 animal families (54 species) were identified, surpassing the taxonomic resolution of traditional morphological methods. Plant composition differed significantly among localities ($p = 0.001$), with strong dominance of Poaceae in BA2, while the animal component revealed substantial participation of invertebrates, especially termites (Termitidae) in addition to wild and domestic vertebrates. Chapter 3 characterizes the intestinal microbiota using 16S rRNA gene *metabarcoding*, identifying 31 bacterial phyla, 282 families, and 789 genera. Alpha diversity did not vary between seasons ($p > 0.05$), indicating seasonal functional stability, whereas beta diversity revealed significant differences among localities ($R^2 = 0.126$; $p = 0.001$), with BA2 presenting the most distinct profile. Indicator taxa reflected associations with diet and environmental context, including *Streptococcus gallolyticus* in more preserved areas, *Clostridium perfringens* in agricultural landscapes, and *Escherichia coli* in protected areas under peripheral pressure.

Taken together, these results demonstrate that geographic location and landscape use exert a stronger influence on the intestinal microbiota of the maned wolf than seasonality, revealing functional resilience associated with dietary plasticity but microbial sensitivity

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Keywords: feeding ecology; wild canids; microbial diversity; land use.

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ESTRUTURA DA TESE

Esta tese foi desenvolvida no formato de capítulos e tem como eixo central a integração entre ecologia alimentar, microbiota intestinal e conservação do lobo-guará (*Chrysocyon brachyurus*) em paisagens do bioma Cerrado. A estrutura adotada permite avançar de uma abordagem conceitual e comparativa em escala global para análises empíricas detalhadas em populações de vida livre, culminando em uma síntese aplicada voltada à conservação.

A Introdução Geral contextualiza o lobo-guará como um canídeo neotropical generalista, destacando sua plasticidade dietética, ampla distribuição espacial e exposição crescente a paisagens antropizadas. Nesse contexto, a microbiota intestinal é apresentada como componente funcional da adaptação ecológica, mediando a relação entre dieta, ambiente e saúde. A introdução delimita a lacuna de conhecimento existente sobre a ecologia microbiana de canídeos sul-americanos e estabelece os objetivos gerais da tese, bem como as hipóteses relacionadas aos efeitos da sazonalidade, da heterogeneidade espacial e do uso da paisagem sobre a dieta e a microbiota intestinal da espécie.

O Capítulo 1, intitulado “*Intestinal microbiota of wild canids: a systematic overview and conservation perspectives*”, apresenta uma revisão abrangente da literatura sobre microbiota intestinal em canídeos selvagens. A partir da síntese de 21 estudos publicados entre 2012 e 2023, abrangendo 15 espécies, o capítulo discute padrões taxonômicos recorrentes, determinantes ecológicos da variação microbiana e os efeitos da dieta, do manejo e da antropização. O capítulo evidencia a ausência de dados para canídeos neotropicais e propõe um arcabouço conceitual no qual a microbiota é tratada como indicador funcional de saúde, qualidade ambiental e resiliência ecológica, estabelecendo a base teórica que orienta os capítulos empíricos subsequentes.

O Capítulo 2, “*DNA metabarcoding reveals the animal and plant diet of maned wolves (Chrysocyon brachyurus) in the Cerrado*”, investiga a composição dietética de populações de vida livre por meio de *metabarcoding* integrado dos marcadores ITS2 e COI. Foram analisadas amostras fecais provenientes de três localidades com diferentes características de paisagem e impacto humano. O capítulo descreve a diversidade e a estrutura dos itens alimentares vegetais e animais, avalia variações espaciais e sazonais e compara os resultados com o

conhecimento clássico obtido por métodos morfológicos. Os achados reforçam a plasticidade dietética do lobo-guará, com forte efeito da localidade sobre a composição da dieta e evidenciam a sensibilidade do método molecular para detectar itens pequenos e altamente digeríveis, fornecendo uma base essencial para a interpretação dos padrões microbianos.

O Capítulo 3, “*Landscape - not season - shapes gut microbiota of free-ranging maned wolves (Chrysocyon brachyurus) in the Cerrado*”, caracteriza a microbiota intestinal por *metabarcoding* do gene 16S rRNA em populações de vida livre. O capítulo avalia a composição taxonômica, a diversidade alfa e beta e identifica gêneros indicadores associados a diferentes localidades. Os resultados revelam estabilidade sazonal da microbiota, contrastando com diferenças geográficas marcantes, especialmente em áreas sob maior influência antrópica. A integração com os dados dietéticos do capítulo anterior permite interpretar a resiliência funcional da microbiota frente à variação alimentar à sensibilidade microbiana às transformações da paisagem.

Por fim, as Considerações Finais integram os principais achados da tese, discutindo a microbiota intestinal como componente funcional da ecologia do lobo-guará e como ferramenta complementar para conservação. São destacadas as implicações teóricas e aplicadas dos resultados, bem como lacunas futuras, incluindo a necessidade de abordagens longitudinais, funcionais e comparativas em outros canídeos neotrópicos, reforçando o papel da microbiota na interface entre dieta, paisagem e saúde populacional.

INTRODUÇÃO GERAL

O lobo-guará, *Chrysocyon brachyurus* (Illiger, 1815), maior canídeo da América do Sul e espécie endêmica do Cerrado, tem sua persistência cada vez mais condicionada à intensificação das pressões antrópicas e à fragmentação progressiva das paisagens naturais [1–5]. Classificado como 'Vulnerável' nacionalmente [6] e 'Quase Ameaçado' pela International Union Conservation of Nature (IUCN) [7], esse carnívoro generalista distingue-se por elevada plasticidade dietética, expressa tanto espacial quanto sazonalmente. Embora tal flexibilidade seja frequentemente associada à resiliência ecológica da espécie, suas implicações diretas para a saúde individual e para a conservação populacional ainda permanecem insuficientemente esclarecidas [8]. Nesse contexto, a microbiota intestinal composta por um conjunto de microrganismos envolvidos na digestão, na modulação do sistema imune e na resposta a distúrbios ambientais, emerge como um componente central para a avaliação da condição fisiológica de populações selvagens [9, 10]. Embora a microbiota intestinal represente um elo promissor entre dieta, ambiente e saúde, faltavam até então estudos que explorassem de forma integrada esses componentes no para o lobo-guará.

Estudos baseados em abordagens moleculares têm demonstrado que a microbiota intestinal de canídeos selvagens apresenta padrões taxonômicos amplamente consistentes, com a identificação de 18 filos bacterianos ao longo de 21 publicações que abrangem 15 espécies estudadas entre 2012 e 2023. De forma recorrente, a estrutura dessas comunidades é dominada pelos filos Bacteroidota, Bacillota, Fusobacteriota, Pseudomonadota e Actinomycetota, com destaque para famílias como Lachnospiraceae, Clostridiaceae, Bacteroidaceae, Fusobacteriaceae e Prevotellaceae. Dentro desse contexto, canídeos generalistas tendem a exibir maior variabilidade microbiana quando comparados a carnívoros estritos, padrão interpretado como reflexo direto da maior plasticidade dietética e da exposição ampliada a diferentes matrizes ambientais [11, 12]. Em contrapartida, ambientes submetidos a perturbações antrópicas recorrentes têm sido associados a estados de disbiose, caracterizados pela redução relativa de Bacillota e pela elevação de Pseudomonadota, grupos frequentemente relacionados a processos

inflamatórios e a condições de estresse nutricional [13]. Em lobos-cinzentos da China, o aumento de Pseudomonadota e Actinomycetota apresentou correlação direta com a intensidade da perturbação humana, indicando que a degradação de habitats favorece a proliferação de anaeróbios facultativos e táxons oportunistas [14, 15]. Esses estudos demonstram que a microbiota intestinal de canídeos selvagens responde de maneira mensurável a gradientes ambientais e de uso da paisagem. No entanto, a escassez de dados para o lobo-guará em contextos de vida livre ainda limitava a extrapolação desses padrões para espécies neotropicais, restringindo o entendimento de como tais processos se manifestam no Cerrado [10, 16].

A aplicação integrada de *metabarcoding* de DNA, utilizando simultaneamente os marcadores ITS2 para táxons vegetais e COI para táxons animais, permitiu a primeira caracterização abrangente da dieta do lobo-guará em populações de vida livre do Cerrado. A partir de 49 amostras fecais coletadas em três localidades —BA1 (Jaborandi, BA) com ~57% de vegetação herbácea e arbustiva e ~27% de área florestal preservada; BA2 (Barreiras e Luís Eduardo Magalhães, BA) dominada por ~57% de áreas agrícolas e apenas ~8% de vegetação nativa; e DF (Distrito Federal) composto por unidades de conservação federais e distritais—foram identificadas 57 famílias vegetais (117 espécies) e 42 famílias animais (54 espécies). O sequenciamento Illumina MiSeq PE300 gerou 15.311 ASVs iniciais para ITS2 e 10.728 para COI, dos quais 13.725 e 8.465, respectivamente, permaneceram após filtragem. A resolução taxonômica alcançada superou sistematicamente métodos morfológicos tradicionais, detectando táxons de corpo mole, altamente digeridos ou consumidos em pequenas quantidades, anteriormente subestimados. A composição vegetal diferiu significativamente entre localidades ($p = 0,001$), com BA1 apresentando dominância de Sapotaceae (16,54%), Poaceae (13,67%) e Asteraceae (11,80%); BA2 exibindo forte predominância de Poaceae (57,07%); e DF revelando maior equitabilidade com Poaceae (23,35%), Asteraceae (18,20%) e Myrtaceae (15,66%). A variação sazonal manifestou-se principalmente na abundância relativa de recursos complementares, com Poaceae mantendo-se recorrente em ambas as estações (21,25% na seca; 22,65% na chuvosa), enquanto Chrysobalanaceae, Sapotaceae e Melastomataceae elevaram-se na estação

chuvosa. A dieta animal revelou ampla gama de invertebrados, com destaque para Termitidae (32,27% na chuvosa), e vertebrados incluindo Dasypodidae (52,30% em BA1; 16,24% na chuvosa), Tinamidae (25,11% no DF) e espécies domésticas como *Gallus gallus* e *Coturnix japonica*.

A caracterização da microbiota intestinal de 47 amostras fecais por *metabarcoding* do gene 16S rRNA identificou 31 filos, 282 famílias e 789 gêneros, representando a primeira avaliação abrangente para *C. brachyurus* em vida livre. Os filos dominantes incluíram Bacillota (68,66% na estação chuvosa; 74,13% na seca), Pseudomonadota (18,15% na chuvosa), Bacteroidota (4,75%), Actinomycetota (4,18%) e Fusobacteriota (3,58%), com famílias predominantes como Clostridiaceae, Lactobacillaceae, Streptococcaceae, Peptostreptococcaceae e Enterobacteriaceae. A diversidade alfa não diferiu significativamente entre estações (riqueza média de $88,00 \pm 55,69$ no período chuvoso vs. $84,67 \pm 44,61$ no seco; $p > 0,05$), indicando a manutenção de um núcleo funcional resiliente independente de variações sazonais. Em contraste, a diversidade beta revelou diferenças significativas entre localidades ($R^2 = 0,126$; $F = 3,18$; $p = 0,001$), com BA2 emergindo como a mais distinta microbiologicamente ($p = 0,001$). A análise de táxons indicadores identificou *Streptococcus* (25,38%) e *S. gallolyticus* (30,95%) como característicos de BA1, associados ao consumo de frutos como a lobeira; *Clostridium perfringens* (25,32%) predominou em BA2, potencialmente refletindo o consumo de recursos antropogênicos em matriz agrícola; e *Escherichia coli* (30,99%) destacou-se no DF, sugerindo que pressões de urbanização e agricultura periférica afetam áreas protegidas. A quantificação da paisagem em *buffers* de 3 km confirmou que a localidade geográfica constitui o determinante primário da estrutura microbiana ($p = 0,001$), superando efeitos sazonais.

As associações dieta-microbiota revelaram que a plasticidade alimentar do lobo-guará age em escalas temporais e espaciais [10, 17], mas suas consequências microbiológicas são mediadas principalmente pelo contexto ambiental [18–20]. A elevação de Lactobacillaceae (36,08%) em BA2, área dominada por agricultura intensiva, e de Enterobacteriaceae (23,21%) no DF, região com pressão antrópica periférica, contrastam com o perfil de BA1, onde

S. gallolyticus correlaciona-se ao consumo de recursos nativos [18, 21]. Essas diferenças sugerem que a exposição a recursos antropogênicos e a degradação de *habitats* promovem perfis microbianos distintos, potencialmente associados a riscos de disbiose [13, 22]. A ausência de diferenças sazonais na diversidade alfa, apesar das variações na composição dietética, indica que o núcleo funcional da microbiota permanece estável, mas, a estrutura taxonômica responde a perturbações espaciais de forma mensurável. Esse padrão alinha-se com observações em outros canídeos selvagens, nos quais ambientes perturbados favorecem anaeróbios facultativos [13, 23] e reduzem táxons produtores de ácidos graxos de cadeia curta, comprometendo a homeostase intestinal [24, 25].

A integração metodológica de *metabarcoding* dietético (ITS2 e COI) e microbiano (16S rRNA) a partir das mesmas amostras fecais estabeleceu um protocolo não invasivo de referência, aplicável a outras espécies de carnívoros ameaçados do Cerrado. A superação das limitações de métodos morfológicos tradicionais, que subestimam sistematicamente a diversidade de itens alimentares [26, 27], permitiu detectar táxons anteriormente não documentados, incluindo cinco espécies de cupins na estação chuvosa (*Nasutitermes corniger*, *Neocapritermes opacus*, *N. talpa*, *Parvitermes bacchanalis*, *Ruptitermes* sp.) e espécies domésticas na dieta. A resolução taxonômica ampliada, alcançando 117 espécies vegetais e 54 animais, fornece a base para avaliar a plasticidade alimentar em contextos de conservação e manejo [10]. A demonstração de que a localidade geográfica determina a estrutura microbiana, independentemente de variações sazonais, reforça a necessidade de abordagens espacialmente explícitas em estudos de ecologia microbiana de carnívoros selvagens [10, 28].

Os objetivos centrais desta tese foram: (i) sintetizar o conhecimento atual sobre microbiota intestinal de canídeos selvagens, examinando implicações para nutrição, saúde, ecologia e conservação; (ii) caracterizar a composição, diversidade e variação espacial e sazonal da dieta do lobo-guará utilizando a técnica *metabarcoding*; e (iii) caracterizar a composição, diversidade e estrutura da microbiota intestinal do lobo-guará, avaliando padrões associados à sazonalidade, localização geográfica e diferentes impactos na paisagem. Esses objetivos foram executados de forma integrada, permitindo avaliar como a plasticidade dietética interage com perturbações ambientais para moldar a

microbiota intestinal, e como essas interações podem informar estratégias de conservação baseadas em evidências.

A tese estrutura-se em três capítulos complementares. O Capítulo 1 apresenta uma revisão direcionada de 21 publicações (2012-2023) abrangendo 15 espécies de canídeos selvagens, sintetizando padrões taxonômicos gerais, efeitos de guilda alimentar, condição de manejo e perturbação ambiental sobre a microbiota intestinal, estabelecendo o contexto teórico para os estudos empíricos subsequentes. O Capítulo 2 aplica *metabarcoding* integrado (ITS2 e COI) a 49 amostras fecais de lobo-guará em três localidades do Cerrado, caracterizando variação sazonal e espacial da dieta, testando a plasticidade alimentar em gradiente de impacto humano e identificando núcleo taxonômico estável e recursos complementares. O Capítulo 3 utiliza *metabarcoding* do gene 16S rRNA em amostras fecais das mesmas localidades, quantificando a paisagem em *buffers* de 3 km e analisando diversidade alfa e beta, táxons indicadores e associações entre composição microbiana, dieta e contexto ambiental. Em conjunto, os capítulos fornecem a primeira caracterização integrada de dieta e microbiota intestinal do lobo-guará em populações de vida livre, demonstrando que a localidade geográfica e o grau de perturbação humana determinam a estrutura microbiana, enquanto a plasticidade dietética confere resiliência funcional. Esses resultados estabelecem base para avaliar a condição fisiológica de populações selvagens e informar estratégias de conservação que considerem não apenas a disponibilidade de recursos alimentares, mas também a integridade de processos microbiológicos essenciais à saúde e persistência da espécie.

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Intestinal Microbiota of Wild Canids: A Systematic Overview and Conservation Perspectives

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ABSTRACT

Intestinal microbiota plays a pivotal role in host nutrition, metabolism, and health, yet its relevance to wildlife conservation and management remains underexplored. Therefore, to illustrate how microbiome science offers a powerful interdisciplinary framework for advancing evidence-based wildlife conservation, we used the relationship between wild canids and their gut microbiomes as valuable models for examining how host diet, environment, and management influence intestinal microbial communities. We reviewed 21 publications from 2012 to 2023 that encompassed 15 wild canid species. Across these publications, 18 bacterial phyla were identified, dominated by Bacteroidota, Bacillota and Fusobacteriota. Microbial composition varied chiefly by feeding guild and management condition: generalist canids exhibited broader microbial variability linked to dietary plasticity and environmental exposure, whereas carnivorous species displayed more stable, protein-metabolizing microbiota enriched for Fusobacteriota. Host captivity and anthropogenic disturbance seem to alter gut profiles, favoring facultative anaerobes, and reducing taxa that produce short-chain fatty acids (SCFAs). Functionally, SCFA and bile acid metabolism underpin gut homeostasis, while dysbiosis characterized by reduced Bacillota and increased Pseudomonadota is associated with inflammation, nutritional stress and degraded habitats. While we found evidence for the use of gut microbial taxa and metabolites as bioindicators of health and environmental quality in wild canids, major research gaps do remain for neotropical canid species and strictly carnivorous taxa, as well as in methodological standardization. We propose ten practical recommendations to integrate microbiota data into conservation strategies, with emphasis on disease control, dietary management, rehabilitation, and ecological monitoring.

Keywords

Microbial composition; Dietary ecology; Host–microbe interactions; Captivity effects; Microbial indicators of health

1. INTRODUCTION

Intestinal microbiota contributes to nutrient processing, immune function, and disease resistance in mammals (Barko et al. 2018; Trevelline et al. 2019). Gut microbial composition is influenced by intrinsic factors, such as sex, age, and genetics, and by extrinsic factors, including diet, habitat, climate, and anthropogenic disturbance (Menke et al. 2017). Diet is considered the main driver of gut microbial community structure, which in turn shapes nutrient absorption, energy balance, and immune responses (Schmidt et al. 2018). These elements guide how we evaluate gut health and how we interpret the effects of nutritional and environmental pressures on microbial stability (Hanning and Diaz-Sanchez 2015; Nishida and Ochman 2018).

In recent years, microbiome research has become increasingly relevant to wildlife conservation. Intestinal microbiota profiles can support health assessments, dietary planning, rehabilitation, and monitoring of environmental change (Trevelline et al. 2019). As human activities continue to alter habitats and food availability (Antwis et al. 2017), microbial indicators provide a more mechanistic basis for conservation decisions (Antwis et al. 2017). Selecting model groups that capture ecological variation is therefore important for linking microbiome patterns to conservation outcomes.

Wild canids are well suited for this purpose, as their gut microbial communities help digest both animal and plant-derived foods and support gut stability under different environmental and dietary conditions (Barko et al. 2018; Menke et al. 2017). As hosts, wild canids occupy a wide range of environments and display diets that vary from strict carnivory to broad omnivory. This diversity offers opportunities to explore how trophic flexibility and environmental conditions influence gut microbial communities and host health (Biles et al. 2021; Castelló 2018; Menke et al. 2017; Wang et al. 2022). The ecological importance and wide geographical distribution of wild canids also make them a useful group for examining microbiome dynamics within conservation contexts (Adams et al. 2021; Colborn et al. 2020; Trevelline et al. 2019).

However, most knowledge about canid intestinal health still comes from studies on domestic dogs (Barko et al. 2018; Bermingham et al. 2017; Deng and Swanson 2015; Hanning and Diaz-Sanchez 2015; Nishida and Ochman 2018; Schmidt et al. 2018; Suchodolski 2011) or must be extrapolated from even more

general contexts. Such reliance introduces bias, as domestic models do not represent the ecological and physiological diversity seen in wild species. Extrapolating knowledge of the gut microbiome from domesticated dogs may obscure microbial adaptations of wild canid hosts tied to specific foraging behavior, diet breadth, and environmental exposure (Biles et al. 2021; Bragg et al. 2020; Sugden et al. 2020). Further, host definitions of a “healthy” microbiota come from studies on humans and domestic animals, where it is described as a balanced and resilient community (Félix et al. 2022; Rinninella et al. 2019). Such definitions may not apply directly to wild canids, which occupy diverse habitats and show varied feeding strategies (Nishida and Ochman 2018) but understanding what gut health means for this group is important for interpreting their ecology and conservation requirements. Recognizing these knowledge gaps is essential for developing management approaches that align with the ecology of wild canids.

The family Canidae comprises 37 extant species distributed across all continents except Antarctica, occupying a remarkable diversity of habitats and trophic niches (Castelló 2018). This global distribution and ecological versatility make canids a valuable group for exploring how diet, environment, and behavior shape intestinal microbiota composition and function (Biles et al. 2021; Wang et al. 2022; West et al. 2019). The family includes species that range from highly social hunters to solitary foragers, and from strict carnivores to broad omnivores, offering natural contrasts for examining microbial adaptation to different ecological and nutritional contexts (Allen 2007; Bornbusch et al. 2023; DeCandia et al. 2021; Soares 2020). Taken together, wild canids represent a globally distributed and ecologically diverse group whose study offers critical opportunities to understand how diet, ecology, and environmental change shape intestinal microbiota. This perspective provides a foundation for interpreting the patterns presented in the following sections.

Social species such as the gray wolf (*Canis lupus*) and the African wild dog (*Lycaon pictus*) exhibit cooperative hunting and a strong dependence on animal prey (Srinivasan and Würsig 2023), whereas species like foxes (*Vulpes* spp.) and raccoon dogs (*Nyctereutes procyonoides*) are dietary generalists that combine animal and plant food sources (Schwemmer et al. 2021). This trophic variability creates a gradient for understanding how macronutrient intake, habitat

characteristics, and anthropogenic exposure influence intestinal microbial communities (Adams et al. 2021; Bornbusch et al. 2023; Menke et al. 2017; Sugden et al. 2020; Youngblut et al. 2019).

Wild canids inhabit environments ranging from pristine ecosystems to highly anthropogenic landscapes, demonstrating notable behavioral and dietary plasticity that enables persistence across varying degrees of habitat disturbance (Biles et al. 2021; Menke et al. 2017; Sugden et al. 2020). This ecological breadth provides a powerful framework for assessing how environmental conditions influence host–microbiota interactions and for identifying adaptive microbial patterns associated with resilience and environmental change (Bragg et al. 2020; West et al. 2019).

Globally, approximately 37% of wild canid species are currently classified as threatened (BRASIL 2022; IUCN 2016), mainly due to habitat degradation, road mortality, disease transmission from domestic animals, and conflict with humans (DeMatteo et al. 2011; Leite-Pitman and Williams 2011; Lemos et al. 2020; Paula and DeMatteo 2015). Because of their ecological diversity, geographical breadth, and sensitivity to environmental change, wild canids serve as sentinel species for assessing the health impacts of habitat modification and for understanding host–microbiota–environment interactions relevant to conservation biology.

Although this review encompasses studies on wild canids from a global perspective, Brazil provides an illustrative regional example due to its exceptional biodiversity and the conservation challenges facing its native carnivores (BRASIL 2022; Castelló 2018; ICMBio 2025). Six wild canid species occur in Brazil: *Cerdocyon thous* (crab-eating fox), *Atelocynus microtis* (short-eared dog), *Speothos venaticus* (bush dog), *Lycalopex vetulus* (hoary fox), *Lycalopex gymnocercus* (Pampas fox), and *Chrysocyon brachyurus* (maned wolf) (Castelló 2018). Among these, only *C. thous* and *L. gymnocercus* are listed as Least Concern on the IUCN Red List, whereas the others are classified as Near Threatened (BRASIL 2022; ICMBio 2025; IUCN 2016).

These Brazilian canids represent a wide ecological gradient, with striking variation in feeding strategies and habitat use. The *S. venaticus* is a pack-hunting carnivore that depends almost exclusively on animal prey (Beisiegel and Ades 2002), while the *C. brachyurus* and the *C. thous* are generalist feeders that

include vertebrates, invertebrates, and fruits in their diets (Gatti et al. 2006; Soares 2020). *Lycalopex* species show intermediate strategies; the *L. vetulus*, for instance, specializes in consuming termites and other arthropods as primary energy sources (Kotviski 2017). Generalist diets are associated with greater ecological flexibility, allowing adaptation to seasonal or anthropogenic resource fluctuations, whereas strictly carnivorous species are more sensitive to prey decline and habitat alteration (Cruz et al. 2022).

As an example of regionally focused conservation initiatives, the Brazilian government implemented the National Action Plan for Wild Canid Conservation (ICMBio 2018), targeting threats such as habitat loss, human–wildlife conflict, and pathogen transmission from domestic dogs. The plan prioritizes research on health, population dynamics, and *ex-situ* management, including rehabilitation and reintroduction programs for orphaned maned wolves. Incorporating microbiota analyses into these conservation actions could provide valuable insights into host health assessment, dietary adaptation, and stress responses, thereby contributing to evidence-based conservation strategies for threatened canids within a broader global framework (Antwis et al. 2017; Trevelline et al. 2019; West et al. 2019).

This review synthesizes current knowledge on the intestinal microbiota of wild canids and examines its implications for host nutrition, health, ecology, and conservation. We evaluate whether a meaningful definition of a “healthy” microbiota exists for these wild canids and propose conceptual and practical guidelines where such a definition remains unclear. Following the framework proposed by Trevelline et al. (2019) Trevelline et al. (2019), we outline ten areas in which microbiome research can support conservation practice, including disease prevention, dietary planning, probiotic use, environmental monitoring, and rehabilitation for translocation. These perspectives highlight the value of incorporating microbiome science into wildlife management to support effective conservation outcomes.

2. SOURCES OF EVIDENCE ON WILD CANID INTESTINAL MICROBIOTA

To summarize the current knowledge on the intestinal microbiota of wild canids, we conducted a targeted search of scientific publications from 2012 to 2023. The goal was not to produce a formal systematic review, but rather to assemble a representative and recent set of studies focused on this taxonomic group. We searched the Web of Science®, Scopus®, and Google Scholar® databases using combinations of the terms *microbiota*, *microbiome*, *intestinal*, and *canid*, as well as the scientific names of the genera within the family Canidae. This broader search returned more than six hundred papers, including studies on domestic dogs, other carnivores, and methodological approaches. From this initial set of papers, we retained only publications that examined the intestinal microbiota of wild canids, either in free-ranging or captive conditions. Only studies employing molecular approaches, such as DNA metabarcoding or shotgun sequencing, were included, as these methods provide higher and more comparable taxonomic resolution than culture-based techniques (Selvin et al. 2019). After excluding theses, conference abstracts, and studies focused exclusively on domestic dogs or on non-fecal microbiota, a final group of 21 articles met the inclusion criteria (Supplementary Table 1). These studies encompass 15 wild canid species from several regions and represent the most complete compilation currently available.

The selection process also revealed notable geographic and taxonomic gaps. Most studies were conducted in North America, Europe, or Asia, and no published data were identified for South American canids. This absence underscores the need for targeted research on neotropical species, whose ecological and dietary diversity may provide important insights for conservation-focused microbiome studies.

3. WHAT WE FOUND FOR WILD CANID MICROBIOMES

3.1. Core composition and ecological drivers of intestinal microbiota structure in wild canids

Across the 21 studies we reviewed, wild canid intestinal microbiota included 18 bacterial phyla, sixteen of which were shared between feeding guilds,

while Gemmatimonadota and Planctomycetota appeared only in generalist species (Supplementary Table 2). The dominant phyla found across wild canid hosts were Bacteroidota, Bacillota, Fusobacteriota, Pseudomonadota, and Actinomycetota. Frequent bacterial families included Lachnospiraceae, Clostridiaceae, Bacteroidaceae, Fusobacteriaceae, and Prevotellaceae; the most common genera were *Bacteroides*, *Fusobacterium*, *Clostridium*, *Blautia*, and *Prevotella* (Figure 1). These patterns summarize the taxonomic profiles reported from studies conducted between 2012 and 2023 in both free-ranging and captive wild canids (Table 1).

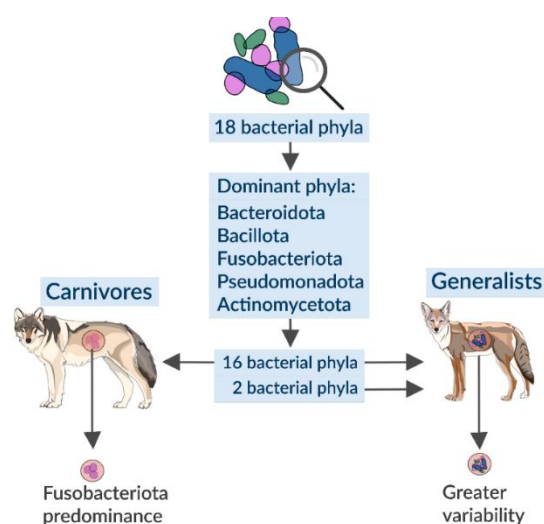


Figure 1. Conceptual summary of gut microbiota composition in wild canids based on published studies.

Table 1. Main bacterial phyla, families, and genera identified in the intestinal microbiota of wild canids

Taxonomic rank	Representative taxa	Occurrence
Phylum	Bacteroidota, Pseudomonadota, Bacillota, Fusobacteriota, Actinomycetota	Common to all
Phylum	Gemmatimonadota, Planctomycetota	Only in generalist species

Family	Lachnospiraceae, Clostridiaceae, Bacteroidaceae, Fusobacteriaceae, Prevotellaceae	Common to all
Genus	<i>Bacteroides</i> , <i>Fusobacterium</i> , <i>Clostridium</i> , <i>Blautia</i> , <i>Prevotella</i>	Common to all

We found that dietary habits of wild canid hosts were especially important in shaping phylum-level profiles of the gut microbiome. Free-ranging generalist hosts showed wide fluctuations in microbial composition, likely linked to seasonal and spatial variation in both animal and plant foods (Adams et al. 2021). Strict carnivore host, whose diets rely heavily on prey tissue, tended to maintain more stable microbial communities throughout the year because prey composition changes less across the seasons (Nardi et al. 2022; Wang et al. 2022). Generalist canids showed greater variation in the relative abundance of the dominant phyla than strictly carnivorous species, whereas Fusobacteriota tended to dominate in strictly carnivorous canids, a pattern consistent with higher animal-protein intake. This relationship between protein availability and Fusobacteriota abundance has also been reported for other carnivorous mammals (Félix et al. 2022; Zoelzer et al. 2021). In contrast, generalists displayed broader shifts across studies, reflecting their wider range of food sources and exposure to diverse environmental microbial inputs (Figure 2A, 2B; Supplementary Table 3, 4).

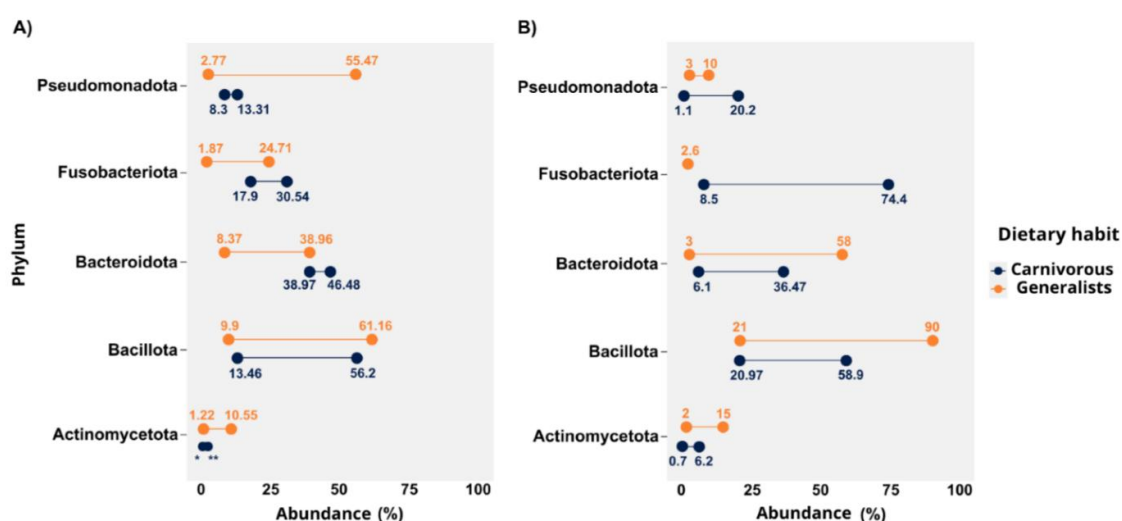


Figure 2. Minimum and Maximum relative abundance (%) reported across all reviewed studies for each of the five major bacterial phyla in the intestinal microbiota of wild (A) and captive (B) canids, according to dietary habit and environment. *0.53; **1.29.

These patterns align with broad microbial trends reported in mammals, in which Bacillota and Bacteroidota form the core of the gut community and support diverse metabolic functions (Finlayson-Trick et al. 2017; Zoelzer et al. 2021). Pseudomonadota, however, showed greater variability in free-ranging generalist species, especially in human-disturbed environments (Biles et al. 2021; Wu et al. 2017). This variability suggests that generalists respond more strongly to habitat differences and resource heterogeneity.

Feeding guilds alone, however, does not fully explain microbiota variation, particularly under captive conditions. Studies reported inconsistent phylum-level patterns when diet composition was not examined in detail. Protein, starch, and fiber sources differed widely among facilities, contributing to variation across captive populations (Barraza-Guerrero et al. 2021; Bragg et al. 2020; Nardi et al. 2022). Accounting for these nutritional variables is essential for interpreting microbiota structure and distinguishing dietary effects from environmental ones. In cases of wild canids receiving standardized food formulations under captive conditions, dietary composition generated clearer phylum-level shifts in intestinal microbiota compared to free-ranging individuals. Captive generalist canids, typically fed diets with higher carbohydrate and starch content (e.g., commercial extruded feeds and plant-derived ingredients), exhibited increased relative abundances of Bacillota and Bacteroidota. In contrast, captive strictly carnivorous canids maintained higher abundances of Fusobacteriota, a pattern consistently associated with protein- and fat-rich, meat-based diets. These trends are concordant with previous studies linking Bacillota and Bacteroidota to carbohydrate fermentation and Fusobacteriota to protein metabolism in canids under human care (Adams et al. 2021; Barraza-Guerrero et al. 2021; Bragg et al. 2020; Nardi et al. 2022).

Environmental context also influenced microbial composition. In gray wolves from China, Pseudomonadota and Actinomycetota increased with greater human interference, while Fusobacteriota decreased (Wu et al. 2017). Coyotes in urban or peri-urban environments showed lower Bacillota abundance than rural

individuals (Biles et al. 2021). These trends indicate that disturbed habitats promote facultative anaerobes and opportunistic taxa associated with environmental exposure and dietary contamination (Figure 3).

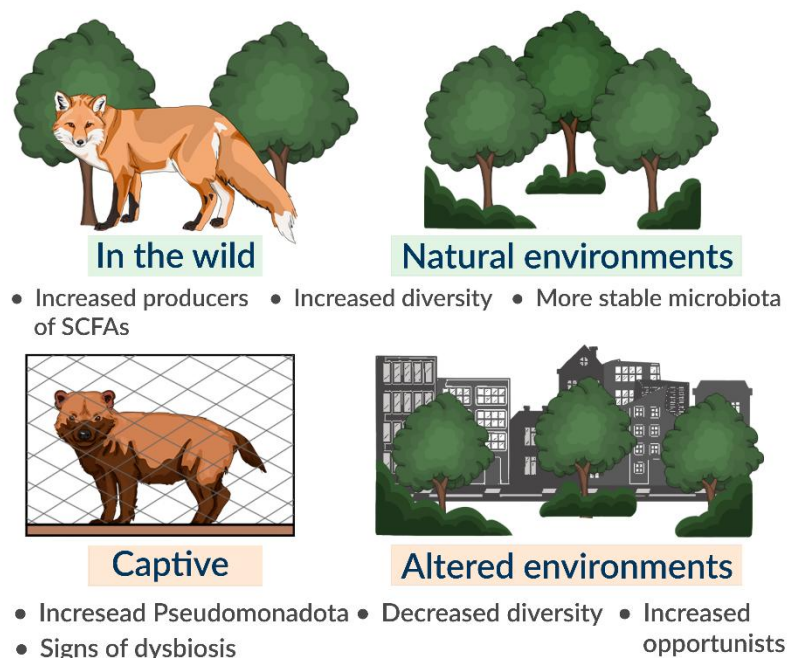


Figure 3. Conceptual overview of environmental influences on the gut microbiota of wild canids. *SCFA: Short-chain fatty acids.

At the genus level, several bacterial taxa have been proposed as indicators of environmental quality (Table 2). In this context, positive or negative relationships reflect directional associations in relative abundance along gradients of habitat quality, human disturbance, and host condition. *Succinivibrio* was linked to highly disturbed settings, while *Turicibacter* was more common in areas with minimal human impact (Wu et al. 2017). Urban coyotes often carried higher proportions of *Blautia* and *Collinsella*, while rural individuals showed more *Fusobacterium*, *Anaerobiospirillum*, and *Sutterella* (Sugden et al. 2020). Black-backed jackals (*Canis mesomelas*) living near livestock operations presented distinct microbial signatures compared with those near game farms (Menke et al. 2017). These species-specific patterns reflect local food availability and contact with livestock-associated microbes. Taken together, these taxa function as candidate bioindicators of habitat disturbance and host condition. Genera such as *Fusobacterium*, *Lachnoclostridium*, *Phascolarctobacterium*, and *Turicibacter*

were more common in high-quality environments, whereas *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Blautia*, *Faecalibacterium*, *Collinsella*, and *Slackia* tended to occur in animals from degraded or urbanized areas and were associated with poor body condition or inflammatory signs (Sugden et al. 2020).

Table 2. Bacterial taxa showing positive (higher relative abundance or frequency) or negative (lower relative abundance or frequency) associations with parameters related to environmental quality, human interference, and body condition in wild canids.

Parameters	Positive relationship	Negative relationship	Reference
High-quality environment and/or low human interference	<i>Fusobacteriota</i> <i>Lachnoclostridium</i> <i>Phascolarctobacterium</i> <i>Turicibacter</i> Bacillota	<i>Pseudomonadota</i> <i>Clostridium_sensu_st</i> ricto_1 <i>Succinovibrio</i> Actinomycetota	(Biles et al. 2021; Wu et al. 2017)
Low-quality environment and/or high human interference – Poor body condition*	<i>Streptococcus</i> <i>Enterococcus</i> <i>Lactobacillus</i> <i>Blautia</i> <i>Faecalibacterium</i> <i>Clostridium</i> cluster XVIII <i>Clostridium</i> XIVa <i>Collinsella</i> <i>Slackia</i> <i>Helicobacter</i>	<i>Allobaculum</i> <i>Clostridium</i> XI <i>Dorea</i> <i>Alloprevotella</i> <i>Fusobacterium</i>	(Sugden et al. 2020)
Low-quality environment and/or high human interference - Game farm	<i>Clostridium tertium</i> <i>Ruminococcus</i> <i>Peptostreptococcus</i> <i>bacterium</i> <i>Peptococcus</i> <i>Blautia obeum</i> <i>Lactobacillus coryniformis</i> <i>Anaerobiospirillum</i> <i>Ignatzschineria</i>		(Menke et al. 2017)
Low-quality environment and/or high human interference – Cattle farm	<i>Blautia glucerasea</i> <i>Lactobacillus reuteri</i> <i>Lactobacillus gasseri</i>		(Menke et al. 2017)

Low-quality environment and/or high human interference – Trophic position	Sphingobacterium Flavobacterium	Bacteroides Fusobacterium	(Colborn et al. 2020)
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* Low body condition score, consumption of anthropogenic diet, enlarged spleen.

Overall, intestinal microbiota composition in wild canids reflects the combined effects of diet, habitat, and management conditions (Barraza-Guerrero et al. 2021; Bragg et al. 2020; McKenzie et al. 2017; Nardi et al. 2022; Wu et al. 2017). Understanding these interactions is essential for conservation, particularly in rehabilitation, reintroduction, and health-monitoring programs. The functional consequences of these compositional changes, especially their roles in short-chain fatty acid and bile-acid production, are addressed in the next section.

3.2. Functional and metabolic pathways of wild canid gut bacteria

Dietary macronutrients strongly shape the functional profile of wild canid intestinal microbiota. Protein-rich diets in the host favor Fusobacteriota and genera such as Fusobacterium (Adams et al. 2021; DeCandia et al. 2018; Menke et al. 2014; Sugden et al. 2020; Wu et al. 2016), whereas host diets rich in starches or fermentable fibers promote Bacillota and Bacteroidota involved in carbohydrate fermentation and SCFA production. Acetate, propionate, and butyrate are the main SCFAs generated, supporting epithelial energy supply, barrier function, and immune modulation (Suchodolski, 2011; Thomas et al. 2011). Phylum-level comparisons of gut bacteria allow broad interpretation of macronutrient effects, but evaluating specific nutritional pathways requires examining key families and genera involved in protein, lipid, and carbohydrate metabolism (Table 3).

Table 3. Principal macronutrient classes, representative bacterial taxa, associated metabolic pathways, and main fermentation products identified in wild canid intestinal microbiota. Information was adapted from Suchodolski (2011).

Microbial activity	Main products	Representative bacterial taxa
Decarboxylation and deamination of amino acids	Ammonia	Clostridium spp., Peptostreptococcus spp., Peptococcus spp., Fusobacterium spp.
Deconjugation and dihydroxylation of bile acids	Bile acids (cholate and deoxycholate)	Clostridium hiranonis, Lactobacillus spp.
Vitamin synthesis	Vitamins K ₂ , B ₁₂ , biotin, folate	Enterococcus spp., Pseudomonas spp., Sphingomonas spp., Lactobacillus spp.
Carbohydrate fermentation	Lactate, propionate, butyrate, acetate	Bacteroides, Clostridium cluster XIVa, Prevotella spp., Faecalibacterium spp., Bifidobacterium spp.
Amino-acid fermentation	Hydrogen, methane, amines, phenols, NH ₃ , organic acids, hydrogen sulfide	Sulfate-reducing bacteria (SRB), Desulfovibrio spp., Clostridium spp., Peptostreptococcus spp.
Oxalate degradation	Formate and CO ₂	Oxalobacter formigenes
Degradation of carbohydrates and inulin	Lactate	Bifidobacterium spp.
Hydrogen, alcohol, and acetic acid metabolism	Methane and CO ₂	Methanobacteria

Within Bacteroidota, *Bacteroides* and *Prevotella* are among the most abundant and metabolically versatile genera of the mammalian gut microbiome and contribute substantially to short-chain fatty acid (SCFA) production (DeCandia et al. 2021; McKenzie et al. 2017). *Bacteroides* species are particularly efficient at degrading complex polysaccharides and soluble proteins derived from animal tissues and tend to be more abundant in carnivorous wild canids, whereas *Prevotella* is specialized in fermenting plant-derived

carbohydrates and is more frequently associated with generalist canids consuming fruits, roots, and other vegetal resources (Adams et al. 2021; DeCandia et al. 2021; McKenzie et al. 2017). In *Urocyon littoralis*, seasonal dietary shifts, marked by fluctuations in the availability of fruits, nectar, and other carbohydrate-rich plant resources, were accompanied by corresponding changes in the relative abundance of *Prevotella* and *Bacteroides*, indicating that temporal variation in carbohydrate source and availability drives restructuring within the Bacteroidota community (Adams et al. 2021).

Clostridiaceae, particularly *Clostridium* clusters XIVa and IV, play major roles in fermenting resistant starches and dietary fibers, generating butyrate and other SCFAs (Barraza-Guerrero et al. 2021; Finlayson-Trick et al. 2017; Suchodolski, 2011) (Table 3). Genera such as *Ruminococcus*, *Faecalibacterium*, *Dorea*, and *Turicibacter* are similarly involved in butyrate production, supporting epithelial health and anti-inflammatory activity (Finlayson-Trick et al. 2017; Suchodolski, 2011). In contrast, proteolytic *Clostridium* species, such as *C. perfringens*, increase under high-protein diets (Menke et al. 2014; Nardi et al. 2022), producing metabolites like ammonia, branched-chain fatty acids, and phenols, which may become harmful when accumulated excessively (Félix et al. 2022).

Other bacterial taxa contribute to lipid metabolism. *Lactobacillus* and *Peptacetobacter* (*C. hiranonis*) participate in bile-acid deconjugation and transformation, processes that regulate lipid absorption and help maintain antimicrobial balance in the intestinal lumen (Breton and Dechelotte 2021; Finlayson-Trick et al. 2017; Rowe and Winston 2024) (Table 3). *Blautia*, a Lachnospiraceae genus, frequently increases in captive canids fed starch-rich diets, where it participates in carbohydrate fermentation but has also been associated with metabolic imbalance under low dietary diversity (Barraza-Guerrero et al. 2021; Bragg et al. 2020; Wu et al. 2016).

In *Canis mesomelas*, the predominance of *Clostridia* and *Fusobacteria*, especially *Fusobacterium mortiferum*, suggests a microbial profile shaped by scavenging behavior. Although the species is omnivorous, its microbiota resembles that of carnivorous or carrion-feeding mammals, underscoring that

feeding behavior may influence microbial structure more strongly than broad dietary categories (Menke et al. 2017). Fusobacteriaceae, thus, represent a functional adaptation to high-protein diets, supporting nitrogen recycling and energy extraction from substrates not fully digested in the small intestine (Adams et al. 2021; DeCandia et al. 2018; Menke et al. 2014; Sugden et al. 2020; Wu et al. 2016). Still, excessive proteolysis can generate harmful metabolites and promote inflammation if not counterbalanced by saccharolytic bacteria (AlShawaqfeh et al. 2017; Menke et al. 2014).

Functionally, the canid intestinal microbiota spans a continuum between protein- and carbohydrate-driven fermentation. Generalist species, whose diets fluctuate between animal and plant resources, harbor more metabolically flexible communities capable of switching between saccharolytic and proteolytic pathways (Adams et al. 2021; McKenzie et al. 2017). Strict carnivores rely more heavily on protein fermentation and exhibit limited contribution from fiber-degrading taxa (Adams et al. 2021; Menke et al. 2017; Sugden et al. 2020; Wu et al. 2017).

These functional differences of the intestinal microbiota have important physiological implications. In domestic dogs with chronic enteropathies, reduced Bacillota and expansion of Pseudomonadota (*Escherichia coli*) are associated with compromised mucosal integrity and intestinal inflammation (AlShawaqfeh et al. 2017; Mann et al. 2024; Quinn et al. 2019). Similar patterns occur in stressed or malnourished coyotes from urban areas (Biles et al. 2021; Sugden et al. 2020), suggesting that SCFAs and bile-acid profiles may serve as functional biomarkers of health and ecological stress in wild canids.

Overall, the balance between animal protein and fermentable carbohydrates determines both the taxonomic and functional structure of the intestinal microbiota. Incorporating metrics such as SCFA levels, bile-acid profiles, and nitrogen metabolites into microbial assessments can improve evaluations of nutritional status and gut homeostasis. These functional insights provide the foundation for exploring whether a consensus definition of a “healthy” microbiome exists for wild canids.

3.3. Is there a consensus “healthy” microbiome for wild canids?

A central question in microbiome research on wild canids is whether it is possible to define a universal “healthy” gut community for the group. Current evidence indicates that this is unlikely. Rather than a single taxonomic pattern, gut health in wild canids appears to depend on functional stability and ecological context. In mammals, a healthy microbiota is typically described as a balanced and resilient community that maintains homeostasis through efficient nutrient metabolism, immune support, and protection against pathogens (Rinninella et al. 2019). In domestic dogs, this balance is often defined as a stable, symbiotic state in which microbial alterations do not compromise health (Félix et al. 2022). These definitions, however, come largely from humans and domestic canids and do not fully capture the ecological and dietary variation found in wild species (Félix et al. 2022; Nishida and Ochman 2018; Rinninella et al. 2019).

Evidence from the reviewed studies suggests that functional attributes, such as SCFA production, bile-acid metabolism, and nitrogen recycling, are more informative than taxonomic composition alone for assessing microbiota health in wild canids. Species living in minimally disturbed habitats typically harbor communities enriched in saccharolytic and SCFA-producing taxa, including *Fusobacterium*, *Lachnoclostridium*, *Phascolarctobacterium*, and *Turicibacter* (Colborn et al. 2020; Wu et al. 2017). These groups support epithelial integrity and metabolic equilibrium and tend to decline in animals exposed to habitat degradation, captivity, or dietary simplification (Biles et al. 2021; Sugden et al. 2020; Wu et al. 2017). In contrast, disturbed environments often correspond with higher abundance of opportunistic or dysbiosis-associated bacteria such as *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Blautia*, *Faecalibacterium*, *Collinsella*, and *Slackia* (Biles et al. 2021; Colborn et al. 2020; Sugden et al. 2020; Wu et al. 2017). These shifts are frequently accompanied by reductions in *Bacillota* and increases in *Pseudomonadota* (e.g., *E. coli*), a pattern linked to mucosal dysfunction and inflammation (AlShawaqfeh et al. 2017; Mann et al. 2024; Quinn et al. 2019).

Such patterns mirror those observed in domestic dogs with chronic enteropathies, where loss of SCFA-producing taxa and expansion of

proteobacteria correlate with dysbiosis and compromised gut barrier function (AlShawaqfeh et al. 2017; Mann et al. 2024; Quinn et al. 2019). Similar microbiota profiles have been documented in urban *C. latrans* experiencing poor nutritional conditions (Biles et al. 2021; Sugden et al. 2020; Sugden 2019). These parallels suggest that microbial imbalance is a common consequence of nutritional or environmental stress across canids and that changes in ecological quality are reflected in intestinal functionality.

Context also influences how specific taxa relate to health. For example, *Blautia* and *Faecalibacterium* often interpreted as beneficial in domestic dogs (Félix et al. 2022; Rinninella et al. 2019), were linked to soft stools in captive *C. rufus* (Bragg et al. 2020) and to poor body condition in urban *C. latrans* (Sugden et al. 2020; Sugden 2019). These divergent associations highlight that microbial roles are species- and environment-dependent and shaped by diet, habitat, and host physiology.

Considering this variability, a practical definition of “healthy” intestinal microbiota for wild canids should incorporate three complementary dimensions:

- (i) Taxonomic diversity and temporal stability, reflecting ecological resilience and microbial turnover (Rinninella et al. 2019; West et al. 2019);
- (ii) Functional capacity, particularly SCFA synthesis, bile-acid transformation, and nitrogen recycling (Suchodolski 2011);
- (iii) Absence of opportunistic proliferation in response to habitat degradation or anthropogenic pressure (Biles et al. 2021; Sugden et al. 2020; Wu et al. 2017).

From a conservation perspective, this multidimensional framework allows microbial data to function as sensitive indicators of nutritional status, immune balance, and environmental quality. Changes in SCFA-producing taxa (e.g., *Fusobacterium*, *Turicibacter*, *Lachnoclostridium*) or increases in dysbiosis-associated groups (e.g., *Streptococcus*, *Enterococcus*, *Blautia*) may serve as early-warning signals of ecological stress or declining health (AlShawaqfeh et al. 2017; Biles et al. 2021; Colborn et al. 2020; Mann et al. 2024; Sugden et al. 2020; Wu et al. 2017). Integrating microbial profiles with metabolite measures

such as SCFA and bile-acid levels may therefore enhance diagnostic accuracy in both free-ranging and captive populations (AlShawaqfeh et al. 2017; Biles et al. 2021; Colborn et al. 2020; Mann et al. 2024; Sugden et al. 2020; Trevelline et al. 2019; Wu et al. 2017).

Defining a “healthy” microbiome for wild canids ultimately requires species-specific baselines supported by longitudinal, functional, and multi-omic approaches capable of capturing seasonal variation, anthropogenic disturbance, and management effects (AlShawaqfeh et al. 2017; Antwis et al. 2017; Félix et al. 2022; Rinninella et al. 2019; West et al. 2019). These baselines will provide the foundation for applying microbiota data to conservation physiology and health monitoring. The next section outlines the remaining challenges for achieving this level of standardization.

3.4. Gaps and Priorities

Research on the intestinal microbiota of wild canids has grown in recent years, but major knowledge gaps persist. These limitations are mainly geographical–taxonomic and methodological, and together they restrict the comparability of studies and the application of microbiome data to conservation.

3.4.1 Geographical and taxonomic gaps

Most available studies come from North America, Europe, and Asia, and no published data exist for South American or Neotropical canids (Biles et al. 2021; Bragg et al. 2020; Colborn et al. 2020; Cruz et al. 2022; Sugden et al. 2020; Wang et al. 2022; Wu et al. 2017) (Figure 4). This is a critical gap, as South America supports six native species - *Cerdocyon thous*, *Chrysocyon brachyurus*, *Speothos venaticus*, *Atelocynus microtis*, *Lycalopex vetulus*, and *L. gymnocercus* - that differ markedly in ecology, diet, and exposure to human disturbances (Gatti et al. 2006; ICMBio 2025; IUCN 2016; Soares 2020). Expanding microbiome studies to these taxa would clarify how environment, climate, and dietary breadth shape microbial diversity.

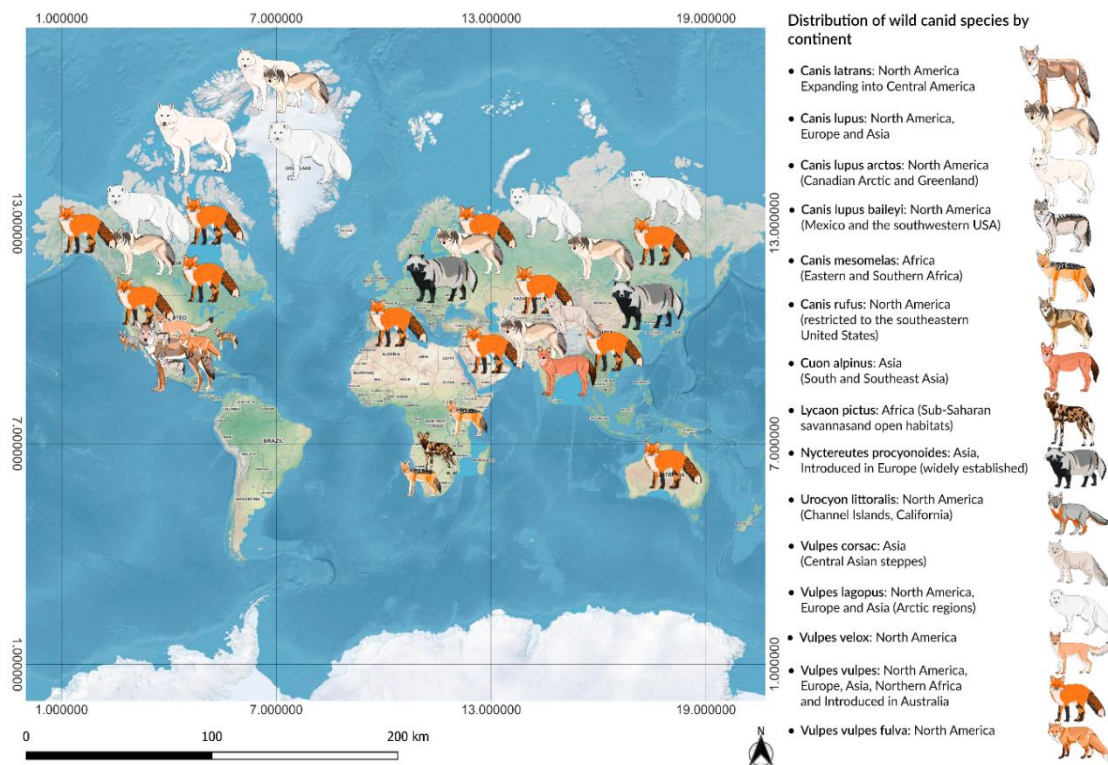


Figure 4. Taxonomic and geographic bias in gut microbiome studies of wild canids. Research effort is disproportionately concentrated on a small number of species, particularly *Canis lupus* and *Canis latrans*. African and Asian canids are represented by only a few isolated studies; no published gut microbiome data are currently available for South American or Neotropical canid species.

Intraspecific variation across subspecies or geographically distinct populations of wild canids remains poorly characterized, despite evidence that intestinal microbiota composition responds to host genetic structure, prey availability, and local environmental pressures (Bragg et al. 2020; DeCandia et al. 2021; Menke et al. 2014; Wang et al. 2022). Broader and more representative sampling across populations and regions is therefore required to assess whether consistent, large-scale patterns in intestinal microbiota composition, such as shifts in the relative abundance of major bacterial phyla are maintained across different ecological contexts. Here, the phylum level is considered as an initial comparative framework, given its consistent reporting across studies, while recognizing that finer taxonomic and functional resolution is necessary for ecological interpretation.

3.4.2 Methodological gaps

Methodological heterogeneity is another major barrier. Studies differ widely in sequencing technologies, bioinformatics workflows, and metadata quality, which complicates comparisons. Most work relies on 16S rRNA sequencing, while few studies incorporate shotgun metagenomics or metabolomics that would better capture functional traits such as SCFA production, bile-acid transformation, and nitrogen metabolism (DeCandia et al. 2021; Félix et al. 2022; Kohl et al. 2015). Only a minority of studies quantify metabolic markers such as SCFAs, bile acids, or fecal calprotectin, despite their value for assessing intestinal function and inflammation (DeCandia et al. 2021; Félix et al. 2022; Kohl et al. 2015; Mann et al. 2024; Schmidt et al. 2018).

Longitudinal data are scarce, limiting insight into temporal stability, seasonal shifts, or microbiota changes during captivity, rehabilitation, or reintroduction (Adams et al. 2021; DeCandia et al. 2021; McKenzie et al. 2017). Host genetic factors add further complexity. Genetic variation in host immune genes such as TLR5, which mediates bacterial flagellin recognition, has been linked to inflammatory bowel disease in both *C. rufus* and *C. brachyurus* (Bragg et al. 2020; Henson et al. 2017; Maia and Gouveia 2002; Marcinczyk et al. 2024; Phipps and Edwards 2009). Including genomic markers in microbiome studies may help identify species- or genotype-specific vulnerabilities relevant to *ex-situ* management.

Future research should prioritize the development of reference baseline datasets that reflect the microbiota of different feeding guilds and biogeographic regions, enabling comparisons across species and landscapes (Bragg et al. 2020; Sugden et al. 2020; Wu et al. 2017). Equally important is the standardization of sampling, sequencing, and analytical protocols, which would reduce methodological inconsistencies that currently hinder cross-study integration (Félix et al. 2022; McKenzie et al. 2017; Suchodolski 2011). Progress in this field will also depend on incorporating functional and host-level biomarkers such as metabolite profiles, immune indicators, and genetic variation into microbiome analyses to improve ecological and physiological interpretation (Félix et al. 2022; Henson et al. 2017; Suchodolski 2011).

Another key priority is the implementation of longitudinal and multi-scale study designs capable of capturing natural temporal variation, seasonal dietary shifts, and the effects of captivity, rehabilitation, and reintroduction on gut microbial communities (Adams et al. 2021; Félix et al. 2022; McKenzie et al. 2017). Addressing these gaps will support the transition from descriptive studies to predictive and applied frameworks, strengthening the links between microbiota composition, animal health, and environmental quality. This foundation is essential for integrating microbial indicators into conservation decision-making, as discussed in the next section.

4. RECOMMENDED INTERVENTIONS AND IMPLICATIONS OF EXISTING KNOWLEDGE GAPS

The synthesis of available studies reveals that intestinal microbiota data offers tangible opportunities for conservation and management, despite the methodological and geographic gaps identified earlier (Figure 5).

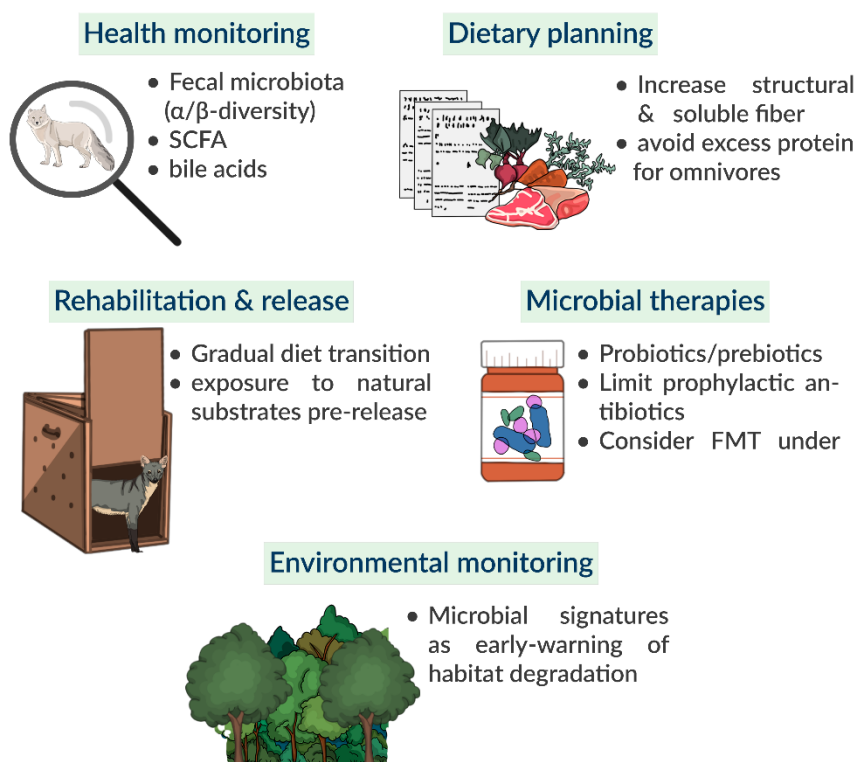


Figure 5. Conceptual framework summarizing conservation and management applications of gut microbiota knowledge in wild canids. *SCFA: Short-chain fatty acids; FMT: Fecal Microbiota Transplants.

Translating these findings into practice requires actions that integrate microbial monitoring with veterinary, nutritional, and ecological strategies. Building upon the framework proposed by Trevelline et al. (2019) and supported by studies on wild and captive canids, ten recommendations for conservation and management are outlined below, organized according to their relevance to ex-situ and in-situ contexts.

4.1. Ex-situ applications

4.1.1 Control of intestinal dysbiosis and chronic gastrointestinal diseases

Intestinal dysbiosis is a central factor underlying gastrointestinal disease in both domestic and wild canids. In captive settings, stress, repeated antibiotic exposure, and diets with limited fermentable fiber frequently disrupt microbial balance, reducing Bacillota and Bacteroidota while favoring opportunistic bacteria such as *E. coli* and *Clostridium perfringens* (AlShawaqfeh et al. 2017; Mann et al. 2024; Quinn et al. 2019). For wild canids under human care, the resulting decline in nutrient assimilation, combined with persistent gastrointestinal instability, can compromise survival and long-term welfare.

Microbial patterns like those observed in domestic dogs with chronic enteropathies have also been reported in free-ranging canids exposed to nutritional stress or anthropogenic disturbance (Biles et al. 2021; Sugden et al. 2020; Wang et al. 2022). Reductions in SCFA-producing taxa such as *Faecalibacterium* and *Turicibacter*, alongside increases in facultative anaerobes such as *Streptococcus* and *Enterococcus*, often correlate with inflammatory markers including elevated fecal calprotectin and altered bile-acid profiles (AlShawaqfeh et al. 2017; Félix et al. 2022; Mann et al. 2024). These microbial signatures could be incorporated into standardized health-monitoring protocols for captive and rehabilitated canids, improving early detection of intestinal dysfunction and supporting more effective long-term treatment, rehabilitation, and release programs.

Generalist canids, such as *C. brachyurus*, naturally consume a wide variety of vertebrates, invertebrates, fruits, and plant material, and therefore ingest substantial amounts of structural and insoluble fiber in free-ranging

conditions. Recent anatomical and dietary analyses revealed that its stomach contents may contain up to 44% neutral-detergent fiber, far higher than the levels in standard extruded canine diets used in captivity (Silva et al. 2025). This high fiber load reflects decades of field studies showing the central role of *Solanum lycocarpum* A.St. Hil. (lobeira) in the species' diet, often representing nearly half of the ingested biomass, along with small vertebrates, insects, and other fruits (Aragona and Setz 2001; Dietz 1984; Garcia 2016; Motta-Junior 1997; Motta-Junior et al. 1996; Motta Junior and Martins 2002; Rodrigues et al. 2007; Santos et al. 2003). Comparable omnivorous strategies are observed in *C. thous*, which combines fruits, insects, and small vertebrates (Cruz et al. 2022; Gatti et al. 2006), and in generalist canids from temperate regions such as the *C. latrans*, where seasonal and anthropogenic resources influence fiber intake and shape microbial composition (Biles et al. 2021; Sugden et al. 2020).

These patterns demonstrate that free-ranging generalist canids ingest a broader range of fibrous substrates than those typically provided in captivity. Their natural diets include plant structural fiber (cellulose, hemicellulose, and pectins) as well as animal-derived components such as hair, feathers, and bone fragments, materials that contribute to intestinal motility, microbial fermentation, and mucosal stimulation. In contrast, ex-situ diets rely heavily on dog kibble supplemented with fruits and vegetables, which are consistently lower in structural fiber and lack indigestible animal matter (Allen 2007; Barboza et al. 1994; Weber et al. 2023). These nutritional mismatches have been linked to chronic diarrhea, soft stools, and mucosal inflammation in captive canids (Bragg et al. 2020; Nardi et al. 2022). Similar patterns have been documented in red wolves and Mexican wolves kept on high-starch formulations, which display shifts toward carbohydrate-fermenting and dysbiosis-associated taxa (Barraza-Guerrero et al. 2021; Bragg et al. 2020; Chen et al. 2022).

Collectively, this evidence indicates that adequate soluble and insoluble fiber intake is essential for maintaining microbial balance and promoting SCFA production in captive generalist canids. Adjusting fiber quantity and quality, incorporating both plant-derived and animal-derived sources, may therefore

enhance gastrointestinal health, reduce dysbiosis, and support long-term welfare under human care.

4.1.2 Development of nutritional protocols and selection of optimal dietary ingredients

The development of nutritionally balanced diets for wild canids requires aligning digestive physiology, trophic ecology, and microbiota needs. Comparisons across carnivorous mammals show that strictly carnivorous species, such as the *L. pictus* and *Cuon alpinus* (dhole), are adapted to high-protein, prey-based diets. In contrast, generalist and omnivorous canids, including the *C. brachyurus*, *C. thous*, and *V. vulpes*, require more complex nutrient profiles that combine moderate protein levels with both fermentable and structural fiber sources (Clauss et al. 2010; Rodden et al. 2012). These components support microbial diversity, intestinal motility, and the production of SCFAs.

Captive feeding practices have not always reflected these differences. Historic diets formulated for maned wolves in North American institutions often exceeded 30% crude protein, largely derived from lean meats and organ tissues, and were associated with cystinuria, chronic diarrhea, and mineral imbalances (Phipps and Edwards 2009). In Brazilian institutions, diets during the 1990s and early 2000s reached 36–53% crude protein; these values were gradually reduced to 21–36% by 2022, aligning more closely with the moderate range of 20–25% recommended for urinary and gastrointestinal health (Faria et al. 2022). These patterns highlight that high-protein, meat-dominant formulations, even when composed of high-quality ingredients, have been associated with gastrointestinal and urinary disorders.

Nutritional protocols for wild canids should therefore adopt an integrative approach that moderates total protein content and includes adequate sources of structural and functional fibers. Insoluble fibers, derived from plant cell walls, hair, feathers, cartilage, and bone fragments, provide mechanical stimulation, extend digesta retention time, and support microbial stabilization (Clauss et al. 2010). Ingredients rich in soluble fiber and oligosaccharides, such as native fruits and

tubers, promote saccharolytic fermentation and SCFA synthesis, reinforcing mucosal integrity. Protein inclusion should emphasize quality rather than excessive quantity: lean meats, whole-prey items, and insect-based meals deliver essential amino acids without overloading nitrogen metabolism.

The AZA, Large Canid Care Manual also stresses the need for flexible feeding protocols that allow seasonal, behavioral, and ecological variability while preventing dietary monotony through enrichment. Beyond proximate nutrient composition, diet evaluation should incorporate fecal scoring, metabolic profiling (e.g., SCFAs, bile acids), and microbiota assessments. Establishing this feedback between diet, gastrointestinal function, and microbial composition will support adaptive nutritional management, reduce chronic enteropathies, and enhance long-term welfare across wild canid species maintained under human care.

4.1.3 Rehabilitation and preparation of individuals for translocation or reintroduction

Captivity, rehabilitation, and eventual release expose wild canids to marked physiological and microbial transitions. During captivity, simplified diets, reduced environmental contact, and routine antibiotic exposure often reduce microbial diversity and remove functionally important taxa (Adams et al. 2021; Barraza-Guerrero et al. 2021; Biles et al. 2021; Félix et al. 2022; Wu et al. 2017). These alterations can weaken the intestinal barrier, compromise nutrient assimilation, and reduce immune resilience, ultimately affecting post-release performance and survival (AlShawaqfeh et al. 2017; Mann et al. 2024). Restoring a diverse and functionally stable intestinal microbiota before reintroduction is therefore essential to ensure that rehabilitated animals can cope with the dietary and ecological demands of natural environments.

Evidence from other vertebrates shows that individuals maintained in highly sanitized or artificial settings often exhibit reduced microbial complexity and lower adaptability after release (Trevelline et al. 2019). Similar outcomes are expected for canids, particularly species with flexible diets, such as *C. brachyurus* and *C. thous*. Rehabilitation should therefore recreate environmental and dietary

conditions that favor microbial recovery. Gradual reintroduction of natural food items to canid hosts like prey fragments containing hair, feathers, or bones, and native fruits and fibrous plant parts, can promote recolonization of the gut by beneficial bacterial taxa and improve mucosal immune function (Clauss et al. 2010; Rodden et al. 2012). Recent rehabilitation trials with maned wolves demonstrated that progressive transitions from processed diets to native fruits and live prey enhance foraging skills, prey handling, and readiness for natural feeding contexts prior to release (Faria et al. 2023). Controlled exposure to soil, vegetation, and organic substrates from native habitats may further facilitate acquisition of environmental microbes important for local adaptation (Trevelline et al. 2019).

The IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN 2013) emphasize that successful translocations depend on thorough pre-release assessment of health, welfare, feasibility, and disease risk, followed by adaptive post-release monitoring. These guidelines align closely with current understanding of host–microbiota interactions, reinforcing that microbial balance is a component of physiological preparedness. Minimizing stress, allowing gradual dietary and environmental transitions, and avoiding excessive sanitation are consistent with IUCN recommendations and support both welfare and ecological functionality.

To guide management decisions, microbiota and metabolite indicators should be integrated into pre-release health evaluations. Non-invasive fecal analyses assessing microbial diversity, SCFA concentrations, bile-acid profiles, and inflammatory markers such as calprotectin, a neutrophil- and macrophage-derived protein widely used as a fecal biomarker of intestinal inflammation and gastrointestinal dysfunction, can help determine intestinal functionality and inflammatory state (AlShawaqfeh et al. 2017; Félix et al. 2022; Suchodolski 2011). Establishing baseline microbiota profiles for each species and population allows meaningful comparison between rehabilitated and wild individuals, providing a clearer assessment of release readiness and post-release adaptability. Table 4 summarizes microbiota-related actions, objectives, and monitoring indicators across the pre-release, translocation, and post-release

phases, based on IUCN IUCN (2013) and Trevelline et al. Trevelline et al. (2019). Together, these rehabilitation strategies establish the physiological and microbial conditions necessary for successful release, creating a bridge to other health-oriented interventions discussed in the following section.

Table 4. Microbiota-related considerations in the rehabilitation and reintroduction of wild canids, summarizing recommended management actions, biological objectives, and monitoring indicators across pre-release, translocation, and post-release phases (adapted from IUCN, IUCN (2013) and Trevelline et al. (2019)).

Phase	Recommended actions	Objectives	Monitoring indicators
Pre-release (rehabilitation and conditioning)	Gradual dietary transition from processed foods to natural items (native fruits, tubers, prey fragments with hair/feathers/bones). Controlled exposure to natural substrates (soil, vegetation, organic litter) from native habitats. Avoid excessive sanitation and prophylactic antibiotic use. Inclusion of environmental enrichment that stimulates foraging and prey-handling behavior.	Reestablish microbial diversity and functional taxa (SCFA producers, bile-acid transformers). Restore digestive motility and mucosal immunity. Enhance behavioral and nutritional adaptation prior to release. Reduce physiological stress and dysbiosis risk.	Fecal microbiota composition (α - and β -diversity, relative abundance of Bacillota and Bacteroidota). SCFA and bile-acid profiles. Fecal pH and calprotectin levels. Body condition and fecal scoring. Behavioral metrics: prey manipulation, foraging, fruit selection.

Translocation and release	Minimize stress during capture, transport, and handling. Maintain dietary and environmental continuity from pre-release phase. Use soft release protocols, when possible, with acclimation enclosures. Monitor for signs of gastrointestinal disturbance.	Prevent acute dysbiosis and stress-induced inflammation. Facilitate microbial and physiological stability during environmental transition. Support immune and metabolic resilience.	Cortisol or fecal glucocorticoid metabolites. Short-term fecal microbiota stability (comparison with pre-release baseline). Feeding behavior and appetite post-release.
Post-release (adaptation and monitoring)	Continue habitat-based dietary availability assessments. Collect fecal samples periodically to evaluate microbiota adaptation. Correlate microbial changes with survival, body condition, and movement patterns. Apply adaptive management if gastrointestinal or nutritional issues arise.	Evaluate long-term microbiota–environment integration. Detect maladaptation or nutritional deficiencies early. Inform management adjustments in future releases.	Longitudinal microbiota monitoring (16S/shotgun metagenomics). SCFA and metabolite analyses. Health status, reproductive success, and survival rates. Habitat diet overlaps (availability vs. consumption).

4.1.4 Reduction in antibiotic use through targeted probiotic or microbial therapies

The frequent use of antibiotics in wildlife care, whether for prophylaxis, stress-related diarrhea, or secondary infections, can disrupt gut microbial stability and compromise long-term health. In canids, antibiotic-induced dysbiosis is commonly characterized by reductions in Bacillota and Bacteroidota, an

expansion of opportunistic *Pseudomonadota* such as *E. coli*, and diminished production of SCFAs (Almeida and Franco 2021; AlShawaqfeh et al. 2017; Mann et al. 2024; Quinn et al. 2019). Repeated antimicrobial exposure may also select for resistant strains and impair immune regulation, creating a recurrent cycle of chronic enteritis and dependency on further antibiotic treatment.

Targeted microbial therapies offer an alternative pathway to restore intestinal balance while reducing antimicrobial reliance. In domestic dogs, probiotic strains such as *Lactobacillus* spp., *Bifidobacterium* spp. (including *B. longum* and *B. animalis*), *Enterococcus faecium*, and *Bacillus coagulans* have been shown to shorten diarrhea duration, modulate inflammatory responses, and stabilize fecal microbial composition (Barko et al. 2018; Félix et al. 2022; Mann et al. 2024). These effects are largely attributed to improved epithelial barrier function, enhanced SCFA production, and competitive suppression of pathogenic bacteria. Evidence for wild canids remains limited, but a controlled trial in *N. procyonoides* and *V. vulpes* demonstrated that a multi-strain formulation containing *Bifidobacterium bifidum*, *Clostridium butyricum*, *Bacillus subtilis*, and *Bacillus licheniformis* can modulate gut communities without the ecological drawbacks associated with routine antibiotics (Peng et al. 2019; Peng et al. 2018). This suggests that probiotic interventions are feasible in conservation settings, if strain selection is tailored to species-specific physiology.

Prebiotic and symbiotic approaches, particularly those incorporating fermentable fibers such as inulin and fructooligosaccharides, may further enhance resilience to stress-induced dysbiosis by acting as substrates for beneficial taxa (Finlayson-Trick et al. 2017; Suchodolski 2011; Thomas et al. 2011). More advanced strategies, including fecal microbiota transplantation (FMT), have shown rapid restoration of eubiosis in dogs with chronic enteropathies and represent a promising avenue for ex-situ wildlife medicine when applied under strict biosafety standards (AlShawaqfeh et al. 2017; Bornbusch et al. 2023; Mann et al. 2024).

To be effective in conservation programs, microbial therapies must follow an evidence-based implementation plan. Probiotic strains should align with the digestive ecology of each species, ideally derived from conspecifics or closely

related taxa. Antibiotic use should be limited to clearly defined clinical needs and complemented by microbiota-informed dietary design, appropriate fiber enrichment, and husbandry practices that preserve microbial diversity. Establishing microbiota reference baselines and standardized evaluation protocols will be essential for safely reducing antimicrobial use while promoting gastrointestinal resilience and overall welfare in wild canids under human care (Adams et al. 2021; Antwis et al. 2017; Trevelline et al. 2019; West et al. 2019).

4.1.5 Identification of bacterial indicators of health and welfare

The intestinal microbiota provides a practical, non-invasive indicator of physiological and welfare status in wild canids, integrating the effects of diet, environment, and host immunity (Biles et al. 2021; Colborn et al. 2020; Sugden et al. 2020; Wu et al. 2017). Several bacterial groups consistently reflect gastrointestinal stability, particularly Firmicutes-associated taxa involved in complex carbohydrate fermentation and SCFA synthesis. *Clostridium* clusters XIVa and IV, which include genera such as *Faecalibacterium* and *Ruminococcus*, play central roles in SCFA production and are linked to epithelial integrity and immune regulation (Barko et al. 2018; Mann et al. 2024; Suchodolski 2011; Thomas et al. 2011). Members of Erysipelotrichaceae, including *Turicibacter*, also respond sensitively to nutritional and environmental conditions and are often associated with higher-quality habitats (Félix et al. 2022; Finlayson-Trick et al. 2017).

Not all microbial indicators behave consistently across species or ecological settings. The genus *Blautia*, for instance, correlates with intestinal stability in domestic dogs but is associated with dysbiosis in several wild and captive canids exposed to high-starch diets or anthropogenic disturbance (Barraza-Guerrero et al. 2021; Bragg et al. 2020). These contrasting patterns reflect functional differences among *Blautia* species and highlight the need to interpret bacterial indicators in the context of local dietary and environmental conditions (Bornbusch et al. 2023; Trevelline et al. 2019).

Opportunistic or inflammation-associated taxa such as *Streptococcus*, *Enterococcus*, *Collinsella*, *Clostridium perfringens*, and *Escherichia/Shigella*,

tend to increase under nutritional stress, low-fiber or high-protein diets, or urbanization (Biles et al. 2021; Mann et al. 2024; Sugden et al. 2020; Wu et al. 2017). These shifts frequently coincide with reduced SCFA production and elevated inflammatory markers, supporting their value as early-warning indicators of intestinal imbalance (AlShawaqfeh et al. 2017; Félix et al. 2022).

Diagnostic precision can be enhanced by using composite measures that integrate microbial abundance ratios with metabolic readouts such as SCFAs and bile acids (Félix et al. 2022; Rowe and Winston 2024). Routine fecal monitoring, especially when paired with dietary and behavioral assessments, may allow early detection of subclinical disturbances in captive individuals (West et al. 2019; Zoelzer et al. 2021). Establishing species- and environment-specific baselines will be essential to distinguish natural ecological variation from clinically relevant dysfunction (Adams et al. 2021; Menke et al. 2017).

Taken together, these patterns reinforce that gut health in wild canids is best assessed through functional markers such as SCFA production, bile-acid metabolism, and mucosal integrity rather than through rigid taxonomic profiles (Mann et al. 2024; Suchodolski 2011). Integrating microbial and metabolic indicators into routine evaluations strengthens welfare assessments and supports evidence-based management in both captive and free-ranging populations.

4.2. In-situ applications

4.2.1 Monitoring wildlife exposed to anthropogenic disturbance

Intestinal microbiota composition shifts in response to habitat degradation and proximity to human activity. In wolves, coyotes, and jackals, disturbed environments are characterized by reduced Fusobacteriota and increased Pseudomonadota and Actinomycetota, patterns that reflect dietary shifts, altered exposure to environmental microbial sources, and stress-related immune modulation (Biles et al. 2021; Menke et al. 2017; Sugden et al. 2020; Wu et al. 2017). Because these changes integrate nutritional, ecological, and physiological pressures, periodic microbiome sampling provides a powerful non-invasive tool

for monitoring ecosystem quality and cumulative stress in wild populations (Trevelline et al. 2019; West et al. 2019; Wu et al. 2017).

When combined with metrics such as fecal glucocorticoid metabolites, immune biomarkers, and behavioral observations, microbiome data can improve the detection of subtle or subclinical impacts of anthropogenic disturbance. Longitudinal and metagenomic approaches further strengthen this framework by identifying microbial signatures linked to environmental degradation and potential pathogen emergence, supporting One Health-oriented conservation strategies across canid populations (AlShawaqfeh et al. 2017; Biles et al. 2021; DeCandia et al. 2018; Mann et al. 2024; Sugden et al. 2020; Trevelline et al. 2019; West et al. 2019). These findings illustrate how microbial data can bridge physiological responses and environmental conditions, providing a foundation for integrating intestinal microbiota into broader assessments of habitat quality and conservation planning.

4.2.2 Adaptation of management practices to climatic and habitat variability

Environmental stressors such as temperature fluctuations, rainfall patterns, and shifts in resource availability directly influence diet composition and intestinal microbiota diversity in wild canids (Wang et al. 2022; Wu et al. 2017). Because the intestinal microbiota supports nutrient assimilation, immune modulation, and metabolic flexibility, it acts as a key mediator of physiological adaptation under variable climatic conditions (Antwis et al. 2017; Barko et al. 2018; Mann et al. 2024; Rinninella et al. 2019; Wang et al. 2022; Youngblut et al. 2019). Climate-driven changes in prey availability and plant phenology can alter microbial community structure, with consequences for host resilience and health.

Integrating microbiota data into ecological and nutritional studies helps clarify how wild canids respond to environmental variability and climate change (Adams et al. 2021; Antwis et al. 2017; Colborn et al. 2020; Veloso 2019; Wang et al. 2022). Longitudinal and metagenomic approaches can further identify microbial signatures linked to adaptive capacity, informing evidence-based decisions for both in-situ and ex-situ management. Such insights may guide

adaptive feeding strategies, refine rehabilitation protocols for translocated individuals, and support conservation planning in the face of increasing climatic instability (Adams et al. 2021; Antwis et al. 2017; Bornbusch et al. 2023; Guo et al. 2020; Rojas et al. 2023). By linking microbial dynamics to climatic and habitat variability, these insights extend beyond individual physiology and begin to intersect with broader ecological processes. This perspective naturally leads to the examination of how social structure and population organization shape microbiome patterns, a key dimension for understanding health and connectivity in wild canid populations.

4.2.3 Characterization of social and population-level microbial patterns

Social structure plays a significant role in shaping intestinal microbiota composition in wild canids. In wolves, pack members frequently share similar microbial profiles, a pattern driven by common diets, physical interactions, scent-marking behaviors, and overlapping territories (DeCandia et al. 2021; Nardi et al. 2022). These processes promote horizontal microbial transmission, forming what has been described as a “social microbiome,” in which microbial exchange reinforces social cohesion and may contribute to kin recognition. Microbiota-mediated production of volatile compounds further supports olfactory communication, an essential component of territoriality and reproductive behavior in canids.

Characterizing these social microbial signatures offers insights into group dynamics, contact networks, and potential routes of pathogen transmission (Bornbusch et al. 2023; Guo et al. 2020). At broader spatial scales, divergence in microbiota composition among packs or populations can reflect ecological boundaries, variation in resource use, or underlying genetic structure. These patterns provide non-invasive tools for monitoring population connectivity, assessing habitat fragmentation, and detecting early signals of disease spread in conservation programs (DeCandia et al. 2021; West et al. 2019). These social and population-level microbial signatures highlight that gut communities are shaped not only by individual physiology and habitat conditions, but also by broader ecological relationships. Extending this perspective to the landscape

scale allows us to examine how geography, resource distribution, and environmental gradient's structure microbiota across populations.

4.2.4 Assessment of geographic and ecological variation in microbiota.

Spatial variation in intestinal microbiota composition reflects key ecological processes, including habitat connectivity, trophic niche breadth, and environmental heterogeneity (Adams et al. 2021; Colborn et al. 2020). Distinct microbial assemblages can function as biogeographic markers, revealing the influence of population isolation (Adams et al. 2021; Menke et al. 2017), local resource availability (Adams et al. 2021; Colborn et al. 2020), and anthropogenic disturbance (Biles et al. 2021; Trevelline et al. 2019). Variation in prey composition (Nardi et al. 2022), vegetation structure, and climatic conditions further shape community profiles, mirroring ecological gradients and host adaptive responses (Antwis et al. 2017).

The integration of microbiota data with genetic, behavioral, and geospatial information can refine landscape-level conservation planning for fragmented or recovering populations. Recognizing how environmental and geographic variation structure microbial communities strengthens our ability to identify connectivity priorities, anticipate population-specific vulnerabilities, and design management strategies that reflect the ecological realities experienced by each wild canid population. A clearer view of how geography and environmental gradient's structure intestinal microbial communities establishes the foundation for interpreting microbial patterns in relation to feeding ecology. Extending this framework to trophic interactions shows that certain microbial signatures function as robust indicators of diet composition and nutrient assimilation.

4.2.5 Evaluation of diet composition and trophic position through microbial signatures.

Functional microbial markers can complement traditional dietary assessments based on stable isotopes, metabarcoding, or behavioral observations. Because gut microbial composition reflects nutrient assimilation and digestive strategy, shifts in key taxa offer indirect evidence of host adaptation

to available resources (Antwis et al. 2017; Thomas et al. 2011). Protein-rich diets, for example, are typically associated with elevated abundance of *Fusobacterium* (Barraza-Guerrero et al. 2021; Bragg et al. 2020; Zoelzer et al. 2021) and proteolytic *Clostridium* clusters I and XI (Suchodolski 2011). In contrast, greater prevalence of *Prevotella*, *Bacteroides*, or *Ruminococcus* is characteristic of diets richer in carbohydrates, fruits, or plant-derived material (Adams et al. 2021; Bermingham et al. 2017; DeCandia et al. 2021; Thomas et al. 2011).

Temporal variation in these microbial signatures can indicate seasonal changes in resource use or increased reliance on anthropogenic food sources (Adams et al. 2021). Combining microbial functional profiles with isotopic and ecological data improves the resolution of trophic models, enabling more precise interpretation of feeding ecology, resource partitioning, and ecosystem roles of wild canids (Colborn et al. 2020; Cruz et al. 2022). These microbial indicators also support non-invasive dietary monitoring in field settings, strengthening adaptive management and conservation strategies for species living in dynamic or degraded environments (Antwis et al. 2017; Trevelline et al. 2019). Together, these applications illustrate the growing relevance of microbiome data for ecological inference and management. Yet, the value of these tools ultimately depends on the consistency and breadth of the underlying evidence base.

4.3. Consequences of remaining gaps

Despite the expanding applications of microbiome research, the lack of standardized methodologies from sample collection to sequencing and bioinformatics continues to restrict cross-study comparability (Barko et al. 2018; Youngblut et al. 2019). The persistent absence of data for neotropical canids further limits the development of reliable baselines for threatened or endemic taxa (Cruz et al. 2022; Soares 2020; Trevelline et al. 2019; West et al. 2019). Without coordinated frameworks, microbiota studies risk remaining primarily descriptive and difficult to translate into conservation practice.

Closing these gaps is essential to produce actionable baselines, strengthen species-specific health monitoring, and integrate microbial indicators

into wildlife management. When microbiota-based indicators are incorporated into broader ecological and physiological assessments, their value becomes directly practical: they support welfare evaluations, reduce disease incidence, and contribute to long-term population viability. With methodological consistency and contextual interpretation, the microbiome can assume a central role in conservation physiology, bridging molecular ecology with applied wildlife management. Progress in this area depends on collaborative, long-term programs that unite microbial ecology, veterinary medicine, nutrition, and conservation planning (Antwis et al. 2017; Trevelline et al. 2019; West et al. 2019). The creation of reference microbiota datasets for key species under *ex-situ* management, such as *C. brachyurus* and *C. rufus*, will provide the basis for benchmarking gut health and guiding reintroduction and rehabilitation protocols (Bornbusch et al. 2023; Bragg et al. 2020; West et al. 2019). These coordinated frameworks are essential for moving microbiome research from descriptive findings toward operational conservation tools.

5. CONCLUSION

This review synthesizes current evidence on the intestinal microbiota of wild canids and highlights how diet, environment, and management conditions shape microbial communities. According to studies, Bacteroidota, Bacillota, and Fusobacteriota dominate the intestinal microbiota, and their relative abundances reflect feeding ecology and habitat characteristics. Generalist species show broader microbial variability driven by dietary flexibility and exposure to diverse environmental sources, whereas strictly carnivorous species maintain more stable, protein-associated microbial profiles. Functionally, the intestinal microbiota supports host homeostasis through short-chain fatty acid production, bile-acid metabolism, and nitrogen processing. Alterations in these pathways, coupled with shifts such as the loss of Bacillota and the rise of Pseudomonadota, mirror nutritional imbalance, stress, and habitat degradation and can serve as early indicators of intestinal dysfunction.

Microbial data offer practical applications for both *ex-situ* and *in-situ* management, including dietary formulation, health monitoring, rehabilitation, and ecological assessment. However, significant gaps remain, especially the lack of

studies on neotropical canids and the absence of standardized methods that allow reliable cross-study comparisons. Future research should prioritize the creation of region- and guild-specific microbiota baselines, the use of longitudinal and functional approaches, and the integration of microbial, physiological, and genetic data. Strengthening these foundations will help position microbiome research as a practical tool for conservation, supporting early detection of health alterations and guiding evidence-based management in both captive and free-ranging populations.

LIST OF ABBREVIATIONS

AZA: Association of Zoos and Aquariums

DNA: Deoxyribonucleic acid

FMT: Fecal microbiota transplantation

IUCN: International Union for Conservation of Nature

SCFA: Short-chain fatty acids

SSC: Species Survival Commission (IUCN)

TLR5: Toll-like receptor 5

DECLARATIONS

Ethics approval and consent to participate

Not applicable. This study is based solely on previously published data and did not involve research on live animals or human participants.

Consent for publication

Not applicable.

Availability of data and materials

All data analyzed in this study are available within the published literature cited throughout the manuscript. No new datasets were generated or analyzed during this review.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ARGF conceived the study, conducted the systematic literature review, and prepared the first draft of the manuscript.

CRRM contributed expertise in conceptual development and provided supervision, supporting the interpretation of the main themes and refining the overall structure of the manuscript.

RC contributed to the conceptual framework and assisted in the interpretation of ecological and evolutionary aspects, reviewing the manuscript to improve clarity and coherence.

ED provided expertise in nutrition and comparative physiology and supported the interpretation of dietary and functional aspects, contributing technical input that strengthened the manuscript.

APF contributed expertise in nutrition and metabolism and assisted in refining dietary and functional interpretations, offering technical feedback that improved analytical accuracy.

APNQ and **WRS** contributed their expertise in ecological field analyses and supported the interpretation of environmental contexts relevant to wild canid microbiota. Both authors also reviewed and refined the sections addressing health, physiology, and disease-related aspects, providing technical input that improved the accuracy and coherence of the manuscript.

AM contributed expertise in microbial community analyses to the interpretation of bacterial taxon data, refined the section on the most relevant bacterial groups, prepared Figure 2, and refined the overall structure of the manuscript.

JM contributed expertise in genetics and microbiome science and reviewed the manuscript for intellectual content, providing technical input that improved analytical rigor and interpretative depth.

All authors reviewed and approved the manuscript.

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DNA metabarcoding reveals the animal and plant diet of maned wolves (*Chrysocyon brachyurus*) in the Cerrado

ABSTRACT

The maned wolf (*Chrysocyon brachyurus*) is a flagship generalist canid of the South American Cerrado, yet its dietary niche breadth has been historically characterized primarily through morphological fecal analysis, which often underestimates cryptic or highly digestible items. This study employed an integrated DNA metabarcoding approach, using ITS (Plantae) and COI (Metazoa) markers, to characterize the diet of free-ranging populations across three distinct areas in the Cerrado. We identified 171 potential dietary items (117 plant and 54 animal taxa), a number that exceeds the cumulative richness of 159 species reported across 35 years of traditional literature. Our results reveal a stable taxonomic core dominated by Solanaceae, Poaceae, and Dasypodidae, showing high compositional overlap across seasons and localities. The molecular approach uncovered a previously underestimated fraction of the diet, including a high diversity of social insects (Termitidae) and birds (Tinamidae). While domestic prey (*Gallus gallus*) was detected, it was restricted to a few individuals in fragmented landscapes, reinforcing the species' opportunistic-generalist nature rather than systematic conflict with livestock. Despite technical challenges related to gaps in genetic reference databases for Cerrado fauna and flora, which necessitated family-level analysis for higher reliability, this study redefines the scale of the maned wolf's trophic plasticity. These findings provide a robust framework for both *in-situ* and *ex-situ* conservation strategies, highlighting the importance of dietary fiber and resource availability for the long-term persistence of the species.

Keywords: Canid conservation; Dietary composition; COI; feeding ecology; ITS

1. INTRODUCTION

Diet is a fundamental driver of animal abundance and population dynamics, shaping key attributes such as body condition, home range size, spatial use, and activity patterns in canids (Jiménez 2012; Paula 2015; Quéméré et al. 2013). Ensuring adequate nutrition, even amid natural environmental fluctuations or human-induced landscape changes, is essential for reproductive success and long-term population viability (Emmert 2012). Studying foraging strategies across time and diverse landscapes offers valuable insights into how these ecological traits respond to habitat fragmentation and disturbance, thereby enabling assessments of species' ecological flexibility (Paula 2015; Quéméré et al. 2013).

Canids, a diverse family of carnivores, exhibit varying degrees of ecological flexibility in response to environmental changes, particularly regarding diet and habitat (de Paula 2016; Soares 2018). This flexibility is a key factor in their ability to survive and adapt, especially in human-altered landscapes (Franceschini 2020). Generalist species tend to be more resilient to environmental changes, whereas specialists, although optimized for specific resources, may face greater extinction risks over macroevolutionary timescales (Balisi and Van Valkenburgh 2020; Leão et al. 2023). Beyond individual survival, this plasticity can impact broader ecological processes, such as seed dispersal, small prey control, and the reorganization of trophic interactions in fragmented landscapes (Quéméré et al. 2013; Rodrigues et al. 2007; Santos et al. 2003). Flexibility in canids should therefore be understood as a hierarchical set of responses that operate across different temporal scales and biological levels. While this flexibility enables persistence in changing environments, it is ultimately constrained by physiological, phylogenetic, and demographic factors that may limit long-term viability (Paula and DeMatteo 2015; Quéméré et al. 2013).

Traditional approaches for determining diet rely on taxonomic methodologies applied to feces or stomach content analysis, which have served as the main methodological framework for decades (Dietz 1984; Pompanon et al. 2012; Sousa et al. 2019). These methods seek to identify and quantify consumed items from recognizable morphological remains (such as seeds, teeth, bones,

and hair), allowing for inferences about dietary composition, seasonal patterns, and the relative importance of different food categories (Amboni 2007; Dietz 1984; Motta-Junior 1997; Rodrigues et al. 2007). Despite their wide application, these methodologies have well-recognized limitations, especially regarding the taxonomic identification of highly digested or small items, such as soft tissues, arthropods, and plant parts that are not resistant to digestion (Castro and Emmons 2011; Pompanon et al. 2012; Sato 2024; Tercel et al. 2021). These limitations are especially significant for generalist and opportunistic species, whose diets often include a wide variety of items consumed in small amounts or sporadically, but which may still have important nutritional and ecological roles (De Barba et al. 2014; Pinho et al. 2023).

Molecular approaches based on DNA metabarcoding have emerged as promising tools to overcome the limitations of traditional methods, providing a non-invasive and accurate technique for determining animal diets (Ando et al. 2020; Pinho et al. 2023; Pompanon et al. 2012; Tercel et al. 2021). By enabling the identification of multiple taxa from DNA fragments in feces, markers such as the Internal Transcribed Spacer (ITS), targeting the plant fraction (Chen et al. 2010), and Cytochrome c oxidase subunit I (COI), focused on the animal fraction (Hebert et al. 2003), allow for the detection of food items that frequently remain invisible to morphological analyses (Ando et al. 2020; Pinho et al. 2023; Sato 2024; Tercel et al. 2021). The integration of traditional and molecular methods represents a more efficient strategy, providing a more comprehensive and refined view of the diet in generalist species while strengthening ecological inferences and practical applications (Ando et al. 2020; Pinho et al. 2023; Pompanon et al. 2012; Quéméré et al. 2013; Tercel et al. 2021).

The maned wolf, *Chrysocyon brachyurus* (Illger, 1815), is the largest canid in South America and an emblematic species of the Cerrado biome, where it performs relevant ecological functions associated with seed dispersal, prey control, and maintenance of functional landscape connectivity (Dietz 1984; Paula 2015). Although widely distributed in South America, the Cerrado hosts most of the species' population (Paula and DeMatteo 2015). Extinction risk assessments indicate that the maned wolf lives under persistent threats, such as habitat loss

and degradation from agricultural conversion (Paula 2015; de Paula 2016), vehicle collisions (Cheida 2005; Rodrigues et al. 2007), direct persecution (Dietz 1984; Paula et al. 2007), and exposure to domestic animal pathogens (Massara 2009; Paula et al. 2007), leading to its global classification as Near Threatened by the IUCN (IUCN 2016) and Vulnerable nationally by ICMBio (ICMBio 2025).

Traditionally, the maned wolf is characterized as a generalist and opportunistic omnivore (Amboni 2007; Michalski et al. 2014; Motta-Junior 1997; Santos et al. 2003; Soares 2020). Its dietary profile exhibits marked seasonal variations, with a tendency toward higher consumption of small vertebrates during the dry season and various fruits during the rainy season (de Arruda Bueno and Motta-Junior 2009; Dietz 1984; Motta-Junior 1997; Soares 2020). Despite this, the literature points to selectivity for *lobeira* (*Solanum lycocarpum*), a fundamental item for maintaining constant plant material consumption throughout the year (de Almeida Jácomo et al. 2009; Amboni 2007; Emmert 2012; Garcia 2016). The prevalence of this fruit is associated with its ability to colonize anthropized environments and roadsides (Lombardi and Motta-Junior 1993). Such dietary patterns are directly impacted by changes in spatial ecology and landscape use (Franceschini 2020).

In this context, this study represents the first integrated application of DNA metabarcoding using ITS and COI markers to evaluate the diet of wild maned wolf populations under different conditions. We sought to overcome a persistent methodological gap and provide an unprecedented view of the species' foraging ecology. The study focuses on two main objectives: assessing the methodological efficiency of metabarcoding and examining trophic variation in response to spatial and temporal gradients. Specifically, we hypothesized that (a) DNA metabarcoding would detect a greater diversity and richness of food items with higher resolution; (b) the maned wolf's diet is organized around a stable taxonomic core, such as *Solanum lycocarpum*; and (c) dietary diversity and composition do not differ significantly between seasons and locations, reflecting high plasticity.

By testing these hypotheses, this study provides a comprehensive assessment of the maned wolf's dietary dynamics. The findings enhance our

understanding of its trophic ecology and provide empirical insights to inform adaptive management and conservation strategies both *in-situ* and *ex-situ*.

2. MATERIALS AND METHODS

2.1. Target species and study areas

We selected the maned wolf (*Chrysocyon brachyurus*) as our focal species, a generalist canid endemic to the Cerrado biome, characterized by high spatial and seasonal dietary plasticity (Soares 2020). Given its sensitive response to landscape changes, the maned wolf serves as an indicator and flagship species for conservation actions (de Paula 2016).

We conducted this study in the Cerrado biome across two regions in western Bahia and one in the Federal District (DF), Brazil. The study areas are classified as tropical savanna climate (Aw) according to Köppen, following the updated Brazilian climate classification of Alvares et al. (2013) (Alvares et al. 2013). Climatic characterization (precipitation and temperature) was based on the most recent 1991–2020 climatological normals from the Brazilian National Institute of Meteorology (INMET 2022). The study areas are characterized by a tropical savanna climate (Aw) with distinct wet (Nov–Apr) and dry (May–Oct) seasons, while encompassing contrasting landscape contexts, land-use intensities, and levels of environmental protection. (Table 1).

To characterize land-use intensities and levels of environmental protection, we used land use and land cover rasters from MapBiomas Collection 10 (30-meter resolution) (MapBiomas 2025). We established 3-km radius zones of influence (buffers) around each sampling point, representing the species' average home range (Paula and DeMatteo 2015). Landscape composition was quantified using relative proportions within these buffers across six functional categories (Forest, Herbaceous/Shrub Vegetation, Agriculture, Non-Vegetated Area, Water, and Not Observed) following a methodology adapted from Souza et al. (2020) (Souza et al. 2020).

The BA1 area, located in Jaborandi (BA), is a large rural property (~33,000 ha) within the Sertão Veredas–Peruaçu mosaic, one of the largest continuous regions of protected areas in the Cerrado. The landscape is dominated by native

vegetation, particularly herbaceous and shrubby formations (~57%). Forested areas occupy ~26%, while agriculture accounts for ~13%, making this the region with the lowest anthropogenic conversion pressure among the study sites. This area is monitored by long-term research projects using camera traps and GPS/VHF radio-collars.

Table 1. Identification and location of study areas sampled in the Cerrado biome, Brazil.

Area ID	Local	City (State)	Latitude (S)	Longitude (W)	Area (ha)	Climate (Aw) rainy Nov–Apr; dry May–Oct;
BA1	Farm	Jaborandi (BA)	14,64377	45,80214	33.000	800–1,400 mm/year; Mean annual temperature 24–26 °C
BA2	Farm	Barreiras (BA)	11,83731	46,28913	1.000	900–1,500 mm/year; Mean annual temperature 24–26 °C
			11,82271	46,02532	1.700	
		Luís Eduardo Magalhães (BA)	12,08418	46,06691	800	
			12,27391	46,08709	900	
DF	ESECAE ¹	Brasília (DF)	15,59132	47,66665	10.550	1,100–1,600 mm/year; Mean annual temperature 21–23 °C
	APAC ²		15,59823	48,16658	46.510	
	PNB ³		15,73471	47,92149	42.355	

Note: ¹Estação Ecológica de Águas Emendadas; ²Área de Proteção Ambiental de Cafuringa;

³Parque Nacional de Brasília.

The BA2 area comprises four private properties in Barreiras and Luís Eduardo Magalhães (BA), totaling ~4,400 ha. It is set within a predominantly anthropized matrix, where agriculture covers over 57% of the landscape. The area retains forest fragments (~35%) and herbaceous-shrubby vegetation (~8%), characterizing an intensive agricultural matrix with remnant native patches. The DF area is in the Federal District and includes conservation units such as the Brasília National Park and the ESEC Águas Emendadas. This area exhibits the highest forest cover (>70%), with agriculture occupying ~23% and non-vegetated areas ~5%, reflecting proximity to the urban infrastructure of the Federal Capital.

Monitoring is conducted through the Cerrado carnivore health assessment project.

2.2. Sample Collection and Processing

We obtained fresh fecal samples (October 2020 to July 2023) through three strategies: capture campaigns, continuous monitoring of free-ranging populations, and opportunistic collection from rescued individuals. During monitoring, the scats were identified by shape, diameter, odor, and the presence of wolf fur, typically found near tracks (de Arruda Bueno and Motta-Junior 2009). Approximately 40g of feces were stored in sterile containers at -20°C , then transferred to -80°C at the University of Brasília (UnB). A 10 g aliquot from each sample was transferred to identified Falcon tubes and stored in duplicate.

2.3. Molecular analysis

A total of 137 fecal samples were collected (BA1 = 102; BA2 = 17; DF = 18). We extracted genomic DNA using the QIAamp DNA Stool Mini Kit (Qiagen). All samples were screened by PCR, and DNA quality was assessed prior to sequencing. Based on amplification success and DNA integrity, 55 samples with the highest DNA quality (BA1 = 25; BA2 = 14; DF = 16) were selected and sent to the Vale Institute of Technology (ITV – Belém, Brazil) for sequencing. At ITV, Genomic DNA was extracted using the DNeasy® PowerSoil® kit (QIAGEN). DNA was quantified by fluorometry using a Qubit® 3.0 (Thermo Fisher Scientific) and stored at -80°C .

2.3.1. Target region amplification and library preparation

To sequence the dietary items, 49 samples were used for library construction by amplifying the ITS (Plantae) and COI (Metazoa) regions. We used ITS2_ILL/ITS3_ILL (Chen et al. 2010; Moorhouse-Gann et al. 2018) primers for the plant fraction and mlCOIintF_ILL/jgHCO2198_ILL (Wang et al. 2023) primers for the animal fraction (Table 2).

Table 2. List of primers tested for the evaluation of the DNA metabarcoding technique.

Primer	Target	Amplicon (bp)	Sequence (5'-3')	Reference
mICOLint-ILL-F	Metazoa	365	GGWACWGGWTGAACWGT	(Wang et al. 2023)
WTAYCCYCC				
jgHCO2198-ILL-R			TAIACYTCIGGRTGICCRAAR AAYCA	
ITS2F_ILL	Plantae	226	TCGTCGGCAGCGTCAGATG	(Chen et al. 2010; Moorhouse-Gan n et al. 2018)
			TGTATAAGAGACAGATGCGA	
			TACTTGGTGTGAAT	
			GTCTCGTGGGCTCGGAGATGT	
ITS3R_ILL			GTATAAGAGACAGGACGCTTCT CCAGACTACAAT	

Reaction conditions and thermocycling parameters followed the original development protocols for each primer set and are detailed in Tables 3 and 4.

Table 3. PCR reaction components (Master Mix).

Reagent	Volume (μL)
5x GoTaq Buffer	5.0
dNTP mix (2 mM)	1.25
MgCl ₂ (25 mM)	2.0
Primer F (10 μM)	0.5
Primer R (10 μM)	0.5
GoTaq DNA Polymerase	0.25
DNA (2 ng/μL)	5.0
Ultrapure water	10.5

Table 4. PCR thermocycling conditions for each primer pair.

mICOLint-ILL-F e			ITS2F_ILL e		
jgHCO2198-ILL-R			ITS3r_ILL		
Temperature	Duration	Cicles	Temperature	Duration	Cicles
95 °C	5 min	1	94°C	5 min	1
95 °C	30 s		94°C	30 s	
51 °C	30 s	45	56°C	30 s	
72 °C	45 s		72°C	45 s	40
72 °C	7 min	1	72°C	10 min	1
4 °C	∞	1	4 °C	∞	1

After indexing, the amplified products were again purified using AMPure XP magnetic beads. Sequencing was carried out on the NextSeq 2000 platform (Illumina, San Diego, CA, USA) using the NextSeq 2000 P1 kit (600 cycles).

2.3.2. Bioinformatics pipeline

Raw data were processed using DADA2 (v1.28.0) in R (v4.3.4) (Callahan et al. 2016). Reads were filtered based on *Phred* > 30, expected errors (Bokulich et al. 2013), minimal 50pb and assigned to Amplicon Sequence Variants (ASVs). Taxonomy was assigned using the BOLD database (v.4) (Ratnasingham et al. 2024) with > 97% similarity.

2.4. Statistical analysis

We performed statistical analyses in R (v4.3.4) (R Core Team 2024) using packages *phyloseq* (McMurdie and Holmes 2013), *microbiome* (v.1.28.0) (Lahti and Holmes 2019), *vegan* (v1.50.0) (Oksanen et al. 2025) and *ggplot2* (v3.5.1) (Wickham et al. 2024). Alpha diversity (Richness, Shannon, and Pielou evenness) was calculated with 5,000 permutations and 95% confidence intervals (Ho et al. 2018). For beta diversity, we filtered taxa for ITS (seasonal analysis: prevalence ≥ 4.44%, min 5 counts; locality analysis: prevalence ≥ 4,08%, min 5

counts) and COI (seasonal analysis: prevalence $\geq 5.26\%$, min 10 counts; locality analysis: prevalence $\geq 4.76\%$, min 10 counts) to remove spurious data.

We evaluated dietary distribution using Principal Coordinates Analysis (PCoA) and Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis distances, followed by PERMANOVA and permutational ANOVA (999 permutations, $p < 0.05$). The *microeco* package (v1.14.0) (Liu et al. 2021) was used for Venn diagrams, and indicator species analysis (9,999 permutations) was performed with the *indicspecies* package (v1.7.14) (Cáceres and Legendre 2009). Given the significant loss of taxonomic resolution at lower levels and the heterogeneity in taxonomic assignment among ASVs, all diversity analyses were conducted at the family level, which was considered the lowest level with sufficient resolution for detecting ecological patterns.

3. RESULTS

3.1. Composition and variation of taxa associated with fecal samples

Next-generation sequencing (NGS) applied to maned wolf fecal samples, using the ITS and COI markers, initially yielded a total of 15,311 ASVs for the ITS marker and 10,728 ASVs for the COI marker. After removing sequences not associated with wild samples, non-target taxa, and singletons, the final set consisted of 13,725 ASVs for ITS and 8,465 ASVs for COI.

For the ITS marker, 13,530 ASVs were classified at the kingdom level, 13,344 at the phylum level, and 13,131 at the class level. However, only 2,848 ASVs (21.69%) could be assigned at the order level, with a significant reduction at lower taxonomic levels: 2,483 ASVs at the family level, 1,565 at the genus level, and 776 ASVs classified at the species level. Regarding the COI marker, 5,690 ASVs were classified at the kingdom level, 1,538 at the phylum level, and 905 at the class level. Of these, 439 ASVs were assigned to the order level, 304 to the family level, 268 to the genus level, and 233 to the species level. In both markers, we observed a sharp decrease in ASVs as classification progressed to more refined taxonomic levels.

After data processing and filtering to remove singletons and non-target taxa, the ITS marker allowed for the identification of plant taxa distributed across

3 phyla, 6 classes, 30 orders, 57 families, 130 genera, and 117 species. The COI marker enabled the identification of animal taxa distributed across 4 phyla, 9 classes, 25 orders, 42 families, 53 genera, and 54 species.

3.1.1. General patterns of taxonomic composition

ITS metabarcoding analysis revealed that the plant items found in the samples are composed of a taxonomically diverse set of plants, with marked variation in the relative abundance of taxa among individual samples (Fig 1, Table S1). A few families concentrated a large part of the relative abundance on several samples, while most occurred in low proportions. We observed the recurrence of a set of widely distributed families, including Poaceae and Solanaceae, which contributed consistently across the samples

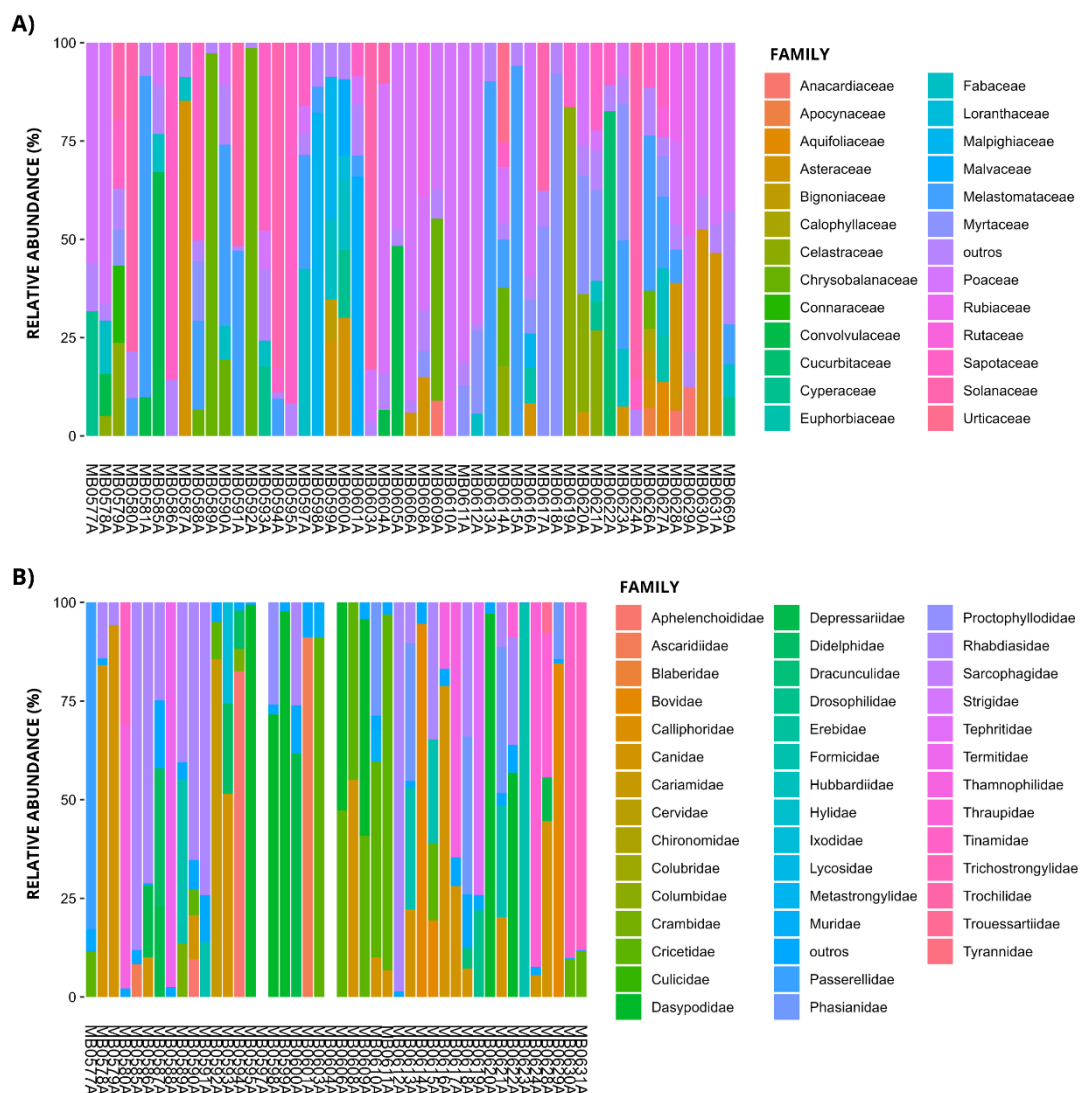


Figure 1. Overall taxonomic composition of the maned wolf (*Chrysocyon brachyurus*) diet via DNA metabarcoding. (A) Relative abundance of plant families identified by the ITS marker; (B) Relative abundance of animal families identified by the COI marker. Each bar represents an individual fecal sample (n=44). Families with relative abundance < 2% are grouped into the "others" category.

COI metabarcoding analysis showed that the animal-derived items found in maned wolf feces also consist of a taxonomically diverse set, with differences in relative abundance observed among samples (Fig 1, Table S2). A pattern marked by the occasional dominance of a few families in certain samples was evident, contrasting with the sporadic and low-abundance occurrence of most other families found.

A significant portion of the sequences assigned to COI corresponds to insects, with the recurrence of families such as Calliphoridae, Drosophilidae, Formicidae, Culicidae, Scarabaeidae, Tephritidae, and Blattidae, which showed the highest accumulated counts and prevalence among samples. These groups were found consistently throughout the sample set, despite variation in relative abundance among individual samples.

In addition to insects, the COI marker detected the presence of vertebrates, including families of small mammals, such as Dasypodidae, Muridae, Cricetidae, and Didelphidae, as well as birds belonging to various families, such as Tinamidae, Columbidae, Thraupidae, and Tyrannidae. Despite their low relative abundance, these groups occurred recurrently in the samples.

3.1.2. Seasonal variation of the plant component

In the dry season, the five identified plant items with the highest relative abundance were the families Poaceae (31.42%), Myrtaceae (10.43%), Convolvulaceae (10.25%), Asteraceae (9.82%), and Sapotaceae (9.08%). In the rainy season, the highest contributions to relative abundance came from the families Poaceae (22.65%), Chrysobalanaceae (14.34%), Sapotaceae (14.02%), Melastomataceae (8.19%), and Cucurbitaceae (6.13%). Solanaceae showed moderate relative abundance in both seasons (4.43% in the dry season; 5.30% in the rainy season), forming part of the set of plant families widely distributed throughout the analyzed period (Fig 2A).

At the species level, the families Poaceae and Solanaceae exhibited a seasonal occurrence pattern with species shared between the dry and rainy seasons, as well as species exclusive to each period. For Poaceae, eight species were recorded in both seasons, such as *Anthaenantia lanata* and *Axonopus longispicus*, while other taxa were restricted to a single period, such as *Arthrostylidium scandens* (exclusive to the dry season) and *Cinnagrostis viridiflavescens* (rainy season only). Similarly, the family Solanaceae was represented by species of the genus *Solanum* with distinct seasonal patterns, including shared taxa like *Solanum crinitum* and exclusive taxa like *Solanum campaniforme*, recorded only during the rainy period (Table 5).

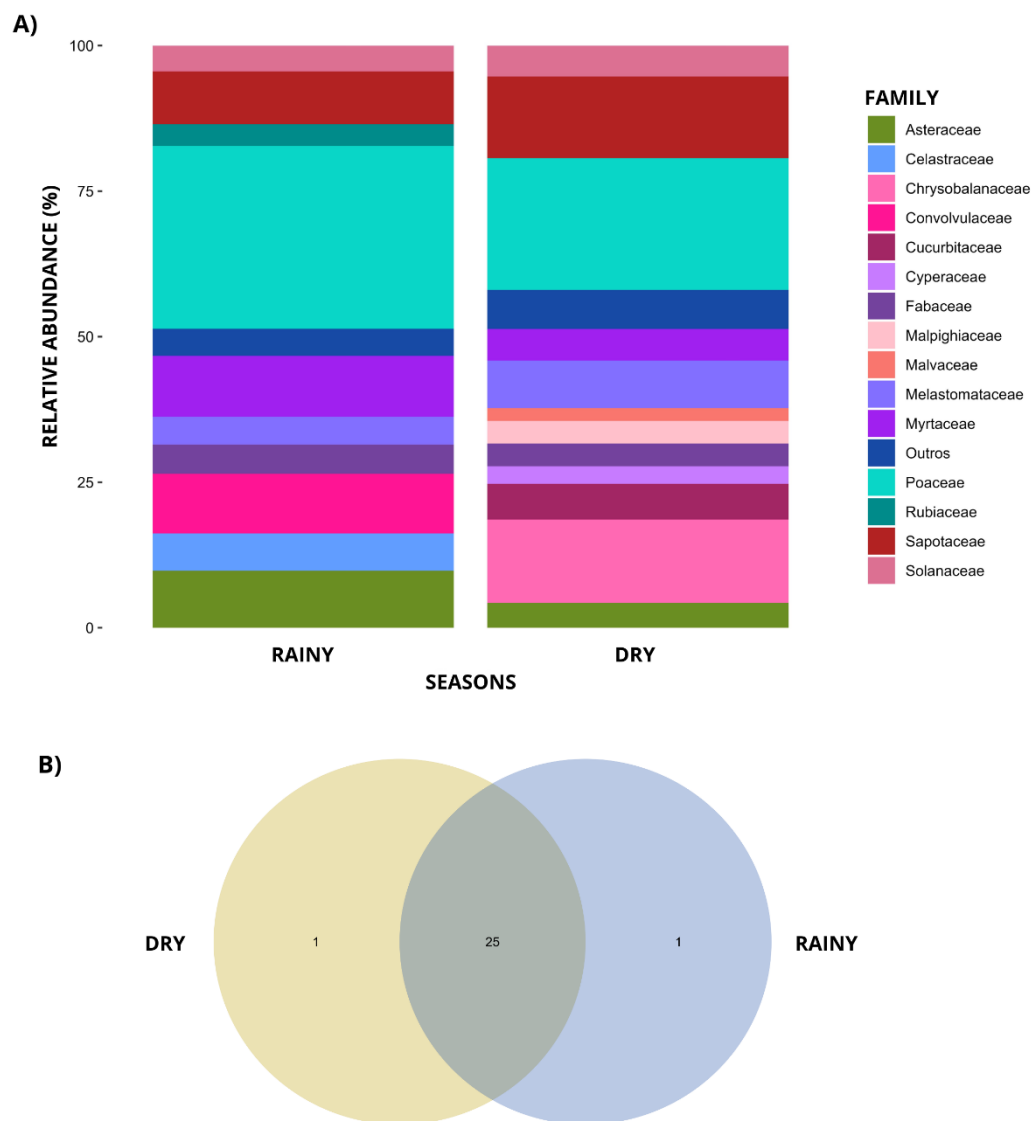


Figure 2. Seasonal dynamics of the plant dietary component (ITS marker). (A) Relative abundance of the main plant families in the dry and rainy seasons. Each bar represents the relative abundance of plant family in the dry and rainy seasons. Relative abundance < 2% are grouped into the "others" category. (B) Venn diagram showing the number of shared ($n = 25$) and exclusive plant families between seasonal periods.

Table 5. Occurrence of genera and species from the Poaceae and Solanaceae families identified in the fecal samples of *Chrysocyon brachyurus* in the dry and rainy seasons. An 'X' indicates the presence of the respective taxon in that season.

Family	Genus	Species	Dry	Rainy
Poaceae	<i>Anthraenantia</i>	<i>A. lanata</i>	X	X
	<i>Arthrostyloidium</i>	<i>A. scandens</i>	X	
	<i>Axonopus</i>	<i>A. longispicus</i>	X	X
	<i>Cinnagrostris</i>	<i>C. viridiflavescens</i>		X
	<i>Echinolaena</i>	<i>E. inflexa</i>	X	X
	<i>Eleusine</i>	<i>E. tristachya</i>	X	X
	<i>Eragrostis</i>	<i>E. maypurensis</i>		X
	<i>Gymnopogon</i>	<i>G. swallenii</i>	X	X
	<i>Hildaea</i>	<i>H. ruprechtii</i>		X
	<i>Ichnanthus</i>	<i>Ichnanthus</i> sp.	X	
	<i>Leptochloa</i>	<i>L. exilis</i>		X
	<i>Oedochloa</i>	<i>O. camporum</i>	X	X
	<i>Panicum</i>	<i>Panicum</i> sp.		X
		<i>P. millegrana</i>	X	
	<i>Paratheria</i>	<i>P. prostrata</i>	X	X
	<i>Paspalum</i>	<i>Paspalum</i> sp.		X
		<i>P. axillare</i>	X	
	<i>Schizachyrium</i>	<i>S. condensatum</i>	X	X
Solanaceae	<i>Solanum</i>	<i>S. campaniforme</i>		X
		<i>S. crinitum</i>	X	X

Alpha diversity was evaluated using the ITS marker, considering metrics of richness, Shannon diversity index, and Pielou's evenness index (Fig 3A). Although tests indicated an absence of statistically significant differences

between the dry and rainy seasons, observed values varied among individual samples, with considerable overlap between the rainy ($n = 21$) and dry ($n = 24$) seasons. Overall, mean family richness was slightly higher in the rainy season (by 0.81), while the Shannon and Pielou indices showed only minor differences between seasons (differences of 0.04 and 0.02, respectively). The mean differences between periods were small for all evaluated metrics, suggesting that the alpha diversity of the plant component remains relatively stable across the seasonal cycle.

For beta diversity assessment, data filtration resulted in the removal of 94.87% of ASVs and 65.95% of reads. Plant family composition did not differ between the dry and rainy seasons. Principal Coordinates Analysis (PCoA) and Constrained Analysis of Principal Coordinates (CAP) did not show evident separation between seasons, as evidenced by PERMANOVA ($p = 0.074$ and $p = 0.068$, respectively) (Fig 3B).

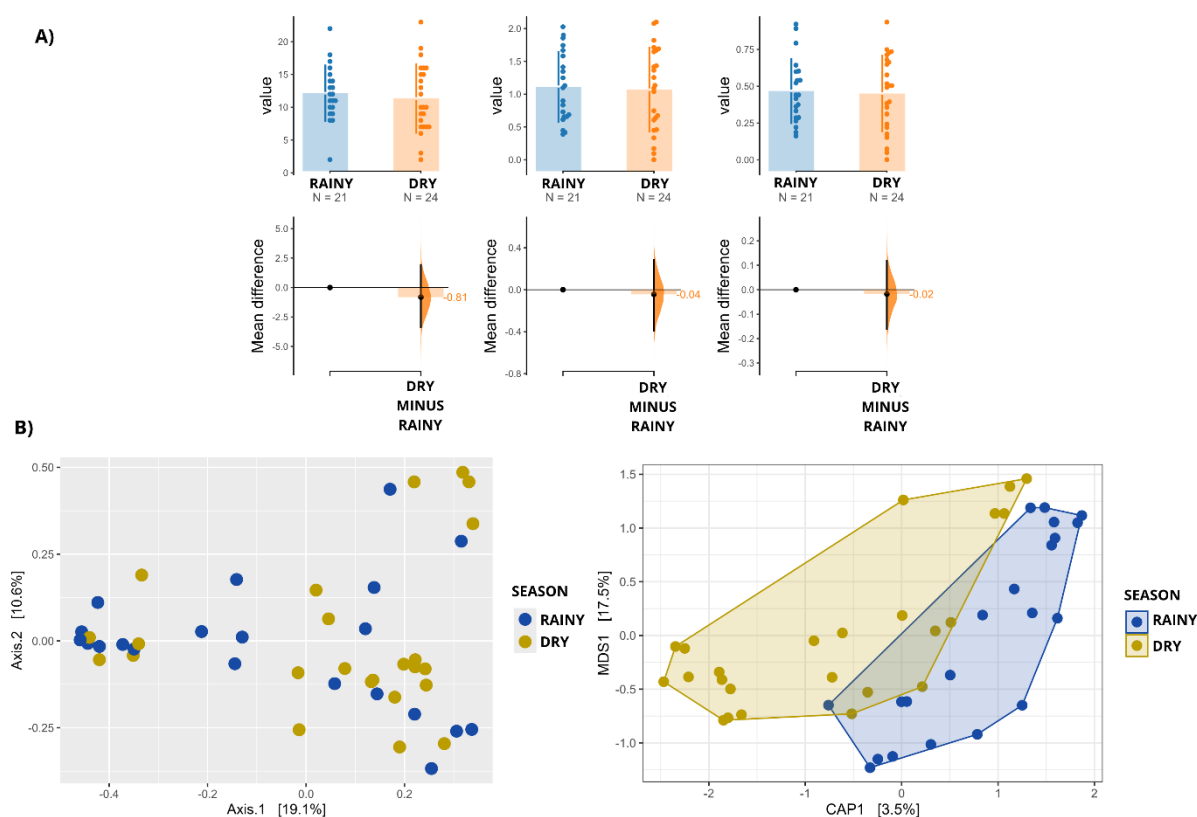


Figure 3. Diversity and structure of the plant diet across seasons in maned wolf (ITS marker). (A) Alpha diversity from dry ($n = 24$) and rainy ($n = 21$) seasons (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated with

5,000 permutations and 95% confidence intervals. (B) Beta diversity analyses based on Bray–Curtis dissimilarity. PCoA explained 29.7% of total variation, and CAP explained 21%.

Complementing the ordination analyses, the Venn diagram demonstrates high similarity in family identity between seasons. With 25 families shared, only one family (Acanthaceae) was observed exclusively in the dry season, and one (Meliaceae) was exclusive to the rainy season samples (Fig 2B).

3.1.3. Seasonal variation of the animal component

In the dry season, families with the highest relative abundance were Dasypodidae (61.85%), Formicidae (16.74%), Bovidae (4.53%), Rhabdiasidae (3.74%), and Passerellidae (3.25%). In the rainy season, the relative contribution shifted, with higher participation of Termitidae (32.27%), followed by Dasypodidae (16.24%), Rhabdiasidae (13.03%), Tinamidae (12.33%), and Bovidae (11.58%) (Fig 4A).

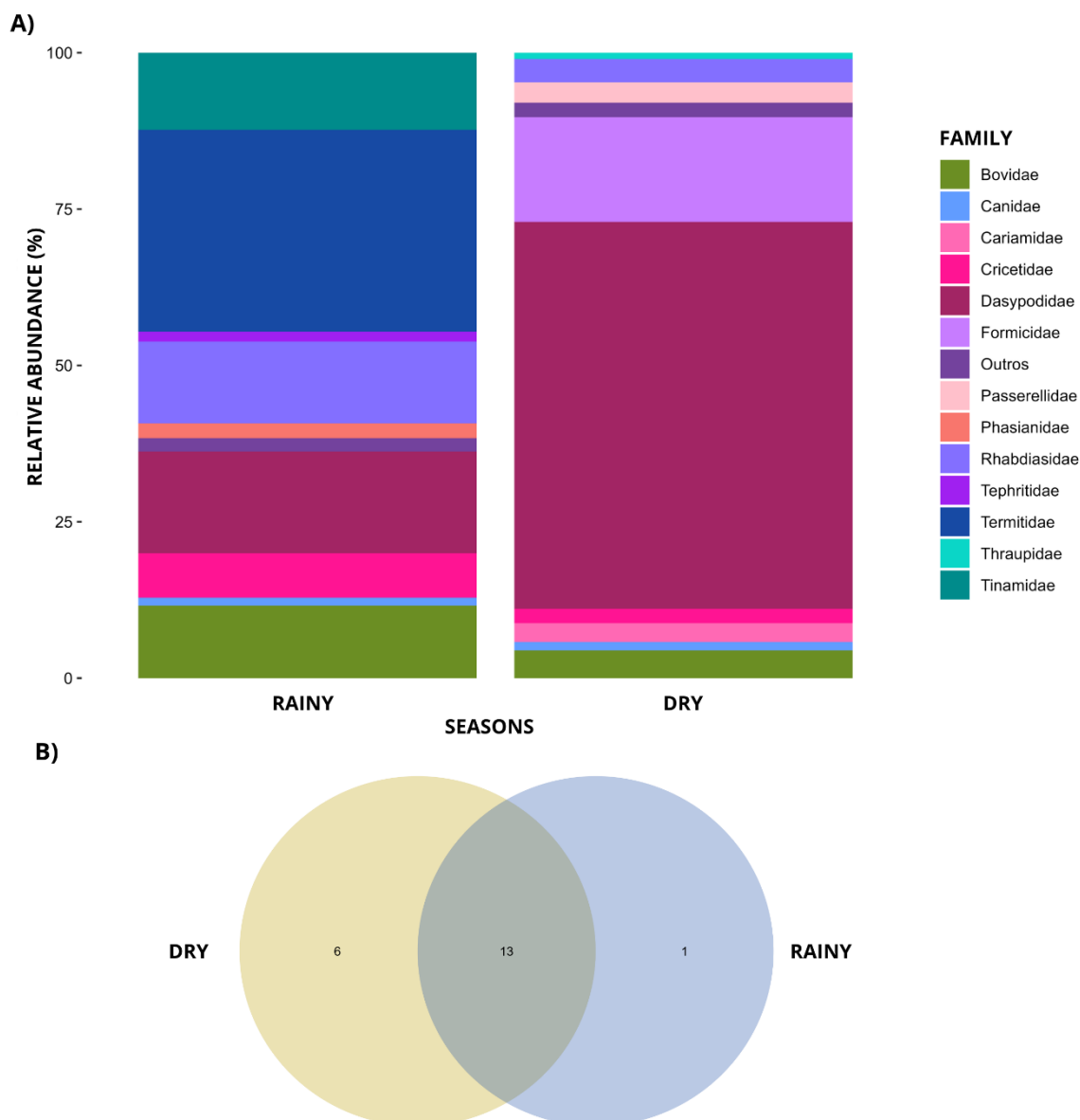


Figure 4. Seasonal dynamics of the maned wolf dietary component (COI marker). (A) Relative abundance of the main animal families in the dry and rainy seasons. Each bar represents the relative abundance of animal family in the dry and rainy seasons. Relative abundance < 1% are grouped into the "others" category. (B) Venn diagram showing the number of shared ($n = 13$) and exclusive animal families between seasonal periods.

Taxonomic identification at the genus and species levels revealed distinct seasonal patterns. Among Cingulata (Dasypodidae), *Dasypus septemcinctus* occurred in both seasons, while *Euphractus sexcinctus* was exclusive to the dry season. Among Blattodea, the family Termitidae showed higher richness in the rainy season, with five specific records (*Nasutitermes corniger*, *Neocapritermes*

opacus, *N. talpa*, *Parvitermes bacchanalis*, and *Ruptitermes* sp.), while only *Nasutitermes* sp. was shared between both seasons (Table 3).

Table 6. Occurrence of genera and species of the Termitidae, Dasypodidae, Phasianidae, Cricetidae, and Muridae families in the dry and rainy seasons.

Ordem	Família	Gênero	Espécie	Estação	
				Seca	Chuva
Blattodea	Termitidae	Nasutitermes	Nasutitermes sp.	X	X
			N. corniger		X
		Neocapritermes	N. opacus		X
			N. talpa		X
		Parvitermes	P. bacchanalis		X
		Ruptitermes	Ruptitermes sp.		X
Cingulata	Dasypodidae	Dasypus	D. septemcinctus	X	X
		Euphractus	E. sexcinctus	X	
Galliformes	Phasianidae	Coturnix	C. japonica		X
		Gallus	G. gallus	X	X
Rodentia	Cricetidae	Akodon	A. paranaensis	X	
		Calomys	C. expulsus	X	X
			C. tener	X	
		Cerradomys	C. scotti		X
		Oligoryzomys	O. fornesi	X	X
			O. mattogrossae	X	
	Muridae	Mus	M. musculus	X	X

In the order Galliformes, domestic species of the family Phasianidae were identified: *Gallus gallus* (domestic chicken) in both seasons and *Coturnix japonica* (Japanese quail) only in the rainy season. *Gallus gallus* was found in samples from only one individual in BA1, four in BA2, and two in DF. Rodentia showed the highest taxonomic diversity among vertebrates. Three species were exclusive to the dry season (*Akodon paranaensis*, *Calomys tener*, and *Oligoryzomys mattogrossae*), one was exclusive to the rainy season

(*Cerradomys scotti*), and three occurred in both periods (*Calomys expulsus*, *Oligoryzomys fornesi*, and *Mus musculus*).

Alpha diversity of the animal fraction (COI) showed no significant differences between seasons (Fig 5A). Mean family richness was slightly higher in the dry season (+0.25). Beta diversity assessment PCoA and CAP similarly showed no evident separation between seasons (PERMANOVA $p = 0.322$ and $p = 0.305$) (Fig 5B).

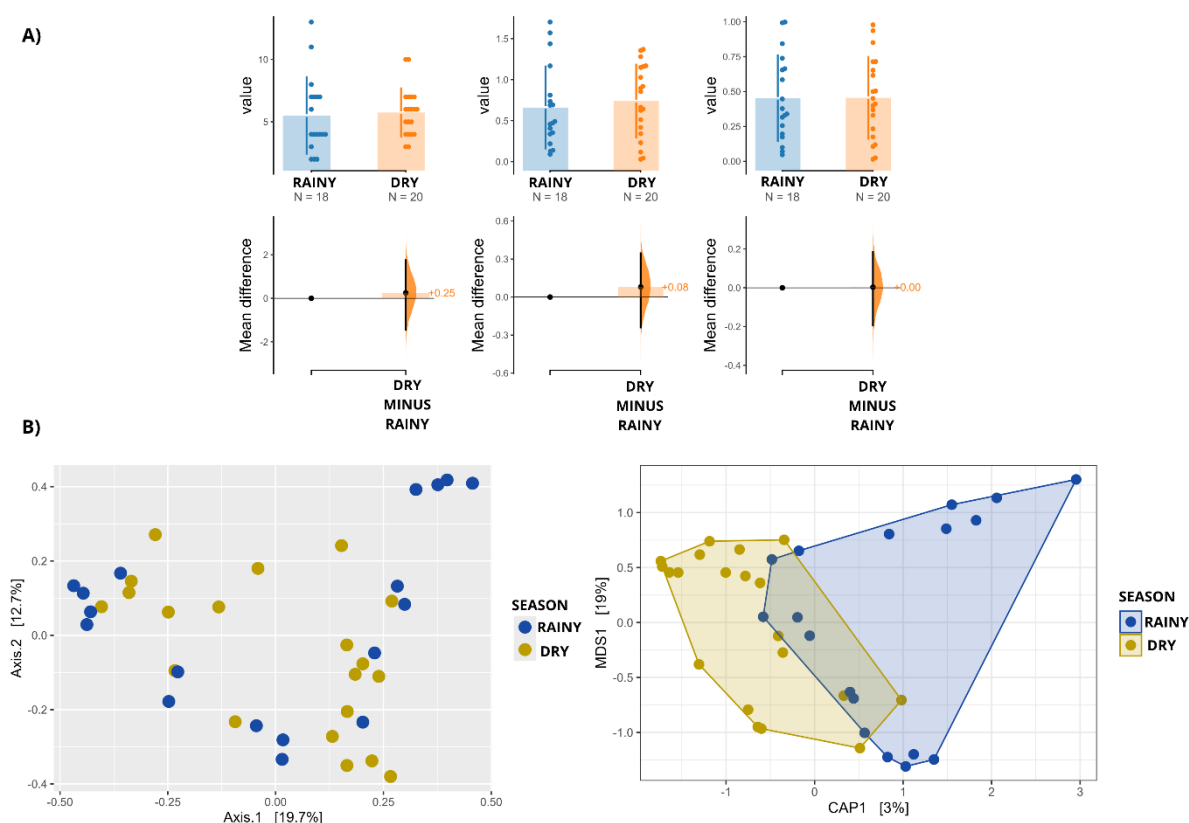


Figure 5. Diversity and structure of the maned wolf diet across seasons (COI marker). (A) Alpha diversity from dry ($n = 20$) and rainy ($n = 18$) seasons (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated with 5,000 permutations and 95% confidence intervals. (B) Beta diversity analyses based on Bray–Curtis dissimilarity. PCoA explained 32.4% of total variation, and CAP explained 22%.

Venn diagram analysis for the animal taxon's (20 families) showed that most families (13) are shared between seasons. Six families were exclusive to the dry season, while only one (*Dracunculidae*) was exclusive to the rainy season (Fig 4B).

3.1.4. Spatial variation of plant taxa associated with fecal samples

In BA1, 41 plant families were identified, with Sapotaceae (16.54%) and Poaceae (13.67%) being the most abundant. BA2 showed a strong predominance of Poaceae (57.07%). In DF, 41 families were identified, with Poaceae (23.35%) and Asteraceae (18.20%) dominant. The family Solanaceae was found at all locations with relative abundances below 6% (Fig 6A).

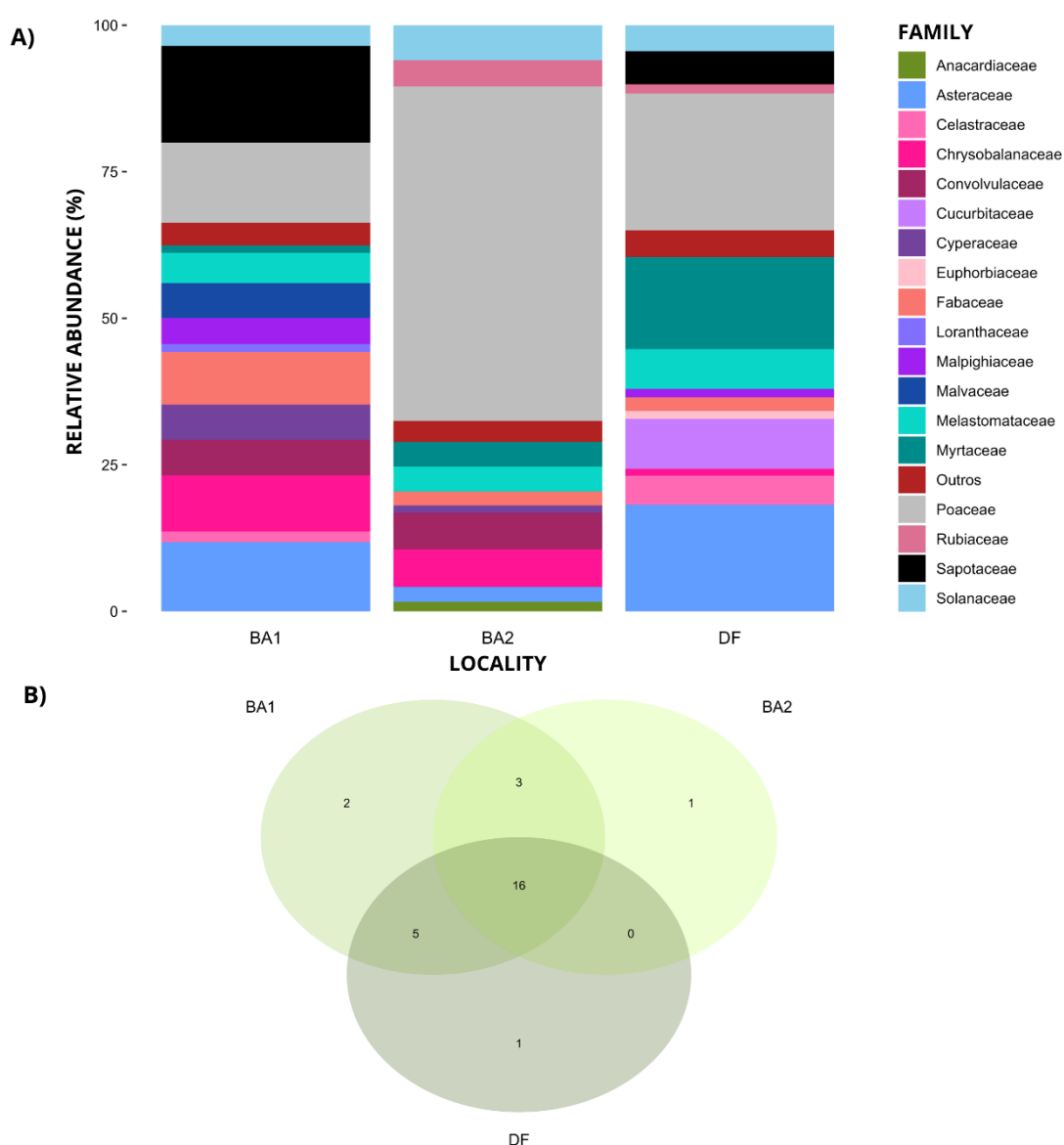


Figure 6. Spatial variation in the plant diet (ITS marker) across Fazenda Jaborandi (BA1), Luís Eduardo Magalhães e Barreiras (BA2) e Distrito Federal (DF). (A) Relative abundance of animal families per locality. Each bar represents the relative abundance of plant family in the localities. Relative abundance < 1% are grouped into the "others"

category. (B) Venn diagram showing the high overlap of plant families shared ($n = 16$) across BA1, BA2, and DF.

Species of the genus *Paratheria* and *Schizachyrium* occurred in at least two locations, while *Oedochloa camporum* was restricted to DF. Alpha diversity metrics showed no differences among locations (Fig 7A). However, beta diversity (PCoA and CAP) showed significant differences between areas ($p = 0.001$). BA2 differed from both BA1 ($p = 0.002$) and DF ($p = 0.018$) (Fig 7B). Indicator species analysis associated Urticaceae with BA2 ($p = 0.0042$) and Cucurbitaceae with the Federal District ($p = 0.0246$).

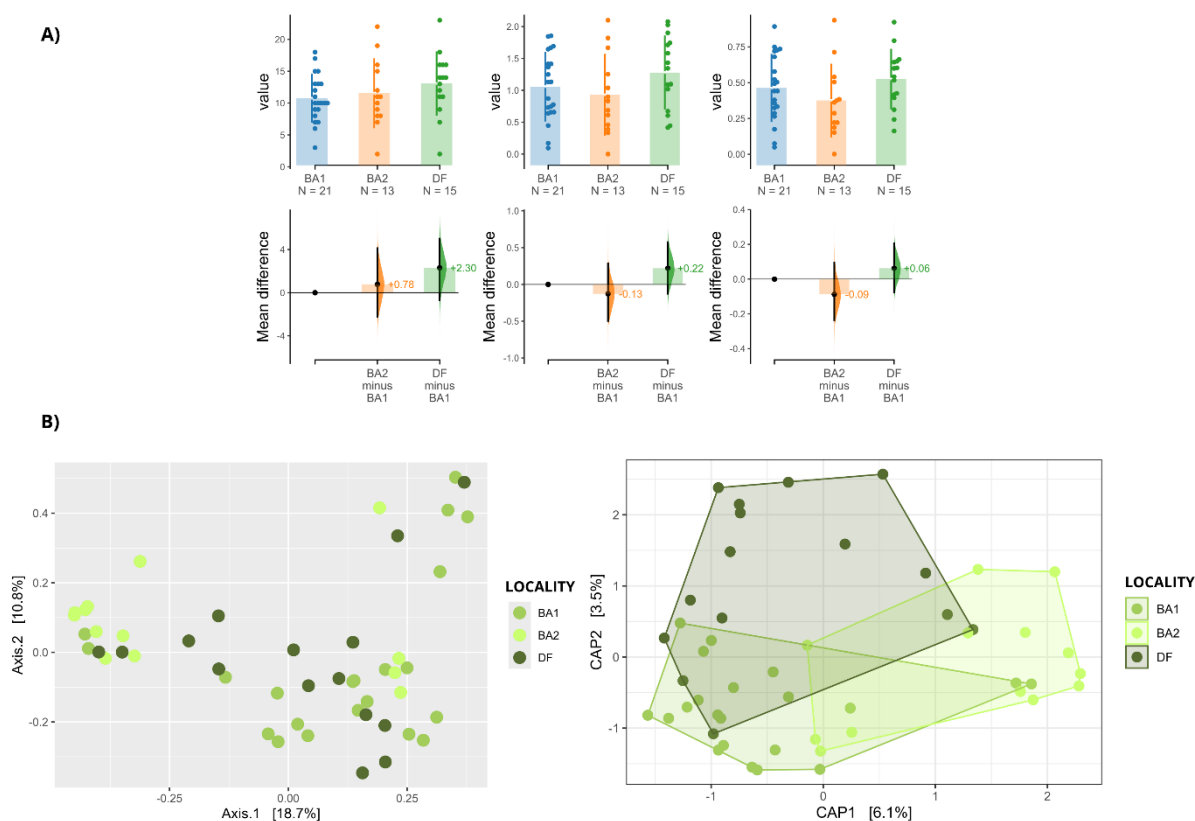


Figure 7. Spatial variation in the plant diet (ITS marker) Fazenda Jaborandi (BA1), Luís Eduardo Magalhães and Barreiras (BA2) e Distrito Federal (DF). (A) Alpha diversity from Fazenda Jaborandi – BA1 ($n = 21$), Luís Eduardo Magalhães and Barreiras – BA2 ($n = 13$), and the Distrito Federal – DF ($n = 15$) (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated using 5,000 permutations with 95% confidence intervals. (B) Beta diversity analyses based on Bray–Curtis dissimilarity. PCoA explained 29.5% of total variation, and CAP explained 9.6%.

3.1.5. Spatial variation of the animal component

In BA1, the animal fraction was dominated by Dasypodidae (52.30%) and Termitidae (22.07%). In BA2, Rhabdiasidae (45.40%) and Cricetidae (25.28%) were most abundant. In DF, Tinamidae (25.11%), Formicidae (23.99%), and Dasypodidae (22.16%) showed similar proportions (Fig 8A).

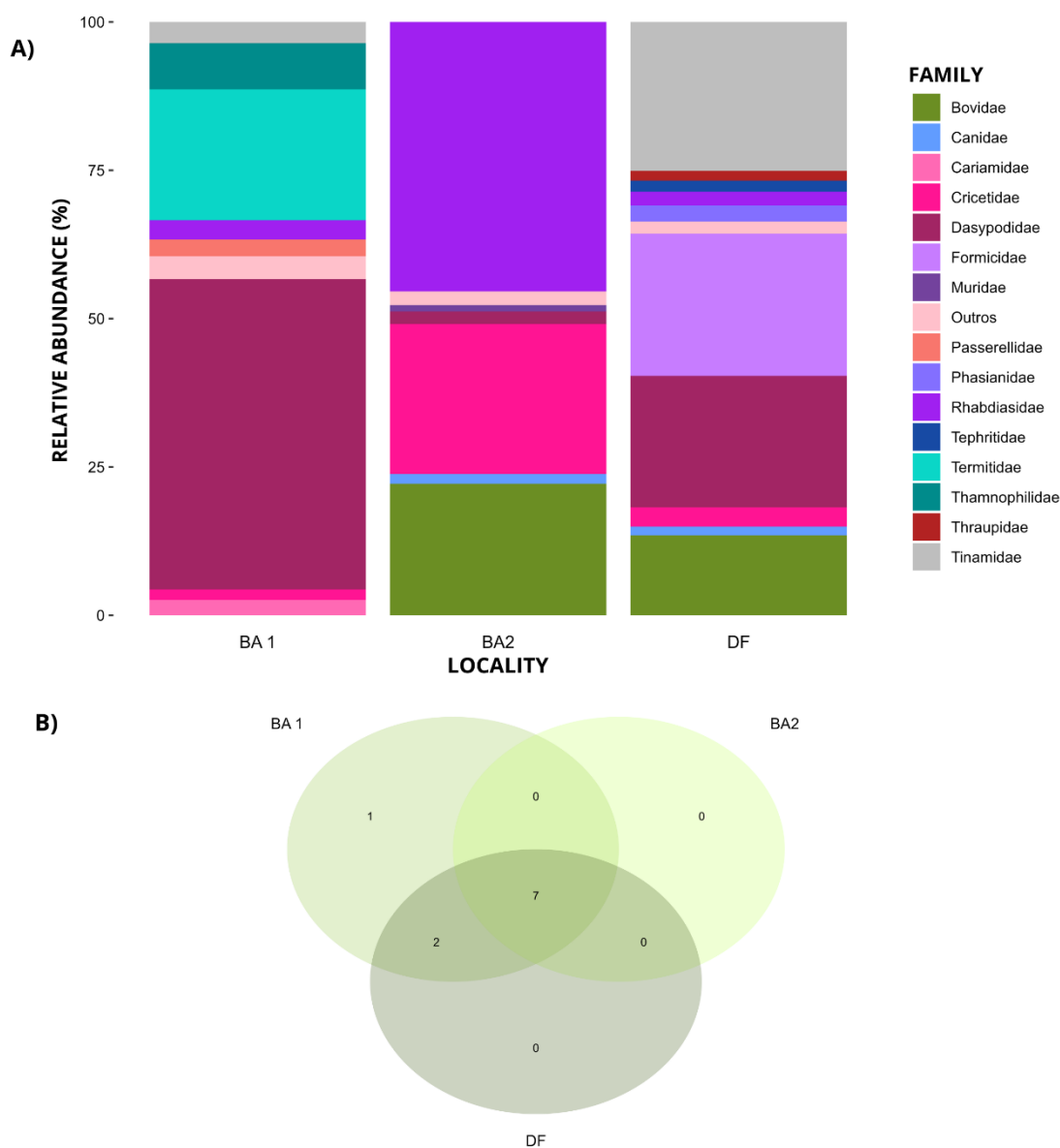


Figure 8. Spatial variation in the animal diet (COI marker) across Fazenda Jaborandi (BA1), Luís Eduardo Magalhães e Barreiras (BA2) e Distrito Federal (DF). (A) Relative abundance of animal families per locality. Each bar represents the relative abundance of animal family in the localities. Relative abundance < 1% are grouped into the "others"

category. (B) Venn diagram showing the high overlap of animal families shared ($n = 7$) across BA1, BA2, and DF.

Animal alpha diversity metrics showed no significant differences among locations (Fig 9A). Beta diversity analyses similarly showed no significant separation (PERMANOVA $p = 0.151$) (9B). Venn diagram analysis showed that seven families were shared across all locations, including Dasypodidae, Cricetidae, and Bovidae (Fig 8B).

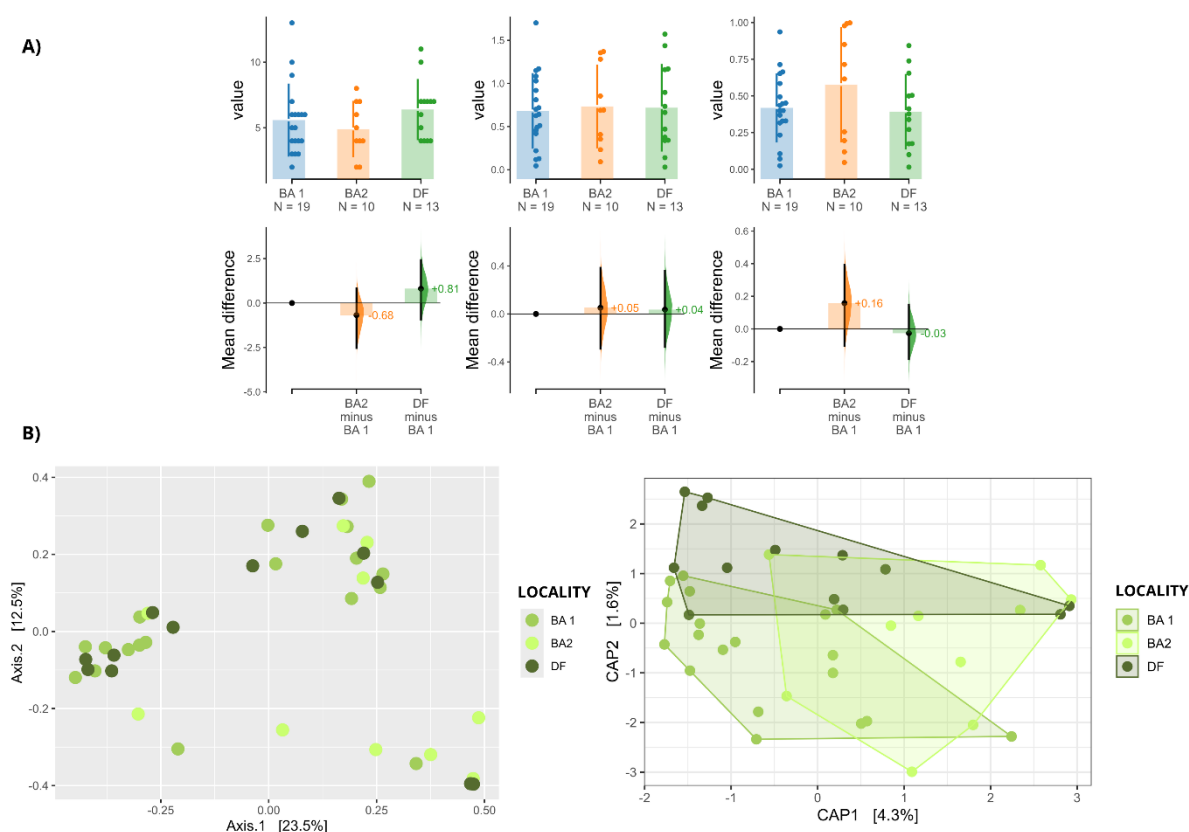


Figure 9. Diversity and structure of the animal diet (COI marker) across Fazenda Jaborandi (BA1), Luís Eduardo Magalhães and Barreiras (BA2) e Distrito Federal (DF). (A) Alpha diversity from Fazenda Jaborandi – BA1 ($n = 19$), Luís Eduardo Magalhães and Barreiras – BA2 ($n = 10$), and the Distrito Federal – DF ($n = 13$) (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated using 5,000 permutations with 95% confidence intervals. (B) Beta diversity analyses based on Bray–Curtis dissimilarity. PCoA explained 36% of total variation, and CAP explained 5.9%.

4. DISCUSSION

This pioneering study reveals that the maned wolf's diet is more diverse than previously described by decades of morphological analyses, identifying 171 potential species (117 plant and 54 animal taxa), a number that exceeds the accumulated richness reported in 35 years of traditional literature. Our results consolidate the maned wolf as a solitary mesopredator of extreme plasticity, whose omnivory is more balanced and diverse than that of strictly carnivorous social canids, such as the gray wolf (*Canis lupus*) (Bosch et al. 2015; Zlatanova et al. 2014), or the bush dog (*Speothos venaticus*) (Zuercher et al. 2005). The metabarcoding technique allowed access to a cryptic fraction of the diet, revealing consumption of insects and birds that are significantly underestimated by conventional methods, although biological precision still faces limitations due to gaps in genetic databases for the Cerrado. The maned wolf maintains a stable taxonomic core (Solanaceae, Poaceae, Dasypodidae, and social arthropods) and exhibits generalist-opportunistic behavior across different landscapes.

DNA metabarcoding proved to be a promising tool for characterizing resource use by the maned wolf, as it allowed for the broad detection of plant and animal items that often go unnoticed in traditional morphological methods (Alberdi et al. 2020; Ando et al. 2020; Pinho et al. 2023; Sousa et al. 2019). Initially, next-generation sequencing revealed a total of 26,039 ASVs (Amplicon Sequence Variants), with 15,311 for plant items (ITS) and 10,728 for animal items (COI). This initial data highlights the power of the technique to capture the biological complexity present in fecal samples, identifying even soft-bodied prey or items consumed in trace amounts (Ando et al. 2020; Pinho et al. 2023; Sousa et al. 2019).

This sensitivity extends to the detection of internal parasites, such as nematodes from the family Dracunculidae, and highlights the "challenge of omnivory," where the technique captures not only deliberate consumption, but also accidental ingestion of insects presents in fruits or DNA from secondary predation (Sato 2024; Sousa et al. 2019; Tercel et al. 2021). Thus, molecular data provides a broader view that includes the wolf's health and ecological interactions, although rigorous criteria are needed to distinguish primary dietary

content from biological noise and indirect interactions (Deagle et al. 2019; Tercel et al. 2021).

Regarding taxonomic resolution, we observed a sharp loss of information as classification progressed to more refined levels. In the ITS marker, although over 13,000 ASVs were classified to the class level, only 2,848 (21.69%) could be assigned to the order level, with even steeper reductions at the genus and species levels. A similar pattern occurred with the COI marker, where thousands of sequences were discarded during the initial kingdom classification due to a lack of matches in databases, resulting in only 233 sequences reaching the species level. This technical bottleneck directly reflects gaps in global genetic databases, such as BOLD and NCBI, which still lack reference sequences for many native and endemic Cerrado species (Ando et al. 2020; Pinho et al. 2023; Quéméré et al. 2013). Many species in the biome have never been sequenced for the markers used, rendering them "invisible" to precise identification (Quéméré et al. 2013). The insufficiency of these references prevents the reliable assignment of scientific names to many sequences, which could lead to erroneous diagnoses if classification were forced at the genus or species level, as algorithms may indicate a "best match" with related species that are not the actual prey (Ando et al. 2020; Pinho et al. 2023). Therefore, while metabarcoding offers unprecedented reach, its current biological precision in the Cerrado is preserved by elevating the analysis to the Family level, ensuring the integrity of inferences about resources used by wild animals and reducing the chances of underestimated or mistaken interpretations (Deagle et al. 2019; Franceschini 2020).

From a quantitative perspective, metabarcoding advances in detecting taxonomic richness become evident when contrasting our results with the Brazilian literature. Using ITS and COI markers, 171 potential species were identified in the maned wolf's diet (117 plant and 54 animal species). This number individually exceeds the accumulated richness of 35 years of traditional studies compiled in the review by Soares (2020) (Soares 2020), which totaled 159 species from 26 works published between 1984 and 2019. Classic studies (Amboni 2007; Michalski et al. 2014; Rodrigues et al. 2007; Rodrigues 2002) reported numbers varying from 11 to 77 items, even with substantial sampling

efforts. These contrasts reinforce that the molecular approach not only complements but redefines the scale at which dietary diversity can be evaluated.

One of the main advances provided by metabarcoding is overcoming taxonomic invisibility (Ando et al. 2020; Pinho et al. 2023; Sousa et al. 2019). In morphological studies, arthropods are often recorded with high frequency but low taxonomic resolution, usually restricted to broad categories like "insects" or "coleopterans" (Amboni 2007; Soares 2020). In this study, the COI marker allowed for the identification of 25 orders and 42 animal families, including a wide diversity of insect families rarely recognized by traditional methods, such as Calliphoridae, Drosophilidae, Culicidae, and Tephritidae. This pattern suggests that the contribution of arthropods to the maned wolf's diet may be more diverse than historically described (Pinho et al. 2023), even though metabarcoding does not allow for direct inferences regarding consumed biomass (Deagle et al. 2019; Sato 2024; Tercel et al. 2021).

A similar situation is observed for birds. The literature frequently points to limitations in identifying this group, as fragmented feathers rarely allow for refined taxonomic assignments (Amboni 2007; Soares 2020). Reviews indicate that over 50% of bird records remain unidentified at the family level (Soares 2020). In this study, the use of COI enabled the detection of families such as Tinamidae, Columbidae, Thraupidae, and Tyrannidae, offering unprecedented resolution for this dietary component and demonstrating the potential of metabarcoding to reveal previously underestimated trophic interactions (Ando et al. 2020; Pinho et al. 2023; Sousa et al. 2019).

Our results using the ITS marker reinforce studies indicating that the plant fraction of the maned wolf's diet is characterized by the coexistence of two complementary patterns: the recurrent dominance of a restricted set of plant families and a wide, yet sporadic, taxonomic diversity (Motta-Junior 1997). This pattern was consistent across locations, with geographical factors explaining only a small portion of the data variation (6.1%), and between seasons, showing a high overlap of taxa, with 25 families shared between dry and rainy periods. These results suggest that the observed pattern reflects the generalist-

opportunistic behavior of the species rather than a methodological or sampling effect.

This "few dominants and many rares" pattern is typical of metabarcoding data in diet studies and has been described for other generalist and omnivorous mammals, such as the brown bear (*Ursus arctos*) (De Barba et al. 2014), the wild boar (*Sus scrofa*) (Robeson et al. 2018), folivorous primates (Quéméré et al. 2013), and various bat species (Alberdi et al. 2020). The observation of these patterns across different groups suggests that the coexistence of a stable resource core with a vast taxonomic periphery may be common in diets with high ecological plasticity revealed by molecular methods (Deagle et al. 2019).

In this study, the stability of alpha diversity indices across seasons and locations indicates that the identity of the primary plant families consumed remains relatively constant over space and time. Although classic literature often points to a marked seasonality, with higher plant consumption in the rainy season and animal consumption in the dry season (Amboni 2007; Dietz 1984; Motta-Junior 1997; Rodrigues et al. 2007; Veloso 2019), studies such as those by Aragona and Setz (2001) and Emmert (2012) (Aragona and Setz 2001; Emmert 2012) are cited as exceptions for not finding such pronounced seasonality. This interpretation is supported by our Venn diagrams, which show broad sharing of families between seasons and locations. Thus, despite the high total diversity detected by ITS, the maned wolf's plant diet appears organized around a stable core of resources widely available in the Cerrado.

The ITS data also shows significant differences between individual samples, reflected in the variation of relative abundance of plant families. This is ecologically consistent with the maned wolf's behavior as a predominantly solitary (Cheida 2005; Dietz 1984) and territorial (Carvalho and Vasconcelos 1995) species with low dietary overlap between individuals (de Almeida Jcomo et al. 2004). Unlike social canids like gray wolves, where group feeding tends to homogenize the diet (de Almeida Jcomo et al. 2004; DeCandia et al. 2021; Nardi et al. 2022), each maned wolf fecal sample more directly represents the recent feeding history of a single individual.

While the sporadic diversity of the 57 identified families reflects the wolf's plasticity, the consistent presence of Solanaceae suggests a continuous contribution to plant biomass throughout the annual cycle. The ecological centrality of this family, especially the genus *Solanum* and the species *Solanum lycocarpum*, is well-documented as a predictable and functionally important resource (Dietz 1984; Motta-Junior 1997; Soares 2020). Although *S. lycocarpum* was not specifically identified due to database gaps, the family Solanaceae showed constant presence across seasons (Dry: 4.43%; Rainy: 5.30%) and all locations (BA1, BA2, and DF).

The genus *Solanum* is described as the most important in the maned wolf's foraging ecology (Dietz 1984; Motta Junior and Martins 2002), representing between 40% and 90% of occurrences in fecal samples in various studies (Azevedo and Gastal 1997; Massara 2009; Rodrigues et al. 2007; Soares 2018). *S. lycocarpum* is particularly vital because it provides continuous availability throughout the year (Dietz 1984; Motta-Junior et al. 1996), unlike most Cerrado flora which fruits seasonally (Oliveira 1991; Peres 2011). Furthermore, a mutualistic relationship exists: the maned wolf is the primary disperser of *Solanum* seeds (Dietz 1984; Santos et al. 2003), and the plant, a pioneer species (Moura 2007; Palaretti et al. 2014), colonizes degraded areas and roadsides (Massara 2009; Rodrigues et al. 2007), facilitating wolf movement between forest fragments (Andrade 2020).

Nutritionally, the consumption of *S. lycocarpum* is significant. During the ripening of this fruit, starch is progressively converted into simple sugars, increasing palatability and energy return (Oliveira Junior et al. 2003). A single fruit can weigh nearly 900 g (Oliveira Junior et al. 2003; Silva 1994); thus, ingesting just a few fruits can meet a substantial portion of the wolf's daily energy requirement, estimated at 1,500 to 1,600 kcal (Castro and Emmons 2011). Moreover, the high fiber content (up to 35.85%) in *S. lycocarpum* (Oliveira Junior et al. 2003) far exceeds that of other native fruits (Silva 1994). Free-ranging maned wolves ingest significant amounts of fibrous plant material, with neutral detergent fiber averages of 44.2% in stomach contents (Silva et al. 2025).

Finally, the family Poaceae (grasses) also integrates the stable core. Traditionally interpreted as accidental or functional (for digestive cleansing) (Amboni 2007; Carvalho and Vasconcelos 1995; Cheida 2005; Dietz 1984), our study shows grasses are an important component, contributing 31.42% in the dry season and 22.65% in the rainy season. In BA2, this value reached 57.07%. ITS resolution revealed active selection of genera like *Paratheria* and *Schizachyrium*. This suggests that grasses may play a more important role in the diet than previously considered.

Although the ITS marker does not allow direct quantification of the nutritional content of ingested items, the taxonomic stability of these groups across seasons and localities provides a consistent functional indication of regular intake of fibrous plant material in the species' natural diet. From a physiological perspective, this dietary pattern is associated with the predominance of saccharolytic fermentative pathways in the large intestine, mediated by the intestinal microbiota, with potential increases in the production of short-chain fatty acids, such as butyrate (Barko et al. 2018; Flint et al. 2012; Louis and Flint 2017; Vital et al. 2015). Disruptions to this diet–fiber–microbiota axis, particularly under *ex-situ* conditions where plant material consumption may be reduced, may be associated with shifts in intestinal metabolism (Clayton et al. 2018; Silva et al. 2025), subclinical inflammation (Henson et al. 2017), and systemic metabolic imbalances previously reported for the species (Childs-Sanford 2005; Phipps and Edwards 2009).

In addition, diets with lower proportions of plant-derived components and higher protein content tend to promote urinary acidification, reducing cystine solubility and increasing the risk of urinary stone formation in a species known to be genetically predisposed to cystinuria (Allen 2007; Barboza et al. 1994; Bovée et al. 1981; Childs-Sanford 2005; Marcinczyk et al. 2024). In this context, the dietary patterns revealed by ITS may inform discussions on how diet composition could influence gastrointestinal health and urinary management in the maned wolf.

The animal component of the maned wolf diet, characterized by using the COI marker, revealed a taxonomically diverse assemblage of invertebrates,

particularly insects. Recurrent families, including Formicidae, Termitidae, Scarabaeidae, Calliphoridae, Drosophilidae, and Blattidae, were consistently detected across seasons and sampling localities, although with marked variation in relative abundance among individual samples. This pattern, characterized by the dominance of a limited number of taxa alongside a high proportion of low-abundance groups, suggests that invertebrates may constitute a recurrent dietary component rather than representing sporadic or incidental ingestion. This interpretation is supported by independent evidence from stomach content analyses of road-killed individuals, which show that although invertebrates account for a small fraction of total consumed biomass (approximately 2.5% by mass), their high frequency of occurrence (around 63%) reflects opportunistic yet consistent consumption over time (Silva et al. 2025).

Metabarcoding allowed for the precise identification of Termitidae (termites), which are often lumped into broad categories in morphological studies (Dietz 1984; Pompanon et al. 2012; Sato 2024). We detected genera such as *Nasutitermes*, *Neocapritermes*, *Parvitermes*, and *Ruptitermes*. Notably, termites represented 32.27% of animal DNA reads during the rainy season. This seasonal increase refines the classic model of maned wolf seasonality (Soares 2020).

Some insect detections likely reflect secondary predation. The recurrent presence of Dasypodidae (armadillos) in samples, a major biomass source (Santos et al. 2003; Silva and Talamoni 2003), suggests that the DNA of the armadillo's insect-based diet is being co-amplified, a known limitation of metabarcoding (Ando et al. 2020; Sousa et al. 2019; Tercel et al. 2021). However, direct insect consumption remains a key bioenergetic strategy. Hunting small items like termites is "energetically more economical" (Amboni 2007) than pursuing large prey, which requires higher physical investment (Franceschini 2020). Insects provide high-quality protein (25–75% dry matter), essential amino acids, and lipids (Oonincx and Finke 2021; Shah et al. 2022). Additionally, chitin acts as an insoluble fiber with prebiotic effects, promoting beneficial bacteria like *Bifidobacterium* (Abenaim and Conti 2025).

Animal alpha diversity showed no significant differences between seasons or locations, indicating a stable structure. However, armadillos (Dasypodidae)

contributed more to the dry season (61.85% vs. 16.24% in the rain). Cricetidae rodents remain the most abundant vertebrate group in 35 years of literature (Soares 2020), and our study confirmed the presence of genera like *Calomys*, *Oligoryzomys*, and *Cerradomys*.

Domestic vertebrates, specifically cattle and chickens (*Gallus gallus*), were recorded. Chickens were found in 1 individual in BA1, 4 in BA2, and 2 in DF. The higher frequency in BA2 likely reflects its intensive agricultural matrix and fragmented habitat, which increases the probability of anthropogenic encounters. Furthermore, the exotic domestic rodent *Mus musculus* was identified exclusively in the BA2 locality. This occurrence reinforces the evidence of the maned wolf's interaction with anthropized areas and its ability to exploit synanthropic resources in highly modified landscape. Our results confirm that domestic item consumption is opportunistic and restricted to a few individuals, rather than systematic predation, contributing to a less conflictual understanding of the species' relationship with rural production.

Beyond dietary changes, habitat fragmentation imposes substantial physiological costs on wildlife populations (de Paula 2016). Individuals inhabiting modified landscapes exhibit significantly higher levels of fecal glucocorticoids (cortisol), indicating chronic physiological stress when compared to animals living in protected areas (de Paula 2016; Sperscoski et al. 2012). In addition, increased proximity to domestic animals in fragmented habitats elevates the risk of transmission of lethal pathogens, such as canine parvovirus and canine distemper virus, which can compromise the long-term viability of wild populations (Consorte-McCrea and Santos 2013; Gomes et al. 2024).

Dietary ecology represents an additional and often underappreciated interface between landscape use and health outcomes (de Paula 2016; Silva et al. 2025). The generalist feeding behavior of the maned wolf, revealed with high resolution through COI metabarcoding, constitutes a primary pathway for exposure to endoparasites. The recurrent consumption of rodents and arthropods exposes individuals to parasite taxa such as Acanthocephala and Trichuridae, which commonly use these prey groups as intermediate or paratenic hosts (Massara et al. 2012). In this sense, parasitic infection is not merely incidental but

emerges as an intrinsic consequence of trophic interactions within the species' natural foraging strategy (Massara et al. 2012).

The application of metabarcoding further underscores both the power and the interpretative challenges of molecular approaches. The detection of parasite families rarely documented through visual inspection methods, such as Dracunculidae, illustrates the heightened sensitivity of DNA-based techniques. However, this sensitivity necessitates ecological caution: the presence of parasite DNA in fecal samples may reflect an active infection in the host, secondary ingestion of parasites presents in consumed prey, or a combination of both processes (Deagle et al. 2019; Tercel et al. 2021). Consequently, parasite detections derived from metabarcoding data should be interpreted within an integrative ecological framework that considers diet composition, prey ecology, and landscape context, rather than unequivocal evidence of host infection (Tercel et al. 2021).

5. CONCLUSION

This study represents the first integrated application of DNA metabarcoding using ITS and COI markers to characterize the plant and animal diet of wild maned wolf (*Chrysocyon brachyurus*) populations in the Cerrado. This approach substantially expanded the identification of resources compared to traditional taxonomic methods, particularly for highly digestible or small-bodied taxa, providing a more comprehensive view of the species' dietary composition.

Our results indicate that while quantitative variations in relative abundance occur across seasons and locations, the central taxonomic identity of the diet remains remarkably consistent. The absence of significant differences in alpha diversity and the high overlap in beta diversity suggest that seasonality and spatial variation manifest as fluctuations in relative resource contribution rather than qualitative replacement. The recurrence of widely distributed families like Poaceae and Solanaceae, alongside consistently detected animal groups, reinforces the maned wolf's generalist-opportunistic character.

Despite these advancements, the loss of taxonomic resolution, particularly for the COI marker, highlights critical gaps in genetic databases for Cerrado

fauna. This necessitates family-level analyses and underscores the urgency of expanding reference sequencing for native species. Overall, integrating molecular markers provides a robust framework for understanding the maned wolf's trophic plasticity, offering crucial insights for both *in-situ* and *ex-situ* conservation and management strategies.

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Landscape, not season, shapes gut microbiota of free-ranging maned wolves (*Chrysocyon brachyurus*) in the Cerrado

ABSTRACT

The Brazilian Cerrado has undergone intense habitat conversion, with more than 50% of its original vegetation already lost, creating a critical conservation scenario for medium- and large-sized carnivores. In rapidly transformed landscapes, gut microbiota emerges as a sensitive integrative indicator of ecological disturbance, reflecting shifts in diet, pathogen exposure, and physiological stress. Despite growing recognition of the microbiome as a valuable tool for conservation biology, data on wild carnivores remain heavily concentrated in the Northern Hemisphere, leaving substantial gaps for Neotropical species such as the maned wolf (*Chrysocyon brachyurus*), a vulnerable Cerrado endemic whose intestinal microbiota has not previously been characterized in free-ranging populations. We aimed to characterize the composition, diversity, and structure of the gut microbiota of free-ranging maned wolves in the Cerrado, evaluating variation associated with seasonality and geographic location across landscapes with distinct degrees of anthropogenic impact. We analyzed 47 fresh fecal samples collected from three Cerrado localities during dry and rainy seasons using 16S rRNA metabarcoding. Sequencing generated 21,055 ASVs, identifying 31 bacterial phyla, 282 families, and 789 genera. The microbiota was dominated by Bacillota and Pseudomonadota. No seasonal differences were detected in alpha or beta diversity, indicating temporal stability. In contrast, geographic location is significantly structured microbial composition. The area embedded in an intensive agricultural matrix (BA2) exhibited the most distinct profile, characterized by high relative abundance of fermentative families, including Lactobacillaceae, Lachnospiraceae, Peptostreptococcaceae, Clostridiaceae and Erysipelotrichaceae. The Federal District showed the highest abundance of Enterobacteriaceae and *Escherichia coli*, suggesting increased exposure to anthropogenic stressors, while BA1 displayed indicators associated with fruit consumption and dietary plasticity. This study demonstrates that landscape heterogeneity, particularly the degree of anthropogenic disturbance, shapes the gut microbiota of the maned wolf more strongly than seasonality, establishing an

empirical foundation for non-invasive health monitoring and conservation of Neotropical populations. The findings highlight the potential of fecal microbiota analysis as a sensitive tool for assessing the interface between wildlife, agricultural activities, and ecological integrity in fragmented landscapes, thereby supporting adaptive management strategies and biodiversity coexistence in the Cerrado.

Keywords: Cerrado biodiversity; Wild canids; Anthropogenic impact; 16S rRNA metabarcoding; Gut microbiota.

1. INTRODUCTION

As South America's second-largest biome, the Cerrado encompasses 2 million km² of structurally heterogeneous vegetation, from open grasslands to dense woodlands, supporting rich biodiversity with over 10,000 plant species and 5% of global fauna (Klink and Machado 2005; Myers et al. 2000). This species richness, combined with high endemism and accelerating habitat loss, positions the Cerrado among Earth's 36 biodiversity hotspots requiring urgent conservation prioritization (Magioli et al. 2025; Strassburg et al. 2017). Agricultural expansion and livestock production have already destroyed over 50% of the Cerrado's original cover, with conversion rates accelerating in recent decades (Andrade 2020; Kuhlmann and Ribeiro 2016). This landscape transformation creates critical conservation challenges for medium and large-bodied carnivores, which require extensive home ranges, intact habitat connectivity, and sufficient prey populations to maintain viable populations (Magioli et al. 2025).

The maned wolf (*Chrysocyon brachyurus*) is the largest canid in South America and the sole representative of the genus *Chrysocyon*. Classified as Near Threatened by the IUCN (IUCN 2016) and in Brazil, is classified as "Vulnerable" (ICMBio 2025), this species is endemic to South American open biomes, with the Cerrado representing its core distribution (Paula and DeMatteo 2015). Maned wolves exhibit a solitary, monogamous social structure and occupy large home ranges (20-115 km²) that encompass diverse habitat types (Dietz 1984). Their diet is remarkably broad, including fruits (particularly *Solanum lycocarpum*, the lobeira), small mammals, birds, reptiles, and invertebrates, with seasonal and geographic variation in resource use reflecting local availability (Dietz 1984; Soares 2020b). This dietary plasticity likely represents an adaptive response to the Cerrado's pronounced seasonality, characterized by a well-defined dry season with minimal rainfall and a rainy season with abundant precipitation and resource pulses (Alvares et al. 2013; Klink and Machado 2005). Despite the maned wolf's conservation relevance and unique ecology, fundamental aspects of its biology remain poorly understood, particularly regarding physiological responses to environmental change (Consorte-McCrea and Santos 2013; de Paula 2016). In this context, it becomes necessary to identify biological indicators

capable of integrating environmental signals and translating them into measurable physiological metrics.

Gut microbial communities function as integrative biosensors that transduce environmental variation such as dietary shifts, pathogen exposure, anthropogenic stressors, into measurable compositional and functional signatures, offering conservation biologists a mechanistic link between landscape-scale disturbance and individual-level physiological consequences (Barko et al. 2018; Sugden et al. 2020; Trevelline et al. 2019; Youngblut et al. 2019). The gut microbiome plays essential roles in nutrient acquisition, immune system development, pathogen resistance, and stress response modulation (Amato 2019; Barko et al. 2018; Trevelline et al. 2019). In wildlife populations, microbial community composition reflects host diet, habitat quality, social interactions, and exposure to anthropogenic disturbances (Amato et al. 2013; Biles et al. 2021; Sugden et al. 2020). Consequently, fecal microbiota analysis has emerged as a powerful non-invasive tool for assessing wildlife health and adaptation to environmental change (Barko et al. 2018; Trevelline et al. 2019; West et al. 2019).

Current microbiome-conservation research exhibits pronounced geographic bias: while North American and European carnivores have received extensive microbial characterization (see Chapter 1), Neotropical species remain critically underrepresented, and the maned wolf exemplifies this gap. Despite its conservation relevance and unique ecology, no baseline gut microbiota data exists for free-ranging populations. Previous microbiome studies on canids have revealed that diet composition, captivity status, geographic location, and anthropogenic disturbance can significantly influence gut microbial communities (Adams et al. 2021; Menke et al. 2017; Wu et al. 2017). However, these studies have focused primarily on species from temperate regions, leaving substantial knowledge gaps for tropical and subtropical carnivores.

Understanding how environmental factors shape the gut microbiota of free-ranging maned wolves is critical for several reasons. First, microbiota composition can serve as an early indicator of environmental stress and habitat degradation, potentially detecting population health declines before demographic changes become apparent (Sugden et al. 2020; Trevelline et al. 2019). Second,

microbial dysbiosis - disruption of normal microbial community structure - has been linked to various health problems in domestic and captive wild canids, including inflammatory bowel disease, obesity, and compromised immune function (Redfern et al. 2017; Suchodolski 2011). Third, characterizing baseline microbiota in free-ranging populations provides a reference for evaluating the impacts of captivity, translocation, and other conservation interventions (McKenzie et al. 2017). Concurrently, agricultural intensification creates landscape mosaics with varying degrees of habitat conversion, from relatively intact protected areas to intensive agricultural matrices (Andrade 2020; Magioli et al. 2025; Strassburg et al. 2017). These gradients in seasonal and spatial environmental variation offer opportunities to test hypotheses about microbial community assembly and stability in wild carnivores.

From a theoretical perspective, this study contributes to the understanding of how environmental factors, such as seasonality and the degree of anthropization, and dietary factors, such as feeding plasticity, modulate the structure of gut microbial communities in generalist canids. Ecological theory predicts that hosts with more diverse diets should harbor more diverse and functionally redundant microbiomes, conferring resilience to environmental disturbances (Amato et al. 2013; Colborn et al. 2020; Youngblut et al. 2019). Practically, this study establishes a reference protocol for the non-invasive monitoring of *C. brachyurus* gut microbiota using field-collected fecal samples, an approach applicable to other threatened carnivore species in the Cerrado (West et al. 2019). Fecal microbiota analysis offers significant advantages over invasive methods, such as blood collection or swabs, including the elimination of the need for capture, reduced stress for the animals, and the possibility of repeated sampling over time (DeCandia et al. 2021; Lapid et al. 2025; West et al. 2019). The integration of metabarcoding for microbiota detection with dietary metabarcoding from the same fecal samples allows for the simultaneous characterization of resource consumption patterns and bacterial community structure, maximizing information yield per sample and facilitating integrated analyses (Biles et al. 2021; Colborn et al. 2020; Silva et al. 2025; Trevelline et al. 2019; West et al. 2019).

The data generated may enhance *ex-situ* management programs, leading to more assertive feeding protocols and veterinary care for maned wolves under human care (Adams et al. 2021; Bornbusch et al. 2023; Silva et al. 2025; Weber et al. 2023). Studies indicate that the type of diet offered in captivity significantly affects microbiota composition and is associated with variations in fecal consistency and gastrointestinal health indicators (Barraza-Guerrero et al. 2021; Bermingham et al. 2013; Bragg et al. 2020; Schmidt et al. 2018). Comparing the microbiota of free-ranging individuals with those under human care can identify key taxa whose abundance is associated with digestive homeostasis (Bornbusch et al. 2023; Chen et al. 2022; Guo et al. 2020; West et al. 2019). This can guide the development of probiotic supplements or dietary adjustments that promote gut health in animals maintained for management, breeding, or translocation purposes.

In this way, the characterization of the *C. brachyurus* gut microbiota across different climatic seasons and landscape contexts allows us to test hypotheses regarding factors modulating microbial variation: (1) the seasonal dietary hypothesis, which posits that diet composition governs gut microbiota structure (Colborn et al. 2020; Ley et al. 2008; Youngblut et al. 2019); and (2) the environmental filter hypothesis, which suggests that habitat quality and fragmentation exert direct effects on the gut microbiota (Adams et al. 2021; Amato et al. 2013; Bennett et al. 2016; Sugden et al. 2020). Treating the gut microbiota as a functional component of the host–environment interaction, this work provides an empirical basis for understanding the ecological mechanisms regulating the host–microbiota–landscape interface in generalist canids. Our findings demonstrate that spatial factors associated with landscape heterogeneity outweigh seasonality in determining microbial structure, supporting the use of fecal microbiota as a complementary tool for ecological and health assessment of maned wolf populations in heterogeneous landscapes.

2. MATERIALS AND METHODS

2.1. Target Species and Study Areas

We conducted this study in the Cerrado biome across two regions in western Bahia and one in the Federal District (DF), Brazil. The study areas are classified as tropical savanna climate (Aw) according to Köppen, with distinct wet

(November–April) and dry (May–October) seasons, as defined by the updated Brazilian climate classification (Alvares et al. 2013) and recent climatological normals (INMET 2022). These regions encompass contrasting landscape contexts, land-use intensities, and levels of environmental protection (Table 1).

To characterize land-use intensities and levels of environmental protection, we used land use and land cover rasters from MapBiomas Collection 10 (30-meter resolution) (MapBiomas 2025). We established 3-km radius zones of influence (buffers) around each sampling point, representing the species average home range (Paula and DeMatteo 2015). Landscape composition was quantified using relative proportions within these buffers across six functional categories (Forest, Herbaceous/Shrub Vegetation, Agriculture, Non-Vegetated Area, Water, and Not Observed) following a methodology adapted from Souza et al. (2020) (Souza et al. 2020).

Table 1. Identification and location of the study areas sampled in the Cerrado biome, Brazil.

Area ID	Local	City (State)	Latitude (S)	Longitude (W)	Area (ha)	Climate (Aw) rainy Nov–Apr; dry May–Oct;
BA1	Farm	Jaborandi (BA)	14,64377	45,80214	33.000	800–1,400 mm/year; Mean annual temperature 24–26 °C
BA2	Farm	Barreiras (BA)	11,83731	46,28913	1.000	900–1,500 mm/year; Mean annual temperature 24–26 °C
		Luís Eduardo Magalhães (BA)	11,82271	46,02532	1.700	
			12,08418	46,06691	800	
DF	ESECA E ¹	Brasília – (DF)	15,59132	47,66665	10.550	1,100–1,600 mm/year; Mean annual temperature 21–23 °C
	APAC ²		15,59823	48,16658	46.510	
	PNB ³		15,73471	47,92149	42.355	

Note: ¹Estação Ecológica de Águas Emendadas; ²Área de Proteção Ambiental de Cafuringa; ³Parque Nacional de Brasília

2.2. Sample Collection and Processing

We obtained fresh fecal samples (October 2020 to July 2023) through three strategies: capture campaigns, continuous monitoring of free-ranging populations, and opportunistic collection from rescued individuals. During monitoring, the scats were identified by shape, diameter, odor, and the presence of wolf fur, typically found near tracks (de Arruda Bueno and Motta-Junior 2009). Approximately 40g of feces were stored in sterile containers at -20°C , then transferred to -80°C at the University of Brasília (UnB). A 10 g aliquot from each sample was transferred to identified Falcon tubes and stored in duplicate.

2.3. Molecular Analyses

A total of 137 fecal samples were collected (BA1 = 102; BA2 = 17; DF = 18). We extracted genomic DNA using the QIAamp DNA Stool Mini Kit (Qiagen), following the manufacturer's recommendations. All samples were screened by PCR, and DNA quality was assessed prior to sequencing. Based on amplification success and DNA integrity, 55 samples with the highest DNA quality (BA1 = 25; BA2 = 14; DF = 16) were selected and sent to the Vale Institute of Technology (ITV – Belém, Brazil) for sequencing. At ITV, DNA extraction was finalized using the DNeasy® PowerSoil® kit (QIAGEN), and DNA concentration was quantified using Qubit® 3.0 (Thermo Fisher Scientific). The reaction conditions and thermocycling parameters followed the original protocols established for each primer set and are described in Table 2.

We amplified the V3 and V4 regions of the 16S rRNA gene using primers 16S_F_ILL/16S_R_ILL (Klindworth et al. 2013) for 47 samples. After purification with AMPure XP beads (Beckman Coulter), we indexed libraries using the IDT for Illumina Unique Dual (UD) Index kit. Sequencing was performed on the NextSeq 2000 platform (Illumina) using the NextSeq 2000 P1 kit (600 cycles) (Table 3).

Table 2. List of primers tested for the evaluation of the DNA metabarcoding approach and PCR conditions used for each primer pair.

		16S_F_ILL e 16S_R_ILL		
Reagent	Volume (μL)	Temperature	Time	Cycle
5x GoTaq Buffer	5.0	95 °C	3 min	1
dNTP mix (2 mM)	1.25	95 °C	30 s	
MgCl ₂ (25 mM)	2.0	57 °C	30 s	25
Primer F (10 μM)	0.5	72 °C	30 s	
Primer R (10 μM)	0.5	72 °C	5 min	1
GoTaq DNA Polymerase	0.25	4 °C	-	1
DNA (2 ng/μL)	5.0			
Ultrapure water	10.5			

Table 3. Primers tested for DNA metabarcoding evaluation.

Primer	Target	Amplicon (bp)	Sequence (5'-3')	Reference
16S_F_ILL	Prokaryota	460	TCGTCGGCAGCGTCAGATGTGTATAAGA GACAGCCTACGGGNGGCWGCAG	(Klindworth et al. 2013)
16S_R_ILL			GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAGGACTACHVGGGTATCTAATCC	

Raw data were processed using DADA2 (v1.28.0) in R (v4.3.4) (Callahan et al. 2016). Reads were filtered based on *Phred* > 30, expected errors (55), minimal 50pb and assigned to Amplicon Sequence Variants (ASVs). Taxonomy was assigned using the SILVA database (v.138.2) (Pruesse et al. 2007) with > 97% similarity.

2.4. Statical Analysis

We performed statistical analyses in R (v4.3.4) (R Core Team 2024) using packages *phyloseq* (McMurdie and Holmes 2013), *microbiome* (v.1.28.0) (Lahti and Holmes 2019), *vegan* (v1.50.0) (Oksanen et al. 2025) and *ggplot2* (v3.5.1) (Wickham et al. 2024). Alpha diversity (Richness, Shannon, and Pielou evenness) was calculated with 5,000 permutations and 95% confidence intervals

(Ho et al. 2018). For beta diversity, we filtered taxa (seasonal analysis: prevalence $\geq 9.30\%$, min 10 counts; locality analysis: prevalence $\geq 6.38\%$, min 15 counts) to remove spurious data.

We evaluated dietary distribution using Principal Coordinates Analysis (PCoA) and Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis distances, followed by PERMANOVA and permutational ANOVA (999 permutations, $p < 0.05$). The *microeco* package (v1.14.0) (Liu et al. 2021) was used for Venn diagrams, and indicator species analysis (9,999 permutations) was performed with the *indicspecies* package (v1.7.14) (Cáceres and Legendre 2009). All diversity analyses were conducted at the genera level, which was considered the lowest level with sufficient resolution for detecting ecological patterns.

3. RESULTS

3.1. Landscape Structure and Study Area Characterization

Landscape characterization revealed marked differences in the distribution of land use and land cover classes across the three sampling sites (Figure 1). In site BA1, "Herbaceous and Shrub Vegetation" predominated, representing approximately 57% of the area, followed by "Forest Area" at approximately 27%. "Agricultural Areas" accounted for 12% of the landscape, while "Non-Vegetated Areas" and "Water" showed lower representation ($\sim 4\%$ and $< 1\%$, respectively). Site BA2 exhibited a predominance of "Agricultural Areas," representing approximately 57% of the landscape composition, followed by "Forest Area" at approximately 35%. "Herbaceous and Shrub Vegetation" occupied a reduced proportion (8%), while "Non-Vegetated Areas" and "Water" values were below 1%. In the Federal District (DF), "Forest Area" occupied about 72% of the landscape, followed by "Agricultural Areas" ($\sim 24\%$). "Non-Vegetated Areas" accounted for approximately 5%, while "Water" and "Herbaceous and Shrub Vegetation" showed the lowest proportions ($\sim 1\%$ each). Comparatively, the sampling sites formed a spatial gradient of land use and cover, with BA2 characterized by a higher proportion of agricultural areas, BA1 by a predominance of herbaceous and shrub vegetation, and the DF by greater forest cover (Figure 1).

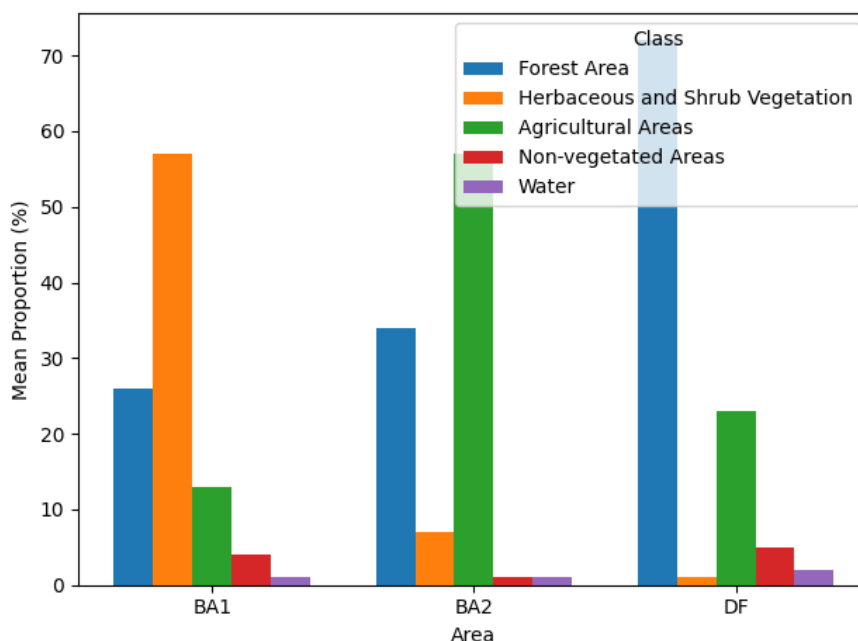


Figure 1. Mean proportion (%) of land use and land cover categories within the zones of influence (3-km radius buffers) of the sampled localities. Columns represent the percentages observed at Fazenda Jaborandi (BA1), Luís Eduardo Magalhães e Barreiras (BA2), and the Federal District (DF) for the following classes: Forest Area (green); Herbaceous and Shrub Vegetation (beige); Agricultural Areas (cream); Non-vegetated Areas (red); and Water (blue). Data were obtained from MapBiomas Collection 10 with a spatial resolution of 30 meters.

3.2. Sequencing Data and Taxonomic Resolution

Sequencing of the 47 samples generated a read volume ranging from 3,604 (sample MB0587A) to 1,129,010 (sample MB0603A). Initial processing identified 41,207 ASVs. After removing sequences classified as chloroplasts or mitochondria and excluding singletons, 21,055 ASVs remained.

Regarding taxonomic resolution, 34,888 ASVs were classified at the kingdom level, 34,559 at the phylum level, 34,125 at the class level, 33,483 at the order level, 31,772 at the family level, 24,891 at the genus level, and 9,578 were identified at the species level. In total, we identified 2 kingdoms (Bacteria and Archaea), distributed across 31 phyla, 70 classes, 160 orders, 282 families, 789 genera, and 1,140 species.

3.3. General Microbiota Structure

Taxonomic distribution across samples revealed a composition dominated by a few high-abundance groups. At the phylum level, the fecal microbiota was predominantly composed of Bacillota, which showed the highest representation in nearly all analyzed samples (Figure 2A). Pseudomonadota was the second most abundant phylum, followed by Actinomycetota, Bacteroidota, and Fusobacteriota. We observed inter-individual variation in the proportion of these phyla, with some samples showing a higher presence of Campylobacterota and Verrucomicrobiota (Figure 2A).

At the family level, Clostridiaceae, Lactobacillaceae, Streptococcaceae, Peptostreptococcaceae, and Enterobacteriaceae concentrated the highest proportion of reads, forming the dominant core of the community (Figure 2B). Although present in all 47 samples, Lachnospiraceae, Fusobacteriaceae, Erysipelotrichaceae, and Bifidobacteriaceae exhibited significant fluctuations in relative abundance, with peaks reaching 13.41%, 8.32%, 5.43%, and 1.73%, respectively.

At the genus level, the community was characterized by the predominance of recurrent taxa across all samples (Figure 2C). *Clostridium sensu stricto 1* was found in all samples, with mean abundances between 8.62% and 15.40%, reaching an individual peak of 25.31%. *Streptococcus* also showed wide distribution, with mean values from 3.80% to 25.38% and peaks up to 30.95%. For *Escherichia-Shigella*, mean abundances ranged from 3.82% to 20.79%, while *Ligilactobacillus* and *Peptoclostridium* presented mean intervals of 2.19–17.31% and 3.82–10.57%, respectively. Other relevant genera included *Lactobacillus* (0.74–11.23%) and *Fusobacterium* (1.91–5.53%). *Bifidobacterium* presented lower mean abundances (1.19–1.69%), although individual peaks were observed, such as *Bifidobacterium choerinum* reaching 4.68% in a single sample (Figure 2C).

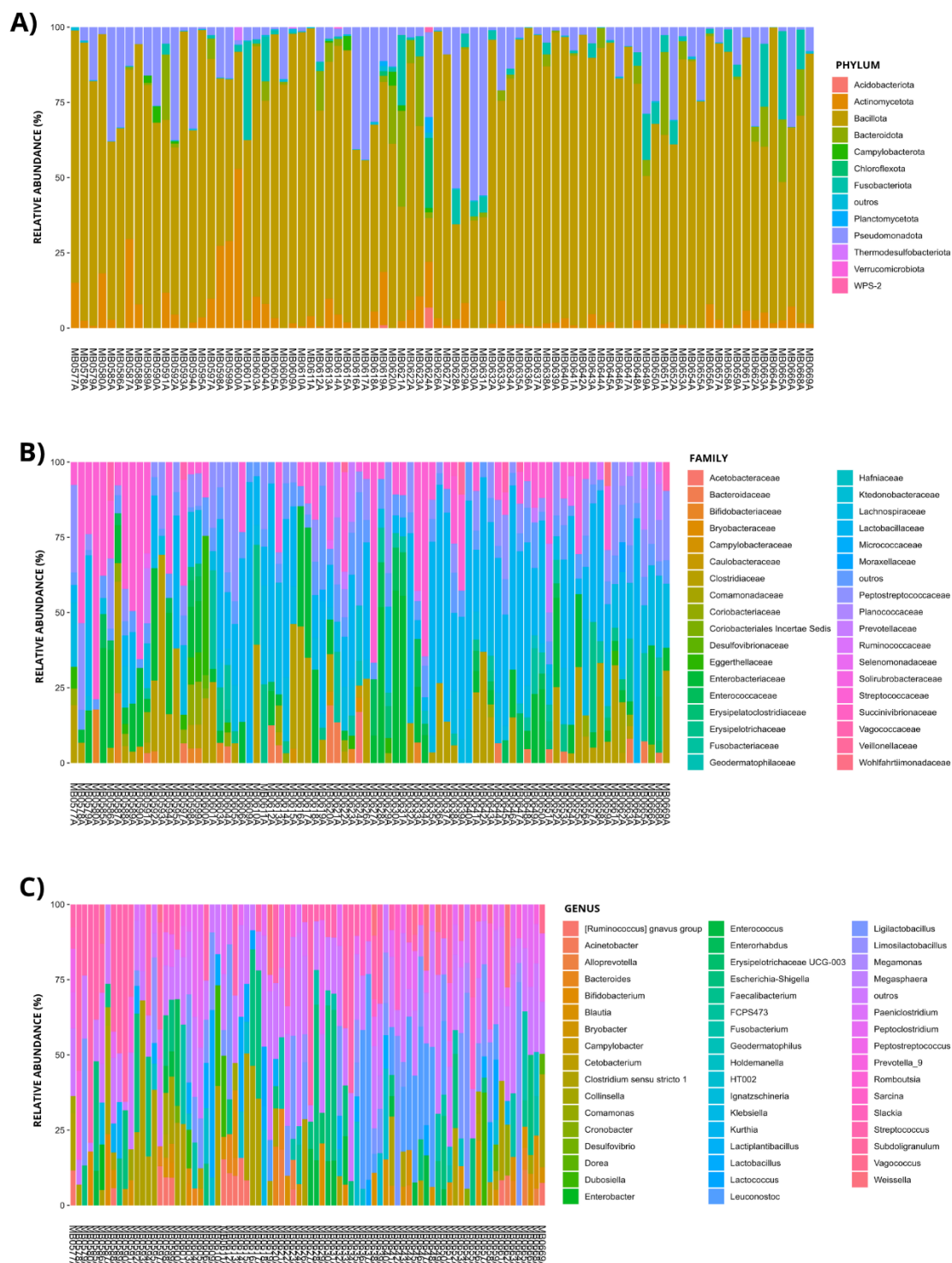


Figure 2. General taxonomic profile detected by 16S metabarcoding in fecal samples of *Chrysocyon brachyurus*. A) Phylum, B) Family, and C) Genus levels for all samples (n=47). Each bar represents an individual fecal sample. Relative abundance < 0.5% (phylum), 3% (family) and 5% (genera) are grouped into the "others" category.

3.4. Microbiota Dynamics across Seasons

Taxonomic composition at the phylum level indicated a consistent predominance of Bacillota and Pseudomonadota in both seasons (Figure 3A). In the rainy season, Bacillota presented the highest abundance (68.66%), followed by Pseudomonadota (18.15%), Bacteroidota (4.75%), Actinomycetota (4.18%), Fusobacteriota (3.58%), Campylobacterota (0,40%), Planctomycetota (0,060%), Chloroflexota (0,058), Verrucomicrobiota (0,049) e Thermodesulfobacteriota (0,049). During the dry season, Bacillota dominance increased to 74.13%, while Pseudomonadota decreased to 11.16%. The phyla Actinomycetota, Fusobacteriota, Bacteroidota, Campylobacterota, Chloroflexota, Acidobacteriota, Planctomycetota e Thermodesulfobacteriota presented relative abundances of 5.58%, 3.92%, 3.42%, 0,65%, 0,63%, 0,18%, 0,14% e 0,05%, respectively (Figure 3A).

At the family level, the rainy season showed similar proportions for Lactobacillaceae (19.00%), Enterobacteriaceae (17.17%), Streptococcaceae (15.99%), Peptostreptococcaceae (12.30%), Lachnospiraceae (7,99%), Enterococcaceae (3,95%), Fusobacteriaceae (3,60), Erysipelotrichaceae (3,47%), Clostridiaceae (3,07%) e Bacteroidaceae (2,92%) (Figure 3B). In the dry season, Clostridiaceae (19.77%) was the most representative, followed by Lactobacillaceae (14.79%), Streptococcaceae (13.58%), Peptostreptococcaceae (10.65%), Lachnospiraceae (10.41%), Enterobacteriaceae (1,02%), Fusobacteriaceae (3,94%), Coriobacteriaceae (2,60%), Prevotellaceae (2,06%), Erysipelotrichaceae (2,02%) (Figure 3B).

At the genus level, *Streptococcus* (15.52%), *Escherichia-Shigella* (13.02%), and *Ligilactobacillus* (10.94%) was the most representative in the rainy season (Figure 3C), followed by *Peptoclostridium* (8,11%), *Enterococcus* (4,08%), *Fusobacterium* (3,72%), *Blautia* (3,32%), *Clostridium sensu stricto 1* (3,13%), *Bacteroides* (3,02%) and *[Ruminococcus] gnavus group* (2,78%). In the dry season, *Clostridium sensu stricto 1* became the most abundant (20.09%), followed by *Streptococcus* (13.23%), *Escherichia-Shigella* (9.93%), *Peptoclostridium* (5,75%), *Ligilactobacillus* (5,33%), *Lactobacillus* (5,10%), *Fusobacterium* (4,04%), *[Ruminococcus] gnavus group* (3,83%), *Blautia* (3,78%), *Paeniclostridium* (3,55%) (Figure 3C).

At the species level, sequences assigned to the *Escherichia-Shigella* clade and classified as *Escherichia coli* (22.57%) and *Streptococcus gallolyticus* (20.60%) predominated during the rainy season. These were followed by sequences classified as *Fusobacterium mortiferum* (16.42%), *Paeniclostridium sordellii* (14.84%), *Enterococcus faecium* (11.30%), *Romboutsia hominis* (11.06%), *Streptococcus hyointestinalis* (10.86%), sequences within *Clostridium sensu stricto 1* classified as *C. baratii* (7.88%), sequences within *Clostridium sensu stricto 1* classified as *C. perfringens* (7.00%), and *Bacteroides fragilis* (6.84%). During the dry season, sequences within *Clostridium sensu stricto 1* classified as *C. perfringens* (23.69%) were highly represented, followed by sequences assigned to *Escherichia coli* within the *Escherichia-Shigella* group (5.48%), *Fusobacterium mortiferum* (24.59%), *Paeniclostridium sordellii* (21.31%), sequences within *Clostridium sensu stricto 1* classified as *C. baratii* (18.82%), *Collinsella stercoris* (8.63%), sequences within *Clostridium sensu stricto 1* classified as *C. colicanis* (7.98%), *Collinsella tanakaei* (7.07%) and *Blautia hansenii* (6.12%).

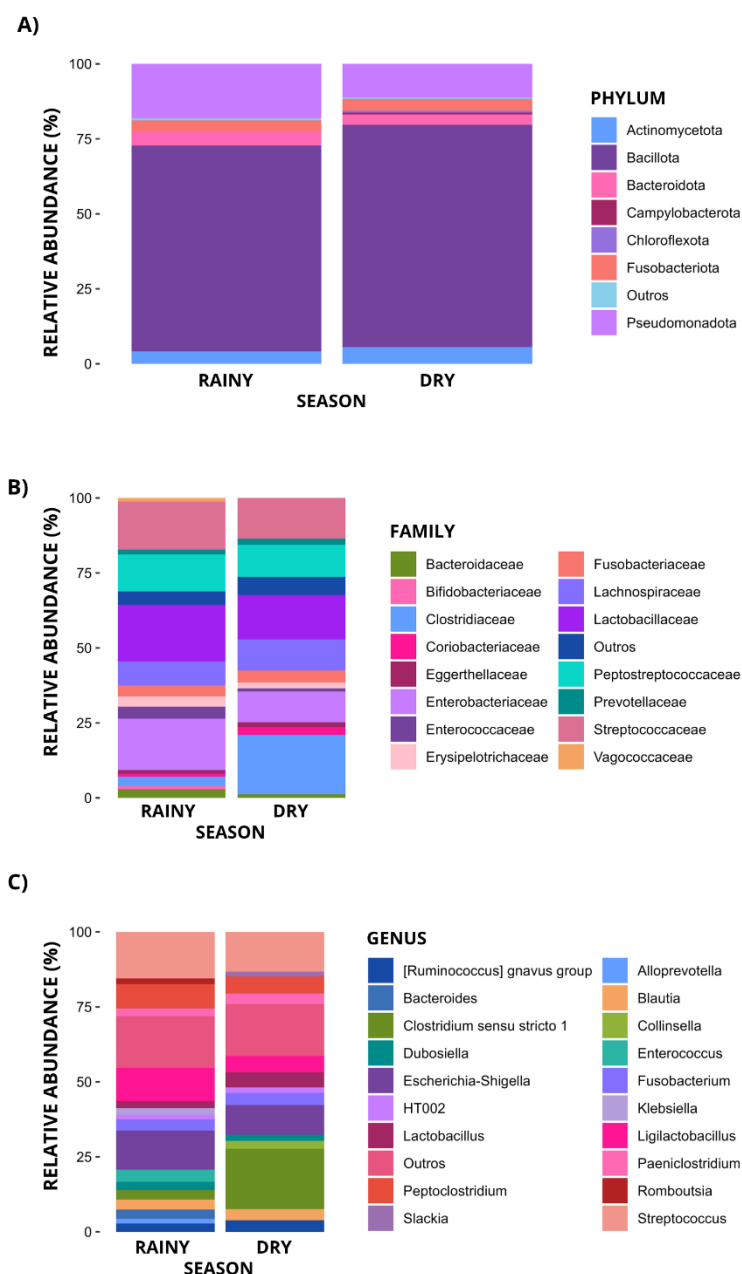


Figure 3. Taxonomic composition across seasons based on 16S metabarcoding of fecal samples from *Chrysocyon brachyurus*. A) Phylum, B) Family, and C) Genus levels categorized by season. Each bar represents the relative abundance of each taxon in the dry and rainy seasons. Relative abundance < 1.5% are grouped into the "others" category.

3.5. Microbiota Variation across Localities

Phylum-level composition revealed Bacillota dominance (>50%) across all localities, with the highest abundance in site BA2 (85.20%), followed by BA1 (71.12%) and DF (54.53%) (Figure 4A). Pseudomonadota was most abundant in

the DF (25.62%) and lower in BA1 (13.97%) and BA2 (4.60%). Actinomycetota showed higher abundance in BA1 (9.13%) compared to BA2 (4.87%) and the DF (4.33%) (Figure 4A).

At the family level, BA1 was characterized by high Streptococcaceae (25.33%) and Clostridiaceae (15.24%), followed by Enterobacteriaceae (13.74%), Peptostreptococcaceae (12.10%), Lachnospiraceae (7.33%), Lactobacillaceae (5.79%), Eggerthellaceae (4.21%), Fusobacteriaceae (3.61%), Coriobacteriaceae (2.56%) and Enterococcaceae (2.27%). Site BA2 showed a distinct pattern, with a predominance of Lactobacillaceae (36.08%) and a higher contribution of Lachnospiraceae (13.41%), followed by Peptostreptococcaceae (10.86%), Clostridiaceae (10.24%), Erysipelotrichaceae (8.32%), Enterobacteriaceae (4.37%), Streptococcaceae (3.94%), Bacteroidaceae (2.46%) and Coriobacteriaceae (1.95%). In the DF, Enterobacteriaceae presented the highest relative abundance (23.21%), followed by Streptococcaceae (12.43%), Lactobacillaceae (10.53%), Lactobacillaceae (10.53%), Peptostreptococcaceae (8.40%), Clostridiaceae (8.37%), Lachnospiraceae (7.10%), Fusobacteriaceae (5.35%) and Enterococcaceae (5.03%) (Figure 4B).

Genus-level variations confirmed these spatial trends (Figure 4C). *Streptococcus* predominated BA1 (25.38%), whereas BA2 was marked by *Ligilactobacillus* (17.32%) and *Lactobacillus* (11.24%). In the DF, *Escherichia-Shigella* reached 20.80%. Other genera detected across the three localities included *Escherichia-Shigella* (11.08%), *Paeniclostridium* (5.01%), *Peptoclostridium* (3.82%), *Fusobacterium* (3.69%), *[Ruminococcus] gnavus group* (3.09%), *Collinsella* (2.62%), *Enterococcus* (2.33%) and *Blautia* (2.28%) in BA1. In BA2, additional representative genera comprised *Clostridium sensu stricto 1* (10.61%), *Peptoclostridium* (10.57%), *Dubosiella* (7.88%), *[Ruminococcus] gnavus group* (5.81%), *Blautia* (4.93%), *HT002* (4.80%), *Escherichia-Shigella* (3.83%) and *Streptococcus* (3.80%). A similar pattern was observed in the DF, where *Clostridium sensu stricto 1* (10.61%), *Peptoclostridium* (10.57%), *Dubosiella* (7.88%), *[Ruminococcus] gnavus group* (5.81%), *Blautia* (4.93%), *HT002* (4.80%), *Escherichia-Shigella* (3.83%) and *Streptococcus*

(3.80%) were also recorded. A distinct pattern was observed in the DF, where the community was dominated by *Escherichia*-

Shigella (20.79%), followed by *Streptococcus* (11.18%), *Clostridium sensu stricto 1* (8.62%), *Peptoclostridium* (6.51%), *Fusobacterium* (5.53%), *Ligilactobacillus* (5.46%), *Enterococcus* (5.20%), and *Blautia* (3.79%).

Species-level assignments showed similar patterns across localities, with sequences classified as *Streptococcus gallolyticus* (30.95%) predominating in BA1, sequences assigned to *Clostridium sensu stricto 1* and classified as *C. perfringens* (25.32%) in BA2, and sequences within the *Escherichia-Shigella* clade classified as *E. coli* (30.99%) in the DF (Figure 4C).

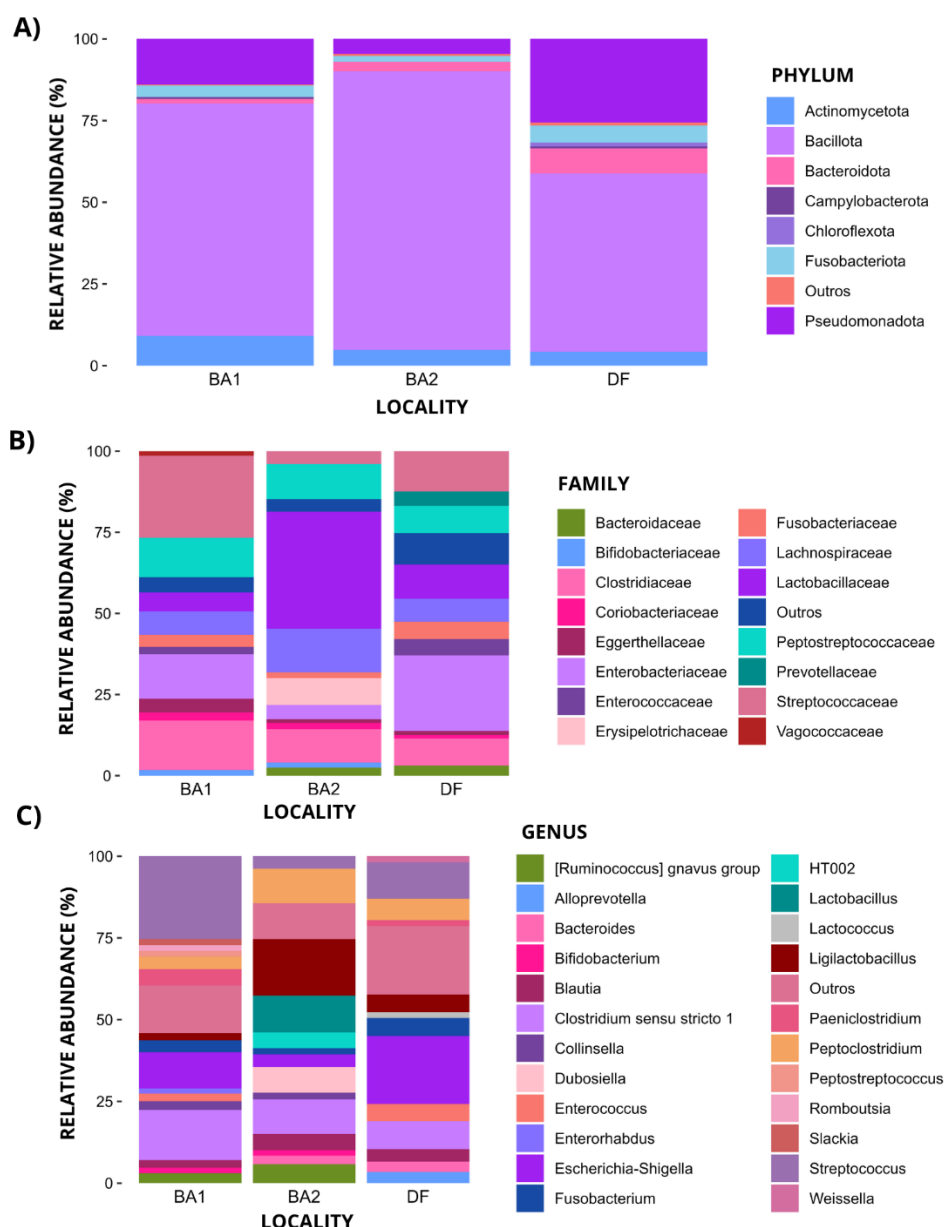


Figure 4. Landscape-associated variation in bacterial community composition based on 16S metabarcoding of fecal samples from *Chrysocyon brachyurus*. A) Phylum, B) Family, and C) Genus levels for Jaborandi (BA1), Luís Eduardo Magalhães e Barreiras (BA2), and Distrito Federal (DF). Each bar represents the relative abundance of each taxon in the localities. Relative abundance < 1.5% are grouped into the "others" category.

3.6. Alpha Diversity

Alpha diversity analyses indicated no differences between the dry and rainy seasons for the evaluated metrics (Figure 5A). Mean observed richness was similar between periods (Rainy: 88.00; Dry: 84.67). Shannon diversity

(Rainy: 2.04; Dry: 2.02) and Pielou evenness (Rainy: 0.47; Dry: 0.46) also remained consistent, indicating stability across the seasonal cycle (Figure 5A).

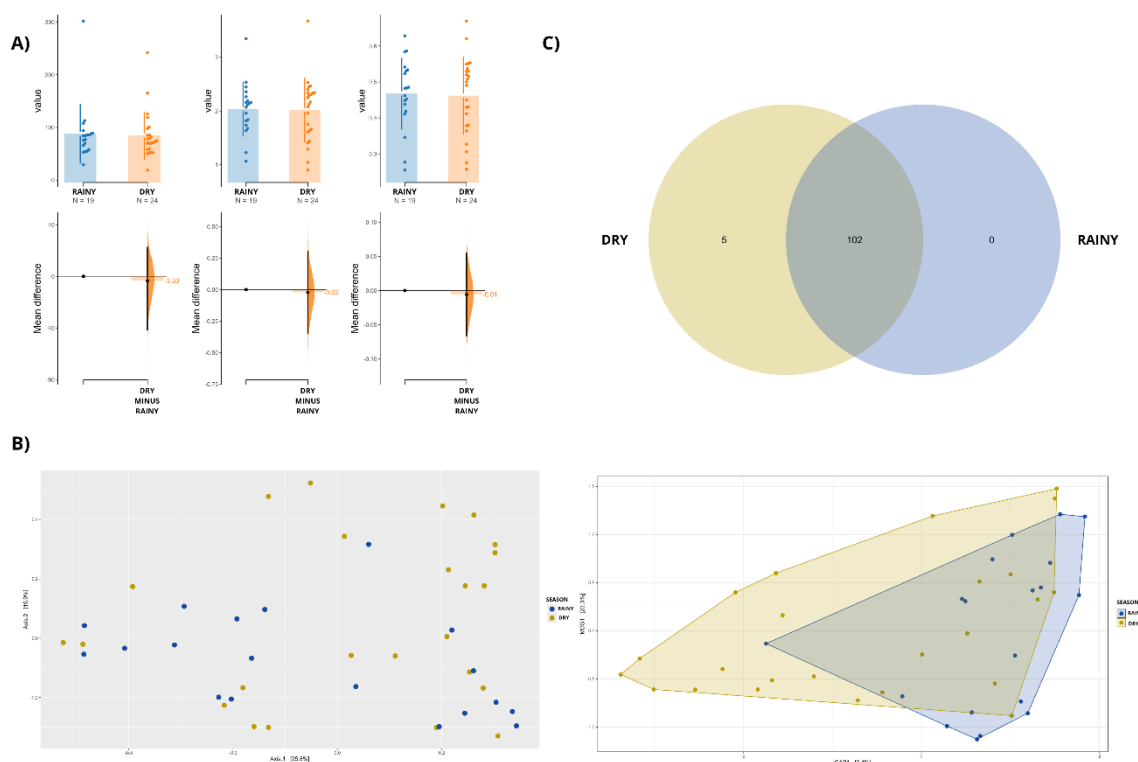


Figure 5. Seasonal patterns of fecal bacterial diversity in *Chrysocyon brachyurus* based on 16S metabarcoding at the genus level. A) Alpha diversity from dry ($n = 24$) and rainy ($n = 19$) seasons (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated with 5,000 permutations and 95% confidence intervals. B) Beta diversity analyses based on Bray–Curtis dissimilarity. PCoA explained 45.7% of total variation, and CAP explained 26.7%. C) Venn diagram of bacterial genera detected in each season, showing dry-exclusive ($n = 5$), rainy-exclusive ($n = 0$), and shared genera ($n = 102$).

Regarding localities, observed richness was lowest in BA1 (73.65), intermediate in BA2 (88.33), and highest in the DF (101.93). Shannon diversity followed a similar trend (BA1: 1.84; BA2: 2.05; DF: 2.19). Evenness also varied gradually (BA1: 0.44; BA2: 0.46; DF: 0.48), indicating a progressive increase in diversity from site BA1 to the DF (Figure 6A).

3.7. Beta Diversity

Beta diversity analyses showed no differences between the dry and rainy seasons. Both PCoA and CAP ordinations indicated an absence of seasonal

clustering (PERMANOVA $p = 0.129$ and $p = 0.167$, respectively) (Figure 5B). The Venn diagram confirmed this similarity, with 102 shared genera and only five genera exclusive to the dry season (Figure 5C).

In contrast, beta diversity revealed significant compositional differences between localities (Figure 6B). Locality explained 12.6% of total variation ($R^2 = 0.126$, $F = 3.18$, $p = 0.001$). CAP analysis also confirmed a significant locality effect ($F = 2.93$, $p = 0.002$), though axis variance was relatively low (11.8%). Pairwise comparisons showed significant differences between BA1 and BA2 ($p = 0.001$) and between DF and BA2 ($p = 0.003$), while BA1 and DF were statistically similar ($p = 0.117$). The Venn diagram for localities highlighted a core of 80 shared genera, with site BA2 exhibiting exclusive taxa such as *Asaccharobacter* and *DNF00809* (Figure 6C).

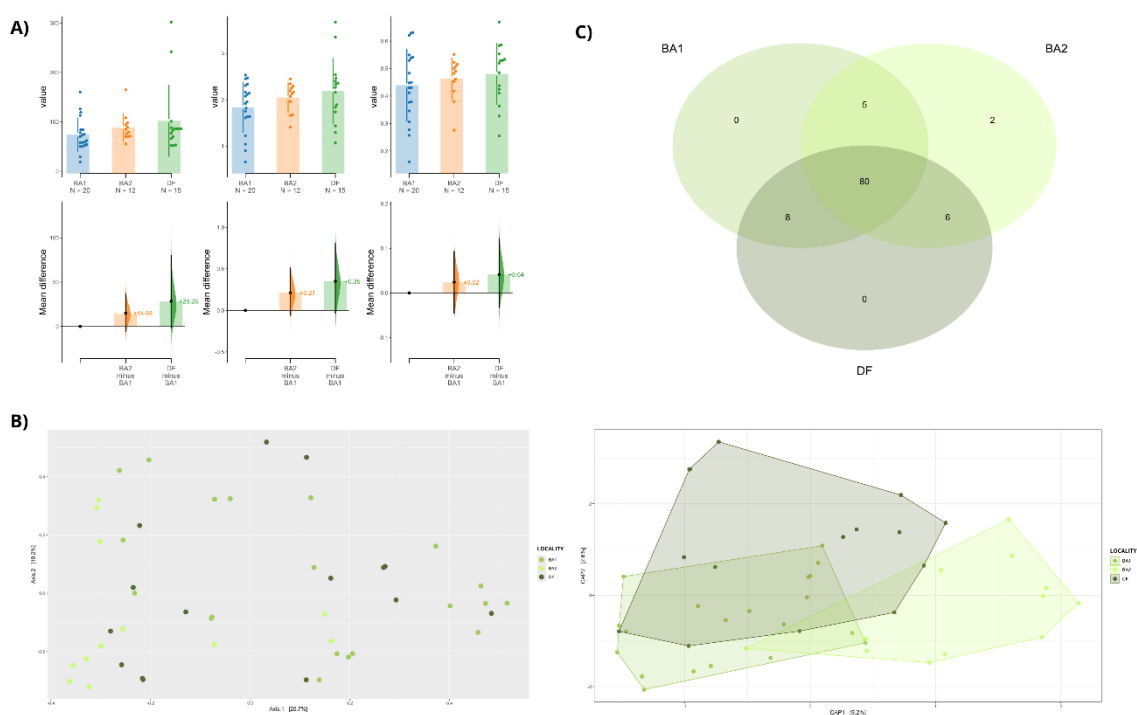


Figure 6. Spatial variation in gut bacterial diversity across localities in *Chrysocyon brachyurus* based on 16S metabarcoding at the genus level. A) Alpha diversity from Fazenda Jaborandi – BA1 (n = 20), Luís Eduardo Magalhães e Barreiras – BA2 (n = 12), and the Distrito Federal – DF (n = 15) (Observed Richness, Shannon Index, and Pielou's Evenness). Estimates were generated using 5,000 permutations with 95% confidence

intervals. B) Beta diversity analyses (BA1 n = 21; BA2 n = 13; DF n = 15) based on Bray–Curtis dissimilarity. PCoA explained 44.9% of total variation, and CAP explained 11.8%. C) Venn diagram of bacterial genera detected in each locality, showing exclusive and shared genera (n = 80).

3.8. Indicator Genera

Indicator species analysis identified 19 genera with significant associations to localities. Site BA2 presented the highest number of associated genera (nine), followed by DF (eight) and BA1 (two). Stat indices ranged from 0.322 to 0.590, indicating weak to moderate local fidelity. All 19 indicator genera presented relative abundances below 0.5% in their respective localities.

4. DISCUSSION

Our study aimed to describe the composition of the gut microbiota of *Chrysocyon brachyurus* across different seasonal and geographic contexts using 16S rRNA metabarcoding. Analyses revealed a microbiota dominated by the phyla Bacillota and Pseudomonadota, with alpha diversity patterns showing no differences between the dry and rainy seasons or among the three study localities (BA1, BA2, and DF). Conversely, beta diversity analysis demonstrated significant differences in bacterial community composition between localities ($p = 0.001$), with site BA2 standing out compared to BA1 and DF. The absence of seasonal differentiation in microbiota structure ($p = 0.129$) suggests that spatial factors exert a greater influence on microbial composition than seasonality.

Landscape characterization reinforces this interpretation by highlighting anthropization impacts among the study areas. Land use and land cover analyses revealed a range from relatively conserved landscapes to scenarios dominated by agricultural matrices, reflecting broad processes of Cerrado transformation, particularly associated with the expansion of the agricultural frontier in the MATOPIBA region (Agostinho et al. 2023; Magioli et al. 2025). Site BA1 exhibited a mosaic configuration, with a predominance of herbaceous-shrub and savanna vegetation, indicating greater availability and diversity of natural resources. In contrast, BA2 represented the most anthropized environment, with a high proportion of agricultural areas and fragmented native vegetation, suggesting resource limitation and increased exposure to environmental stressors. The Federal District, although composed of legally protected areas with high forest

cover, is embedded in a regional context of intense urbanization and agricultural use, which imposes anthropogenic pressures, particularly in edge areas (Andrade and Steinke 2023; Andrade 2020).

The fecal microbiota of *Chrysocyon brachyurus* presented a taxonomic profile dominated by the phyla Bacillota and Pseudomonadota, which comprise most of the bacterial community in all analyzed samples. This pattern is typical of generalist canids, in which Bacteroidota, Actinomycetota, and Fusobacteriota also contribute complementarily to the microbial structure (Barko et al. 2018; DeCandia et al. 2021; Peng et al. 2018). In the present study, the dominance of Bacillota and Pseudomonadota in *C. brachyurus* partially differs from the general pattern observed in wild canids, where Bacteroidota frequently exceeds Pseudomonadota in abundance, as seen in wolves (*Canis lupus*), golden jackals (*Canis aureus*), and black-backed jackals (*Canis mesomelas*) (Chen et al. 2022; Lapid et al. 2025; Menke et al. 2017; Wu et al. 2017). The inter-individual variation observed in the relative abundance of these phyla, including sporadic representation of Campylobacterota and Verrucomicrobiota in some samples, is also a recurrent trait in carnivores (Handl et al. 2013; Lapid et al. 2025; Peng et al. 2019; Sugden et al. 2020).

This intraspecific heterogeneity is consistent with comparative studies demonstrating high microbiota variability in carnivorous species, in contrast to the greater uniformity observed in herbivores (Zoelzer et al. 2021). The individual variation and taxonomic fluctuation observed in the maned wolf find scientific support in the species' dietary plasticity and opportunistic nature, traits that allow generalist canids to exploit heterogeneous resources in response to spatial and temporal environmental variations (Colborn et al. 2020). This adaptive capacity results in highly dynamic microbial communities, as the gut microbiome responds rapidly to changes in dietary macronutrient composition (Sugden et al. 2020). This phenomenon is evidenced by "prey-switching" behavior, common in canids that alternate between protein-rich prey and anthropogenic resources or seasonal fruits, generating selective pressures in the digestive tract that favor microbial dispersal and diversity (Sugden et al. 2020; Sugden 2019). Nevertheless, the recurrence of major phyla such as Bacillota, Pseudomonadota, Actinomycetota, Bacteroidota, and Fusobacteriota across different populations

and free-ranging conditions suggests the existence of a relatively stable functional microbial core (Chen et al. 2022; Nishida and Ochman 2018).

At the family level, Clostridiaceae, Lactobacillaceae, Streptococcaceae, Peptostreptococcaceae, and Enterobacteriaceae dominated the maned wolf gut microbiota. This set of taxa is associated with the functional capacity to process omnivorous diets and reflects the expected pattern for canids ingesting both animal and plant biomass (Silva et al. 2025). The recurrent presence of Lactobacillaceae and Clostridiaceae has been widely documented in canids, including the black-backed jackal (*Canis mesomelas*), in which the relative abundance of *Lactobacillus* and *Clostridium* varies between cattle farm habitats and hunting areas (Menke et al. 2017). Similarly, the Streptococcaceae family, primarily represented by the genus *Streptococcus*, is frequently reported in studies based on culture and sequencing of wild and domestic canids (Félix et al. 2022; Peng et al. 2019). The Enterobacteriaceae family, including the genus *Escherichia*, constitutes a recurrent component of fecal microbiota in various canid species, often associated with a stable core that can expand under stress conditions or access to anthropogenic diets (Félix et al. 2022; Zoelzer et al. 2021).

The detection of families such as Lachnospiraceae, Erysipelotrichaceae, Fusobacteriaceae, and Bifidobacteriaceae in specific maned wolf samples reinforces the functional diversity of this community. Fusobacteriaceae, particularly the genus *Fusobacterium*, has been reported as abundant in golden jackals and wolves (Lapid et al. 2025; Nardi et al. 2022), playing important roles in protein and amino acid degradation in canids (Chen et al. 2022). Conversely, the Bifidobacteriaceae family, represented by the genus *Bifidobacterium*, is classically associated with responses to probiotic interventions and maintenance of the intestinal barrier in canids (Barko et al. 2018).

The variation in the abundance of these taxa among maned wolf individuals reflects the species' dietary plasticity and opportunistic nature, where exploitation of heterogeneous resources results in dynamic microbial communities that respond rapidly to dietary changes (Rojas et al. 2023; Sugden et al. 2020). However, this microbial core is effectively preserved despite dietary differences, indicating that host phylogeny and evolutionary history impose

biological constraints that ensure the persistence of taxa essential for homeostasis (Chen et al. 2022; Colborn et al. 2020).

The absence of seasonal differentiation in the gut microbiota observed in this study finds parallels and contrasts in the literature on wild canids, reflecting the combined influence of dietary, physiological, and environmental factors on microbial community structure (Adams et al. 2021; Biles et al. 2021; Rojas et al. 2023). In strict carnivores, such as the gray wolf (*Canis lupus*), analyses indicated a relatively constant and stable microbiota composition, maintaining phyla and families associated with a diet based on animal tissues (Nardi et al. 2022). This stability pattern is often associated with the fact that the nutritional niche of these predators, composed of proteins and fats, varies little in terms of macronutrient composition throughout the year, regardless of the taxonomic identity of the prey consumed (Kohl et al. 2015; Nardi et al. 2022; Rojas et al. 2023). In contrast, generalist canids subjected to drastic variations in precipitation or utilizing anthropogenic resources may present more dynamic and heterogeneous microbiotas (Adams et al. 2021; Biles et al. 2021).

Studies on coyotes (*Canis latrans*) in temperate ecosystems demonstrate that dietary consumption patterns and trophic niche vary significantly both seasonally and spatially among populations (Colborn et al. 2020). Generalist canids exhibit high microbial plasticity in response to the exploitation of heterogeneous resources, which increased consumption of anthropogenic items rich in fibers and starches, promotes structural changes in the gut microbiota (Colborn et al. 2020; Magioli et al. 2019; Sugden et al. 2020). At a functional level, this dietary transition in canids is associated with the enrichment of taxa and metabolic pathways adapted to processing complex polysaccharides (Chen et al. 2022; Lyu et al. 2018; Sugden et al. 2020). Variations in the microbiota of red foxes (*Vulpes vulpes*) also reflect adaptation to different ecological niches, showing an abundance of genes focused on the degradation of simple and complex carbohydrates, such as chitin from arthropods, which aids in nutrient bioavailability in varied diets (Wang et al. 2022). Beyond direct dietary influence, the structure of microbial communities in omnivorous carnivores is shaped by physiological and metabolic adjustments of the host in response to the environment and resource availability (Bornbusch et al. 2023; Wang et al. 2022).

In the case of the maned wolf, the absence of seasonal differentiation in the gut microbiota observed here may reflect the maintenance of a functionally equivalent dietary base throughout the year, whereby nutritional consistency buffers fluctuations in the bacterial community (Aragona and Setz 2001; Sugden 2019). Additionally, the lower thermal amplitude characteristic of the Cerrado favors greater host physiological stability, as reduced metabolic activity and microbiome alterations are common adaptive responses to animals in environments with more rigorous thermal seasonality (Bornbusch et al. 2023; Sepulveda and Moeller 2020).

This interpretation is consistent with the review by Soares (2020) (Soares 2020a), which pointed out divergent results regarding the species' dietary seasonality, noting that some studies did not detect differences in item consumption between seasons. Thus, the stability in gut microbiota composition can be explained by the species' generalist nature through three main factors: 1) Broad dietary plasticity: the maned wolf exhibits high flexibility to exploit heterogeneous resources and adapt its consumption to alternative items according to spatial and environmental availability (de Paula 2016; Silva et al. 2025); 2) Constant availability of wolf-apple (*Solanum lycocarpum*): this item, present in 100% of samples in some studies and available year-round, ensures an uninterrupted source of complex carbohydrates, acting as a gut ecosystem stabilizer (Childs-Sanford 2005; Dietz 1984; Garcia 2016; Silva et al. 2025); and 3) Presence of arthropods and small vertebrates in both seasons: although the taxonomic identity of prey may vary, the consumption of animal-derived proteins and fats is a recurrent trait throughout the year, maintaining the functional microbial core associated with protein metabolism (Barko et al. 2018; Silva et al. 2025; Soares 2020a).

The dominance of Bacillota in both seasons reflects a pattern consistent with the consumption of complex carbohydrates and dietary fiber, as this phylum is significantly enriched in canids fed starch-rich or commercial diets (Barraza-Guerrero et al. 2021; Bragg et al. 2020). Comparative studies demonstrate that Bacillota and Bacteroidota are the dominant phyla in generalist carnivores, while strict carnivores exhibit greater representation of Fusobacteriota in response to animal tissue digestion (Nardi et al. 2022;

Youngblut et al. 2019). In the maned wolf, the consistent presence of Fusobacteriota and a moderate proportion of Bacteroidota reflect an intermediate dietary strategy. Although animal protein is a major component, the regular consumption of fruits and plant material requires additional fermentative capacities supported by taxa specialized in complex carbohydrate degradation (Lapid et al. 2025; Youngblut et al. 2019).

Stability at the phylum level contrasts with more pronounced variations at family and genus levels, reflecting functional redundancy within large taxonomic groups (Antwis et al. 2017; Barko et al. 2018). Different families and genera within Bacillota, for instance, can perform equivalent metabolic functions, such as protein fermentation and production of short-chain fatty acids (SCFAs), allowing taxonomic substitution among individuals without altering the global functional capacities of the microbial community (Antwis et al. 2017; Barko et al. 2018; Bermingham et al. 2017; Youngblut et al. 2019). Seasonal variations were most evident at the family level. Clostridiaceae showed an increase in the dry season, while Streptococcaceae demonstrated a reduction. Lactobacillaceae, Enterobacteriaceae, and Peptostreptococcaceae maintained relatively stable representations.

The increase in Clostridiaceae during the dry season deserves special attention due to this family's central role in canine protein metabolism (Bermingham et al. 2017). Members of this family are recognized for possessing robust proteolytic capabilities and are stimulated by animal protein ingestion in systems with low carbohydrate availability (Bermingham et al. 2017; Hanning and Diaz-Sanchez 2015). In strict carnivores, Clostridiaceae is identified as one of the most abundant families, positively correlating with the digestibility of animal tissue-based diets (Bermingham et al. 2017; Nardi et al. 2022).

For the maned wolf, the increase in Clostridiaceae during the dry season may reflect the higher vertebrate consumption observed during this period, when animal items become a critical component for supplying energy demands (Childs-Sanford 2005; Soares 2020a). Although commensal strains are prevalent components in healthy canid gut microbiomes, the genus *Clostridium sensu stricto 1* presents the greatest taxonomic variation in the dry season and includes

species recognized for proteolytic capacity (Bermingham et al. 2017; Hanning and Diaz-Sanchez 2015). In carnivores, *Clostridium spp.* plays a central role in protein metabolism, fermenting amino acids (such as lysine and glutamate) to produce SCFAs like acetate and butyrate through specialized biochemical pathways (Bermingham et al. 2017; Hanning and Diaz-Sanchez 2015; Vital et al. 2015). Vertebrates have high muscle protein and connective tissue content, requiring intensified proteolytic activity from the microbiota (Bermingham et al. 2017). The ability of Clostridiaceae members to perform protein and amino acid breakdown in low-carbohydrate systems confers a competitive advantage during periods of predominantly carnivorous diet (Bermingham et al. 2017; Lapid et al. 2025; Zoelzer et al. 2021).

In contrast, the relative reduction of this family in the rainy season may coincide with increased consumption of fruits and arthropods, particularly termites (Isoptera), which are recurrent dietary items in maned wolves but often underrepresented in traditional morphological analyses (Silva et al. 2025; Soares 2020a). Fruits from the Cerrado biome are rich in complex plant polysaccharides such as starch and pectin, substrates efficiently degraded by members of the genus *Bacteroides*, which are functionally versatile and recognized as key degraders of structurally diverse carbohydrates (Miranda 2010; Wang et al. 2022). Concurrently, arthropod proteins, although nutritionally rich, possess a distinct structural matrix frequently associated with chitin, which may further select for microbial communities enriched in chitin-degrading capacities, including taxa within *Bacteroides* and other specialized fermenters (Wang et al. 2022).

Lactobacillaceae was of the most abundant families found in *C. brachyurus* feces in both seasons. Within this family, *Ligilactobacillus* (formerly *Lactobacillus*) performs important functions in simple carbohydrate metabolism and lactic acid production (Hanning and Diaz-Sanchez 2015). *Lactobacillus* integrates into networks correlating with high-carbohydrate resource consumption (Sugden et al. 2020). While traditionally associated with carbohydrate-rich diets, genomic evidence shows that species in this family, such as *Lactobacillus johnsonii*, can produce proteases and peptidases acting in the final stages of protein digestion (Hanning and Diaz-Sanchez 2015). In canids, the

genus *Lactobacillus* has been reported with higher abundance in individuals fed high-protein raw meat diets compared to processed diets (Bermingham et al. 2017; Schmidt et al. 2018).

In the maned wolf, the diet comprises about 50% plant biomass, highlighted by wolf-apple fruits, whose phytochemical analyses reveal high levels of polysaccharides such as starch, pectin, and mucilage (Childs-Sanford 2005; Silva et al. 2025). Its persistence in the maned wolf microbiota regardless of the season is also justified by the year-round availability of wolf-apple, ensuring an uninterrupted source of complex carbohydrates (Childs-Sanford 2005; Garcia 2016; Silva et al. 2025). Additionally, grass consumption (Poaceae) introduces complex fibers like cellulose and hemicellulose into the digestive tract (Hanning and Diaz-Sanchez 2015; Silva et al. 2025). In parallel, the constant detection of Lachnospiraceae in the *C. brachyurus* microbiota reflects the importance of this plant fraction, as this family is recognized for its specialization in plant sugar hydrolysis and SCFA production in mammals (Zoelzer et al. 2021).

The third most abundant family, Streptococcaceae, showed a reduction in the dry season compared to the rainy period. Members of this family are widely recognized in canids for their polysaccharolytic capacities, fermenting various sugars and soluble starch (Hanning and Diaz-Sanchez 2015). In generalist and opportunistic canids, the genus *Streptococcus* is recognized for its polysaccharolytic capacities and positively correlates with the consumption of carbohydrate-rich resources, suggesting a central role in processing diets that diverge from strict carnivory (Hanning and Diaz-Sanchez 2015; Sugden et al. 2020).

The reduction of Streptococcaceae in the dry season may reflect variations in fruit availability or the diversity of plant items consumed (Sugden et al. 2020). However, dietary data for the maned wolf in the Cerrado indicate that the total fruit biomass ingested (especially wolf-apple) might not show expressive seasonality, suggesting that microbial fluctuation could be associated with changes in the chemical composition or secondary compounds of the fruits available in each season rather than just total quantity (Garcia 2016). Thus, the decrease in Streptococcaceae during the dry season likely reflects a shift from a

dietary profile richer in seasonal fruits and soluble carbohydrates to a comparatively more protein-oriented composition (Sugden et al. 2020).

Less abundant but functionally relevant genera include *Fusobacterium* and the *[Ruminococcus] gnavus* group. *Fusobacterium* is recognized for protein fermentation and butyrate production, representing a characteristic taxon of strict carnivores (Hanning and Diaz-Sanchez 2015; Zoelzer et al. 2021). In canids on high-protein diets, *Fusobacterium* becomes a dominant component (Barraza-Guerrero et al. 2021; Bermingham et al. 2017). In the maned wolf, the stability of *Fusobacterium* reinforces the maintenance of constant animal protein intake year-round (Silva et al. 2025). This reflects the evolutionary adaptation of the carnivore gastrointestinal tract, which lacks the expanded fermentation sites necessary to sustain cellulolytic bacteria populations (Silva et al. 2025; Youngblut et al. 2019).

Beta diversity analysis evidenced differences in the maned wolf gut microbiota composition among the three localities, with BA2 (Luís Eduardo Magalhães and Barreiras) standing out as the most distinct. This spatial differentiation aligns with BA2's characterization as an intensive agricultural area (57% agricultural cover, 35% forest), in contrast to BA1 (12% agriculture, 58% herbaceous-shrub vegetation) and the DF (predominantly 72% forest).

At the phylum level, global taxonomic architecture remained conserved across localities, consistent with patterns previously described for wild canids (DeCandia et al. 2021; Zoelzer et al. 2021). However, relative abundances revealed a structured ecological gradient among sites. Bacillota predominated in BA2, followed by BA1 and DF, indicating an important dominance of fermentative lineages within the intensive agricultural matrix of BA2. In contrast, Pseudomonadota (formerly Proteobacteria) reached its highest abundance in the Federal District, with intermediate values in BA1 and minimal representation in BA2. Elevated proportions of this phylum have frequently been associated with environmental disturbance, increased exposure to anthropogenic interfaces, and a higher prevalence of facultative anaerobes, suggesting that DF may represent a more ecologically unstable or human-influenced context (AlShawaqfeh et al. 2017; Biles et al. 2021). Actinomycetota was most pronounced in BA1, with lower

and comparable proportions in BA2 and DF. This configuration positions BA1 as an intermediate scenario, potentially reflecting greater environmental heterogeneity or site-specific responses to moderate levels of human interference.

The Cerrado is one of the world's most threatened biomes. Projections indicate the biome could lose 31% to 34% of its native cover by 2050 due to agricultural expansion (Strassburg et al. 2017). Intensive agricultural landscapes, like BA2 in the MATOPIBA region, impact maned wolf physiology and health by affecting resource availability (Magioli et al. 2025; Soares 2018). Environmental disturbances and the resulting physiological stress can significantly alter gut microbiota structure (Biles et al. 2021; Sugden et al. 2020; West et al. 2019). The distinct bacterial composition in BA2 revealed the most divergent bacterial profile among all sampled areas, with a markedly altered composition consistent with dysbiosis induced by habitat degradation and the environmental pressures of intensive agricultural landscapes (Bornbusch et al. 2023; Sugden et al. 2020).

This site exhibited a dominance of fermentative taxa with family Lactobacillaceae reaching 36.08% - the highest single-family abundance recorded across all sites - followed by Lachnospiraceae, Peptostreptococcaceae, Clostridiaceae and Erysipelotrichaceae. This distinctive taxonomic signature, with nearly 80% of the bacterial community concentrated in five fermentative families, represents a profound restructuring of the gut microbiome that likely reflects both the physiological costs of surviving in a highly simplified agricultural matrix and metabolic adaptations toward enhanced carbohydrate fermentation and energy extraction pathways (Hanning and Diaz-Sanchez 2015; Sugden et al. 2020). Such pronounced taxonomic simplification and the overwhelming prevalence of fermentative lineages may indicate a stress-induced reduction in microbial diversity and a functional reorganization toward anaerobic metabolism, potentially compensating for reduced dietary quality or diversity in the fragmented, resource-limited landscape surrounding this population (Biles et al. 2021).

At the genus level, BA2 reinforced its distinctive profile through the pronounced dominance of fermentative lineages, particularly those within

Lactobacillaceae, with *Ligilactobacillus* and *Lactobacillus* collectively representing nearly 30% of the community. Additional representative genera included *Clostridium sensu stricto* 1, *Peptoclostridium* and *Dubosiella*, all of which are associated with anaerobic fermentation and carbohydrate metabolism (Bermingham et al. 2017; Chen et al. 2022). BA2 was the only locality to harbor exclusive genera in the Venn diagram analysis, *Asaccharobacter* and *DNF00809*, and exhibited the highest number of indicator genera (n = 9) among all sampled areas. This taxonomic signature, characterized by both the extreme dominance of specific fermentative genera and the presence of site-exclusive taxa, reinforces that the simplified habitat matrix in BA2 imposes distinct environmental constraints that drive microbial community assembly toward a functionally specialized, stress-adapted configuration.

In urban coyotes, consumption of anthropogenic resources has been linked to structural shifts in gut microbiota, including increased overall diversity and enrichment of taxa such as *Streptococcus* and Lachnospiraceae, commonly associated with processed diets (Sugden et al. 2020). Despite this apparent increase in diversity, such microbial profiles were correlated with reduced body condition and elevated stress indicators, suggesting that higher diversity in anthropized environments does not necessarily equate to functional stability and may instead reflect dysbiosis or adaptive responses to sub-optimal nutritional resources (Biles et al. 2021; Bornbusch et al. 2023; Pilla and Suchodolski 2021; Sugden et al. 2020). Similarly, studies on black-backed jackals in Namibia identified differences in *Lactobacillus* and *Clostridium* abundance between individuals inhabiting cattle farms and those in natural reserves (Menke et al. 2017). However, host intrinsic factors, including sex, age, and body mass index, explained a significantly greater proportion of microbial variation than land use, and no consistent enrichment of pathogenic taxa was observed in livestock-associated environments (Menke et al. 2017).

Elevation of Clostridiaceae in BA2 may also reflect higher domestic protein consumption (livestock carcasses, opportunistic prey) and reduced native fruit carbohydrates, shifting metabolism toward proteolytic routes (Bermingham et al. 2017; Marcinczyk et al. 2024). The dominance of *Clostridium perfringens* in this region supports functional adjustment to anthropogenic pressures (Biles et al.

2021; Wang et al. 2022). These findings indicate that agricultural exposure does not necessarily translate into immediate health impairment, although the cumulative effects of prolonged anthropogenic pressure on gut microbial stability and host physiology remain insufficiently understood.

Lactobacillaceae and Lachnospiraceae illustrate the interface between plant carbohydrate availability and intestinal homeostasis (Sugden et al. 2020; Zoelzer et al. 2021). Lactobacillaceae ferment sugars from fruits and agricultural waste, producing lactic acid and inhibiting opportunistic pathogens (Barko et al. 2018; Hanning and Diaz-Sanchez 2015). Its abundance in BA2 could be compatible with access to cultivated fruits/grains (maize, soy) and eventually ruminant carcass ingestion. However, its dominance over butyrate producers like Lachnospiraceae could signal functional dysbiosis and reduced SCFA production essential for gut health (Sugden et al. 2020; Zoelzer et al. 2021). Lachnospiraceae tends to be more abundant in high-habitat-integrity contexts and complex carbohydrate-rich diets (Nardi et al. 2022; Vital et al. 2015). The Lactobacillaceae/Lachnospiraceae ratio in BA2 can be interpreted as an indicator of metabolic adjustment to simple carbohydrate supply in an agricultural frontier scenario (Bornbusch et al. 2023; Sugden et al. 2020).

Although landscape analyses indicate a substantial proportion of forest formations within the region in the Federal District (DF), the scenario is critical, with most of the original Cerrado vegetation already lost and approximately 30% of plant species considered locally extinct (Rodrigues et al. 2016). Urban sprawl from Brasília has been a major driver of landscape fragmentation between 1953 and 2013, progressively reducing habitat connectivity and increasing isolation of remaining natural areas (Jacob et al. 2021). This fragmentation poses severe challenges for wide-ranging species such as the maned wolf, which faces habitat suppression, reduced movement corridors, and high mortality rates from roadkills in the region (Andrade and Steinke 2023). The markedly elevated proportion of Pseudomonadota and Enterobacteriaceae observed in this locality may therefore be interpreted as a biological reflection of chronic physiological stress and sustained contact with human-modified environments.

Enterobacteriaceae serves as a clearer marker of environmental stress and health risk (Barko et al. 2018; Biles et al. 2021). In canids exposed to anthropized environments or captivity, increased Enterobacteriaceae coupled with reduced SCFA producers is a dysbiosis pattern linked to inflammation and habitat degradation (Barko et al. 2018; Biles et al. 2021; Félix et al. 2022). High abundance of *E. coli* in the Federal District (30,99%), even within protected units, is concerning. This pattern evidences these areas' porosity to peripheral pressure from urbanization and agriculture, suggesting legal designation does not ensure functional isolation. *E. coli* is a fecal contamination indicator acquired via contaminated water, domestic/human feces, or livestock carcasses (Barko et al. 2018; Félix et al. 2022). In canids from anthropized areas, *E. coli* carrying resistance genes suggests exposure to human/veterinary sources (West et al. 2019). Enterobacteriaceae expansion reinforces the need for monitoring as a potential early indicator of ecological degradation and immunological vulnerability (Biles et al. 2021; Pilla and Suchodolski 2021). The maintenance of Enterobacteriaceae and genera *Escherichia-Shigella* at moderate levels in the present study could reflect the degree of anthropization in the species' home ranges, showing significant variation between localities, with dominance significantly higher in the DF compared to other areas.

In site BA1, *Streptococcus* (specifically *S. gallolyticus*, 30.95%) was a major indicator of taxon (25.38% abundance), possibly aiding in the degradation of phenolic compounds and glycosylated alkaloids such as solasonine present in wolf-apple fruits (Childs-Sanford 2005; Hanning and Diaz-Sanchez 2015). This metabolic specialization may favor the efficient exploitation of a key resource that represents nearly half of the dietary biomass. In addition, increased *Streptococcus* abundance may also be associated with diets that are less digestible or contain higher loads of complex carbohydrates and secondary plant metabolites (Barraza-Guerrero et al. 2021; Sugden et al. 2020), as described in domestic dogs consuming lower-digestibility formulations (AlShawaqfeh et al. 2017; Félix et al. 2022). Under such conditions, *Streptococcus* spp. can proliferate due to greater substrate availability in the distal gut, reflecting altered fermentative dynamics rather than strict ecological disturbance (Hanning and Diaz-Sanchez 2015; Sugden et al. 2020). Therefore, its dominance in BA1 may

not solely indicate detoxification of fruit-derived compounds or anthropogenic exposure. At the same time, *Streptococcus* species are common inhabitants of ruminant gastrointestinal tracts and can be transferred to carnivores through carcass consumption or contact with soil and pasture (Adams et al. 2021; Bornbusch et al. 2023; Rowe and Winston 2024). Thus, the high relative abundance observed in BA1 may represent a combined signal of dietary specialization, substrate digestibility patterns, and potential environmental or domestic-associated microbial inputs (Bornbusch et al. 2023; Sugden et al. 2020).

In this study, we observed substantial variation between samples, with standard deviations in alpha diversity metrics reaching 60–70% of the mean. This high variability suggests that individual factors such as age, sex, body condition, and pathogen exposure history contribute significantly to microbial heterogeneity (DeCandia et al. 2021; Menke et al. 2017; Sugden et al. 2021). The absence of differences between BA1 and the DF indicates that factors like landscape configuration, habitat connectivity, fragment size, and edge effects, rather than simply the proportion of agricultural land, play a key role in shaping gut microbiota structure by influencing movement, resource use, and exposure to anthropogenic pressures (Adams et al. 2021; Biles et al. 2021; Paula et al. 2007; Sugden et al. 2021). Relative stability of fermentative families (Fusobacteriaceae, Clostridiaceae, Lactobacillaceae, Lachnospiraceae) combined with dietary plasticity and resource availability suggests that the maned wolf microbiota may maintain a resilient functional core (Barko et al. 2018; Silva et al. 2025; Sugden et al. 2020). This supports vital metabolic functions (SCFA production, protein/fat metabolism), which may contribute to survival in modified landscapes (Bermingham et al. 2013; Pilla and Suchodolski 2021; Thomas et al. 2011).

The gut microbiota serves as an integrative biosensor that synthesizes information from diet, pathogen exposure, physiological stress, and environmental quality into a single biological system, positioning it as a central tool for wildlife conservation and health monitoring (Adams et al. 2021; Guo et al. 2020; West et al. 2019). Specific microbial signatures observed in this study, such as elevated Enterobacteriaceae in the Federal District and the Lactobacillaceae in BA2, demonstrate how anthropogenic pressures can be quantitatively tracked

and compared across populations through fecal microbiome profiling (Biles et al. 2021; Sugden et al. 2020). Moreover, the reduction of butyrate-producing families (Lachnospiraceae and Ruminococcaceae) observed in highly disturbed sites may signal the loss of critical protective functions, including gut barrier integrity and anti-inflammatory activity, potentially predisposing individuals to clinical disease before overt symptoms manifest (Barko et al. 2018; Félix et al. 2022; Lapid et al. 2025; Mann et al. 2024). These findings underscore the diagnostic potential of microbiome-based biomarkers for early detection of dysbiosis and stress-related health deterioration in wild populations, offering a non-invasive approach to monitor environmental degradation and its physiological consequences (Gomes et al. 2024; Lapid et al. 2025; Sugden et al. 2020; Trevelline et al. 2019).

Beyond wild population monitoring, microbiome profiling holds critical implications for *ex-situ* management and translocation programs (Bornbusch et al. 2023; Bragg et al. 2020). Captive canid microbiota differs substantially from that of free-ranging individuals, typically showing reduced diversity, increased abundance of Enterobacteriaceae, and decreased representation of SCFA-producing taxa-patterns associated with artificial diets, sanitized environments, and chronic stress (Adams et al. 2021; Barraza-Guerrero et al. 2021; Bornbusch et al. 2023; McKenzie et al. 2017; West et al. 2019). Post-release microbiota convergence toward wild-type profiles depends on multiple interacting factors, including individual body condition, stress levels, and habitat quality at the release site (Bornbusch et al. 2023; Chong et al. 2019; Yao et al. 2019). Recognizing these dynamics, modern conservation guidelines increasingly propose "microbial rewilding" strategies that facilitate natural dietary transitions and promote exposure to native substrates prior to and following translocation, thereby enhancing the likelihood of successful microbiome restoration and, ultimately, individual fitness (Bornbusch et al. 2023; Guo et al. 2020; West et al. 2019).

To strengthen mechanistic understanding of how microbial community shifts translate into physiological and health outcomes, future research should integrate advanced metagenomic approaches with targeted metabolomic profiling. Specifically, shotgun metagenomics can provide gene-level functional resolution, while fecal metabolomics (particularly quantification of SCFAs and bile

acids) can directly link microbial composition to host metabolic processes and immune function (Barko et al. 2018; Knight et al. 2018; Rowe and Winston 2024). Furthermore, integrating microbiome data with clinical and hormonal biomarkers, such as fecal glucocorticoid metabolites, can elucidate the biological mechanisms through which chronic stress and habitat degradation compromise host health (Amato et al. 2013; Vynne et al. 2014). Such integrative frameworks are particularly urgent in the context of accelerating environmental change across the Cerrado, where climate change and intensified land-use transformation are projected to drive substantial native vegetation loss and hydrological alterations (de Paula 2016; Strassburg et al. 2017). These landscape-scale changes will inevitably modify resource availability and quality, with cascading indirect effects on maned wolf physiology, behavior, and population viability (Amboni 2007; Grande et al. 2020; Torres et al. 2013). In this rapidly changing scenario, integrated non-invasive fecal monitoring emerges as a strategic tool for early detection of environmental stress and health deterioration, thereby providing the empirical foundation necessary to design and implement adaptive conservation measures that can respond dynamically to ongoing and future threats (Gomes et al. 2024; Lapid et al. 2025; Sugden et al. 2020; Trevelline et al. 2019).

5. CONCLUSIONS

This study provides the first comprehensive characterization of the gut microbiota of free-ranging *Chrysocyon brachyurus*, revealing that microbial community structure is predominantly shaped by spatial factors associated with landscape heterogeneity in the Cerrado biome. The dominance of Bacillota and Pseudomonadota, combined with the observed seasonal stability, suggests a resilient functional core adapted to the maned wolf's broad dietary plasticity. However, the microbial divergence found in intensive agricultural matrices suggests that anthropization may act as an environmental filter, promoting the expansion of bacterial taxa associated with environmental stress and exposure to pathogens of domestic origin.

The integration of microbiota and landscape data establishes a robust empirical basis for the non-invasive monitoring of the health and conservation of Neotropical canid populations. Our results support the potential of the gut microbiota as a sensitive tool for evaluating the complex interface between

wildlife, agricultural activities, and ecological integrity in fragmented landscapes. Ultimately, these findings provide essential insights into management strategies aimed at promoting the coexistence and resilience of biodiversity within the Cerrado.

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CONSIDERAÇÕES FINAIS

Esta tese apresenta a primeira caracterização integrada da dieta e da microbiota intestinal do lobo-guará (*Chrysocyon brachyurus*) em populações de vida livre do bioma Cerrado, contribuindo de forma inédita para o entendimento da ecologia microbiana de canídeos neotropicais e de seus vínculos com a conservação. Ao integrar revisão sistemática, metabarcoding dietético e caracterização da microbiota intestinal em diferentes contextos espaciais e ambientais, o estudo avança além de descrições taxonômicas isoladas e propõe uma abordagem funcional para compreender a adaptação da espécie a paisagens heterogêneas.

Os resultados demonstram que a microbiota intestinal do lobo-guará apresenta uma arquitetura taxonômica compatível com o perfil de canídeos generalistas, dominada principalmente por Bacillota e Pseudomonadota, refletindo adaptações metabólicas associadas a uma dieta omnívora e altamente plástica. Apesar da variação sazonal documentada na composição da dieta, especialmente entre fontes proteicas de origem animal e vegetal, não foram detectadas diferenças sazonais consistentes na diversidade ou estrutura da microbiota intestinal. Esse padrão indica elevada estabilidade funcional ao longo do ano, sustentada por redundância metabólica e pela substituição funcional de recursos alimentares, sem necessidade de reestruturação microbiana profunda.

Em contraste, a localidade geográfica emergiu como o principal determinante da variação microbiana, evidenciando que o uso da paisagem e o grau de antropização exercem influência mais pronunciada sobre a microbiota intestinal do que a sazonalidade climática. A distinção observada na localidade inserida em matriz agrícola intensiva reforça o papel da paisagem como filtro ambiental primário, capaz de moldar assinaturas microbianas associadas à disponibilidade de recursos, ao contato com animais domésticos e à exposição a ambientes modificados. A detecção de sinais de antropização mesmo em áreas legalmente protegidas ressalta que a proteção formal não elimina completamente a influência de pressões periféricas, destacando a importância da gestão de zonas de amortecimento e da conectividade da paisagem.

Do ponto de vista teórico, os achados desta tese contribuem para o debate sobre os determinantes da estrutura da microbiota em vertebrados selvagens, sustentando a hipótese de que fatores espaciais e históricos superam variações temporais na modulação de comunidades microbianas em carnívoros generalistas. A estabilidade microbiana observada, apesar da elevada plasticidade dietética do lobo-guará, sugere que a manutenção de um núcleo funcional resiliente é um componente-chave da adaptação da espécie a ambientes sazonais e antropizados.

Sob a perspectiva aplicada, o protocolo não invasivo estabelecido para análise de microbiota fecal mostrou-se robusto, replicável e diretamente transferível para outras espécies de carnívoros ameaçados do Cerrado. A integração entre metabarcoding da microbiota e da dieta a partir das mesmas amostras amplia significativamente o potencial de monitoramento ecológico e sanitário, permitindo a identificação de biomarcadores associados à degradação ambiental e à saúde populacional. Esses resultados oferecem subsídios relevantes para programas de manejo *in situ* e *ex situ*, vigilância sanitária e formulação de estratégias de conservação baseadas em evidências funcionais.

Por fim, esta tese reforça o potencial da microbiota intestinal como ferramenta integradora na conservação da fauna silvestre, ao conectar dieta, uso da paisagem, saúde e persistência populacional. Estudos futuros que incorporem abordagens longitudinais, metagenômica funcional, metabolômica fecal e integração com dados fisiológicos e espaciais poderão aprofundar a compreensão dos mecanismos que regulam a interface hospedeiro–microbiota–ambiente, consolidando a microbiota intestinal como componente central na ecologia e conservação do lobo-guará e de outros canídeos neotropicais.