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Retornando à floresta: avaliação do papel ecológico de uma ave frugívora reintroduzida

Maria Paula Miranda Xavier Rufino
Doctor Scientiae

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Thesis submitted to the Forest Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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“A vida é pra quem sabe viver
Procure aprender a arte
Pra quando apanhar não se abater
Ganhar e perder faz parte
Levante a cabeça, amigo, a vida não é tão ruim
Um dia a gente perde
Mas nem sempre o jogo é assim
Pra tudo tem um jeito
E se não teve jeito ainda não chegou ao fim
Mantenha a fé na crença
Se a ciência não curar
Pois se não tem remédio
Então remediado está
Já é um vencedor
Quem sabe a dor de uma derrota enfrentar
E a quem Deus prometeu, nunca faltou
Na hora certa o bom Deus dará
Deus é maior!
Maior é Deus, e quem tá com Ele
Nunca está só
O que seria do mundo sem Ele?
Deus é maior!
Maior é Deus, e quem tá com Ele
Nunca está só
O que seria do mundo sem Ele?
Chega de chorar
Você já sofreu demais, agora chega
Chega de achar que tudo se acabou
Pode a dor uma noite durar
Mas um novo dia sempre vai raiar
E quando menos esperar
Clareou ô, ô, ô
Clareou, ô, ô, ô”
(Rodrigo Leite e Serginho Meriti – Clareou)

ABSTRACT

RUFINO, Maria Paula Miranda Xavier, D.Sc., Universidade Federal de Viçosa, December, 2025. **Back to the Forest: evaluating the ecological role of a reintroduced frugivore bird**. Adviser: Carlos Moreira Miquelino Eleto Torres. Co-advisers: Fabiano Rodrigues de Melo, Peter Van Der Sleen and Marielos Peña Claros.

Defaunation in tropical forests has compromised not only the persistence of threatened species but also key ecological processes such as frugivory and seed dispersal. The reintroduction of extirpated species has increasingly been used as a tool to restore population viability and the interactions disrupted by defaunation. In this work, the reintroduction of the red-billed curassow (*Crax blumenbachii*) in the Atlantic forest was evaluated as a case study to understand how the loss and return of large frugivores influence plant–animal interactions and forest regeneration. This research was carried out in a 753-ha High Conservation Value Area in Minas Gerais, southeastern Brazil, where *C. blumenbachii*, a large-bodied frugivore, was reintroduced. By combining ecological niche modeling, floristic surveys, diet characterization, and seed-germination experiments, this thesis examined how landscape and forest attributes, together with the behavior of the focal species, shape habitat selection, seed dispersal, germination, and seedling recruitment. In Chapter 1, habitat selection by the reintroduced population was assessed through ecological niche modeling. The results showed that proximity to the headquarters and slope were the main predictors of habitat suitability. This indicates that habitat use is shaped more strongly by anthropogenic influences and behavioral traits than by forest structure, and that in more human-modified areas the species may expand its potential occupancy within fragmented landscapes. In Chapter 2, the diet of *C. blumenbachii* was characterized for the first time, and its potential role in seed dispersal was evaluated. The results revealed high richness of consumed plant species, with seasonal variation and fruit and seed traits indicating selection based on availability and preference. Germination trials and estimated dispersal-distance analyses corroborated the species' potential as an effective seed disperser capable of transporting viable seeds over hundreds of meters. In Chapter 3, the effects of reintroduction on forest regeneration were evaluated by comparing plant-species composition across forest strata. Greater dissimilarity between canopy and understory assemblages was observed in areas with higher densities of *C. blumenbachii*, suggesting that, although subtle, the effects of reintroduction on the diversification of species composition in younger forest layers can be detected. However, these effects were not supported by analyses of seedling richness or the abundance of species recorded in the diet, highlighting the challenges of assessing plant–animal interaction outcomes in diverse environments and the need for long-term, multistage monitoring. Taken together, the findings from these chapters underscore the importance of integrating multiple lines of evidence; such as habitat quality, species behavior, functional traits, and community composition; to evaluate the ecological outcomes of species reintroductions. They also reveal how anthropogenic landscapes can influence both the success and the functional role of reintroduced species. More broadly, this thesis illustrates the challenges of restoring, and even detecting, plant–animal interactions, particularly in the presence of functional redundancy and diffuse ecological responses.

Keywords: Atlantic forest ; rewilding ; Cracidae; Crax blumenbachii; animal-plant interaction

RESUMO

RUFINO, Maria Paula Miranda Xavier, D.Sc., Universidade Federal de Viçosa, dezembro de 2025. **Retornando à floresta: avaliação do papel ecológico de uma ave frugívora reintroduzida**. Orientador: Carlos Moreira Miquelino Eleto Torres. Coorientadores: Fabiano Rodrigues de Melo, Peter Van Der Sleen e Marielos Peña Claros.

A defaunação em florestas tropicais tem comprometido não apenas a persistência de espécies ameaçadas, mas também processos ecológicos fundamentais, como a frugivoria e a dispersão de sementes. A reintrodução de espécies-chave têm sido uma ferramenta utilizada para recuperar viabilidades populacionais e as interações afetadas pela defaunação. Nesse trabalho, a reintrodução do mutum-do-sudeste (*Crax blumenbachii*) na Mata Atlântica foi avaliada como um estudo de caso, para compreender como a perda e o retorno de grandes frugívoros influenciam interações animal-planta e a regeneração florestal. Esta pesquisa foi conduzida em uma Área de Alto Valor de Conservação de 753 hectares em Minas Gerais, sudeste do Brasil, onde *C. blumenbachii*, um frugívoro de grande porte, foi reintroduzido. Ao combinar modelagem de nicho ecológico, levantamentos florísticos, caracterização da dieta e experimentos de germinação de sementes, esta tese examinou como as características da paisagem, da floresta e o comportamento da espécie focal influenciam a seleção de habitat, a dispersão de sementes, a germinação e o recrutamento de plântulas. No Capítulo 1, a seleção de habitat da população reintroduzida foi avaliada através de modelagem de nicho ecológico. Os resultados mostraram que, a proximidade da sede e a declividade são os principais preditores de adequação do habitat. Isso indica que, o uso do habitat é moldado por influências antrópicas e características comportamentais mais do que pela estrutura florestal, e que, em áreas mais antropizadas, a espécie pode expandir sua ocupação potencial em paisagens fragmentadas. No Capítulo 2, a dieta de *C. blumenbachii* foi caracterizada pela primeira vez, e foi avaliado seu potencial papel na dispersão de sementes. Os resultados indicaram uma alta riqueza de espécies consumidas, que variam sazonalmente, e com características de frutos e sementes que demonstram uma seleção baseada em disponibilidade e preferência. Os testes de germinação e a análise de distância de dispersão estimada corroboram o potencial da espécie como uma eficaz dispersora de sementes, capaz de transportar sementes viáveis por centenas de metros. No Capítulo 3, os efeitos da reintrodução na regeneração florestal foram avaliados, através da comparação entre a composição de espécies de plantas nos diferentes estratos da floresta. Observou-se uma maior dissimilaridade entre dossel e sub-bosque em áreas com maior densidade de *C. blumenbachii*, indicando que, embora sutis, os efeitos da reintrodução na diversificação da composição de espécies no estrato mais jovem podem ser observados. No entanto, esses efeitos não foram confirmados nas análises de riqueza de plântulas ou na abundância de espécies registradas na dieta, o que destaca os desafios de se avaliar resultados de interações animal-planta em ambientes diversos e a necessidade de monitoramento multiestágio de longo prazo. Em conjunto, as descobertas desses capítulos ressaltam a importância de integrar múltiplas linhas de evidência, como qualidade do habitat, comportamento da espécie, características funcionais e composição da comunidade, para avaliar os resultados ecológicos das reintroduções de espécies. Elas também revelam como as paisagens antropogênicas podem influenciar tanto o sucesso quanto o papel funcional das espécies reintroduzidas. De forma mais ampla, esta tese ilustra os desafios de restaurar, e até mesmo detectar, interações animal-planta, particularmente na presença de redundância funcional e respostas ecológicas difusas.

Palavras-chave: Mata Atlântica; Refaunação; Cracidae; Crax blumenbachii; Interação animal-planta

SUMÁRIO

INTRODUÇÃO GERAL

As florestas tropicais constituem os ecossistemas mais biodiversos do planeta e fornecem serviços ecossistêmicos fundamentais para o bem-estar humano e a integridade dos sistemas naturais (Borma et al., 2022; Brouwer, 2024). No entanto, muitas dessas funções dependem de processos ecológicos que têm sido severamente perturbados pela mudança do uso da terra e pelos efeitos das mudanças climáticas (Arroyo-Rodríguez et al., 2017; Diniz et al., 2023; Fu et al., 2015; Markl et al., 2012). O desmatamento e a degradação promovem a fragmentação florestal (Laurance et al., 2007; Netzel et al., 2024), reduz o tamanho e a conectividade dos fragmentos, intensifica os efeitos de borda, e compromete dispersão, fluxo gênico e qualidade do habitat (Honnay et al., 2005; Mengist et al., 2022; Vaughn et al., 2014; Zambrano et al., 2020). Em conjunto com a pressão de caça, esses processos agravam a defaunação ao isolar populações, limitar a recolonização e facilitar o acesso humano, o que leva à extinção local de espécies chave, como frugívoros de grande porte (Boiten et al., 2023; Dirzo et al., 2014; Galetti et al., 2021; Jansen et al., 2010).

A perda desses frugívoros interrompe interações mutualísticas e tróficas essenciais, como a dispersão de sementes, o recrutamento de plantas, a polinização e herbivoria (Carlo et al., 2024a; Culot et al., 2017; Galetti et al., 2013; Landim et al., 2024; Pires and Galetti, 2023; Villar and Medici, 2021), comprometendo, ao longo do tempo, processos mais amplos, como a ciclagem de nutrientes, o armazenamento de carbono e dinâmicas evolutivas (Bello et al., 2015; Galetti and Dirzo, 2013; Herrera and García, 2010; Villar et al., 2021). Portanto, os efeitos em cascata da fragmentação ameaçam não apenas a conservação da biodiversidade, mas também a resiliência e a capacidade das florestas de fornecer serviços ecossistêmicos essenciais.

Esforços substanciais tem sido realizados por empresas, organizações e governos para prevenir o desmatamento e a perda de biodiversidade (Crouzeilles et al., 2016; Lipsey and Child, 2007; Shaw et al., 2025; Valdivia et al., 2019), por meio de políticas de conservação, restauração de ecossistemas e reintrodução de espécies. Apesar dos avanços no planejamento e na implementação dessas ações, persistem lacunas importantes, especialmente sobre a perda e a recuperação de interações ecológicas em ecossistemas megadiversos e estruturalmente complexos, como as florestas tropicais (Chazdon and Brancalion, 2019; Genes and Dirzo, 2022; Tabarelli and Peres, 2002). Reintroduções fornecem oportunidades únicas para comparar sistemas com e sem espécies-chave, revelando as consequências de sua ausência e o potencial de restauração funcional com o seu retorno (Armstrong et al., 2007; McCarthy et al., 2012; Wilson et al., 2020). Na Mata Atlântica, onde resta apenas 12,4% da cobertura original, majoritariamente fragmentada em pequenos remanescentes (Ribeiro et al., 2009; SOS Mata Atlântica and INPE, 2024), reintroduções eficazes de espécies animais constituem estratégia crítica de conservação (MMA, 2025). Nesse cenário, reintroduzir espécies funcionalmente importantes em fragmentos defaunados atua simultaneamente na prevenção de extinções e na restauração de interações e processos ecológicos (Fernandez et al., 2017; Genes et al., 2019; Landim et al., 2022; Mittelman et al., 2020; Sobral-Souza et al., 2017).

Nesta tese, investigou-se as interações animal-planta e suas consequências para o funcionamento florestal após a reintrodução do mutum-do-sudeste (*Crax blumenbachii* Spix, 1825). *Crax blumenbachii* é uma ave florestal de grande porte e predominantemente terrestre (IBAMA, 2004). Como outros cracídeos, pode atuar tanto como dispersora quanto como predadora de sementes, dependendo das plantas consumidas; portanto, sua extinção local compromete processos ecológicos centrais. *Crax blumenbachii* é ameaçada sobretudo pela caça e pela perda de habitat decorrente do desmatamento e da fragmentação florestal (Rios et al., 2021), pressões agravadas por traços intrínsecos da história de vida dos cracídeos, como monogamia, maturidade sexual tardia, baixa taxa reprodutiva (máximo de dois ovos por estação reprodutiva) e necessidade de grandes áreas de vida (Araujo, 2015; Rios et al., 2021; Srbek-Araujo et al., 2012). Por esses motivos, a espécie tem sido foco de programas de reintrodução, como o Projeto Mutum, criado em 1990, em uma Área de Alto Valor de Conservação (AAVC) em Minas Gerais, que visa conservar tanto suas populações quanto as funções ecológicas que desempenha (Araujo, 2015; Bernardo et al., 2014; Brooks and Fuller, 2006).

O objetivo principal desta tese consiste em compreender como a defaunação e a reintrodução de espécies influenciam as interações animal-planta em fragmentos de florestas tropicais. Utilizando uma população reintroduzida de *C. blumenbachii* como sistema modelo, esta tese aborda os seguintes objetivos específicos:

- Avaliar como fatores ambientais e características florestais influenciam a distribuição espacial da população reintroduzida de *C. blumenbachii*;
- Caracterizar a amplitude da dieta de *C. blumenbachii*, incluindo variação sazonal, preferências por traços de frutos e sementes, e potencial de dispersão de sementes;
- Avaliar como a presença de *C. blumenbachii* influencia a composição florística por meio de comunidades de plântulas em regeneração.

A hipótese é de que a reintrodução de um frugívoro funcionalmente importante contribui para restaurar processos ecossistêmicos-chave, como a dispersão de sementes e a regeneração florestal, particularmente em áreas nas quais as condições ambientais favorecem sua ocorrência e persistência (Genes et al., 2019; Landim et al., 2024; Polak et al., 2014). Adicionalmente, a tese postula que tais efeitos de restauração se tornam detectáveis por meio de mudanças na composição das comunidades de plântulas, na dissimilaridade florística entre estratos florestais e nos padrões de uso de recursos pela espécie reintroduzida. Ao focalizar os mecanismos que vinculam a presença animal aos padrões das comunidades vegetais, esta tese aprofunda a compreensão das interações ecológicas em florestas tropicais e fornece evidências empíricas para amparar estratégias de conservação voltadas à restauração de interações bióticas em ecossistemas defaunados.

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CAPÍTULO I:

ENVIRONMENTAL DRIVERS OF A SUCCESSFUL REINTRODUCTION OF A LARGE FRUGIVOROUS BIRD IN THE BRAZILIAN ATLANTIC FOREST

Environmental drivers of a successful reintroduction of a large frugivorous bird in the Brazilian Atlantic forest

Abstract

Deforestation and fragmentation pose major threats to biodiversity and ecological processes, particularly in the Brazilian Atlantic forest, where most remnants are small and isolated. The reintroduction of large-bodied frugivores, such as the red-billed curassow (Crax blumenbachii), can help restore degraded landscapes, but requires detailed knowledge of habitat preferences. We used ecological niche modelling to evaluate the environmental drivers of habitat selection in a reintroduced population of C. blumenbachii in a 753-ha forest fragment, part of a reintroduction project active for over 30 years. Habitat suitability was best explained by landscape (e.g., proximity to the release area and head office, and distance to water resources) and topographic factors (e.g., slope and altitude), while forest structure variables had limited influence. Our results suggest that steep slopes may act as barriers to movement and limit habitat suitability for this ground-dwelling species, which is an important consideration given that many remaining Atlantic forest fragments are located on steep terrain. Conversely, the species' potential tolerance to more open or anthropogenically influenced areas, which is likely context-dependent, suggests that forest plantations could function as movement corridors when integrated with native forest. This may enhance landscape connectivity and support population viability, particularly considering the species' large home range requirements and the scarcity of continuous forest patches in the Atlantic forest. However, this potential must be interpreted with caution, as the use of plantations was not directly assessed in our study. Overall, our findings highlight the importance of accounting for topographic constraints and post-release behavior in reintroduction planning, particularly to avoid long-term site fidelity near human facilities. This study contributes empirical evidence on how a threatened frugivore responds to landscape features, offering insights for forest restoration, landscape management (e.g., corridor design), and reintroduction strategies in fragmented tropical environments.

Keywords: rewilding, ecological niche modeling, landscape connectivity, habitat selection.

1. INTRODUCTION

Understanding the ecological factors that drive animal habitat selection is fundamental to conservation science, as it informs predictions about species distributions and responses to environmental change (Boyce and McDonald, 1999; Matthiopoulos et al., 2015; Sohl, 2014). Animal habitat selection reflects the interplay between species-specific ecological requirements and the spatial availability of resources (e.g., food, water, and breeding habitats) and susceptibility risks (e.g., predation and hunting) within landscapes (Beyer et al., 2010; Hurtado et al., 2020; Stamps, 2001). Consequently, identifying the environmental variables that shape habitat use is crucial for designing effective conservation strategies, particularly for threatened species with declining populations (Bonfim et al., 2019; Finn et al., 2024).

In tropical forests, land use change has led to fragmentation and deforestation, which have emerged as major challenges to biodiversity conservation (Edwards et al., 2019; Fu et al., 2015; Matricardi et al., 2020). The Brazilian Atlantic forest exemplifies this scenario, with over 85% of its original cover lost, making it one of the most fragmented biodiversity hotspots in the world (Rezende et al., 2018; SOS Mata Atlântica, 2023). Fragmentation and deforestation affect forest structure and dynamics, reducing habitat availability and quality, and ultimately leading to declines in animal populations (Bello et al., 2015; Dirzo et al., 2014; Vaughn et al., 2014; Zambrano et al., 2020). Beyond increasing extinction risks, fragmentation and deforestation also disrupt ecological processes through limitations on species dispersal and the breakdown of biotic interactions, especially for large-bodied, forest-dependent animals (Boiten et al., 2023; Galetti et al., 2021; Holbrook and Loiselle, 2009; Pires and Galetti, 2023), such as Cracidae birds (Brooks and Fuller, 2006; Brooks and Strahl, 2000).

Cracidae birds are an ecologically important group of birds in the Atlantic forest biome, especially guans and curassows, which rely on forests for their survival and play a vital role in forest dynamics through seed dispersal and predation, thereby contributing to plant regeneration and ecosystem functioning (Brooks and Strahl, 2000; Chapter 4). The red-billed curassow (*Crax blumenbachii* Spix, 1825) is a large frugivorous bird, weighing up to 3.5 kg, endemic to the Brazilian Atlantic forest and currently classified as Endangered by the IUCN (BirdLife International, 2016). Large frugivores, including *C. blumenbachii* and other Cracidae species, are particularly vulnerable to hunting pressure and habitat loss resulting from fragmentation, due to their large body size, extensive spatial requirements and low reproductive rates (Amorim, 2014; Brooks and Fuller, 2006; Brooks and Strahl, 2000). Historically distributed widely in eastern Brazil, the species has suffered a drastic reduction in its range and numbers, with only a few remnant populations persisting in fragmented forest patches (BirdLife International, 2016). Conservation efforts, such as captive breeding and reintroduction programs, have been implemented to counteract these declines (Bernardo et al., 2011; CENIBRA, 2023a; IBAMA, 2004). Yet understanding the ecological requirements and habitat preferences of reintroduced populations remains critical to ensure their long-term persistence and guide effective management interventions (Alves et al., 2017; Bernardo et al., 2014; McCarthy et al., 2012).

In this context, our study aimed to evaluate the environmental factors and anthropogenic effects that drive the distribution of a reintroduced population of *C. blumenbachii*. To achieve this, we employed Ecological Niche Modelling (ENM) as a tool to quantify and predict habitat suitability based on key environmental variables, which encompassed topographic, forest structure, and landscape attributes. The latter included anthropogenic factors such as distances to the release site, head office, and forest edge. We hypothesized that the distribution of the reintroduced population is non-random within the forest fragment, increasing with proximity to water resources, in areas with low slopes, and farther from human disturbances. By disentangling the environmental drivers of habitat use, we seek to inform targeted conservation strategies, enhance reintroduction success, and contribute to the broader understanding of species' ecological responses in human-modified tropical forests. This research underscores the necessity of integrating habitat selection studies into conservation planning, particularly for species inhabiting biodiversity hotspots where anthropogenic pressures are intense, and conservation resources are limited.

2. METHODS

2.1. Study site

The research was carried out in a 753-ha High Conservation Value Area (HCVA) located within the Fazenda Macedônia in Ipaba, Minas Gerais, Brazil (Figure 1). The Fazenda Macedônia is a 3,000 ha property is owned by Celulose Nipo-Brasileira (CENIBRA S.A.), and contains several fragments of natural forests (totaling about 1,500 ha). The HCVA, the largest of these fragments, was designated as such due to the occurrence of endemic, rare, threatened, and endangered species, including the red-billed curassow (*Crax blumenbachii*) (CENIBRA, 2023b). This HCVA fragment includes the Fazenda Macedônia Private Reserve of Natural Heritage (RPPN; IBAMA–Ordinance No. 111, of October 14, 1994). The RPPN covers 560 ha and consists of two regions, hereafter referred to as North and South regions, that are connected by a central forest corridor of 71 ha (Figure 2; Rufino et al., 2023). Additional contiguous areas contribute another 122 ha, completing the 753-ha HCVA fragment.

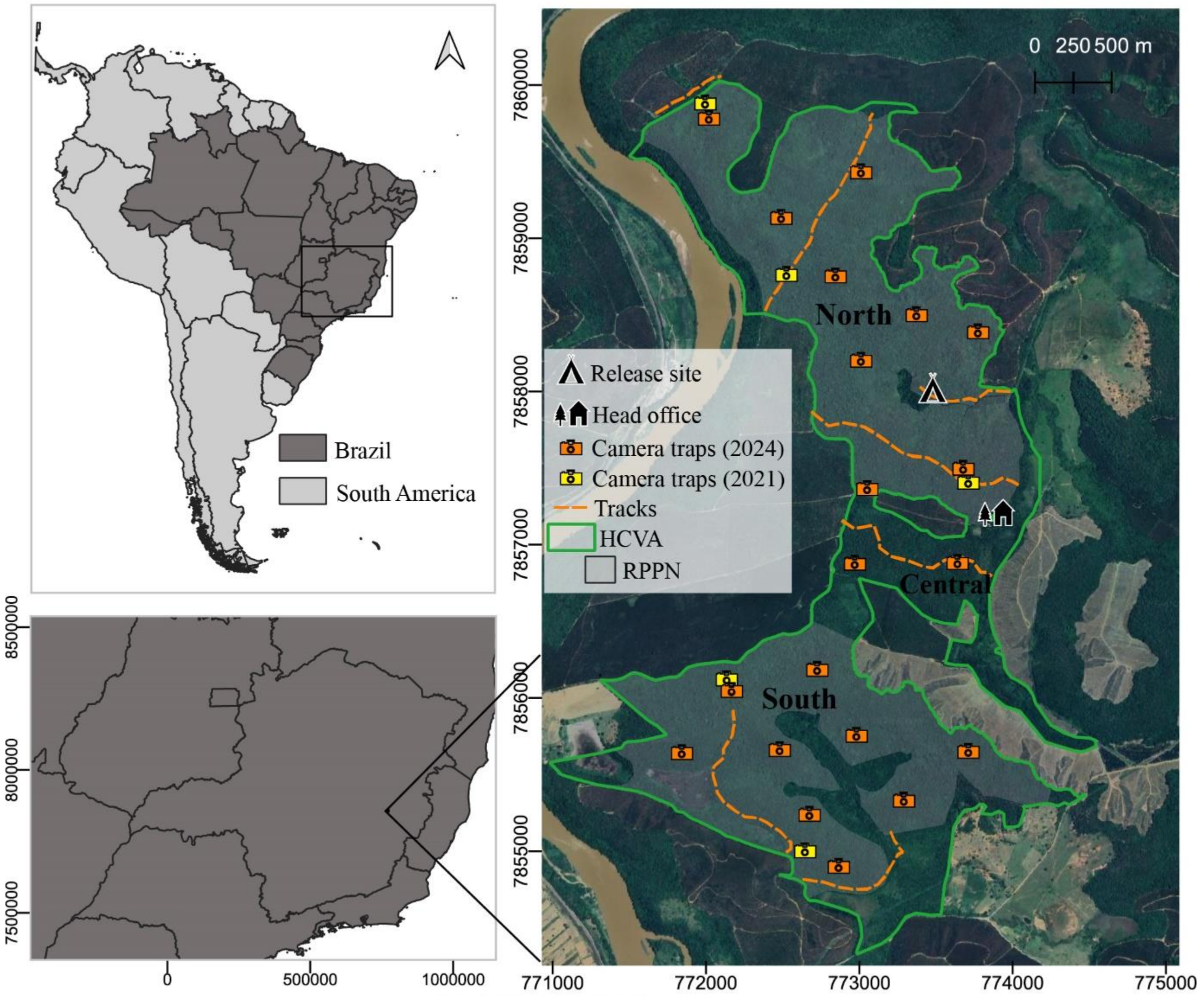

Figure 1. Location of the study area. The right panel shows the boundaries of the High Conservation Value Area (HCVA; green line), a 753-ha forest fragment within the Fazenda Macedônia, Ipaba, Minas Gerais, Brazil. The HCVA includes the Fazenda Macedônia Private Natural Heritage Reserve (RPPN; grey area). Also shown are the survey tracks used for *Crax blumenbachii* faeces sampling and demographic censuses, as well as the locations of camera traps installed in 2021 and 2024. The left panels show the location of the study site at national and continental scales. Coordinate system: SIRGAS 2000 datum, UTM Zone 23S.

The native vegetation is classified as Submontane Seasonal Semideciduous Forest (IBGE, 2012) and covers about 50% of the property, while the remaining 1,500 ha are planted with eucalyptus. The average altitude is 230 m, with an annual temperature range of 18.7–30.2 °C and mean annual precipitation of 1,440 mm, according to data from the Lagoa Perdida meteorological station operated by CENIBRA S.A.

The Mutum Project has been active at the study site since 1990, with a central focus on the reintroduction of threatened bird species (CENIBRA, 2023a), particularly *C. blumenbachii*. This species has been extirpated from over 99% of its original distribution (BirdLife International, 2016), mainly due to habitat loss from deforestation and fragmentation, as well as intense hunting pressure (Rios et al., 2021). In the region surrounding the study site*, C. blumenbachii* had been locally extinct since at least the 1970s. However, through the efforts of the Mutum Project, over 200 individuals have been reintroduced to the area to date (Table S1.; CENIBRA, 2023).

2.2. Data collection

2.2.1. Monitoring of Crax blumenbachii occurrence

Occurrence records (presence points) of *C. blumenbachii* were obtained through field monitoring using three complementary methods: (i) demographic censuses, (ii) faeces collection, and (iii) camera traps. Demographic censuses were conducted along the established tracks (Figure 1), between 7am and 11am and 2pm and 5pm, during 30 monthly field campaigns from December 2020 to June 2024, looking for both direct sightings and indirect evidence such as vocalizations and feathers. Opportunistic observations made outside the tracks, but within the study area, were also recorded. Fecal samples were collected along the same tracks during 25 monthly campaigns from January 2021 to June 2024. The geographic coordinates of all detections and faeces were recorded using GPS.

Five camera traps were initially installed between April and November 2021 (Figure 1). To enhance sampling coverage across the entire forest fragment, 20 additional camera traps were deployed between April and July 2024 (dry season), and the same were reinstalled between October and December 2024 (wet season) (Figure 1). These devices were placed in 20 of the 23 permanent forest inventory plots established in the area (Rufino et al., 2023), maintaining a minimum distance of 500 meters between them.

2.2.2. LiDAR data and environmental variables

The airborne laser scanner used was a LaserScan Optech ALTM Gemini, integrated with a GPS system and mounted on a helicopter; the average point density was approximately 21 points per square meter, with an absolute accuracy of 1 cm for a single point. We used this point cloud data to derive a set of environmental variables potentially influencing the habitat selection of *C. blumenbachii*, grouped into three categories: the topographic variables (i) altitude, (ii) aspect and (iii) slope; the forest structure variables (iv) the 90th percentile of the point cloud height (H_{90}), as a proxy for canopy height, (v) the standard deviation of point cloud height (H_{SD}), representing vertical structural complexity, (vi) the proportion of points above 2 meters relative to the total number of points (H_{CC}), used as a proxy for canopy cover; and the landscape variables given as the distances to (vii) the forest edge (D_{FE}), (viii) the head office (D_{HO}), (ix) the species' release site (D_{RS}) and (x) water resources (D_{WR}). The water resources mapping used to calculate the D_{WR} was provided by the CENIBRA S. A. and includes springs, streams, rivers, and three artificial drinking fountains, which are filled by precipitation and maintained within the forest fragment to support wildlife.

2.3. Data analysis

2.3.1. Monitoring of Crax blumenbachii occurrence

To perform the Ecological Niche Modelling (ENM), we compiled a presence database for *C. blumenbachii* that included the geographic coordinates of all occurrence records obtained through the different survey methods conducted within the forest fragment.

2.3.2. Environmental variables

All environmental variables were derived from airborne LiDAR data processed using the lidR package (Roussel et al., 2020) in R (R Core Team, 2025). Initially, ground points were classified to generate a Digital Terrain Model (DTM). This model served as the basis for calculating the topographic variables and for deriving the landscape variables in QGIS (QGIS Association, 2023). The DTM was also used to normalize the point cloud, removing terrain influence, and enabling accurate characterization of vegetation structure. From the normalized point cloud, we computed the structural metrics (H_{90}, H_{SD}, and H_{CC}) using lidR (Roussel et al., 2020) in R (R Core Team, 2025). All environmental variables were generated as raster layers with a spatial resolution of 10 meters. This resolution was chosen to ensure sufficient detail for modelling at a local spatial scale (Ficetola et al., 2014), while avoiding excessive environmental heterogeneity that could arise from using finer resolutions (e.g., 1 meter). Finer resolutions may introduce artificial variability not relevant to the biological behavior of *C. blumenbachii*, as it is unlikely that individuals would select or avoid habitat patches based on micro-scale differences of just a few meters.

We assessed collinearity among the selected environmental variables by performing Pearson correlation tests using the ENMTools package (Warren et al., 2021) in R (R Core Team, 2025). To reduce multicollinearity and enhance model interpretability, variables with high correlation coefficients ($r > 0.7$) were excluded from subsequent analyses. Based on this criterion, two variables were removed: H_{SD}, which showed a correlation of 0.93 with H_{90}, and D_{RS}, which showed a correlation of 0.86 with D_{HO} (Figure S1).

2.3.3. Ecological niche modelling

ENM was conducted using the maximum entropy algorithm (MaxEnt), which combines presence-only occurrence data with a set of environmental predictors to estimate habitat suitability for the target species (Phillips et al., 2006). We used the ENMeval package (Muscarella et al., 2014) in R (R Core Team, 2025) to optimize the model settings selection, testing all possible combinations of feature classes across a range of regularization multipliers (1, 2, 3, 4 and 5) to control model complexity and prevent overfitting (Merow et al., 2013), which generates 30 candidate models. Model selection was based on the Akaike Information Criterion (AIC), comparing models by the difference between each model's AIC and the lowest AIC observed (ΔAIC). In cases of equivalent AIC values, the model with the simplest feature combination was selected as a tiebreaker (Lee et al., 2021; Merow et al., 2013). The parameterization from the best-performing model was then used to run the final ecological niche model in MaxEnt (version 3.4.4) (Phillips et al., 2006).

Background points were randomly sampled across the study area to represent the available environmental space (Laszlo et al., 2022). Although sampling biases are a common issue in presence-only distribution models (Kramer-Schadt et al., 2013; Syfert et al., 2013), we did not consider our dataset biased for two main reasons: (i) the survey design ensured balanced sampling across the different geoenvironmental conditions within the forest fragment (Rufino et al., 2023), and (ii) the higher density of presence records in the central and north regions corresponds to the known historical distribution of the reintroduced population, which also motivated this study. To minimize potential bias due to detectability, we restricted presence data to records strictly within the forest fragment, deliberately excluding areas outside the fragment where indirect evidence (e.g., faeces and feathers) is more easily detected, thus avoiding an artificial inflation of presence records. Finally, although methods such as spatial filtering or background manipulation could be applied to mitigate clustering effects (Kramer-Schadt et al., 2013), we opted against them, as the spatial concentration of records accurately represents the current distribution of the species within the study area.

The MaxEnt model was evaluated using a 10-fold cross-validation procedure. In each iteration, nine folds were used for model training and the remaining fold for evaluation (Laszlo et al., 2022; Merow et al., 2013; Phillips et al., 2006). This process was repeated 10 times, ensuring that 90% of the data was used for training and 10% for testing in each run. The performance of the MaxEnt model was evaluated based on the area under the curve (AUC) for a receiver operating characteristic plot, which quantifies the probability that a randomly chosen presence site is ranked above a randomly chosen background site (Merow et al., 2013; Phillips and Dudík, 2008). Higher AUC values (closer to 1) indicate better model performance, with values above 0.7 generally considered useful (Huntley et al., 2006; Laszlo et al., 2022).

3. RESULTS

During the monitoring, we recorded 483 direct and indirect occurrences of *Crax blumenbachii* (Figure S2). To minimize potential bias, we excluded 354 records collected within the built-up area of the head office and along roads, where detections, especially of faeces, are easier. Thus, the analysis was conducted using a database of 129 occurrence points (Figure 2), including occurrence points in the forest surrounding the head office. We observed an irregular distribution of *C. blumenbachii* within the forest fragment, with only six occurrences recorded in the south region throughout the entire monitoring period. Since these records did not fall within exclusion zones, all were retained in the analysis (Figure 2).

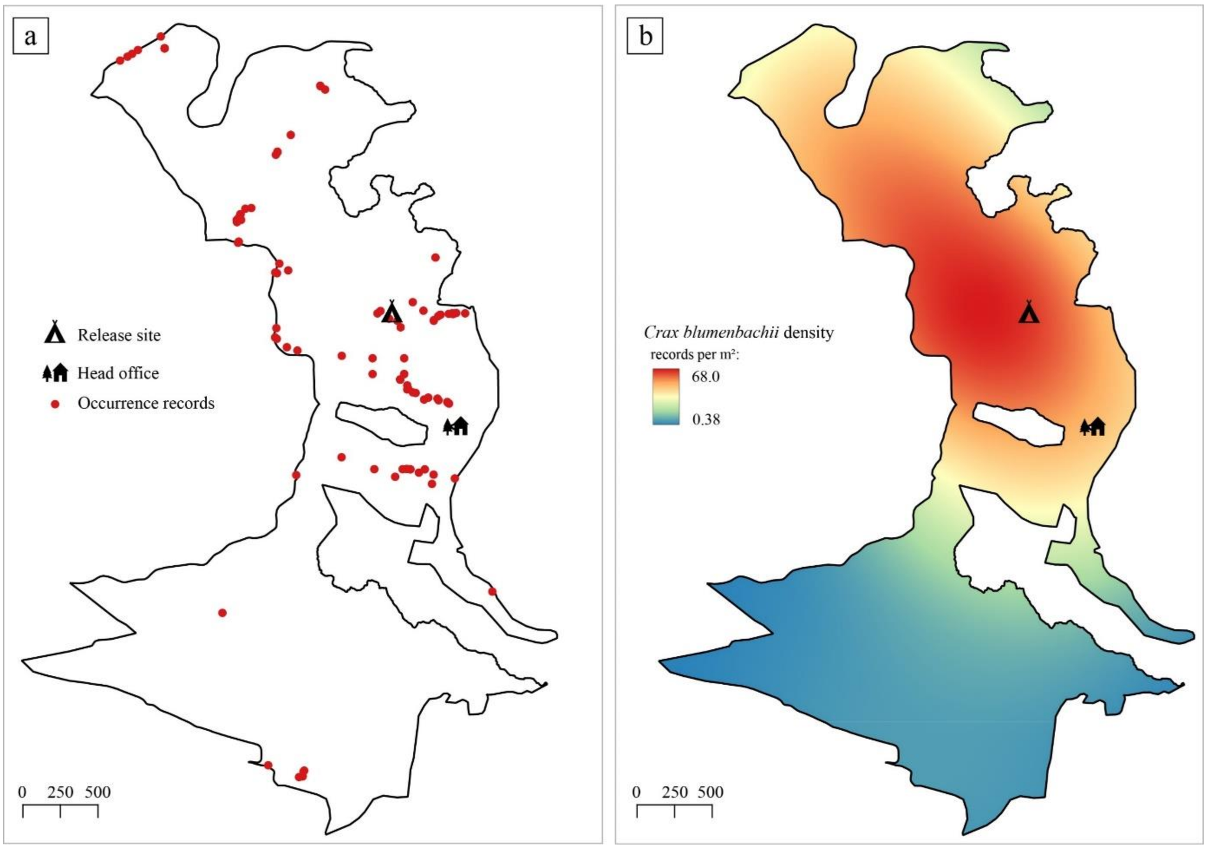

Figure 2. Spatial distribution of *Crax blumenbachii* occurrence records and kernel density estimation within the forest fragment of the study area. (A) Locations of all 129 occurrence records used in the ecological niche modelling. (B) Kernel density estimation of occurrence records, generated from the points shown in panel A, using a search radius of 2 km and an output spatial resolution of 1 m. Warmer colors indicate areas with higher relative occurrence density. The scale values should not be interpreted as absolute counts within a single square meter, but rather as an index of record distribution across the landscape.

The ecological niche modelling was conducted using eight selected environmental variables: altitude, slope, aspect, H_{90}, H_{CC} D_{FE}, D_{HO}, and D_{WR} (Figure 3). These variables, retained after the collinearity analysis, represent a combination of topographic, structural, and landscape predictors.

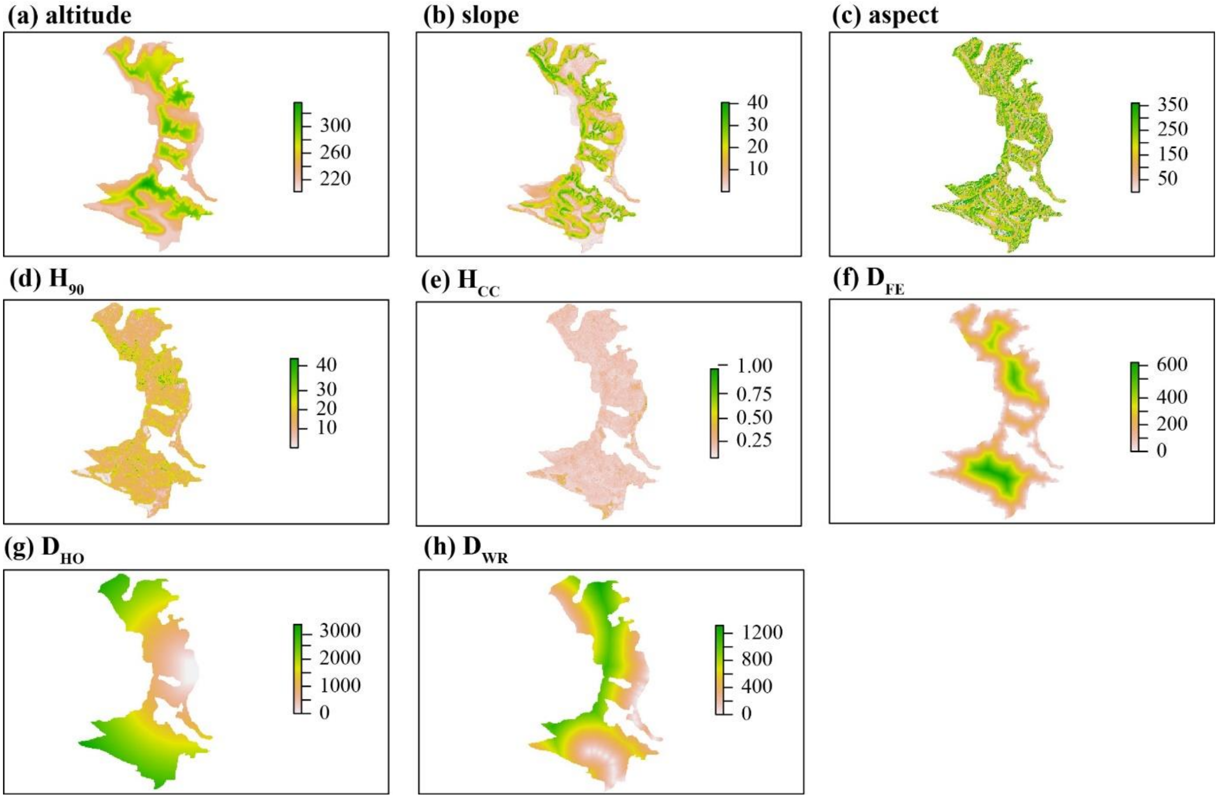

Figure 3. Environmental variables used in the ecological niche modelling for *Crax blumenbachii*. Panels (a) to (h) represent the spatial distribution of each environmental variable included in the model: (a) altitude (masl); (b) slope (degrees); (c) aspect (degrees); (d) the 90th percentile of point cloud height (H_{90}) (m), used as a proxy for canopy height and vertical structural complexity (see Figure S1 for more information); (e) the proportion of points above 2 meters relative to the total number of points (H_{CC}), as a proxy for tree abundance; (f) distance to the forest edge (D_{FE}) (m); (g) distance to the head office (D_{HO}) (m), and (h) distance to water resources (D_{WR}) (m). All raster layers were generated at a spatial resolution of 10 meters and standardized to the extent of the study area.

Among the models with the lowest ΔAIC values, the final model (LQHP5) was selected for its simplest configuration. It incorporates linear (L), quadratic (Q), hinge (H), and product (P) features, and applies a regularization multiplier of 5 (Table S2). This model demonstrated high predictive performance, with an average AUC of 0.818 during model training. In the testing phase, it achieved an average AUC of 0.797 ± 0.070 (Table S3). Among the explanatory variables, the most influential in terms of both percent contribution and permutation importance were the D_{HO}, slope, altitude, and D_{WR} (Table 1). The model indicated a strong influence of the head office on the distribution of *C. blumenbachii*; habitat suitability declined with increasing distance from the head office, up to a maximum D_{HO} value of 3,300 m (Figure 4a). Topographic variables also affected habitat suitability. Slope ranked as the second most important variable (Table 1), with suitability values falling below 0.5 at slopes greater than 15 degrees (Figure 4b). Altitude had a high percent contribution but low permutation importance (Table 1), with suitability decreasing along the altitudinal gradient (Figure 4c). Suitability increased with distance to water resources up to approximately 300 meters but subsequently declined, reaching values around 0.5 at about 1 km from water sources (Figure 4d).

Table 1. Percent contribution and permutation importance of each environmental variable in the final ecological niche model (ENM) for *Crax blumenbachii*, generated using the MaxEnt algorithm. Percent contribution represents the relative influence of each variable on model gain during the training process, while permutation importance reflects the decrease in training AUC when the variable's values are randomly permuted, normalized to percentages. Variables include: D_{HO} = distance to the head office; Slope; Altitude; D_{WR} = distance to water resources; D_{FE} = distance to the forest edge; H_{CC} = proxy for tree abundance; H_{90} = proxy for canopy height; and aspect.

Variable	**Contribution (%)**	**Permutation importance**
D_{HO}	32.9	45.0
Slope	25.2	32.5
Altitude	18.9	2.5
D_{WR}	15.8	15.0
D_{FE}	2.6	2.6
H_{CC}	2.4	1.0
H_{90}	2.1	1.2
Aspect	0.1	0.3

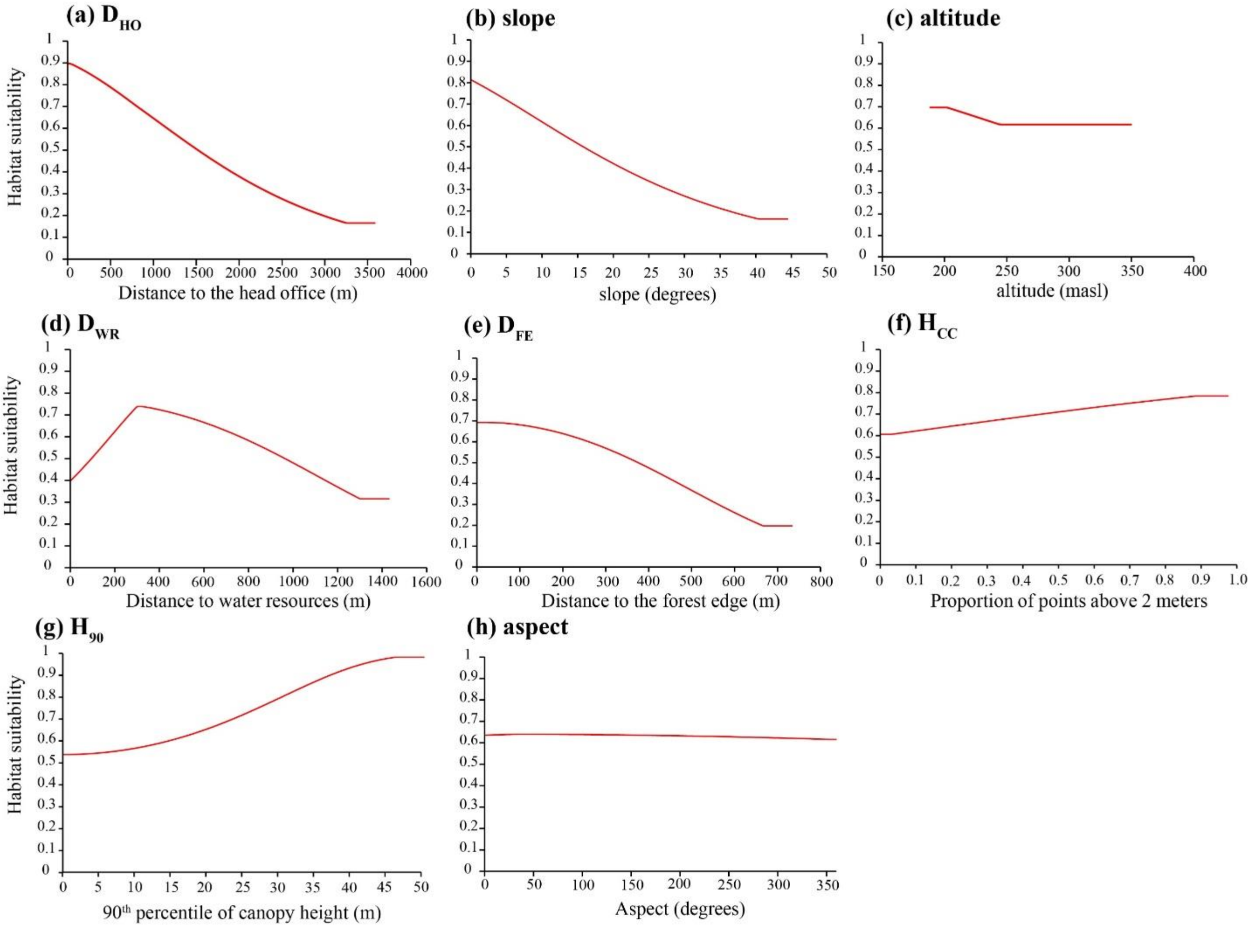

Figure 4. Response curves of the MaxEnt model generated using only each corresponding variable, illustrating their isolated effects on the predicted habitat suitability for *Crax blumenbachii*. The plots reflect the dependence of suitability on both the selected variable and on potential correlations with other predictors. Variables are reported based on Table 1. (A) Distance to the head office (D_{HO}); (B) slope; (C) altitude; (D) distance to water resources (D_{WR}); (E) distance to the forest edge (D_{FE}); (F) proportion of points above 2 meters (H_{CC}), used as a proxy for tree abundance; (G) 90th percentile of canopy height (H_{90}), used as a proxy for canopy height; and (H) aspect. Habitat suitability values range from 0 (lowest suitability) to 1 (highest suitability).

The MaxEnt model predicted habitat suitability closely aligned with the observed distribution, with the central-north regions exhibiting higher suitability compared to the south and far north areas, reinforcing the strong influence of proximity to the D_{HO} on the spatial distribution of *C. blumenbachii* (Figure 5). Nevertheless, slope modulated habitat suitability in areas located farther from the head office, where flatter terrains provide more suitable conditions despite the increased distance to the head office (Figure 3b; Figure 5).

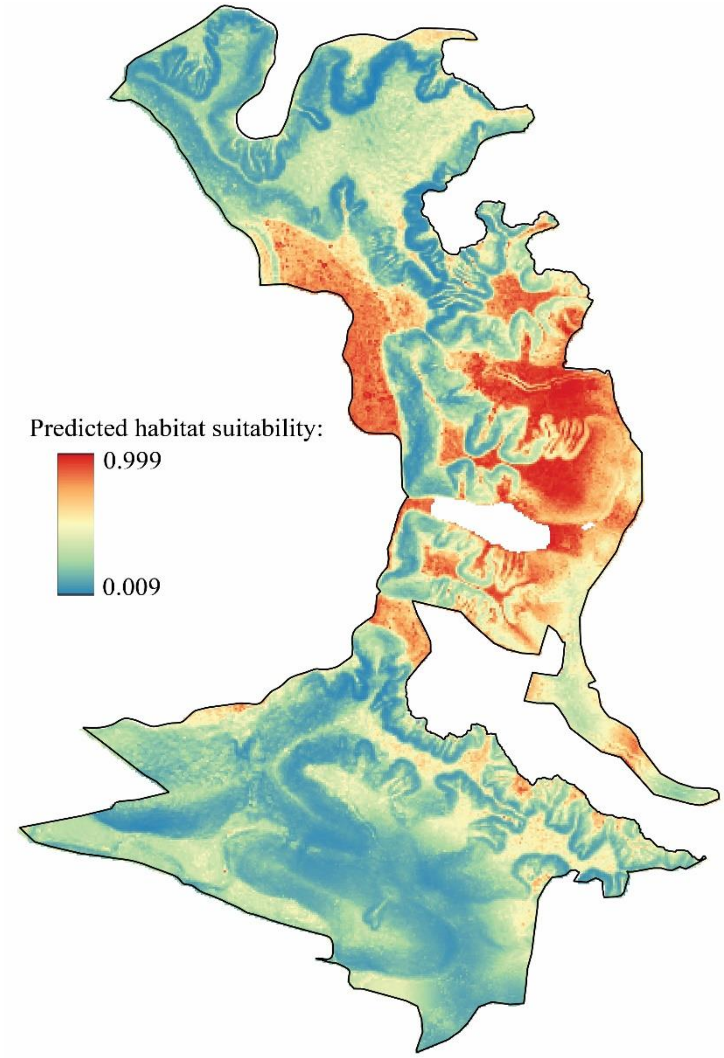

Figure 5. Predicted habitat suitability for *Crax blumenbachii* across the study area generated by the MaxEnt model. Suitability values range from 0 (lowest suitability; dark blue) to 1 (highest suitability; red). Warmer colors indicate regions with higher predicted suitability.

4. DISCUSSION

We confirmed previous observations that the reintroduced population of *Crax blumenbachii* has a heterogeneous distribution within the forest fragment (Figure 2; Araujo, 2015). Therefore, we investigated the factors that could drive habitat selection by performing ecological niche modelling. Our hypothesis was that the distribution of the reintroduced population is non-random within the forest fragment, increasing with proximity to water resources, in areas with low slopes, and farther from human disturbances. The results partially support our initial hypothesis. We found that the distribution of *C. blumenbachii* at Fazenda Macedônia is strongly shaped by landscape variables (D_{HO} and D_{WR}) and topographic factors (slope and altitude), while forest structural variables do not appear to affect habitat suitability.

4.1. Landscape variables determine the distribution of reintroduced species

We identified that distance to the head office (D_{HO}) had the strongest influence on the species' distribution (Table 1), with the suitability decreasing with D_{HO} (Figure 4a), suggesting that anthropogenic factors are shaping the species' spatial distribution within the study area. Because the head office and the release site are ~900 m apart and are highly correlated ($r = 0.93$; Figure S1), the observed pattern could be interpreted as release-site fidelity. However, we believe that this is not the case for two main reasons. First, supplementary feeding that previously occurred at the release site was stopped in 2015 as recommended by Araujo (2015); since then, provisioning has been restricted to the acclimatation stage of the birds right after release ($n = 141$ birds released since then; Table S3) complying with the new protocol. Secondly, the head office has characteristics that may make the area attractive to individuals of *C. blumenbachii* regardless of the distance to the release site. These characteristics are: (i) the presence of a fruit orchard, which serves as a source of fleshy fruits for the birds; (ii) the existence of captive individuals, which may attract free-ranging ones (Swaisgood, 2007); (iii) sporadic feeding, either unintentionally, due to spillage of food for captive birds outside enclosures, or by tourists and visitors; and (iv) protection from predators and hunters, due to the constant human presence and active surveillance. Consistent with this interpretation, another reintroduced population of *C. blumenbachii* did not exhibit site fidelity as a behavioral pattern (Bernardo et al., 2011). We therefore infer that, at this stage of the reintroduction, characteristics of the head office area, rather than proximity to the release site, are the dominant drivers of the observed concentration.

While long-distance movements post-release are a common concern in reintroduction programs, often signaling failure to settle in suitable habitat, reduced post-release fitness, and heightened mortality risk (Stamps and Swaisgood, 2007), our results highlight the opposite challenge: a persistent aggregation around the head office. Such limited displacement can reflect site fidelity or social attraction (Merkle et al., 2022) and, in turn, constrain range expansion and delay colonization of otherwise suitable habitat within Fazenda Macedônia. Given the head office characteristics listed above, we think that conspecific presence may act as an indirect cue of habitat quality (Danchin et al., 2004; Doligez et al., 2003; Muller et al., 1997; Stamps and Swaisgood, 2007), explaining the persistence of the species in the area, even after additional reintroductions. Another concern regarding the strong association between *C. blumenbachii* and the head office region is its relatively tame behavior as a consequence of imprinting in captive-bred individuals, although tame behavior has also been observed in juveniles born in the area (Araujo, 2015; Izquierdo et al., 2025). High tolerance to human presence can become a threat to this population, as it makes individuals more susceptible to hunting and predation (Geffroy et al., 2015; Griffin et al., 2000).

The distance to water resources also affected *C. blumenbachii* habitat suitability (Table 1). Suitability increases up to approximately 200 m from the nearest water source and decreases considerably beyond this threshold (Figure 4d). This unexpected pattern up to 200 m is likely related to the minimum distance at which our sampling units (camera traps or tracks) were installed in the central and north regions of the study area. Although important, D_{WR} does not appear to be a limiting factor for *C. blumenbachii* distribution within the study area. The maximum distance from any location within the forest fragment to the nearest water source was approximately 1.2 km, and habitat suitability in relation to D_{WR} only drops below 0.5 beyond roughly 1 km (Figure 4d). However, it is possible that, at larger spatial scales or in areas where the hydrographic network is more heterogeneously distributed, D_{WR} may become a more determinant factor, as observed for different cracid populations, particularly during the dry season (Bernardo et al., 2011; Hill et al., 2008; Leite, 2020; Setina et al., 2012).

4.2. Steeper slopes may limit the expansion of the area used by reintroduced species

Topographic variables were also identified as key drivers of *C. blumenbachii* distribution, with slope being the second most important predictor, with both high contribution and permutation importance (Table 1). Although altitude showed considerable contribution (Table 1), the narrow altitudinal range within the study area (approximately 200–330 m) suggests that the species' occurrence pattern is more likely associated with the steepness of slopes than with altitude per se, which may explain the low permutation importance of altitude. Additionally, slope appears to be a physical barrier that prevents the distribution of *C. blumenbachii* towards the south region, at least in terms of movement within the forest fragment. Although the south region contains considerably flat habitat patches (Figure 3b), the narrow corridor connecting the central and south regions ends in highly steep slopes, which may prevent access by *C. blumenbachii*.

The effect of slope identified in our analysis is consistent with the species' ecological traits. *Crax blumenbachii* is a large-bodied bird with predominantly terrestrial habits, spending most of its time foraging on the ground (IBAMA, 2004; Srbek-Araujo et al., 2012). In addition, it has low flight capacity and makes only short-distance flights. Therefore, the reduction in habitat suitability in steeper areas (>15°) (Figure 3b) is probably related to the high energy expenditure required for uphill movement, which increases considerably with body weight (Halsey and White, 2017). This constraint may be even more pronounced in the understory of tropical forests, where dense vegetation, such as shrubs and lianas, further hinders locomotion.

Finally, the predicted habitat suitability summarizes the requirements of habitat selection by *C. blumenbachii* (Figure 5), with higher suitability values observed in areas more affected by human presence, particularly around the head office, likely reflecting behavioral traits shaped by the reintroduction process. Moderate suitability was also found in flat terrain areas farther from the head office region. This suggests that although anthropogenic factors associated with reintroduction practices exert a lasting influence, certain environmental features, such as topographic gradients, also play a relevant role in shaping the potential distribution of the species across the landscape.

4.3. Forest structure variables do not affect the species distribution

Surprisingly, forest-related variables were not major determinants of habitat suitability for *C. blumenbachii*, with D_{FE}, H_{CC}, and H_{90} each contributing only about 2% to our model (Table 1). Although of limited influence, the response of *C. blumenbachii*'s habitat suitability to distance from the forest edge (Figure 4e) provided important insights. The negative relationship between these variables indicates that, despite being considered a typically forest species (BirdLife International, 2016; IBAMA, 2004), the reintroduced population tolerates more open habitats, possibly as an adaptive response to anthropogenic influences. Typically, large-bodied frugivorous tend to prefer denser forests as a defense mechanism, especially against hunting pressure (Ferreiro-Arias et al., 2024; Martínez-Penados et al., 2024; Scabin and Peres, 2021). However, in Fazenda Macedônia, edge use may result from the protective effect of human presence, which discourages illegal hunting activities. The area is regularly frequented by company staff, security personnel, visitors, and environmental researchers, whose presence likely reduces poaching activities. A similar pattern was observed in another reintroduced population, where edge use was associated with greater availability of lianas and vines for nest construction, as well as insects that supplement the diet (Bernardo, 2010). These findings suggest that the actual habitat used by *C. blumenbachii* at Fazenda Macedônia may exceed the forest area assessed in this study (753 ha), given that the property includes eucalyptus plantations connected to other forest fragments. It is likely that *C. blumenbachii* uses these plantations as movement corridors between fragments, an assumption supported by occasional but unrecorded field observations of the species within eucalyptus stands. Evidence of habitat flexibility was also found in a wild population of *C. blumenbachii,* where a preference for open forest habitats was apparent (Alves et al., 2017). Other cracid species also reportedly use forest plantations, pastures, and cultivated areas when connected to native forest fragments (Bernardo, 2010; Bertsch and Barreto, 2008; Londoño et al., 2007; Muñoz et al., 2007; Ríos et al., 2021, 2005; Sick, 1970).

Forest structure variables (H_{CC} and H_{90}) were not decisive for *C. blumenbachii* distribution (Table 1). However, habitat suitability increased with both H_{CC} and H_{90} (Figs. 4f, 4g), with minimum suitability values around 0.6, reinforcing the before mentioned tolerance to more open habitats. This finding highlights the potential role of forest plantations as functional corridors between fragments, since structural forest complexity does not appear to be essential for the species' occurrence. Moreover, plantations are typically established in flatter areas to facilitate mechanized management, and their reduced understory may facilitate more energy-efficient movement (Halsey and White, 2017).

4.4. Outlook and implications

Our study evaluated the environmental factors influencing habitat selection by *C. blumenbachii* and offer valuable insights for conservation strategies, not only for *C. blumenbachii* but also for other cracid species inhabiting tropical forests, particularly in landscapes characterized by severe fragmentation. Although our findings are based on a single reintroduced population, there are some wider implications emerging. First, steep slopes emerged as a limiting factor for habitat suitability, which is particularly relevant given that many remaining Atlantic forest fragments and potential ecological corridors are situated in areas of steep topography (Brancalion et al., 2016; Ribeiro et al., 2011). These areas are generally less suitable for agricultural use due to the challenges of mechanization (De Jong, 2025; Freitas et al., 2010) and are legally protected under Brazilian law (for slopes >45°) to prevent soil erosion and landslides (Brancalion et al., 2016). This highlights the difficulty of identifying suitable reintroduction sites for large-bodied, ground-dwelling species and underscores the importance of considering topographic constraints when prioritizing areas for restoration and connectivity, particularly for large terrestrial birds with limited flight capacity.

Second, the apparent tolerance of *C. blumenbachii* for more open or anthropogenically influenced habitats suggests a potential role for forest plantations, such as eucalyptus, in facilitating movement across fragmented landscapes. Although such environments likely do not fulfill the resource requirements of the species, such as dietary needs (as discussed in Chapters 2 and 4) or reproductive suitability (IBAMA, 2004), they may function as steppingstones when integrated with native forest remnants. This could support species dispersal, maintain genetic variability, and promote seed dispersal among forest fragments (Diniz et al., 2023; Wang et al., 2022). Home range size is generally linked to body size, posing additional challenges for large-bodied species. For instance, the estimated home range for *C. blumenbachii* is approximately 125 ha (Bernardo et al., 2011), while the minimum forest fragment size required to sustain a viable population is around 3,141 ha (Bonfim et al., 2019). Such extensive, continuous habitats are increasingly rare in the highly fragmented landscapes of the Brazilian Atlantic forest, where most remnants are smaller than 50 ha (Ribeiro et al., 2011; Scarano and Ceotto, 2015), underscoring the importance of connectivity as a key component of species recovery and ecological function restoration. However, we acknowledge that this potential use of forest plantations was not explicitly tested in our study and should therefore be interpreted with caution. Future research is needed to verify their actual effectiveness as movement corridors. Furthermore, the utility of open or anthropogenic habitats is likely context-dependent, particularly reliant on the absence or effective control of hunting pressure, which could otherwise negate the benefits of increased landscape connectivity (Bonfim et al., 2019; Rios et al., 2021). Finally, it is important to reiterate that while forest plantations may support connectivity, they do not replace the critical ecological functions and biodiversity supported by native forests, which remain essential for the long-term conservation of *C. blumenbachii*, other forest-dependent species, and overall ecosystem integrity (Diniz et al., 2023; Wang et al., 2022).

Our findings reinforce key paradigms in reintroduction ecology, particularly the importance of carefully selecting release sites to avoid long-term site fidelity that may constrain dispersal. This is especially relevant for charismatic species with the potential of becoming tame, where proximity to humans may shape behavior and create unintended dependencies. By advancing the understanding of habitat selection and movement ecology of a reintroduced population of *C. blumenbachii*, this study offers practical insights for planning reintroductions and managing endangered frugivores in fragmented tropical landscapes. These insights can inform habitat restoration, connectivity enhancement, and the development of conservation strategies that reconcile species persistence with human-modified environments, ultimately contributing to more effective biodiversity conservation in the Atlantic forest and similarly degraded ecosystems.

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Supporting Information

Four of the variables initially considered for modelling exhibited high collinearity with others. Hsd and H_{90} were highly correlated (Pearson correlation coefficient, r=0.93) with (Figure S1). While Hsd is a statistical descriptor derived from the LiDAR point cloud, H_{90} is directly extracted from the point cloud. Therefore, we opted for keeping H_{90} in the analysis. D_{RS} and D_{HO} were also highly correlated (r = 0.86) (Figure S1). We chose to retain D_{HO}, as it is likely to exert a stronger influence on the spatial distribution of *C. blumenbachii* for three main reasons: (i) the presence of a fruit tree orchard, (ii) the maintenance of *C. blumenbachii* breeding pairs in captivity, and (iii) occasional supplementary feeding at this location.

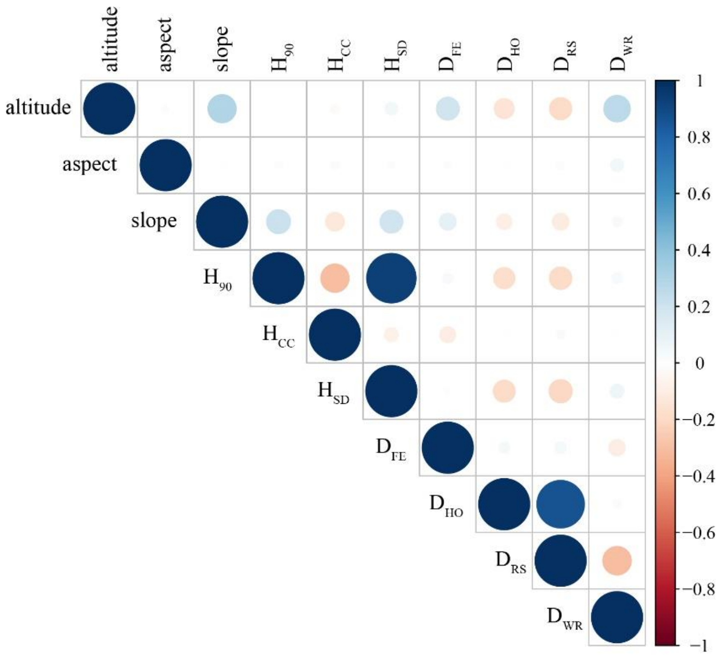

Figure S1. Pearson's correlation matrix between (a)biotic variables. Dark blue circles indicate positive correlations, while dark red circles indicate negative correlations; both the size of the circles and the color intensity represent the strength of the correlation. The matrix includes the topographic variables: (i) altitude, (ii) aspect and (iii) slope; the structural variables (iv) the 90th percentile of the point cloud height (H90), as a proxy for canopy height, (v) the standard deviation of point cloud height (Hsd), representing vertical structural complexity, (vi) the proportion of points above 2 meters relative to the total number of points (Hcc), used as a proxy for canopy cover. It also includes the landscape variables, represented by the distances to (vii) the forest edge (DFE), (vii) the head office (DHO), (viii) the species' release site (DRS) and (ix) water resources (DWR).

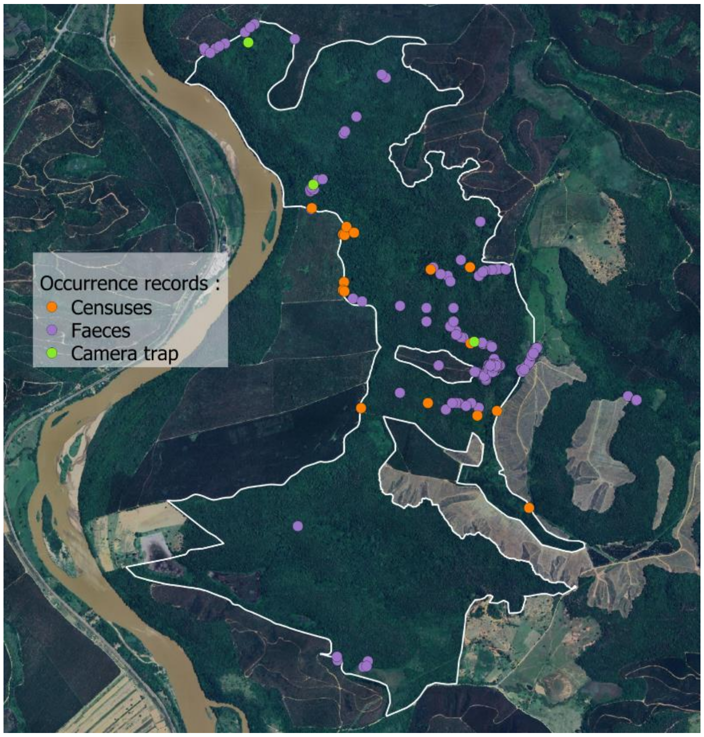

Figure S2. Spatial distribution of *Crax blumenbachii* occurrence records within the forest fragment of the study area. Orange dots represent direct observations obtained through censuses, purple dots indicate records from fecal samples, and green dots correspond to camera trap detections. The white line delineates the forest fragment boundary used for ecological niche modelling and habitat analysis.

Table S1. Number of *Crax blumenbachii* individuals reintroduced per year in Mutum Project in Fazenda Macedônia, Brazil.

Year	***Crax blumenbachii*** **individuals reintroduced**
1990	30
1991	37
2007	5
2010	6
2015	60
2017	40
2018	41
Total	**219**

Table S2. Results of candidate model evaluations. The table shows the performance metrics for 30 candidate models, each defined by a combination of feature classes (fc: linear—L, quadratic—Q, hinge—H, product—P, threshold—T) and regularization multiplier (rm: values ranging from 1 to 5). Model performance was assessed based on the training area under the receiver operating characteristic curve (AUCtrain), Akaike's Information Criterion corrected for small sample sizes (AICc), and ΔAIC (the difference in AIC relative to the best model). Models using only hinge features (H), or hinge combined with low regularization (rm = 1), did not return AICc and ΔAIC values (NA), likely due to overfitting or convergence issues that prevent reliable AICc computation in ENMeval. The best model selected for final predictions was LQHP5 (in bold), which exhibited the lowest ΔAICc among the simplest feature combinations, while maintaining a high AUCtrain.

Model	fc	rm	$\text{AUC}_{\text{train}}$	AICc	ΔAIC
L1	L	1	0.781	1,755,908	24,458
LQ1	LQ	1	0.811	1,738,757	7,306
H1	H	1	0.873	NA	NA
LQH1	LQH	1	0.867	NA	NA
LQHP1	LQHP	1	0.881	NA	NA
LQHPT1	LQHPT	1	0.908	3,619,900	1,888,450
L2	L	2	0.781	1,756,267	24,816
LQ2	LQ	2	0.805	1,740,805	9,355
H2	H	2	0.852	NA	NA
LQH2	LQH	2	0.839	1,897,787	166,337
LQHP2	LQHP	2	0.849	1,898,620	167,170
LQHPT2	LQHPT	2	0.854	1,870,733	139,283
L3	L	3	0.782	1,756,822	25,372
LQ3	LQ	3	0.801	1,740,693	9,242
H3	H	3	0.839	2,634,404	902,954
LQH3	LQH	3	0.818	1,772,268	40,818
LQHP3	LQHP	3	0.83	1,748,576	17,126
LQHPT3	LQHPT	3	0.83	1,761,329	29,879
L4	L	4	0.782	1,757,580	26,130
LQ4	LQ	4	0.797	1,746,102	14,652
H4	H	4	0.822	2,183,747	452,296
LQH4	LQH	4	0.812	1,741,130	9,679
LQHP4	LQHP	4	0.824	1,737,772	6,322
LQHPT4	LQHPT	4	0.824	1,741,046	9,596
L5	L	5	0.782	1,758,545	27,094
LQ5	LQ	5	0.793	1,751,629	20,179
H5	H	5	0.811	2,201,907	470,457
LQH5	LQH	5	0.807	1,736,313	4,862
LQHP5	**LQHP**	**5**	**0.818**	**1,731,450**	**0**
LQHPT5	LQHPT	5	0.818	1,731,450	0

Table S3. Summary of performance metrics for the 10 replicates of the selected best model (LQHP5). For each replicate, the number of training samples, number of iterations until convergence, training area under the receiver operating characteristic curve (AUCtrain), number of test samples, test gain, test area under the curve (AUCtest), standard deviation of AUCtest (AUCSD), and the number of background points used in model calibration are shown. The average values across all replicates are presented in the last row.

Individual model	**Training samples**	**Iterations**	**AUC$_{\text{train}}$**	**Test samples**	**Test gain**	**AUC$_{\text{test}}$**	**AUC$_{\text{SD}}$**	**Background points**
1	73	220	0.8135	9	0.8371	0.8425	0.0738	10065
2	73	260	0.8196	9	0.5431	0.7699	0.0892	10063
3	74	280	0.8087	8	10.528	0.8787	0.0526	10065
4	74	260	0.8103	8	0.9425	0.8825	0.0373	10064
5	74	380	0.8315	8	0.0757	0.661	0.0924	10065
6	74	280	0.8126	8	0.9927	0.8492	0.0865	10065
7	74	180	0.8096	8	10.334	0.889	0.0528	10065
8	74	280	0.8213	8	0.6531	0.759	0.1028	10065
9	74	280	0.8425	8	-0.3641	0.5774	0.093	10067
10	74	180	0.8121	8	11.629	0.8655	0.0643	10064
Average	73.8	260	0.8182	8.2	0.6929	0.7975	0.0745	10064.8

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CAPÍTULO II:

DIET COMPOSITION OF THE RED-BILLED CURASSOW (*Crax blumenbachii*) AND ITS ROLE AS SEED DISPERSER IN THE ATLANTIC FOREST: CONSERVATION INSIGHTS

Submetido para publicação

Diet composition of the red-billed curassow (*Crax blumenbachii*) and its role as seed disperser in the Atlantic forest: conservation insights

Abstract

Forest fragmentation and defaunation are major drivers of biodiversity loss, particularly in tropical regions. Animal reintroduction can help restore ecological functions, as 70–90% of tree species rely on animal-mediated seed dispersal. The Cracidae family comprises key Neotropical seed dispersers but has been severely impacted by habitat loss and hunting. In the Brazilian Atlantic forest, efforts such as reintroduction programs seek to restore populations of five Cracidae species. However, many aspects of their ecology and natural history, particularly dietary composition, remain poorly understood, hindering effective management strategies. We assessed, for the first time, the diet of *Crax blumenbachii*, one of the last remaining large-bodied frugivorous bird in this biome. Fecal samples from a reintroduced population were analyzed from January 2021 and June 2024 in a 753-ha forest fragment in Minas Gerais. Our findings reveal that it have a diverse diet, consuming 80 plant species, mainly fleshy-fruited plants with fruit traits (type, colour, consistency, dehiscence, size) distinct from those most available in the fragment. During the dry season, when fruit availability decreased, the species expanded its dietary breadth, indicating generalist behavior. We also found that *C. blumenbachii* disperses seeds over long distances, with most seeds deposited 300–500 m from ingestion point. Some dispersed seeds exhibited high germination rates (up to 73%). Our results suggest that the loss of *C. blumenbachii* could significantly impact plant recruitment across a wide range of species, reinforcing its ecological importance in maintaining forest diversity and dynamics.

Keywords: reintroduction, tropical forest, defaunation, dispersal distance, frugivorous, Brazil, Cracidae.

1. INTRODUCTION

Forest fragmentation and defaunation are major drivers of biodiversity loss, particularly in tropical regions, where they cause substantial alterations in environmental conditions and, consequently, in ecological interactions (Bovendorp et al., 2019; Ma et al., 2023; Michel et al., 2020; Valiente-Banuet et al., 2015). Fragmentation generates edge effects, reduces genetic diversity, and compromises habitat quality, while hunting pressure further intensifies defaunation, often resulting in the loss of key species (Dirzo et al., 2014; Jansen et al., 2010; Pfeifer et al., 2017; Vaughn et al., 2014; Zambrano et al., 2020). Ecological restoration of forests and animal species reintroductions are initiatives aiming to restore ecosystem functioning (Crouzeilles et al., 2016; Marino et al., 2024; Shaw et al., 2025; Strier et al., 2021).

Animal reintroduction projects often prioritize flagship species that represent broader groups, which helps raising public awareness and enhances our understanding of the ecological roles of both target species and their associated species (Genes et al., 2019; Gibbs et al., 2008; Mittelman et al., 2020; Seddon et al., 2007). Such an approach is particularly valuable in highly biodiverse systems, where assessing each species individually is impractical (Flyvbjerg, 2006; Holling, 1992). In tropical forests, animal reintroduction projects are very important, as around 70-90% of tree species rely on animal-mediated dispersal, with birds being particularly effective due to their abundance and mobility across fragmented landscapes (Almeida-Neto et al., 2008; Chaves et al., 2018; Corlett, 2017). Birds of the Cracidae family, including chachalacas, guans, and curassows, are key seed dispersers in the Neotropics but have been severely impacted by habitat loss and hunting (IUCN, 2025; Pacheco et al., 2021). Their decline threatens forest regeneration, as these large-bodied (up to 3.5 kg (BirdLife International, 2016)), predominantly terrestrial, birds consume a wide variety of fruits, including large-seeded species, which are particularly vulnerable to disperser loss (Galetti et al., 2021; Kattan et al., 2016; Wotton & Kelly, 2011).

In the Brazilian Atlantic forest, the five threatened Cracidae species are included in the National Action Plan (PAN) for Birds of the Atlantic forest, which seeks to restore populations through collaborative conservation efforts, including reintroduction projects (ICMBio, 2023). However, many aspects of their ecology, particularly dietary composition, remain poorly understood, hindering effective management. For instance, in the Atlantic forest, diet studies are scarce, with *Aburria jacutinga* Spix, 1825 being the only species systematically analyzed (Galetti et al., 1997). The red-billed curassow (*Crax blumenbachii* Spix, 1825) (Figure 1) is a species, recognized as one of the five Brazilian bird species saved from extinction through conservation efforts (Bolam et al., 2021), yet its diet remains poorly documented, with only two brief field observations available (Sick, 1970; Teixeira & Snow, 1982).

Food availability and food selection directly influence survival and population persistence (Briga et al., 2017; Oro & Furness, 2002). This is particularly true for reintroduced populations, which often consist of individuals raised in captivity with limited exposure to natural food resources and foraging conditions (Armstrong & Seddon, 2008; Parish & Sotherton, 2007; Sarrazin & Barbault, 1996). Diet variations in frugivorous species often result from the interaction of intrinsic preferences with spatial and seasonal fruit availability, fruit and seed traits, and the presence of alternative food sources (Araujo et al., 2020; Blendinger & Villegas, 2011; Carlo, 2005; Sasal & Morales, 2013; van Schaik et al., 1993). Thus, understanding dietary breadth and food preferences in reintroduction programs is essential for selecting suitable habitats to increase reintroduction success and informing adaptive management strategies that support long-term population establishment (Leite, 2020; Moegenburg, 2002; Walker, 2007). In the case of the *C. blumenbachii* population reintroduced by the Mutum Project since 1990 at Fazenda Macedônia, Minas Gerais, Brazil (CENIBRA, 2023), understanding dietary preferences also provides key insights into plant-animal interactions, species' adaptive capacity to environmental changes, and potential resource limitations (Atkins & Travis, 2010; Evans & Moustakas, 2018; Mohammed et al., 2018).

In this study, we assessed, for the first time, the diet composition of *C. blumenbachii*, the last remaining large-bodied frugivorous bird in the Brazilian Atlantic forest (Bonfim et al., 2019). We analyze fecal samples from a reintroduced population of *C. blumenbachii* between January 2021 and June 2024. We hypothesize that *C. blumenbachii* has a diverse diet, consuming a wide range of fruits and seeds, and acting as an important seed disperser, potentially over long distances. We also hypothesize that this bird exhibits seasonal preference for a selected group of species, which varies with resource availability seasonality. Finally, we expect that it contributes to high germination rates of consumed seeds for some species, potentially influencing plant recruitment and forest regeneration.

2. METHODS

2.1. Study site and target species

The research was carried out in a High Conservation Value Area (HCVA) located within the Fazenda Macedônia in Ipaba, Minas Gerais, Brazil (Figure 1). This forest fragment, covering 753-ha and owned by Celulose Nipo-Brasileira (CENIBRA S.A.), is classified as

Submontane Seasonal Semideciduous Forest (IBGE, 2012), and it is mostly covered by old secondary forests. The climate is humid tropical with dry and rainy seasons (climate of type Aw according to the Köppen classification (Alvares et al., 2013). The mean annual precipitation is 1,440 mm, with a dry season (< 100 mm) from April to September.

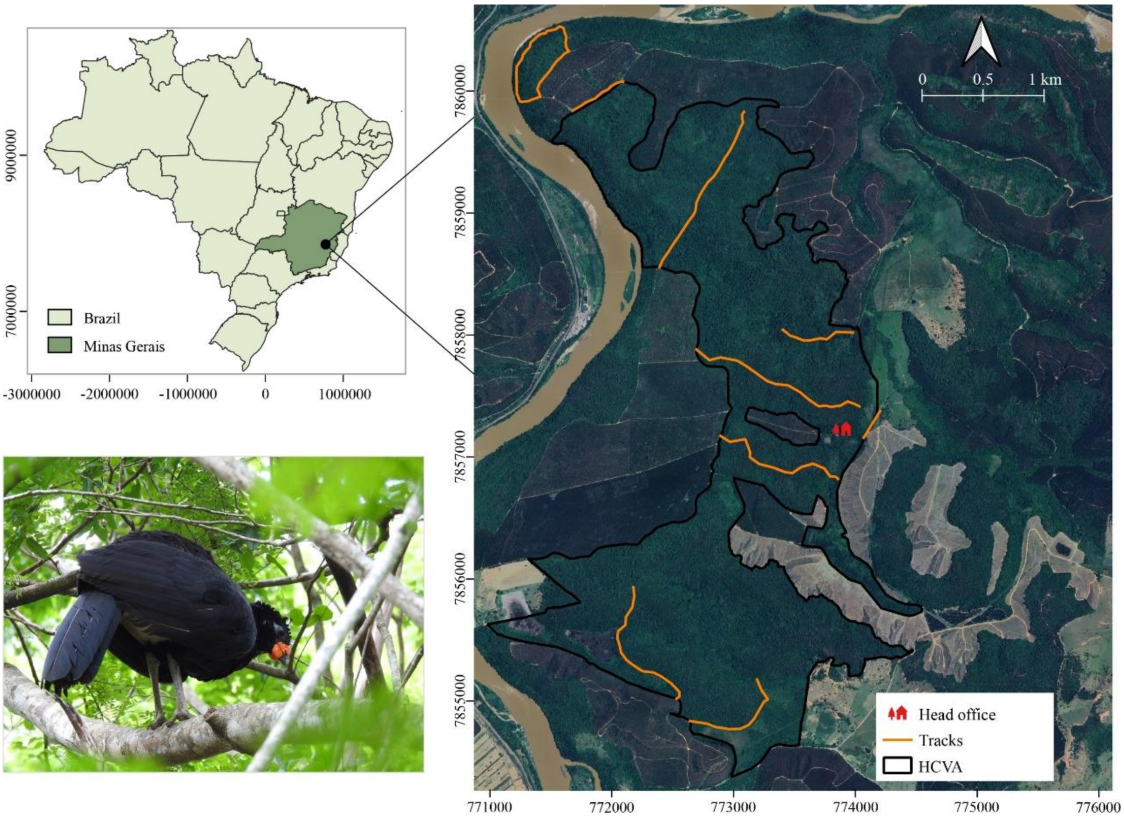


Figure 1. Location of the study area. The High Conservation Value Area (HCVA), a 753-ha forest fragment located within Fazenda Macedônia, Minas Gerais, Brazil, and a photograph of the studied species *Crax blumenbachii* at the study site (Photo by Roney Assis). The tracks used to survey *C. blumenbachii* faeces for diet characterization are also shown. Coordinate system: SIRGAS 2000, UTM Zone 23S.

The studied population is part of a reintroduction project started in 1990, successfully releasing more than 200 individuals of *Crax blumenbachii* into a region where the species had been absent since at least the 1970s (CENIBRA, 2023a), primarily due to hunting and habitat loss (Rios et al., 2021). Until now, the diet of *C. blumenbachii* had only been briefly mentioned in observational notes, reporting that individuals were seen consuming insects, seeds, tender leaves, buds, and fruits, including *Virola bicuhyba* (Schott ex Spreng.) Warb. and *Eugenia* spp. L. (Sick, 1970; Teixeira and Snow, 1982).

2.2. Fecal sampling and analysis

Diet characterization was conducted using fecal samples collected between January 2021 and June 2024, across 25 monthly sampling events. Faeces were collected along linear transects, pre-existing tracks, roads at the forest fragment edges and areas near to the head office (Figure 1). The coordinates of each fecal sample were recorded. Faeces identification was based on morphological characteristics: (i) the presence of uric acid and lack of hair, which differentiates them from mammal faeces, and (ii) faeces size, since *C. blumenbachii* is the only large-bodied bird (over 3 kg) in the study area, and the presence of large seeds, distinguishing them from those of smaller birds. To improve faeces attribution, we trained with captive birds and free-living individuals that frequented the head office region, from which we collected reference faeces. During the first campaign, we also followed ~10 free-living individuals for about a week and directly observed several defecation events; those samples formed a small qualitative validation subset. We contrasted morphology and context with similar frugivorous cracids (e.g., *Penelope*, *Aburria*), noting that *C. blumenbachii* typically leaves larger, cohesive ground boluses, whereas guans and piping guans often defecate from perches, producing smaller, more dispersed deposits. We applied a conservative rule of not collecting samples when attribution was uncertain, and observed that collection sites coincided with independent records from demographic censuses and camera traps.

After collecting, the fecal samples were stored in labeled individual plastic bags and subsequently washed under flowing water using a 1 mm mesh sieve to remove fecal content while retaining the seeds. The seeds were then separated per morphotypes (i.e., seeds with the same morphological characteristics), dried at room temperature, and stored at 5°C, with labels identifying the corresponding faeces and morphotypes. The number of seeds per faeces and morphotype were counted and recorded. To identify species seeds were photographed, and germination tests were conducted. The germination tests followed two methodologies based on seed size: (i) small seeds were placed in a Petri dish on two sheets of Germitest paper; (ii) large seeds were placed in a roll of Germitest paper packed in a plastic bag to increase the contact surface and maintain humidity. The seeds were kept hydrated with distilled water and stored in a BOD germination chamber at 25°C, for at least six months, under constant light to accelerate metabolism and, consequently, the germination of viable seeds (Campagnoli et al., 2016). Germinated seeds were transferred to tubes filled with a commercial plant substrate (composed of peat, vermiculite, class A agro-industrial organic waste, and limestone) and monitored until seedling development. For taxonomic identification, we combined (i) intensive searches in standard floras and seed-identification manuals for the Atlantic forest and Brazil (e.g., Frigleri, 2016; Lorenzi, 2020, 2021, 2022; Souza Junior & Brancalion, 2021), followed by targeted searches of indexed articles; (ii) germination and seedling development to reveal diagnostic traits, and (iii) consultations with botanical specialists. In addition, all seed species identifications were cross-checked by two co-authors with expertise in Atlantic forest plant species and validated against known geographic occurrence of tree species. We applied conservative confidence thresholds and retained morphotypes at genus/family or labelled as "indeterminate" when certainty was insufficient; consequently, 27.5% of morphotypes were deliberately left unidentified.

2.3. Diet and plant species traits

We classified the identified plant species found in the bird's diet based on their ecological characteristics: (i) growth form (tree; shrub; liana or herb), (ii) species origin (native, exotic, naturalized or cultivated) and (iii) successional group (early or late successional). Additionally, the plant species were classified according to fruit traits, including: (i) fruit type (achene, aggregate, berries, capsule, caryopsis, drupe, follicle, legume, multiple or samara), (ii) fruit consistency (dry or fleshy), (iii) fruit dehiscence (dehiscent or indehiscent), (iv) fruit colour (brown, black, green, orange, purple, red and yellow), (v) fruit size and (vi) seed size. When available, size was determined based on fruit and seed width; otherwise, length was used. Traits classification was performed using the Flora do Brasil (Reflora, 2025) virtual herbarium, the Atlantic frugivory database (Bello et al., 2017), FRUBASE (Jordano, 2013), TraitData (Petrocelli et al., 2024) and additional literature search when insufficient information was found. To assess if *C. blumenbachii* has a preference for certain seed or fruit traits, we also recorded the characteristic described above for all plant species recorded in the forest fragment based on the forest inventory data from 23 permanent plots (10 x 50 m) of adult trees (DBH ≥ 5 cm) (Rufino et al., 2023). In the absence of realized fruit availability data, "preference" is here treated as disproportionate representation of trait categories in the diet relative to the adult community pool. Finally, we also categorized the diet species based on the season in which they were observed, classifying them as occurring during the dry season, the wet season, or both.

2.4. Data analysis

To evaluate whether the plant-based diet characterization was complete given the applied sampling effort, we assessed sampling completeness using sample-coverage analysis

(Chao & Jost, 2012) implemented in the iNext package (Hsieh et al., 2016) in R software (R Core Team, 2025), treating each campaign as a sampling unit and standardizing seasonal comparisons at a common sample coverage.

We evaluated the variability in the plant species composition observed in the diet between seasons using permutational multivariate dissimilarity-based analysis of variance (PERMANOVA) (Anderson & Walsh, 2013). To visualize this variability, we employed non-metric multidimensional scaling (NMDS). The analysis were conducted using the adonis2 and metaMDS functions from the vegan package (Oksanen et al., 2019) in R software (R Core Team, 2025).

We compared the fruit trait distributions of plant species identified in the diet, during the total sampling period, referred to as "total diet" hereafter, (e.g., fruit type, fruit color, fruit consistency, and fruit dehiscence) with the corresponding trait distributions in the forest plant community, representing the available pool of species. This comparison aimed to determine whether the diet's composition differed from that of the forest species pool as a whole. For categorical traits, we used a chi-squared test to compare the distributions between the total diet and forest population. For numerical traits, such as fruit size and seed size, we compared the mean values between these two groups using a Welch t-test. These analyses were performed using the stats package in R software (R Core Team, 2025).

To understand feeding preferences across seasons, we calculated the relative importance (RI) of each plant species consumed by *C. blumenbachii*. The RI was determined by combining the relative abundance of seeds (N%) and the relative frequency of faeces in which the species were observed (F%), using the formula RI (%) = (N% + F%) / 2. We selected the ten species with the highest RI values and assessed their monthly consumption by *C. blumenbachii* based on the number of seeds recorded in the faeces. This data was compared with the theoretical fruiting periods of these species, derived from literature, to identify the months when each species was fruiting. Additionally, we calculated the germination rate (GR%) for these most important species based on the number of germinated seeds (Ngerm) and the total number of seeds (N) of each species, using the formula: GR (%) = (Ngerm / N) × 100. Finally, we estimated potential dispersal distance by pairing each fecal record that contained seeds of the most-consumed species with the nearest mapped fruiting conspecific tree and measuring the straight-line distance between these two points in QGIS (QGIS Association, 2023).

3. RESULTS

We collected 429 *Crax blumenbachii* fecal samples within the study area, of which 50.8% contained seeds (Table 1). The number of faeces collected during the dry season was higher than during the wet season, despite greater sampling effort during the latter. However, the proportion of seedless samples was lower in the wet season, potentially indicating increased fruit consumption. This pattern is further supported by the higher number of seeds found in faeces during the wet season compared to the dry season.

Table 1. General information on the diet of *Crax blumenbachii* based on fecal sample collection in the High Conservation Value Area (HCVA) within the Fazenda Macedônia, Brazil. The number of fecal samples, samples containing seeds, seeds, plant species, proportion of faeces without seeds and sampling effort (in number of sampling events) are provided, categorized by season (dry season from April to September) and in total.

Season	**Number of faeces**	**Faeces with seeds**	**Seedless ratio**	**Number of seeds**	**Number of species**	**Sampling effort**
Dry	222	82	0.6	2,300	51	11
Wet	207	136	0.3	4,230	45	14
Total	429	218	0.5	6,530	80	25

A total of 80 plant species were observed in the fecal samples, of which 49 were identified at the species level, three at the genus level, six at the family level, and 22 remained unidentified (Table S1). Among the 49 species identified, 33 were tree species, seven were shrubs, four were palms, three were lianas, and four were herbs. Furthermore, of these identified species, 34 were classified as early successional species, 13 as late successional species, and two could not be classified due to their lack of natural occurrences (Table S1).

The sample-coverage analysis indicates that, despite the high sampling effort, diet characterization was not fully achieved for either season (Figure 2). Observed coverage was moderate (Total C = 0.672; Dry C = 0.565; Wet C = 0.615), and, when standardized at a common coverage (C* = 0.56), the estimated richness was higher in the dry than in the wet season (50.0 vs 37.8 species), with overlapping 95% CIs.

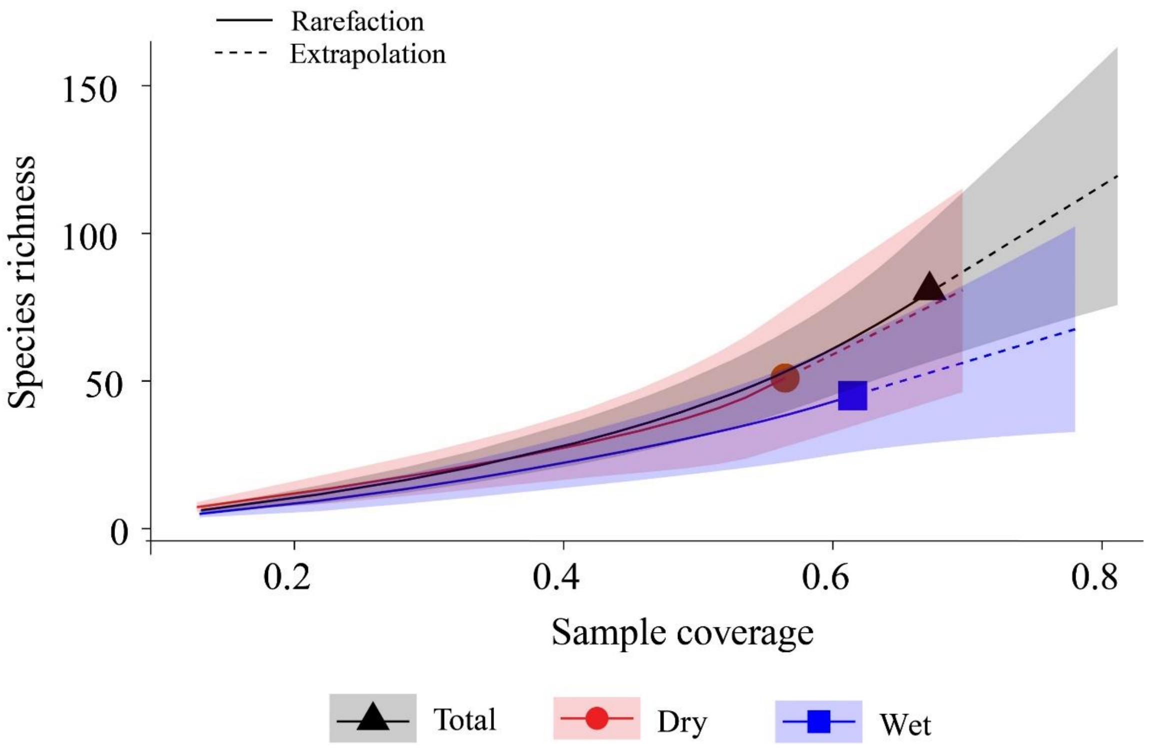


Figure 2. Species richness (q=0) of the *Crax blumenbachii* diet as a function of sample coverage. Curves are shown for: (i) all data combined (Total, gray), (ii) dry season (red), and (iii) wet season (blue). Solid lines show rarefaction (observed range) and dashed lines show extrapolation; symbols mark the observed coverage for each dataset. Shaded bands indicate 95% confidence intervals.

Significant changes in the traits of the fruits and seeds consumed were observed between the seasons. Berries were more common during the wet season, along with larger fruits and seeds, whereas drupes were more frequent during the dry season (Table 2). These seasonal shifts were further supported by significant differences in diet composition as illustrated by the dispersion of each season relative to its centroid (Figure 3). The dry season exhibited greater variability in recorded species (Dmean = 0.69) compared to the wet season (Dmean = 0.61) based on the PERMANOVA analysis (pseudo-F2 = 10.056; p = 0.001).

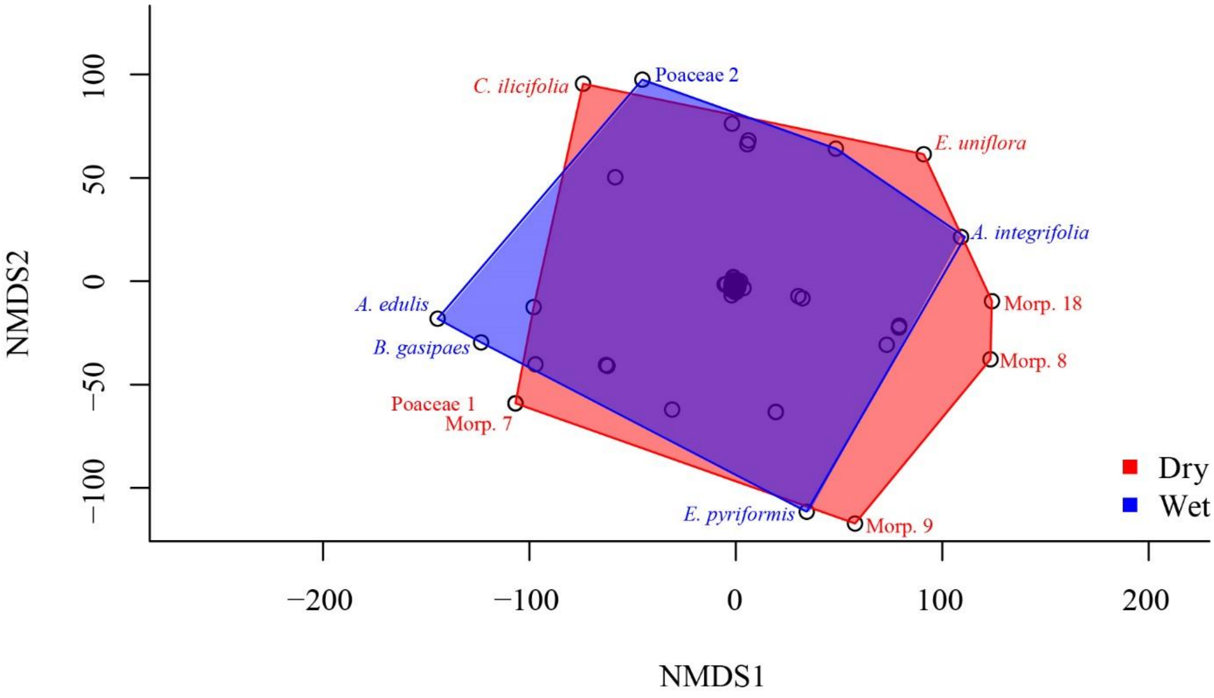
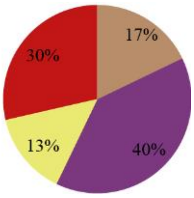
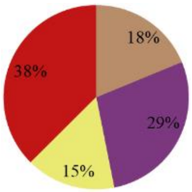
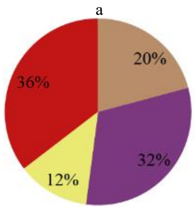
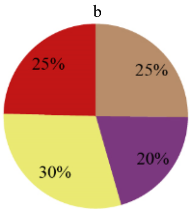
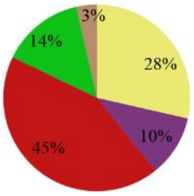
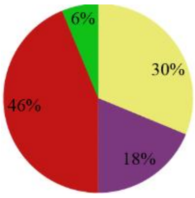
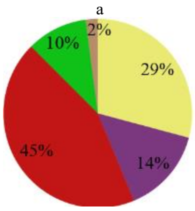
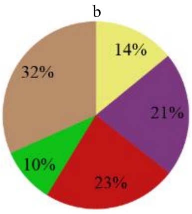


Figure 3. Non-metric multidimensional scaling (NMDS) diagram illustrating the composition of plant species consumed by *Crax blumenbachii* during the dry and wet seasons. Non-shared species between seasons are labeled with their names, with colors indicating the season in which they were observed. Shared species are represented solely by dots. Stress = 0.002.

Birds exhibited some degree of feeding preference. Fruits in the bird diet exhibited significant differences in their trait distribution (for categorical variables) or mean trait values (for numerical variables) over the entire sampling period, compared to the forest community at large (Table 2).

Table 2. Fruit traits of plant species observed in the diet of Crax blumenbachii, per season and during the total sampling period, compared to that of all trees in the forest fragment. Data from the forest fragment are based on 23 permanent plots established in the fragment (Rufino et al., 2023). Fruits traits are provided either using pie charts (fruit type and fruit colour distributions), percentages (fruit consistency, fruit dehiscence) or average (± SD) for fruit and seed size (both in mm). Traits of species in the total diet were compared to those of forest species using χ^2 tests for categorical variables and Welch t-tests for numerical variables; different letters (a or b) indicate significant differences in statistical analyses (at 95% probability). Black numbers in pie charts represent the percentage of species per category. "Other" fruit type category includes aggregate, caryopsis, follicle, legume, multiple, achene and samara.

Trait	**Diet**			**Forest**	**Statistical results**
	Dry	**Wet**	**Total**		
Fruit type 🟥 Berries 🟨 Capsule 🟪 Drupe 🟫 Other					χ^2= 11.779 p= 0.008
Fruit colour 🟫 Brown 🟩 Green 🟥 Orange/Red 🟪 Purple/Black 🟨 Yellow					χ^2= 32.346 p= 0.000
Fruit consistency	Fleshy (77%)	Fleshy (85%)	Fleshy (82%)^{a}	Dry (52%)^{b}	χ^2= 22.923 p= 0.000
Fruit dehiscence	Indehiscent (77%)	Indehiscent (74%)	Indehiscent (76%)^{a}	Indehiscent (58%)^{b}	χ^2= 6.937 p= 0.008
Fruit size (mm)	23.80 ± 22.75	25.80 ± 28.40	25.00 ± 26.39^{a}	36.41 ± 48.31^{b}	$t_{(140.48)}$= -2.190 p= 0.030
Seed size (mm)	8.04 ± 5.60	8.24 ± 5.27	8.24 ± 5.40^{a}	10.61 ± 8.92^{b}	$t_{(206.09)}$= -2.461 p= 0.015

We focused on the ten species with the highest relative importance (Table S1), hereafter referred to as "important species", to evaluate whether their consumption varies with fruit availability. *Psidium guajava* L. was the most important species, being the most frequently observed in fecal samples and containing the highest number of seeds (Table S1). The high preference for *P. guajava* fruits was confirmed by correlating the number of seeds found in fecal samples per month with the fruiting period of this species, as described in phenology literature (Table S2). This analysis revealed that, when *P. guajava* fruits are available, they become the dominant component of the birds' plant-based diet (Figure 4a-b). For instance, *Solanum palinacanthum* Dunal was highly consumed in the absence of *P. guajava*; however, once *P. guajava* fruits became available, the birds ceased consuming *S. palinacanthum*, despite its continued availability.

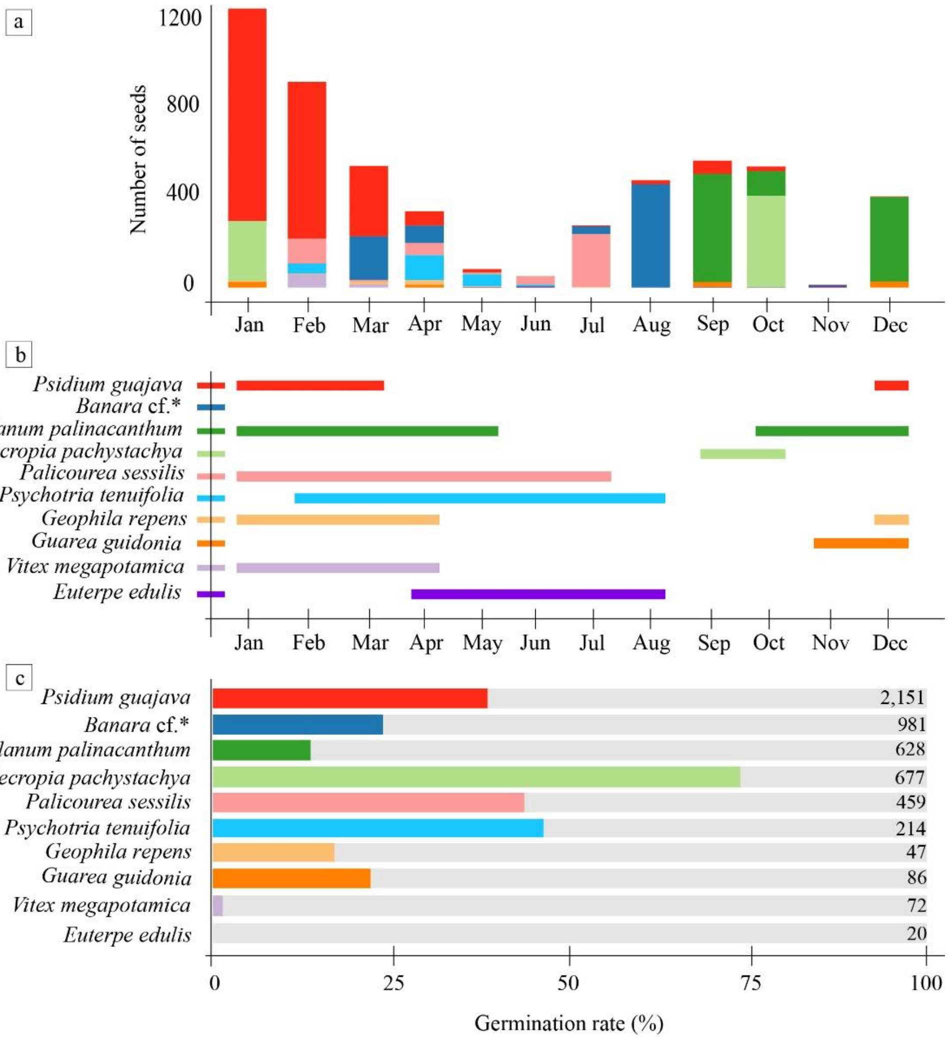


Figure 4. Monthly distribution of the number of seeds observed in fecal samples (a) and theoretical fruiting periods based on literature (b) for the ten most important species in the diet of *Crax blumenbachii*. Species not identified at the species level (*) were excluded from the determination of fruiting periods. Germination rate (in percentage) and the number of seeds tested of each species are also presented (c).

The germination rate of the most important species varied widely, with higher rates observed in *Cecropia pachystachya* Trécul (73.9%), *Psychotria tenuifolia* Sw. (46.3%), *Palicourea sessilis* (Vell.) C.M.Taylor (43.6%), and *P. guajava* (38.5%), while lower rates were recorded for *Euterpe edulis* Mart. (0%), *Vitex megapotamica* (Spreng.) Moldenke (1.4%), and *S. palinacanthum* (13.7%) (Figure 4c; Table S2).

The high consumption of *Psidium guajava* provided an opportunity to evaluate the potential seed dispersal distance by *C. blumenbachii*, as the location of cultivated *P. guajava* trees at Fazenda Macedônia is well-documented. Most *P. guajava* seeds were observed in faeces collected between 300 and 500 meters from the location of the cultivated trees, with only a few seeds found at greater distances of up to four kilometers (Table S3; Figure 5).

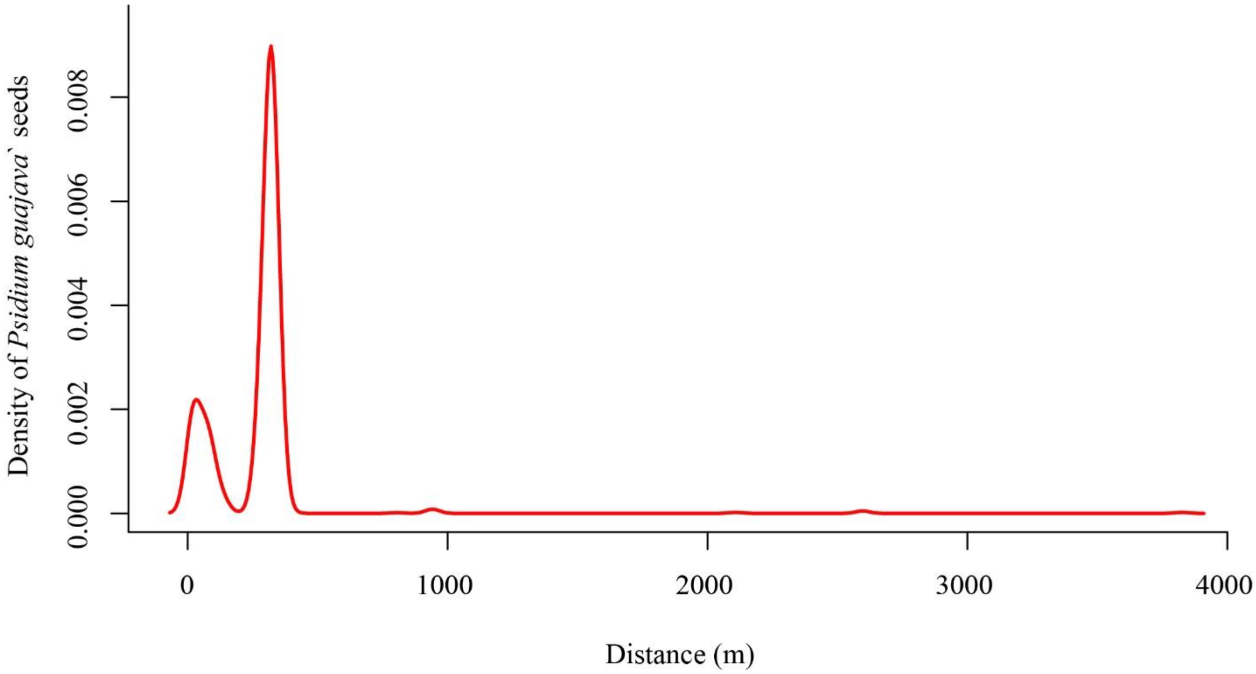


Figure 5. Kernel density estimation of *Psidium guajava* seed dispersal distances (in meters) by *Crax blumenbachii* from cultivated *P. guajava* trees at Fazenda Macedônia, Brazil.

4. DISCUSSION

We evaluated the diet, feeding preferences, and seasonal shifts of a large-bodied, reintroduced frugivorous bird in the Brazilian Atlantic forest. We found that *Crax blumenbachii* has a rich and diverse plant-species diet, primarily composed of species with fruit traits that differ from the most abundant traits within the forest fragment. However, during seasons of lower fruit availability, the bird expands its diet, consuming a broader range of fruits and seeds; as also supported by the higher estimated species richness in the dry season, which indicates a degree of dietary flexibility. This ability to shift feeding habits in response to resource scarcity is a key trait of generalist frugivores (Chaves & Bicca-Marques, 2016; Krishnadas et al., 2011). We also found that *C. blumenbachii* is capable of dispersing seeds over medium and long distances, and that germination responses of defecated seeds were species-specific(Tables S2 and S5); exceeding controls in some species and falling below in others (Table S5). Accordingly, the loss of *C. blumenbachii* could impact plant recruitment across multiple species, with the direction and magnitude of effects depending on species traits, reinforcing its ecological importance in maintaining forest diversity and dynamics.

4.1. Dietary composition and diversity

We recorded seeds of 80 plant species in the diet of *C. blumenbachii* (Table 1). This represents a higher dietary diversity than recorded for any other *Crax* species (see Table S4 for a review of *Crax* diet species lists). Tree species were the most frequently consumed (Table S1), suggesting that, despite spending most of their time on the forest floor, these birds rely heavily on tree fruits for dietary intake, likely consuming them after they have fallen. By feeding on fallen fruits, the birds remove seeds from beneath parent trees and deposit them elsewhere, thereby potentially enhancing seed survival and seedling establishment (Dent & Estrada-Villegas, 2021; Rumeu et al., 2020; Stevenson, 2011). Consequently, *C. blumenbachii* may play a significant role in tree seed dispersal within the forest fragment. Furthermore, early successional species constituted the majority of the diet (Table S1), underscoring the species' importance as a key agent in plant recruitment, particularly at forest edges, canopy gaps, and within secondary forests (Camargo et al., 2020; Foresta et al., 1984).

Over the entire sampling period, half of the fecal samples were seedless (Table 1). However, since this study focused on fruit consumption, other dietary items were not systematically evaluated. Nonetheless, the presence of leaves and insect traces were rarely observed. We suggest that these alternative food sources are consumed, especially when fruit availability is low (Faccio et al., 2018; Rodrigues et al., 2010). Additionally, pieces of soft-fleshed fruits with seeds larger than the birds' gape size may be consumed, while the seeds are not ingested (Valtuille et al., 2024).

Crax blumenbachii has been confirmed as a generalist species consuming a high diversity of species, as evidenced by the sample-coverage results (Figure 2). The concept of frugivore specialization and generalization has been inconsistently defined in ecological studies. In line with most avian studies, we define "specialization" as occurring only in cases of highly predictable and invariant interactions over time and space, conditions that rarely apply to most frugivores. Thus, generalist frugivores consume a wide variety of fruiting species without exclusive or dependent relationships with specific taxa, displaying contextual preferences that shift according to resource abundance and availability (Carlo, Messeder, Allbee, et al., 2024; Kattan et al., 2016). The sample-coverage analysis indicate that, despite the substantial number of plant species observed, a complete characterization of their dietary breadth was not fully achieved. Moreover, when comparisons were standardized at a common sample coverage, estimated richness tended to be higher in the dry season; suggesting a broader dietary scope consistent with seasonal shifts in resource composition; though we regard this as a trend given the overlapping confidence intervals.

4.2. Seasonal shifts in diet

The difference in dietary species richness between seasons was also significant (Figure 3), with 51 species recorded in the dry season and 45 in the wet season, 16 of which were shared between both periods (Table 1; Figure S1). When standardized at a common sample coverage, estimated richness was also higher in the dry season (Figure 2). We attribute this increased richness in the dry season to the reduced availability of preferred fruits during this period, such as *Psidium guajava* and *Cecropia pachystachya*. Zoochoric plant species in Atlantic forest tend to fruit more frequently when water and light availability are higher, as is the case in the wet season (Freire et al., 2013; Leiner & Silva, 2007; Staggemeier et al., 2017). Although generalist birds may strongly prefer some fruit species over others, these preferences can vary in response to fluctuations in resource abundance (Carlo, Messeder, Espíndola, et al., 2024; Muñoz et al., 2007; Pizo et al., 2021), so that, under resource scarcity, they likely adjust their foraging behavior by shifting their diet (Malanotte et al., 2019; Pires & De Melo, 2020), meaning that dietary preferences do not necessarily imply evolutionary-driven specialization (Carlo, Messeder, Allbee, et al., 2024). Notably, rarely consumed species (fewer than 10 seeds) were more frequently observed during the dry season (31 species) than in the wet season (24 species; Table S1), reinforcing the idea that, under resource scarcity, birds expand their diet to include less preferred species.

4.3. Fruit traits and dietary preferences

We found that the fruit traits of the tree species consumed by *C. blumenbachii* differ from those observed in the forest as a whole. This suggests that these birds do not select their food solely based on the most abundant fruits, but also on their preferences (Carlo, Messeder, Allbee, et al., 2024). Capsule-type fruits are the most common in the forest fragment; these are dry fruits that typically have a brown coloration (Table 2). Although capsule fruits were recorded in the diet, they were consumed in a considerably small proportion (Table 2; Table S1). Despite their higher availability, the most frequently consumed fruit types were berries and red/orange-colored fruits (Table 2). Berries are fleshy fruits that are generally rich in carbohydrates and low in protein and fat, making them highly preferred by frugivorous birds due to their easy digestion and rapid absorption (Levey & Rio, 2001; Snow, 1981). In contrast, dry fruits tend to have higher protein, fat, and fiber content, which reduces their digestibility and slows absorption (Debussche et al., 1987; Powers et al., 2015; Quintero et al., 2020).

4.4. The guava eater: Psidium guajava in the diet

We observed a strong preference for *Psidium guajava* fruits, which comprised the majority of seeds (32.9%) found in fecal samples when these fruits are available. *P. guajava* fruits are berries, between 50 mm and 100 mm in size, rich in nutrients and antioxidants, and commonly exhibit high productivity per tree (Lorenzi 1992; Singh et al. 2023; Tuler et al. 2025). Due to these characteristics, they are frequently consumed by frugivorous birds (Berens et al. 2008; Marciniak et al. 2024). *P. guajava* is a non-native species that has become naturalized in the Atlantic forest, as it can reproduce and establish a self-sustaining population (Jiang et al. 2011; Tuler et al. 2025). There are reports of *P. guajava* acting as an invasive species in tropical and subtropical regions, particularly in restoration areas (Jordaan et al. 2011; Marciniak et al. 2024). However, this does not seem to be the case in the study site, where *P. guajava* trees were not recorded in the forest inventory (Rufino et al. 2023), and only in open areas next to the head office. This is likely because *P. guajava* is a pioneer species, and in an old secondary forest, light availability is insufficient for seed germination and seedling development, except at forest edges and in canopy gaps (Barbosa Da Silva et al. 2013; Goodale et al. 2012; Pearson et al. 2003).

4.5. The seed-dispersing curassow

We found evidence that *C. blumenbachii* could act as an important seed disperser, as we observed a high germination rate for some species, such as *C. pachystachya*. A control assay using fruits collected from adult trees and the same germination methods indicated that gut passage did not reduce seed viability and is compatible with successful post-dispersal establishment (De Azevedo et al. 2013; Jordano & Schupp 2000). On the other hand, some species exhibited low germination rates, which does not necessarily imply a negative effect of *C. blumenbachii* consumption. For instance, *Euterpe edulis* had no germinated seeds in either gut-passed or control samples, and is known to have naturally low germination success (Tavares et al. 2008). Other factors unrelated to gut passage may also affect seed germination, including fruit maturity, maternal plant condition, and seed age (Bewley et al. 2013; Kuswantoro & Li'aini 2024). Additionally, most seeds recovered from fecal samples were defecated intact; an essential trait for efficient seed dispersal by cracids (Santamaría & Franco 2000; Valtuille et al. 2024); which reinforces the potential dispersal role of *C. blumenbachii*. Unlike reports for other *Crax* species that often act as seed predators (Erard et al. 2007; Leite 2020), we found intact seeds across size classes, including large-seeded taxa that germinated after defecation (e.g., *Virola bicuhyba* and *Swartzia myrtifolia*).

We used the high consumption of *P. guajava* to estimate the potential seed dispersal distance of *C. blumenbachii*, as the location of cultivated *P. guajava* trees is known. Most *P. guajava* seeds were found in fecal samples between 300 and 500 meters from the adult trees, while a few were found at greater distances, up to four kilometers, likely originating from fruits of *P. guajava* trees not recorded during our surveys, indicating a long-distance dispersal range. While the concept of long-distance dispersal varies across taxa and landscapes (Jordano 2017), our findings suggest that *C. blumenbachii* tends to deposit seeds away from parent trees. This pattern is expected, as both the time spent in fruiting trees and seed retention time generally increases with body mass, leading to longer dispersal distances for larger birds, as *C. blumenbachii* (Vidal et al. 2013; Wotton & Kelly 2011). Although moving seeds farther does not guarantee higher probabilities of establishment, escaping the disproportionately high mortality beneath the parent tree can be crucial for forest regeneration (Augspurger 1984; Jansen et al. 2014; Jordano & Schupp 2000). Furthermore, long-distance dispersal can facilitate the colonization of new areas, contributing to metapopulation dynamics and enhancing genetic connectivity across fragmented landscapes (Camargo et al. 2020; Jordano 2017; Purves & Dushoff 2005).

4.6. Conservation implications

Our study provides important insights into the diet of the last large-bodied frugivorous bird in the Brazilian Atlantic forest, *C. blumenbachii*, which, together with other Cracidae species, has experienced alarming population declines (IUCN, 2025; Leite, 2020; Muñoz &

Kattan, 2007). We also highlight its potential role as seed disperser, consuming a wide variety of fruits and seeds with different traits and dispersing them across the landscape, over medium-long distances, with a high seed germination rate for some species. Although our focal population is partly captive-bred and habituated to human presence -factors that may influence diet and foraging - remaining wild populations are small and highly localized (BirdLife International, 2016), and there are no comparable dietary studies from wild or other reintroduced populations.

The knowledge generated can help refine guidelines for reintroduction projects, both for *Crax* and other Cracidae species, as well as for frugivores in general, particularly regarding habitat suitability in relation to dietary preferences. Given the close relationship observed between *P. guajava* and *C. blumenbachii*, enrichment planting of related native species; such as *Psidium oblongatum* O. Berg or *Psidium cattleyanum* Sabine, which are threatened plant species; could be incorporated into management plans. This strategy would enhance the availability of preferred species, improving habitat suitability and increasing the likelihood of success across the project area. Finaly, we have found strong evidence that, unlike other *Crax* species, *C. blumenbachii* is likely an important seed disperser, reinforcing the need to assess the ecological characteristics of threatened species, in particular, to mitigate the ecological effects of their decline

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Supporting Information

Table S1. Diet characterization of *Crax blumenbachii* at Fazenda Macedônia, Brazil. The table provides the list of plant species consumed, including the number of faeces containing each species, the number of seeds, and the seasons in which the species were observed in the faeces (D = dry, W = wet). Ecological information is also provided: the successional group (SG; E = early successional, L = late successional), life form, and origin (Nat. = naturalized, Cult. = cultivated); as well as fruit and seed traits: fruit type (FT), fruit consistency (FC), fruit dehiscence (FD; D = dehiscent, I = indehiscent), fruit colour (Fcolour; colours marked with * refer to aril colour), fruit size (FS, in mm), and seed size (SS, in mm). Ecological information and fruit trait data were obtained from the literature. The species are ordered by their relative importance (RI, in percentage, was determined by combining the relative abundance of seeds (N%) and the relative frequency of faeces in which the species were observed (F%), using the formula RI (%) = (N% + F%) / 2.).

Family	**Species**	**Faeces**	**Seeds**	**Season**	**SG**	**Life form**	**Origin**	**Traits**						**RI (%)**
								FT	**FC**	**FD**	**Fcolour**	**FS (mm)**	**SS (mm)**	
Myrtaceae	*Psidium guajava* L.	99	2,151	D/W	E	Tree	Nat.	Berries	Fleshy	I	Yellow	100	4	39.2
Salicaceae	*Banara* Aubl. cf.	11	981	D/W	-	-	-	Berries	Fleshy	I	-	-	2	10
Solanaceae	*Solanum palinacanthum* Dunal	10	976	D/W	E	Tree	Native	Berries	Fleshy	I	Yellow	22.4	3.5	9.8
Urticaceae	*Cecropia pachystachya* Trécul	14	677	W	E	Tree	Native	Multiple	Fleshy	I	Yellow	5.3	1.1	8.4
Rubiaceae	*Palicourea sessilis* (Vell.) C.M.Taylor	15	459	D/W	E	Shrub	Native	Drupe	Fleshy	I	Black	4	3	7
Rubiaceae	*Psychotria tenuifolia* Sw.	9	214	D/W	E	Shrub	Native	Berries	Fleshy	I	Red	45	5	3.7

Family	**Species**	**Faeces**	**Seeds**	**Season**	**SG**	**Life form**	**Origin**	**Traits**						**RI (%)**
								FT	**FC**	**FD**	**Fcolour**	**FS (mm)**	**SS (mm)**	
Rubiaceae	*Geophila repens* (L.) I.M.Johnst.	13	47	D/W	L	Herb	Native	Drupe	Fleshy	I	Red	8	2	3.3
Meliaceae	*Guarea guidonia* (L.) Sleumer	7	86	D/W	L	Tree	Native	Capsule	Dry	D	Red*	16.3	9.4	2.3
Lamiaceae	*Vitex megapotamica* (Spreng.) Moldenke	7	72	W	E	Tree	Native	Drupe	Fleshy	I	Purple	20	8.9	2.2
Arecaceae	*Euterpe edulis* Mart.	8	20	D/W	L	Palm	Native	Drupe	Fleshy	I	Purple	13.1	11.3	2
Rubiaceae	*Psychotria carthagenensis* Jacq.	5	102	D/W	E	Shrub	Native	Drupe	Fleshy	I	Red	8	5.1	1.9
Siparunaceae	*Siparuna guianensis* Aubl.	6	26	W	E	Tree	Native	Multiple	Fleshy	D	Red	9	4.3	1.6
Rubiaceae	*Posoqueria acutifolia* Mart.	5	30	D	E	Tree	Native	Drupe	Fleshy	I	Yellow	40	6	1.4
Fabaceae	*Senna alata* (L.) Roxb.	5	12	D	E	Tree	Native	Legume	Dry	D	Brown	14.2	4.9	1.2
Rutaceae	*Murraya paniculata* (L.) Jack	4	40	D	E	Tree	Cult.	Drupe	Fleshy	I	Red	10	5	1.2
Unclassified	Morphotype 16	4	7	D/W	-	-	-	-	-	-	-	-	5	1
Annonaceae	*Xylopia sericea* A.St.-Hil.	4	6	D/W	E	Tree	Native	Aggregate	Fleshy	D	Yellow	12.3	5	1
Cucurbitaceae	*Momordica charantia* L.	3	29	W	E	Liana	Nat.	Berries	Fleshy	D	Orange	150	5	0.9
Solanaceae	*Solanum asperum* Rich.	2	58	D	E	Shrub	Native	Berries	Fleshy	I	Green	9	1.9	0.9
Unclassified	Morphotype 4	2	57	W	-	-	-	-	-	-	-	-	-	0.9

Family	Species	Faeces	Seeds	Season	SG	Life form	Origin	Traits						RI (%)
								FT	FC	FD	Fcolour	FS (mm)	SS (mm)	
Apocynaceae	*Tabernaemontana laeta* Mart.	2	49	W	E	Tree	Native	Follicle	Fleshy	D	Green	33.7	5.9	0.8
Annonaceae	*Annona squamosa* L.	3	15	D	-	Tree	Cult.	Aggregate	Fleshy	I	Green	90	15	0.8
Myristicaceae	*Virola bicuhyba* (Schott ex Spreng.) Warb.	3	13	D	L	Tree	Native	Capsule	Dry	D	Red*	19	15.7	0.8
Passifloraceae	*Passiflora alata* Aiton	3	11	D	E	Liana	Native	Berries	Fleshy	I	Yellow	57.2	7.4	0.8
Malpighiaceae	*Bunchosia armeniaca* (Cav.) DC.	3	5	W	E	Shrub	Native	Drupe	Fleshy	I	Orange	30	20	0.7
Myristicaceae	*Virola gardneri* (A.DC.) Warb.	3	4	D/W	L	Tree	Native	Capsule	Dry	D	Red*	24.3	20.3	0.7
Unclassified	Morphotype 10	2	31	W	-	-	-	-	-	-	-	-	10	0.7
Moraceae	*Maclura tinctoria* (L.) D.Don ex Steud.	1	60	W	E	Tree	Native	Multiple	Fleshy	I	Yellow	2	3	0.7
Unclassified	Morphotype 22	2	19	D	-	-	-	-	-	-	-	-	5	0.6
Poaceae	*Olyra latifolia* L.	2	14	D	E	Herb	Native	Caryopsis	Dry	I	Green	2.7	1.1	0.6
Myrtaceae	*Psidium cattleyanum* Sabine	2	11	W	L	Tree	Native	Berries	Fleshy	I	Purple	40	2.5	0.5
Moraceae	*Brosimum guianense* (Aubl.) Huber	2	9	D/W	E	Tree	Native	Drupe	Fleshy	I	Red	14.3	9.8	0.5
Arecaceae	*Dypsis lutescens* (H.Wendl.) Beentje & J.Dransf.	2	6	D/W	-	Palm	Exotic	Drupe	Fleshy	I	Yellow	22	20	0.5

Family	Species	Faeces	Seeds	Season	SG	Life form	Origin	Traits						RI (%)
								FT	**FC**	**FD**	**Fcolour**	**FS (mm)**	**SS (mm)**	
Rutaceae	*Esenbeckia leiocarpa* Engl.	2	3	D/W	L	Tree	Native	Capsule	Dry	D	Green	29.5	7.3	0.5
Sapindaceae	*Cupania vernalis* Cambess.	2	2	W	E	Tree	Native	Capsule	Dry	D	Orange	13.4	7.5	0.5
Unclassified	Morphotype 1	2	2	D/W	-	-	-	-	-	-	-	-	10	0.5
Unclassified	Morphotype 5	2	2	D	-	-	-	-	-	-	-	-	-	0.5
Myrtaceae	*Eugenia pyriformis* Cambess.	1	30	W	E	Tree	Native	Berries	Fleshy	I	Orange	13.4	13.3	0.5
Lamiaceae	*Aegiphila integrifolia* (Jacq.) Moldenke	1	28	W	E	Tree	Native	Berries	Fleshy	I	Red	8	7	0.4
Arecaceae	*Bactris gasipaes* Kunth	1	17	W	E	Palm	Native	Drupe	Fleshy	I	Orange	45	15	0.4
Poaceae	Poaceae Barnhart 1	1	11	D	-	Herb	-	-	-	-	-	-	-	0.3
Rutaceae	Rutaceae A.Juss.	1	11	D	-	-	-	-	-	-	-	-	12	0.3
Myrtaceae	Myrtaceae Juss. 1	1	10	D	-	-	-	-	-	-	-	-	20	0.3
Rutaceae	*Zanthoxylum L.*	1	10	W	-	-	-	-	-	-	-	-	6	0.3
Unclassified	Morphotype 21	1	8	D	-	-	-	-	-	-	-	-	2	0.3
Myrtaceae	*Eugenia florida* DC.	1	7	W	E	Shrub	Native	Berries	Fleshy	I	Black	12.5	9.8	0.3
Smilacaceae	*Smilax elastica* Griseb.	1	7	D	-	Liana	Native	Berries	Fleshy	I	Black	8	5	0.3
Cordiaceae	*Cordia magnoliifolia* Cham.	1	6	W	E	Tree	Native	Drupe	Fleshy	I	Red	8	5	0.3
Malvaceae	*Bastardiopsis densiflora* (Hook. & Arn.) Hassl.	1	6	W	E	Tree	Native	Capsule	Dry	D	Yellow	30	7	0.3

Family	Species	Faeces	Seeds	Season	SG	Life form	Origin	Traits						RI (%)
								FT	FC	FD	Fcolour	FS (mm)	SS (mm)	
Poaceae	Poaceae Barnhart 2	1	6	W	-	Herb	-	-	-	-	-	-	-	0.3
Rubiaceae	Rubiaceae Juss.	1	6	D	-	-	-	-	-	-	-	-	2	0.3
Unclassified	Morphotype 3	1	5	W	-	-	-	-	-	-	-	-	-	0.3
Fabaceae	*Swartzia myrtifolia* Sm.	1	4	D	E	Tree	Native	Legume	Dry	D	Orange	25	15	0.3
Sapindaceae	*Sapindus saponaria* L.	1	4	D	E	Tree	Native	Berries	Fleshy	I	Red	18.2	11.6	0.3
Sapindaceae	*Allophylus edulis* (A.St.-Hil. et al.) Hieron. ex Niederl.	1	4	W	E	Tree	Native	Berries	Fleshy	I	Red	8.7	6	0.3
Unclassified	Morphotype 2	1	4	W	-	-	-	-	-	-	-	-	-	0.3
Unclassified	Morphotype 7	1	4	D	-	-	-	-	-	-	-	-	-	0.3
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman	1	3	D	E	Palm	Native	Drupe	Fleshy	I	Yellow	20.9	15.6	0.3
Sabiaceae	*Meliosma chartacea* Lombardi	1	3	D	L	Tree	Native	Drupe	Fleshy	I	Yellow	20	15	0.3
Unclassified	Morphotype 13	1	3	D	-	-	-	-	-	-	-	-	2	0.3
Vitaceae	*Cissus* L.	1	3	W	-	-	-	-	-	-	-	-	4	0.3
Cordiaceae	*Cordia ecalyculata* Vell.	1	2	D	L	Tree	Native	Drupe	Fleshy	I	Red	9.9	7.7	0.2
Moraceae	*Clarisia ilicifolia* (Spreng.) Lanj. & Rossberg	1	2	D	L	Tree	Native	Drupe	Fleshy	I	Red	11.2	9.5	0.2
Myrtaceae	*Eugenia uniflora* L.	1	2	D	E	Shrub	Native	Berries	Fleshy	I	Red	17	10.6	0.2

Family	Species	Faeces	Seeds	Season	SG	Life form	Origin	Traits						RI (%)
								FT	**FC**	**FD**	**Fcolour**	**FS (mm)**	**SS (mm)**	
Sapotaceae	*Pouteria glomerata* (Miq.) Radlk.	1	2	W	L	Tree	Native	Berries	Fleshy	I	Yellow	38.4	18.8	0.2
Unclassified	Morphotype 6	1	2	D	-	-	-	-	-	-	-	-	-	0.2
Annonaceae	*Annona dolabripetala* Raddi	1	1	W	E	Tree	Native	Aggregate	Fleshy	I	Yellow	25	6.5	0.2
Myrtaceae	Myrtaceae Juss. 2	1	1	D	-	-	-	-	-	-	-	-	20	0.2
Myrtaceae	*Eugenia bahiensis* DC.	1	1	W	L	Tree	Native	Berries	Fleshy	I	Purple	21	14.8	0.2
Sapotaceae	*Chrysophyllum gonocarpum* (Mart. & Eichler ex Miq.) Engl.	1	1	W	E	Tree	Native	Berries	Fleshy	I	Yellow	19.8	10	0.2
Unclassified	Morphotype 8	1	1	D	-	-	-	-	-	-	-	-	-	0.2
Unclassified	Morphotype 9	1	1	D	-	-	-	-	-	-	-	-	6	0.2
Unclassified	Morphotype 12	1	1	D	-	-	-	-	-	-	-	-	2	0.2
Unclassified	Morphotype 14	1	1	D	-	-	-	-	-	-	-	-	8	0.2
Unclassified	Morphotype 15	1	1	D	-	-	-	-	-	-	-	-	3	0.2
Unclassified	Morphotype 17	1	1	D	-	-	-	-	-	-	-	-	4	0.2
Unclassified	Morphotype 18	1	1	D	-	-	-	-	-	-	-	-	2	0.2
Unclassified	Morphotype 19	1	1	W	-	-	-	-	-	-	-	-	16	0.2
Unclassified	Morphotype 20	1	1	W	-	-	-	-	-	-	-	-	8	0.2
Unclassified	Morphotype 23	1	1	D	-	-	-	-	-	-	-	-	12	0.2

Table S2. Fruiting period of the ten species with the highest relative importance in the diet of *Crax blumenbachii*, based on literature and corresponding references. Species not identified at species level (*) could not be evaluated. The number of seeds tested, and the germination rate (in percentage) are also presented.

Species	**Fruiting**	**Reference**	**Seeds tested**	**Germination rate (%)**
Psidium guajava	Dec - Mar	Lorenzi, 1992	2,151	38.5
Banara cf.*	-	-	981	24.1
Solanum palinacanthum	Sep - Mar	Mentz and de Oliveira, 2004	628	13.7
Cecropia pachystachya	Sep - Oct	Lorenzi, 1992	677	73.9
Palicourea sessilis	Jan - Jul	do Carmo and Simões, 2017	459	43.6
Psychotria tenuifolia	Feb - Aug	do Carmo and Simões, 2017	214	46.3
Geophila repens	Dec - Apr	Pereira and Kinoshita, 2013	47	17.0
Guarea guidonia	Nov - Dec	Lorenzi, 1992	86	22.1
Vitex megapotamica	Nov - Dec	Embrapa, 2006	72	1.4
Euterpe edulis	Apr - Aug	Lorenzi, 1992	20	0.0

Table S3. Number of *Psidium guajava* seeds observed in the faeces of *Crax blumenbachii* categorized by distance (in meters) from *Psidium guajava* trees in Fazenda Macedônia, Brazil.

Distance (m)	*Psidium guajava* **seeds (n)**
0 – 300	702
300 – 500	1422
500 – 1,000	14
1000 – 2,000	0
2000 – 3,000	10
3000 – 4,000	3
Total	2,151

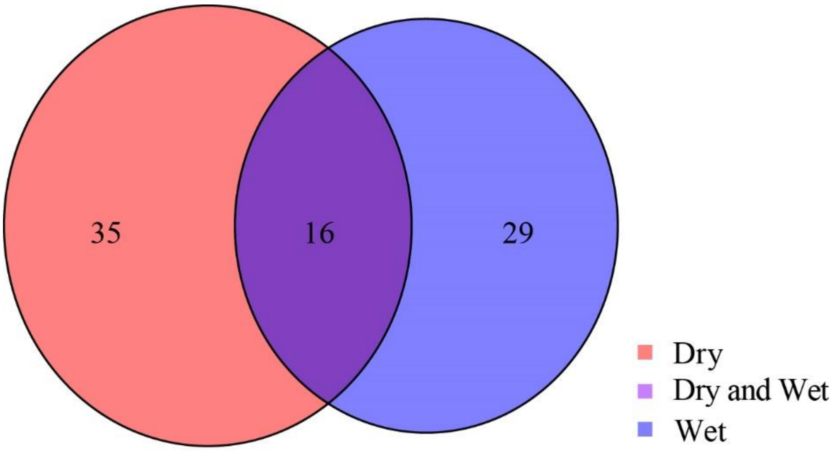

Figure S1. Venn diagram showing the number of plant species observed in the diet of *Crax blumenbachii* per season, including the number of species shared between the dry (light red circle) and wet (light blue circle) seasons (light purple intersection).

Table S4. Diet characterization of *Crax* species according to a literature review. The table provides the *Crax* species that was the study object, the list of plant species consumed, the country and vegetation type where the study was conducted, the research period and methods, and references. Numbers in parentheses represent the number of samples in each method when available in the paper.

Crax **species**	**Plant species**	**Country**	**Vegetation**	**Research period and methods**	**Reference**
Crax blumenbachii	*Virola bicuhyba* (Schott ex Spreng.) Warb. *Lecythis pisonis* Cambess. *Byrsonima crassifolia* (L.) Kunth *Geonoma* Willd.	Brazil	Atlantic forest	Notes based on field observations in Feb.–Mar. 1954 and Feb.–Mar. 1961	Sick, 1970
Crax blumenbachii	*Eugenia* L. *Ferdinandusa* Pohl *Eschweilera* Mart. ex DC.	Brazil	Atlantic forest	Notes based on field observations in Nov. 1979	Teixeira and Snow, 1981
Crax globulosa	*Annona* L. *Malmea* R.E.Fr. Apocynaceae Juss. 1 Apocynaceae Juss. 2 Apocynaceae Juss. 3 *Euterpe precatoria* Mart. *Oenocarpus bacaba* Mart. *Maytenus* Molina *Calophyllum brasiliense* Cambess. *Sloanea rufa* Planch. ex Benth. *Sloanea* L. *Hevea guianensis* Aubl. *Mabea nitida* Spruce ex Benth. *Piranhea* Baill. *Campsiandra* Benth.	Brazil	Amazon	Field observations (119 foraging records), stomach contents (10), and interviews (7) with local people betweeb Aug. 2014 and Oct. 2015.	Leite, 2020

Crax species	Plant species	Country	Vegetation	Research period and methods	Reference
	Dialium L.				
Crax globulosa	*Inga* Mill.	Brazil	Amazon	Field observations (119 foraging records), stomach contents (10), and interviews (7) with local people betweeb Aug. 2014 and Oct. 2015.	Leite, 2020
	Macrolobium acaciifolium (Benth.) Benth.				
	Macrolobium Schreb.				
	Vatairea guianensis Aubl.				
	Vantanea Aubl.				
	Ocotea cymbarum Kunth				
	Ocotea Aubl.				
	Eschweilera Mart. ex DC.				
	Lecythis Loefl.				
	Abarema Pittier				
	Byrsonima Rich. ex Kunth 1				
	Byrsonima Rich. ex Kunth 2				
	Pseudobombax munguba (Mart.) Dugand				
	Bellucia Raf.				
	Miconia Ruiz & Pav.				
	Trichilia P.Browne				
	Ficus L.				
	Ficus maxima Mill.				
	Sorocea duckei W.C.Burger				
	Virola surinamensis (Rol. ex Rottb.) Warb.				
	Eugenia inundata DC.				
	Eugenia patrisii Vahl				
	Eugenia L.				
	Drypetes Vahl				
	Calycophyllum spruceanum (Benth.) K.Schum.				
	Duroia L.f.				
	Banara guianensis Aubl.				

Crax species	Plant species	Country	Vegetation	Research period and methods	Reference
Crax globulosa	*Cupania macrostylis* (Radlk.) Acev.-Ror.	Brazil	Amazon	Field observations (119 foraging records), stomach contents (10), and interviews (7) with local people betweeb Aug. 2014 and Oct. 2015.	Leite, 2020
	Manilkara elata (Allemão ex Miq.) Monach.				
	Manilkara Adans				
	Pouteria Aubl.				
	Sapotaceae Juss.				
	Cecropia Loefl.				
	Vitex cymosa Bertero ex Spreng.				
	Vochysia Aubl.				
Crax rubra	*Spondias purpurea* L.	El Salvador	Rain forest	Field observations between Jan. 1977 and May 1979.	Sermeño, 1986
	Garcinia intermedia (Pittier) Hammel				
	Ardisia paschalis Donn.Sm.				
	Maytenus chiapensis Lundell				
	Drypetes lateriflora (Sw.) Krug & Urb.				
	Ficus L. 1				
	Ficus L. 2				
	Dichapetalum donnell-smithii Engl.				
	Cecropia peltata L.				
	Cecropia obtusifolia Bertol.				
	Geobalanus retifolius (S.F.Blake) Sothers & Prance				
	Manilkara chicle (Pittier) Gilly				
	Brosimum alicastrum Sw.				
	Sideroxylon capiri subsp. *tempisque* (Pittier) T.D.Penn.				
Crax alector	*Duguetia* A.St.-Hil.	French Guiana	Lowland rain forest	Stomach contentes (69) between 1977 and 1985.	Erard et al., 2007
	Ephedranthus guianensis R.E.Fr.				
	Guatteria Ruiz & Pav. 1				

Crax species	Plant species	Country	Vegetation	Research period and methods	Reference
	Guatteria Ruiz & Pav. 2				
Crax alector	*Unonopsis guatterioides* (A.DC.) R.E.Fr.	French Guiana	Lowland rain forest	Stomach contentes (69) between 1977 and 1985.	Erard et al., 2007
	Xylopia nitida Dunal				
	Oreopanax capitatus (Jacq.) Decne. & Planch.				
	Bactris acanthocarpoides Barb.Rodr.				
	Bactris cf *gastoniana* Barb.Rodr.				
	Euterpe oleracea Mart.				
	Geonoma stricta (Poit.) Kunth				
	Protium tenuifolium subsp. *tenuifolium*				
	Protium subserratum (Engl.) Engl.				
	Protium altissimum (Aubl.) Marchand				
	Cecropia obtusa Trécul				
	Cecropia sciadophylla Mart.				
	Coussapoa latifolia Aubl.				
	Coussapoa microcephala Trécul				
	Pourouma Aubl.				
	Goupia glabra Aubl.				
	Licania Aubl.				
	Cayaponia Silva Manso				
	Drypetes fanshawei Sandwith				
	Drypetes variabilis Uittien				
	Hieronyma alchorneoides Allemão				
	Sacoglottis cydonioides Cuatrec.				
	Sacoglottis guianensis Benth.				
	Poraqueiba guianensis Aubl.				
	Ocotea Aubl.				
	Ocotea floribunda (Sw.) Mez				
	Smilax L.				

Crax species	Plant species	Country	Vegetation	Research period and methods	Reference
	Goeppertia cf elliptica (Roscoe) Borchs. & S.Suárez				
Crax alector	*Guarea gomma* Pulle	French Guiana	Lowland rain forest	Stomach contentes (69) between 1977 and 1985.	Erard et al., 2007
	Guarea grandifolia DC.				
	Guarea kunthiana A.Juss.				
	Trichilia septentrionalis C.DC.				
	Abuta cf *grandifolia* (Mart.) Sandwith				
	Abuta Aubl.				
	Orthomene Barneby & Krukoff				
	Inga Mill.				
	Parkia ulei (Harms) Kuhlm.				
	Brosimum parinarioides Ducke				
	Brosimum rubescens Taub.				
	Naucleopsis guianensis (Mildbr.) C.C.Berg				
	Iryanthera sagotiana (Benth.) Warb.				
	Virola michelii Heckel				
	Eugenia sinemariensis Aubl.				
	Minquartia guianensis Aubl.				
	Dussia discolor (Benth.) Amshoff				
	Sarcomphalus cinnamomum (Triana & Planch.) Hauenschild				
	Psychotria bahiensis DC.				
	Psychotria ficigemma DC.				
	Eumachia kappleri (Miq.) Delprete & J.H.Kirkbr.				
	Eumachia guianensis (Bremek.) Delprete & J.H.Kirkbr.				
	Palicourea oblonga (DC.) Delprete & J.H.Kirkbr.				
	Psychotria L.				
	Cupania scrobiculata Rich.				
	Cupania L.				

Crax **species**	**Plant species**	**Country**	**Vegetation**	**Research period and methods**	**Reference**
	Pouteria bilocularis (H.J.P.Winkl.) Baehni				
Crax daubentoni	*Enterolobium cyclocarpum* (Jacq.) Griseb. *Samanea saman* (Jacq.) Merr. Bignoniaceae Juss. 1 Bignoniaceae Juss. 2 *Lantana camara* L. *Lantana trifolia L.* *Genipa americana* L. *Cordia tetrandra* Aubl. *Libidibia coriaria* (Jacq.) Schltdl. *Guazuma ulmifolia* Lam. *Allophylus racemosus* Sw. *Tabernaemontana cymosa* Jacq. *Combretum decandrum* Jacq. *Hecatostemon completus* (Jacq.) Sleumer *Sarcomphalus cyclocardius* (S.F.Blake) Hauenschild *Mangifera indica L.* *Moquilea pyrifolia* (Griseb.) R.O.Williams *Morisonia odoratissima* (Jacq.) Christenh. & Byng *Copernicia tectorum* (Kunth) Mart. *Aquarius paniculatus* (Micheli) Christenh. & Byng *Coccoloba caracasana* Meisn. *Scleria* P.J.Bergius *Poaceae* Barnhart	Venezuela	Tropical dry forest	Faeces collection (90), field observations (92) and stomach content between Nov. 2001 and Jul. 2002.	Bertsch and Barreto, 2008

Table S5. Number of seeds and germination rate (in percentage) for seven plant species, comparing seeds manually extracted from fruits (control samples) with seeds that passed through the digestive tract of *Crax blumenbachii* (gut passage). Control samples were collected when ripe fruits of plant species consumed by *C. blumenbachii* were found during fieldwork. Seeds were manually extracted from the fruits, and the same germination test procedure was applied to both control seeds and those that passed through the digestive tract of *C. blumenbachii*.

Species	**Control samples**		**Gut passage**	
	Seeds tested	**Germination rate (%)**	**Seeds tested**	**Germination rate (%)**
Cecropia pachystachya	30	73.3	677	73.9
Eugenia uniflora	2	0.0	2	100.0
Euterpe edulis	2	0.0	20	0.0
Momordica charantia	4	75.0	29	3.4
Psidium guajava	30	33.3	2,151	38.5
Solanum asperum	40	82.5	58	5.2
Solanum palinacanthum	30	26.7	628	13.7

CAPÍTULO III:

RESTORING PLANT-ANIMAL INTERACTIONS: THE ROLE OF THE RED-BILLED CURASSOW (*Crax blumenbachii*) IN SHAPING TREE-SEEDLING COMMUNITIES IN AN ATLANTIC FOREST FRAGMENT

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Restoring plant-animal interactions: the role of the red-billed curassow (Crax blumenbachii) in shaping tree-seedling communities in an Atlantic forest fragment

Abstract

The decline of large frugivores negatively affects tropical forests by disrupting plant-animal interactions, particularly seed dispersal. Reintroduction projects aimed at restoring animal populations offer valuable opportunities to study the importance of these interactions. We used a 33-year-old reintroduction project to investigate the effect of the frugivorous bird Crax blumenbachii on tree-seedling communities in a Brazilian Atlantic forest fragment. We hypothesized that C. blumenbachii alters forest understory species composition, particularly where the species is most abundant. We also expected that dissimilarity in plant species composition between forest strata would correlate with C. blumenbachii density, with an increase in understory species commonly in their diet. The study was conducted in a 753-ha forest fragment in Minas Gerais, Brazil, using 23 forest inventory plots. We assessed the reintroduced population through demographic censuses and trap camera data, and their diet through fecal analysis. We found that C. blumenbachii density is moderately associated with variation in plant species composition, as indicated by ordination analysis. Additionally, floristic dissimilarity between canopy and understory tended to increase in areas with higher C. blumenbachii density. Contrary to expectations, we did not observe an increase in the abundance of diet plant species in the understory. This may be due to a mismatch between faeces composition, rich in early-successional species, and floristic sampling areas, located in shaded old-growth forest. Despite the modest effects observed, our findings underscore the challenges of detecting vegetation-level responses to species reintroductions and highlight the importance of integrative approaches for understanding and restoring plant-animal interactions.

Keywords: frugivorous, birds, seed dispersal, defaunation, reintroduction, tropical forest.

1. INTRODUCTION

Frugivorous animals are responsible for dispersing up to 90% of the woody plant species in tropical forests, making them one of the primary drivers of forest regeneration in these ecosystems (Almeida-Neto et al., 2008; Chaves et al., 2018; Terborgh et al., 2008). This process is fundamental for maintaining plant diversity and ecosystem resilience in tropical landscapes (Emer et al., 2018). By distributing seeds away from the parent tree, animals can increase seed survival rates, since mortality near the parent tree is commonly high due to the presence of species-specific pathogens and intraspecific competition (Dent and Estrada-Villegas, 2021; Janzen, 1970; Stevenson, 2011; Webb and Peart, 2001). Large vertebrates can play a particularly important role in this process, because of their capability to (1) consume and disperse larger fruits and seeds compared to small frugivores; (2) ingest a greater quantity of fruits, consequently dispersing more seeds; and (3) disperse seeds over greater distances, including crossing different habitats, owing to their larger home ranges and longer gut-passage time (Bueno et al., 2013; Donoso et al., 2020; Graham, 2001; Holbrook and Loiselle, 2009).

Large frugivorous animals are, however, also greatly impacted by habitat loss, fragmentation, and hunting across tropical forests (Gardner et al., 2019), with many species undergoing strong population declines and facing high extinction risk (Allan et al., 2019; Markl et al., 2012). Defaunation of already highly fragmented forest landscapes further exacerbates the loss of plant species, especially endemic and rare ones (Carlo and Morales, 2016; Poulsen et al., 2013). Among the multiple ecological processes involved in forest regeneration, seed dispersal and pollination—especially those mediated by animals—are the most negatively affected by human disturbance (Neuschulz et al., 2016). Studies indicate that the population decline of large frugivores can be directly linked to reduced tree recruitment and the loss of species, especially of species with large seeds (Figure 1) (Bender et al., 2018; Galetti et al., 2021; Wheelwright, 1985; Wotton and Kelly, 2011), as well as a reduction in forest biomass and carbon stocks (Peres et al., 2016).

The declining abundance of large frugivores has significant consequences in the Brazilian Atlantic forest, which is recognized as one of the top five biodiversity hotspots globally (Mittermeier et al., 2005; Rezende et al., 2018). The Brazilian Atlantic forest has been significantly degraded during the last century and only 12% of its original coverage remains, most of it in small, often highly isolated, patches (Ribeiro et al., 2009; SOS Mata Atlântica, 2023). Consequently, the endemic fauna of the Atlantic forest has been severely impacted, resulting in alarming population declines and the extinction of several species at global, national, or local levels (Develey and Phalan, 2021; Galetti et al., 2021; Pires and Galetti, 2023). Among the affected species is one of the last large-bodied frugivorous birds in this biome, the red-billed curassow (*Crax blumenbachii* Spix, 1825), with a wild population of around 250 individuals concentrated in less than 1% of its original range (Bonfim et al., 2019; IBAMA, 2004; IUCN, 2022).

Several reintroduction projects aiming to recover the population of *C. blumenbachii* are underway with the main goal of preventing its extinction (Bernardo et al., 2014; IBAMA, 2004). Within the 753-ha forest fragment at the Private Reserve of Natural Heritage (RPPN) Fazenda Macedônia, *C. blumenbachii* was completely absent since at least the 1970s, but likely much earlier (IBAMA, 2004); however, a small population has been successfully reintroduced since 1990. This reintroduction project may, therefore, have led to the restoration of plant-animal interactions that were absent for decades (Genes et al., 2019; Mittelman et al., 2020; Oliveira-Santos and Fernandez, 2010). Nevertheless, as it is the case for many threatened large frugivores, our understanding of the role of these frugivorous animals for tree recruitment patterns and forest dynamics remains limited. Reintroduction projects, therefore, provide a unique opportunity to investigate plant-animal interactions, and the consequences of their restoration (Landim et al., 2022; Markl et al., 2012; Mittelman et al., 2020; Vidal et al., 2013).

Here, we took advantage of a 33-year-old reintroduction project to investigate the effect of a reintroduced population of *C. blumenbachii* on plant species composition in an Atlantic forest fragment. We combined different sources of plant and animal data to assess the distribution of the reintroduced *C. blumenbachii* population and its diet, as well as floristic composition in 23 plots across the fragment. Our hypothesis is that *C. blumenbachii* alters the species composition of the forest understory, specifically in the parts of the fragment where the bird is most abundant. In addition, we expect that differences in plant species composition among the various forest strata are related to the density of *C. blumenbachii* (Figure 1), along with an increase in the abundance of understory plant species included in their diet.

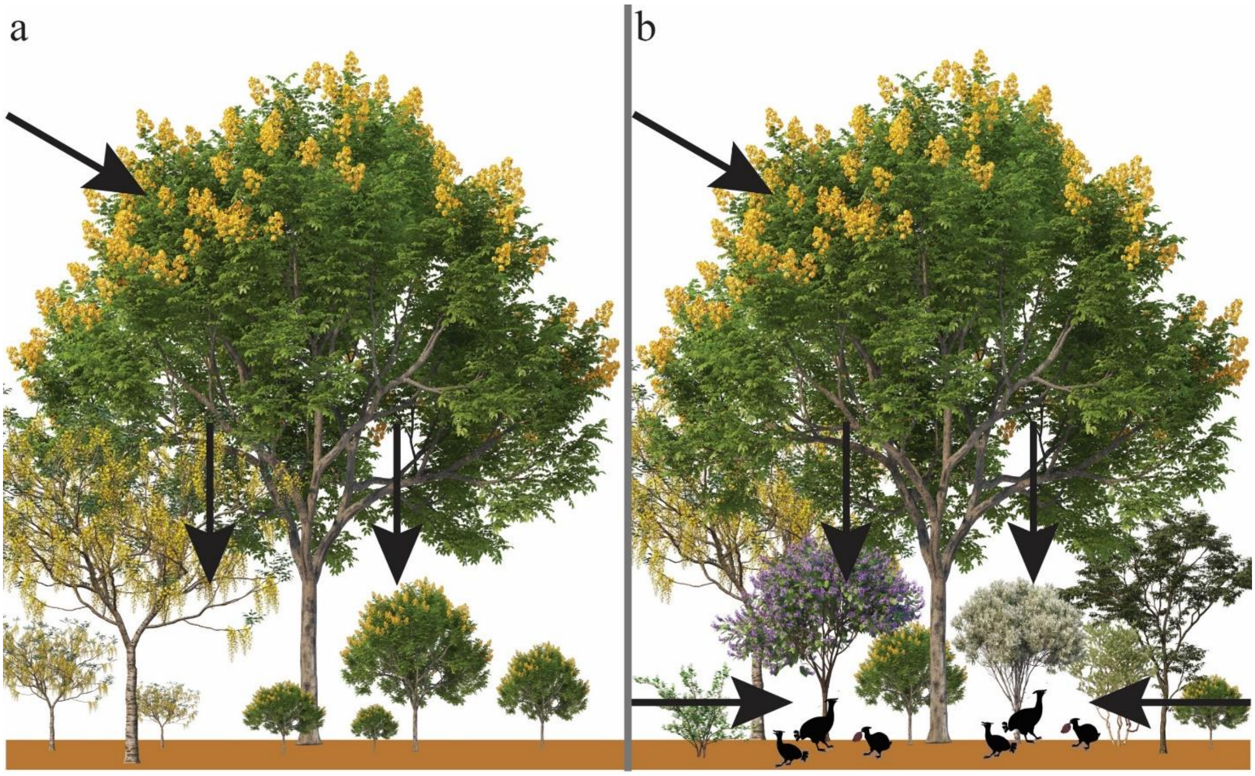

Figure 1. Schematic representation of our hypothesis regarding the impact of Crax blumenbachii on species recruitment. (a) When frugivores are absent, seed dispersal primarily occurs through anemochory (indicated by diagonal arrow) and autochory (indicated by vertical arrows), resulting in relatively high similarity of tree species in the canopy, sub-canopy and understory layers. (b) When frugivores are present, zoochory (indicated by horizontal arrows) also occurs, with animals spreading seeds over relatively large distances across the forest. This may potentially lead to increased dissimilarity between the canopy and understory layers.

2. METHODS

2.1. Study site and target species

The research was carried out in a High Conservation Value Area (HCVA) located within the Fazenda Macedônia in Ipaba, Minas Gerais, Brazil (Figure 2). This 753-ha Atlantic forest fragment is considered a HCVA due to the presence of at least 23 bird and 10 mammals species classified as endemic, rare, threatened, or endangered species, such as *Crax blumenbachii* (CENIBRA, 2023b). This HCVA fragment includes the Fazenda Macedônia Private Reserve of Natural Heritage (RPPN; IBAMA–Ordinance No. 111, of October 14, 1994). The RPPN is 560 ha in size, and consists of two regions, hereafter the North and South regions, that are connected by a central forest corridor of 71 ha (Figure 2; Rufino et al., 2023). Additional areas contiguous with this largest patch contribute a further 122 ha, completing the 753-ha HCVA fragment.

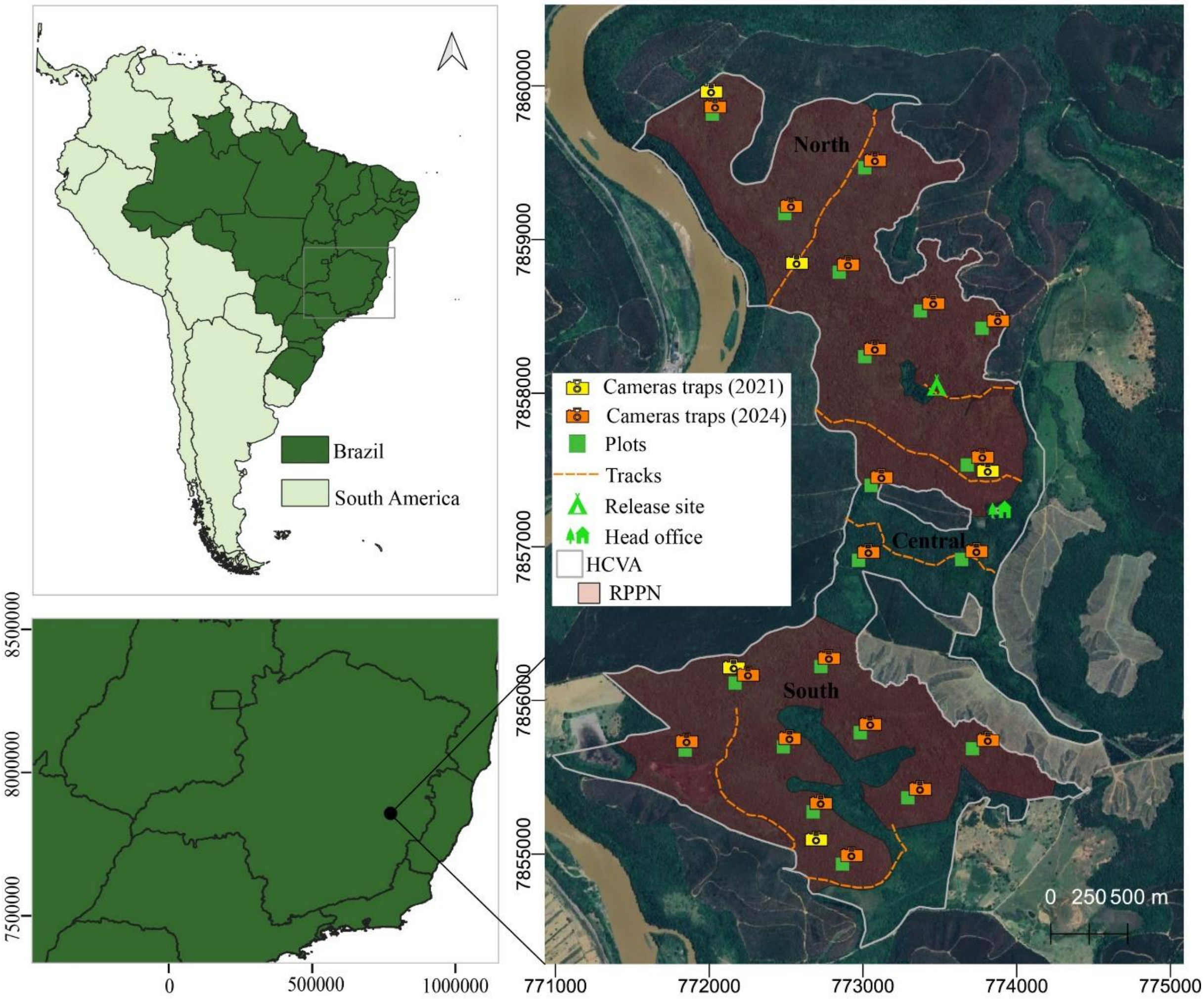

Figure 2. Location of the study area. The study took place in the High Conservation Value Area (HCVA; grey line), located within the Fazenda Macedônia, Ipaba, Minas Gerais, Brazil. This 753-ha Atlantic forest fragment hosts a 33-year old reintroduction project focusing on *Crax blumenbachii*, among other species. The HCVA includes a 560-ha Private Reserve of Natural Heritage (RPPN; highlighted in brown). The locations of forest inventory plots, camera traps and survey tracks are shown. Coordinate system: DATUM SIRGAS 2000, UTM ZONE 23 S.

The vegetation is Submontane Seasonal Semideciduous Forest (IBGE, 2012) with predominant climate of type Aw, humid tropical with dry winter and rainy summer according to the Köppen classification (Alvares et al., 2013). The maximum and minimum temperature are 30.2 °C and, 18.7 °C and mean annual precipitation is 1440 mm (based on data from the Lagoa Perdida meteorological station owned by CENIBRA S.A.); average altitude is 230 m.

At the study site, the Mutum Project started in 1990 with the focus of reintroducing endangered birds (CENIBRA, 2023a). The main target species of this project is *C. blumenbachii*, a large-bodied (up to 90 cm and 3.5 kg; (BirdLife International, 2016; Srbek-Araujo et al., 2012)) and endemic cracid from the Atlantic forest classified as Endangered (EN) by the IUCN (BirdLife International, 2016) mainly due to hunting and habitat loss caused by deforestation and fragmentation (Rios et al., 2021). Recovery of the species is relatively slow because of its late sexual maturity, monogamy, maximum of two eggs per reproductive season, and requirement of large home ranges (Araujo, 2017; Rios et al., 2021; Srbek-Araujo et al., 2012). It is a forest-dependent, ground-dwelling frugivore that spends most of the day foraging on the forest floor and perches in the tree canopy in the late afternoon, where it remains overnight (IBAMA, 2004). Between 1990 and 2018, 219 birds of this species were released (Table S1), most of them in pairs. Additional males were occasionally released to balance the sex ratio and encourage reproduction (Personal communication provided by the CENIBRA S. A.).

Crax blumenbachii was one of the five Brazilian birds species considered saved from extinction over the past thirty years as a result of conservation efforts (Bolam et al., 2021; CENIBRA, 2023b). The longevity and success of the Mutum Project makes it an important study site for developing knowledge about the species and reintroduction ecology (Costante et al., 2023; Martins et al., 2022; Phalan and Fernandes, 2020).

2.2. Forest inventory

In 2021, twenty-three plots were installed throughout the 753-ha forest fragment (Figure 2), ten in the North region, three in the Central region and another ten in the South of the study area (Rufino et al., 2023). In 2024, additional subplots were newly established — three for the sub-canopy and five for the understory — in each plot to increase sampling effort for smaller plants. Trees, shrubs and palms were inventoried using a nested design with three levels, focusing on different forest strata by measuring different plants sizes (Figure S1): (i) Canopy: all plants with diameter at breast height (DBH) ≥ 5 cm were measured and identified in 10 x 50 m plots (500 m^2). These plots were divided into 20 subplots of 5 x 5 m (25 m^2); (ii) Sub-canopy: plants with total height (Ht) ≥ 2.0 m and DBH < 5 cm were measured and identified in three 5 x 5 m subplots, with a total area of 75 m^2 per plot; (iii) Understory: plants ranging in height from 0.3 m to < 2.0 m were measured and identified in five 2.5 x 2.5 m subplots (Figure S1). The total area surveyed was 31.25 m^2 per plot.

Additional details regarding the distribution of plots and the schematic representation of the nested design used in the 2021 measurements can be found in Rufino et al. (2023).

2.3. *Crax* blumenbachii distribution and diet

The monitoring of the *Crax blumenbachii* population was carried out between 2020 and 2024, using a combination of censuses surveys, camera traps and faeces collection throughout the area (Figure 2). Demographic censuses were made along six tracks within the forest fragment looking for *C. blumenbachii*' vocalizations, footprints, and faeces (Figure 2). We recorded the location of every observation using GPS. Five camera traps were installed between April and November 2021 (Figure 2). To enhance sampling coverage across the entire forest fragment, 20 additional camera traps were deployed between April and July 2024, and the same were reinstalled between October and December 2024 (Figure 2). These devices were placed in 20 of the 23 permanent forest inventory plots, maintaining a minimum distance of 500 meters between them. Fecal samples were collected during 25 monthly campaigns from January 2021 to June 2024, along the tracks and forest edges (Figure 2). The location of each faeces sample was recorded by GPS.

The population diet was analyzed based on the seeds extracted from the faeces collected. In addition to the previously mentioned locations, fecal samples were also collected within the head office area exclusively to enhance diet characterization (Figure 2). The head office area was included for diet characterization due to three main reasons: (a) existence of a fruit tree orchard, (b) presence of *C. blumenbachii* couples in captivity, and (c) sporadic feeding. Additionally, the head office is located in the region with the highest density of *C. blumenbachii* within the fragment, making it an important area for understanding current resource use by the population. Notably, only fecal samples from wild individuals were included in the analysis; no samples from captive animals were collected, ensuring that the diet characterization reflects only free-ranging individuals. The faeces were washed in flowing water, dried at room temperature, and stored at 5°C. Species identification was performed based on morphological characteristics of the seeds or, for those that germinated and developed into seedlings, vegetative parts. Reference collections or specialists were consulted for accurate identification.

2.4. Data *analysis*

We generated a Kernel density heat map to calculate the density of *Crax blumenbachii* records across the study area (Parzen, 1962; Rosenblatt, 1956) using all censuses surveys, camera trap records and fecal samples collected within the fragment, but excluding fecal data from the head office to avoid overestimation, as faeces are easier to detect on dirt or grass than in forest litter. We included data from the entire sampling period, as no substantial variation (i.e. seasonality) in detection was observed within and across years. In this study, we interpreted density as density of use, defined as the number of independent records per unit area, rather than the number of individuals. We opted to estimate the density of records rather than population size because our focus was on understanding the spatial distribution and intensity of habitat use by *C. blumenbachii* rather than obtaining demographic estimates. Each record— regardless of source— was treated as an observation of forest use intensity, based on the assumption that detection frequency reflects variation in habitat use across the landscape. Sampling effort was standardized within each data type, ensuring consistent detectability across the area. Although some fecal samples may originate from the same individual, we consider this data source ecologically meaningful, as faecal deposition is directly linked to the species' seed dispersal activity. Variation in the number of feces across plots may therefore reflect differences in frugivory behavior or habitat use, rather than bias. The Kernel density heat map was created using QGIS software (QGIS Association, 2023) using a fixed radius of 2 km and pixel size of 1 m².

To assess whether the density of other frugivorous species covaries spatially with that of *C. blumenbachii*, we generated a Kernel density heat map using all camera trap records of frugivorous mammals and birds (Table S2) obtained throughout the entire monitoring period. All animal species for which (partial) consumption of seeds and fruits was known from literature were selected. The Kernel density map was generated in QGIS (QGIS Association, 2023), using the same parameters applied to the *C. blumenbachii* analysis (fixed radius of 2 km and pixel size of 1 m²), to ensure spatial comparability. We then assessed the spatial association between the two density maps using Pearson's correlation coefficient, calculated with the ENMTools package (Warren et al., 2021) in R .

We calculated the dissimilarity in plant species composition between the forest strata for each plot in order to evaluate the effect of the reintroduced birds. To this end, we used the Jaccard dissimilarity index, which is based on the presence or absence of species in both the understory and (sub)canopy layers. We chose to use presence-absence data rather than abundance because abundance-based indices, such as Bray-Curtis, are sensitive to highly dominant species and may obscure compositional patterns, particularly when comparing structurally distinct communities with different species-abundance distributions (Anderson et al., 2006). Since our goal was to assess floristic dissimilarity and species turnover in relation to *C. blumenbachii* density, presence-absence data provided a more conservative and ecologically meaningful approach for comparing assemblages. The Jaccard dissimilarity index assumes that the sampling is adequate to characterize the floristic composition of the population evaluated (Chao et al., 2005). Despite variations in the total sampled area per strata for each plot, we assumed that the area sampled for the sub-canopy and understory strata was sufficient for characterization because although the sampled area at these levels was smaller, the size criteria for plant inclusion were also reduced, resulting in a greater number of individuals sampled, per area, in the subplots than in the plots (Table S3). To validate this assumption and assess whether differences in sampling effort between strata could bias species composition comparisons, we estimated sample completeness using the coverage-based approach proposed by Chao et al. (2020). We calculated sample coverage for each forest strata using Shannon diversity index ($q = 1$), which accounts for both species richness and relative abundances, in iNEXT.4steps package (Chao et al., 2020) in R (R Core Team, 2024). Plot-level sample completeness values indicated relatively high coverage across strata (Table S4), with means ± SD of 0.71 ± 0.10 for the canopy, 0.61 ± 0.24 for the sub-canopy, and 0.70 ± 0.15 for the understory. Additionally, to test whether sample completeness varied along the *C. blumenbachii* density gradient, we calculated coverage at the plot level per strata and evaluated its correlation with *C. blumenbachii* density using Spearman's test. No significant relationship was found ($\rho = –0.014$, $P = 0.906$), suggesting that sampling completeness did not systematically vary across the density gradient. Coverage sampling values per plot and strata are presented in the supplementary material (Table S4).

We used non-metric multidimensional scaling (NMDS) to visualize the dissimilarity between the forest strata per plot based on the indices generated in the Jaccard presence-absence matrix. To test for significant differences among strata, we applied both a permutational multivariate dissimilarity-based analysis of variance (PERMANOVA) and an analysis of similarity (ANOSIM), each based on the same Jaccard dissimilarity matrix and performed with 999 permutations, as implemented in the vegan package in R (Anderson and Walsh, 2013; Gutow et al., 2015). Because both the understory and sub-canopy strata were sampled using distinct subplots nested within the same canopy plot, permutations in the PERMANOVA were restricted within plots using the strata argument in the adonis2 function. This approach accounts for nested sampling design and prevents pseudoreplication by preserving the spatial dependence of the samples (Anderson, 2008).

To assess the influence of *C. blumenbachii* density and environmental factors on species composition, we used all environmental variables previously identified by Rufino et al. (2023) as key drivers of floristic composition in this forest fragment. The density of *C. blumenbachii* was subsequently added to this set and Pearson correlation analysis was conducted, using the corrplot package (Wei and Simko, 2021) in R software (R Core Team, 2024), to identify and remove variables with high collinearity ($r > 0.6$) (Figure S2). Altitude, remaining phosphorus (P-rem), silt fraction, and density of *C. blumenbachii* were fitted in the NMDS, using the envfit function of the vegan package (Oksanen et al., 2019) in R software (R Core Team, 2024), to assess their influence on species composition. The forest strata was fitted as a variable to assess whether the vertical structure of the forest influences floristic composition independently of the environmental factors tested. Additionally, no spatial differences in food availability for Cracidae birds or in forest quality were found across the forest fragment (Rufino et al., 2023; Rufino et al., in prep.). Methodological details regarding the environmental factors can be found in Rufino et al. (2023).

The impact of the reintroduced birds on forest regeneration were evaluated by calculating the Jaccard dissimilarity index between pairs of the three forest strata (canopy and sub-canopy; canopy and understory; sub-canopy and understory) for each inventory plot, and then, using linear regression to evaluate whether the density of *C. blumenbachii* increased the dissimilarity of the floristic composition between the different forest strata. We found that one of our plots (plot 23) had high canopy-level mortality, with almost half of the trees being dead (Table S3), making species identification for comparison with other strata impossible. Consequently, we removed this plot from these analyses.

Using the diet characterization data (Rufino et al., in prep), we focused specifically on the distribution of species consumed by *C. blumenbachii* within the fragment. We used linear regression to examine whether the abundance of plants consumed by *C. blumenbachii* increases in the understory of the forest plots where *C. blumenbachii* density was higher. To do so, all plant species identified in the diet of *C. blumenbachii* and observed in the understory subplots of the forest inventory were evaluated (Table 1). In addition, we tested whether *C. blumenbachii* density was associated with understory plant species richness at the plot level. Species richness in the understory was calculated for each plot, and a linear regression was performed using *C. blumenbachii* density as the predictor.

Table 1. List of species identified in *Crax blumenbachii* faeces and present in understory subplots within the 753-ha Atlantic forest fragment in Fazenda Macedônia, Brazil. The table provides the successional group (SG; E = early successional, L = late successional), the plots in which each species was recorded (Plots), number of faeces containing each species and the number of seeds found. A total of 429 faeces (219 containing seeds) were found in the study within 2021 and 2024.

Species	Family	SG	Plots	Faeces	Seeds
Allophylus edulis (A.St.-Hil. et al.) Hieron. ex Niederl.	Sapindaceae	E	21	1	4
Brosimum guianense (Aubl.) Huber	Moraceae	E	2, 3, 4, 5, 6, 8, 9, 10,1 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23	2	9
Clarisia ilicifolia (Spreng.) Lanj. & Rossberg	Moraceae	L	1, 16	1	2
Eugenia bahiensis DC.	Myrtaceae	L	4, 5, 7, 8, 9, 10, 11, 13, 16, 18, 19, 20, 22	1	1
Guarea guidonia (L.) Sleumer	Meliaceae	L	11, 13, 21	3	47
Maclura tinctoria (L.) D.Don ex Steud.	Moraceae	E	20	1	60
Meliosma chartacea Lombardi	Sabiaceae	L	6	1	3
Psychotria carthagenensis Jacq.	Rubiaceae	E	19	5	102
Siparuna guianensis Aubl.	Siparunaceae	E	2, 7, 13, 22	6	26
Vitex megapotamica (Spreng.) Moldenke	Lamiaceae	E	8, 9	7	72
Xylopia sericea A.St.-Hil.	Annonaceae	E	6, 21, 22	4	6

3. RESULTS

The highest density of *Crax blumenbachii* was recorded in the North region, followed by the Central region and the area surrounding the Head office (Figure 3). In contrast, the South region consistently exhibited extremely low densities, with only six records registered over more than four years of monitoring. Although the absence of a formal control site outside the release fragment is a limitation, the pronounced spatial disparity allows the South region to serve as a quasi-control area within the study fragment. To assess whether this spatial pattern might reflect broader frugivore activity rather than a *C. blumenbachii*-specific distribution, we generated a kernel density map for other frugivorous birds and mammals recorded by camera traps (Figure S3). We found non-significant correlation between the density maps of *C. blumenbachii* and other frugivores (Pearson's $r = 0.54$), suggesting that the distribution of *C. blumenbachii* does not simply mirror the overall spatial pattern of frugivore habitat use within the study area. Notably, unlike *C. blumenbachii*, other frugivores were frequently recorded in the South region (Figure S3), indicating that the low density of *C. blumenbachii* in this area does not reflect a general absence of frugivore activity.

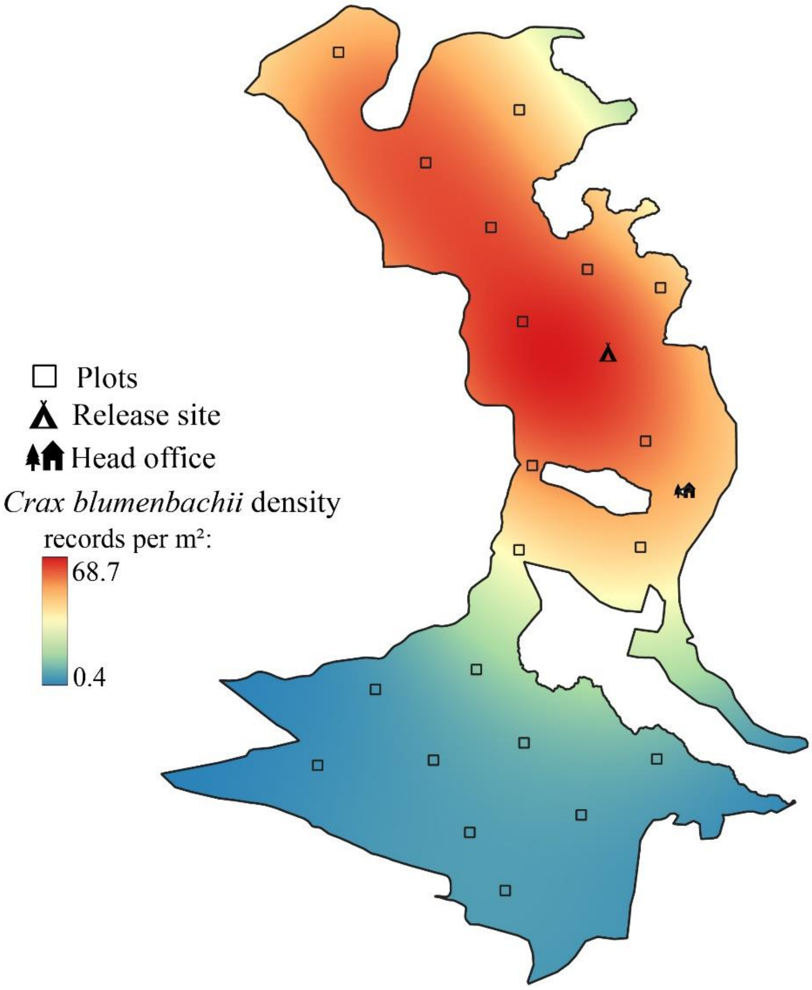

Figure 3. Density heat map, in records per square meter, of *Crax blumenbachii* in the High Conservation Value Area (HCVA), located within the Fazenda Macedônia, Brazil. Warm colors indicate the highest density of *C. blumenbachii*, while cool colors indicate lower density. The squares represent the location of forest inventory plots. The density values represent the kernel-smoothed estimate of records per unit area, considering a search radius of 2 km. These values should not be interpreted as absolute counts within a single square meter, but rather as an index of records distribution across the landscape. The analysis was based on data from camera traps (2021 and 2024), demographic censuses along tracks, and faeces recorded along tracks and forest edges, excluding fecal data from the head office to avoid overestimation.

The non-metric multidimensional scaling (stress = 0.227) of plant species composition across three forest strata in 23 inventory plots does not show clearly defined clusters, with plots from different regions of the fragment being scattered along the ordination (Figure 4). However, most canopy plots appear centralized in the ordination and are surrounded by sub-plots from other strata, with subcanopy subplots being more dispersed in the multidimensional space than the understory subplots. Similarly, plots located in the North and Central regions tend to be more to the left of the ordination, while plots of the South regions tend to be in the right portion (Figure 4). We found a relatively high stress value, but since it remains below 0.35, it suggests a non-random distribution (Clarke, 1993; Dexter et al., 2018). Further analyses confirm the differences between strata, as ANOSIM indicated significant differences in species composition among forest strata (*R*= 0.229, *P*= 0.001), which was further supported by PERMANOVA results (*pseudo-F*= 2.794, *P*= 0.001). These findings suggest that, even though the NMDS does not exhibit sharply defined clusters and needs to be interpreted cautiously, the composition of plant species varies significantly across strata.

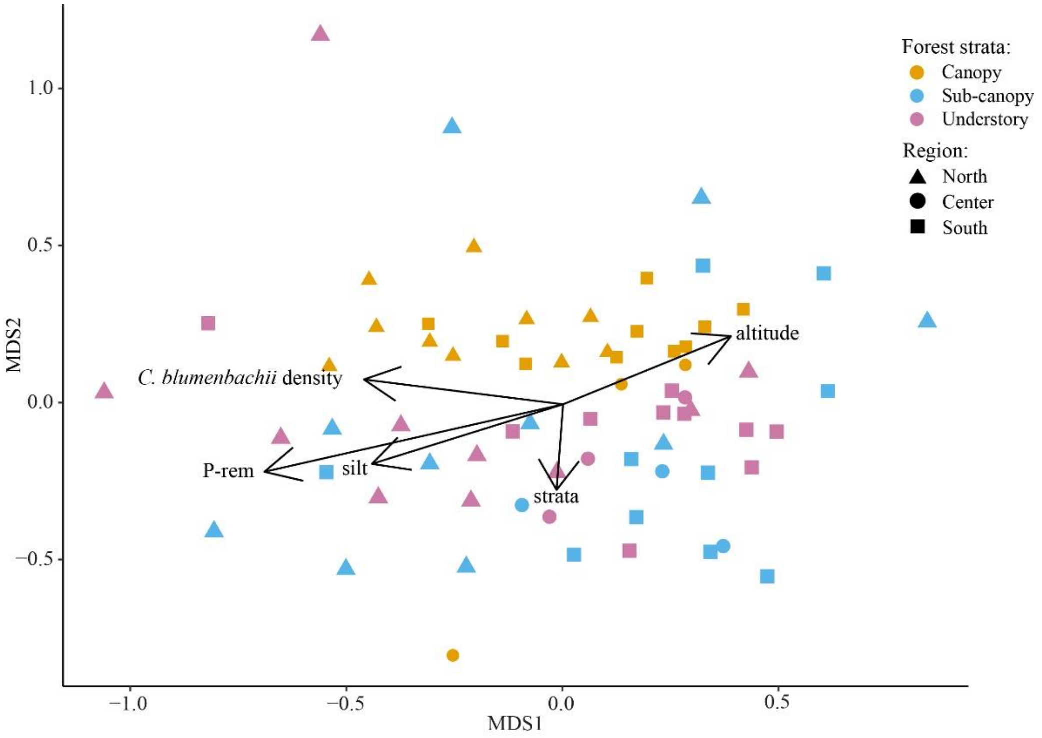

Figure 4. NMDS diagram of plant species composition across three forest strata based in 23 inventory plots using a nested design. Colors represent the level of the forest strata, and symbols represent the region where the plot is located (see Figure 2). Stress = 0.227. Significant variables (at 90% probability) are included: altitude, P-rem (remaining phosphorus), silt (fraction of silt in the soil), *C. blumenbachii* density and strata. Arrow lengths indicate the magnitude of the

correlation between the factor and species composition. The direction in which an arrow points indicates the axis with which the environmental factor is most strongly correlated.

Floristic composition was also significantly associated with environmental variables fitted individually to the NMDS ordination. Remaining phosphorus (P-rem) (P= 0.001; R^2= 0.52), *C. blumenbachii* density (P= 0.001; R^2= 0.22), altitude (P= 0.001; R^2= 0.20) and silt fraction (P= 0.003; R^2= 0.23). The variable "strata" was only marginally significant (P= 0.081; R^2= 0.08). Plots in the South region are at higher altitudes and have lower silt content and remaining phosphorus than those in the North region (Figure 4). P-rem has the strongest influence on floristic composition variability, explaining half of the variability shown in the NMDS. These results indicate that, except for strata, the other variables exhibited moderate correlations with the NMDS configuration, suggesting that they help explain key gradients in plant species composition as captured by the ordination axes. To further explore whether the effects of *C. blumenbachii* on floristic composition were specific to animal-dispersed species, we performed a complementary NMDS analysis separating animal-dispersed and non-animal-dispersed species groups. Interestingly, *C. blumenbachii* density was significantly associated with species composition in both groups (Figure S4).

If *C. blumenbachii* density indeed affects forest composition, as the NMDS results indicate, then we expected that the dissimilarity between forest strata levels would increase with *C. blumenbachii* density, especially when comparing the canopy to the understory (Figure 5a).

While dissimilarity between canopy and understory species composition increases with *C. blumenbachii* density (P= 0.061, adjusted R^2= 0.123), comparisons between canopy and sub-canopy (P= 0.477, adjusted R^2= -0.023), and between sub-canopy and understory (P= 0.734, adjusted R^2= -0.044), show a non-significant decreasing pattern (Figure 5b). These negative adjusted R^2 values in linear regression indicates that the fitted model explains less variability than a null model (i.e., the mean of the response variable provides a better fit than the model itself (Nakagawa and Schielzeth, 2013; Wildt, 1976)).

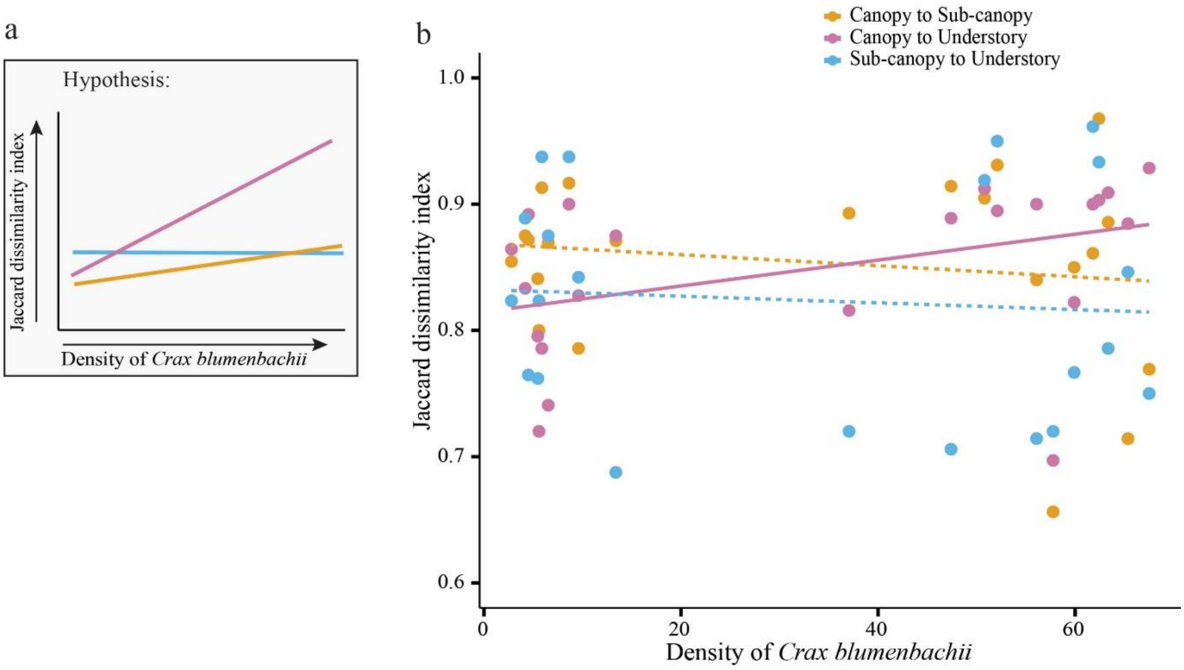

Figure 5. The relation between the Jaccard dissimilarity index in plant species composition, across 22 (sub)plots, with the *Crax blumenbachii* density. The hypothesis (a) and the observed (b) results are provided. Each dot represents a comparison between different forest strata levels within a plot, with colors indicating the strata-pair compared. Dissimilarity values of 1 represent the maximum dissimilarity between plots. In Figure 5b, solid lines represent slopes that are significantly different from zero, while dashed lines indicate slopes that are not significantly different from zero, at the 90% confidence level.

Next, we assessed the effects of *C. blumenbachii* on the abundance of understory plants found in the diet of this bird species. We identified 11 species in *C. blumenbachii* faeces that are present in understory subplots within the forest fragment (Table 1). Although the abundance of these plant species increased slightly with *C. blumenbachii* density, none of these trends were significant (P= 0.869, adjusted R^2= -0.046; Figure 6a). Similarly, we found no significant relationship between *C. blumenbachii* density and understory seedling species richness at the plot level. Although richness also showed a slightly increasing trend, the regression was not significant (P= 0.959, adjusted R^2= -0.047; Figure 6b).

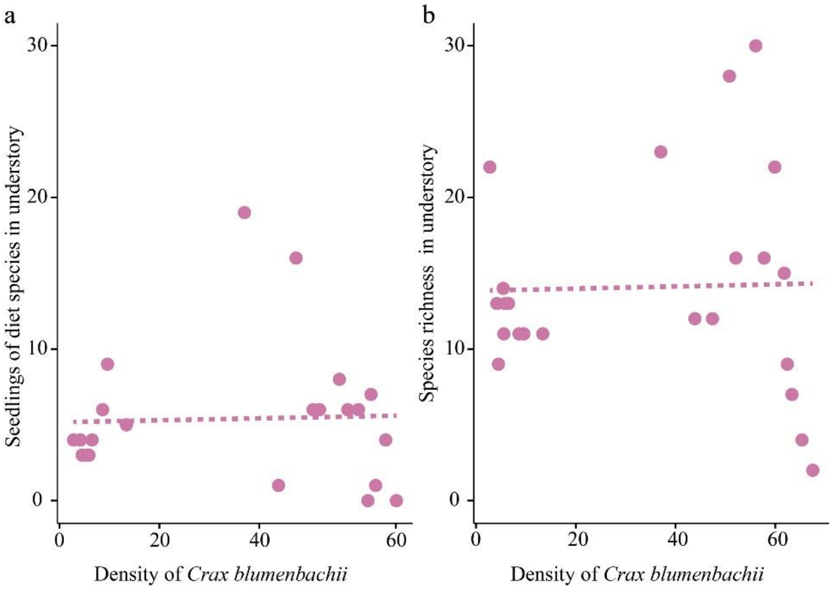

Figure 6. Relationship between *Crax blumenbachii* density and two aspects of understory plant communities: (a) Abundance of seedlings belonging to species identified in the diet of *C. blumenbachii*, and (b) Total seedlings species richness (S). In both cases, the regression slope is not significantly different from zero. Each dot represent the mean value based on five 2.5 × 2.5 m subplots established in 23 plots in the 753-ha Atlantic Forest fragment designated as a High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil.

4. DISCUSSION

We evaluated the potential effects of reintroducing a large-bodied frugivorous bird species (*Crax blumenbachii*) on forest composition in the Brazilian Atlantic forest by examining the understory (where short-term responses to seed deposition are most likely to occur) and the sub-canopy (which may reflect mid-term compositional change as seedlings grow and recruit into higher strata). Our hypothesis was that *C. blumenbachii* alters the species composition of the forest understory, particularly in areas where they are most abundant. Our results provide limited and context-dependent support for this hypothesis. We found a statistically significant, yet modest, association between *C. blumenbachii* presence and plant species composition, with greater floristic dissimilarity between the canopy and understory in areas with higher *C. blumenbachii* density. These patterns may reflect early or subtle effects of *C. blumenbachii* reintroduction on forest structure, but also highlight the challenges of detecting the ecological consequences of frugivore loss and restoration in complex tropical systems.

4.1. Drivers of species composition

The main drivers of plant species composition were soil properties, namely remaining phosphorous and silt content (Figure 4), with a moderate contribution of altitude. Similar results were obtained in an earlier study looking at all forest strata collectively (Rufino et al., 2023). In this study, we also found that *C. blumenbachii* density contributes to 21% of the variability explained by the NMDS (Figure 4). Yet, interactions between plant communities and frugivores are mutualistic (Campagnoli et al., 2024), suggesting that the presence of *C. blumenbachii* could act either as a driver of plant species composition, through seed dispersal (Jordano et al., 2007), or as a consequence, with the reintroduced birds selecting habitats based on its diet preferences (Martínez and García, 2015; Prasad and Sukumar, 2010). Our finding that *C. blumenbachii* density was associated with both animal- and non-animal-dispersed species (Figure S4) suggests that the observed patterns may reflect habitat selection. Moreover, other forest structure variables were not major determinants of *C. blumenbachii* habitat selection in the study area (Chapter 3), reinforcing the possibility that the observed patterns may result from dietary preferences or frugivore–plant interactions. Both scenarios may, however, occur simultaneously, as food availability for Cracidae birds is likely consistent across the fragment (Rufino et al., 2023). Therefore, we cautiously interpret this result as a potential effect of *C. blumenbachii* on species composition by influencing plant recruitment through seed dispersal. Consequently, we expected to find a greater dissimilarity between strata in plots with higher *C. blumenbachii* densities, a pattern that was partially supported by the linear regression between dissimilarity and *C. blumenbachii* density among strata (Figure 5b).

4.2. *Crax blumenbachii* effects on plant recruitment

As *C. blumenbachii* was absent from the forest fragment for decades before their reintroduction, the plant species dispersed by this bird species may have experienced restricted dispersal during the period of its absence (Pérez-Méndez et al., 2016; Wotton and Kelly, 2011). However, the reintroduction of *C. blumenbachii* may increase the dispersal of some tree species and potentially increase floristic dissimilarity among forest strata, in particularly between the canopy and understory.

We indeed found a slight and marginally significant association (regression analysis, P= 0.06) between *C. blumenbachii* density and dissimilarity between the canopy and understory (Figure 5b), suggesting a possible influence of the reintroduced population on seedling species composition. Yet, this association was weak and should therefore be interpreted with caution.

We also explored if dissimilarity patterns could be related to environmental gradients, such as those caused by microclimatic variation, light availability (Brenes-Arguedas et al., 2011; Murakami et al., 2022), disturbance regimes (Döbert et al., 2017; Kissa et al., 2025; Kusuma et al., 2018) or ecological succession (Muñoz et al., 2017). We incorporated into the model of vertical dissimilarity, the environmental variables previously identified as significant drivers of floristic composition in the study area (Rufino et al., 2023), namely: altitude, remaining phosphorus (P-rem), and silt content (see Figure 4), along with the density of other frugivorous species (Figure S3). A stepwise linear regression model selected P-rem as the only significant predictor of vertical dissimilarity ($P = 0.0019$) between understory and canopy (adjusted $R^2 = 0.359$), while *C. blumenbachii* density and all other variables were excluded. This result reinforces the notion that the effect of *C. blumenbachii* on vertical dissimilarity is, at best, weak and may be confounded by other unmeasured factors. However, we would also like to point out that although phosphorus is known to influence floristic composition across broader spatial gradients (Becknell et al., 2012; Neri et al., 2017), we lack a mechanistic hypothesis for how it would affect within-plot vertical dissimilarity. In contrast, the hypothesis that frugivore-mediated seed dispersal promotes vertical structural differentiation is well supported by ecological theory (González-Castro et al., 2019; Norden and Stevenson, 2015; Velho et al., 2012). In addition, our environmental predictors are static and measured only once per plot, while vertical dissimilarity likely reflects dynamic recruitment processes occurring over time (Muñoz et al., 2017; Quero et al., 2011). Another limiting factor in these multiple regression models is that our relatively small sample size (n = 22 forest plots) restricts statistical power, possibly making it difficult to detect subtle ecological effects in multivariate models. For these reasons, we believe it remains plausible that *C. blumenbachii* exerts influence on seedling recruitment patterns and vertical floristic dissimilarity, albeit weak or incipient. While this potential effect may be obscured by environmental variability or limited by the constraints of our dataset, we consider it a hypothesis still worth investigating with more targeted and long-term approaches.

In recent decades, the effects of defaunation have been widely studied, particularly through comparisons between forest fragments containing key frugivores and those lacking them, resulting in presence-absence assessments (Effiom et al., 2013; Terborgh et al., 2008; Vanthomme et al., 2010). Although results vary, most studies report an effect of defaunation on plant species composition and recruitment (Heymann et al., 2019; Wright et al., 2007), while others have found no detectable impact (Chaves et al., 2015). This variability in results underscores the complexity of these plant-animal relationships and the inherent difficulty of quantifying them, especially in megadiverse tropical habitats where multiple ecological drivers operate simultaneously (Wandrag et al., 2017). Further complicating this picture is the possibility of interaction rewiring after defaunation and reintroduction, whereby frugivores may establish novel or altered ecological links in response to modified community contexts (Emer et al., 2018; Mittelman et al., 2020). Additionally, the outcomes are influenced by methodological approaches, taxonomic groups, the existence of functional redundancy among dispersers, and the temporal and spatial scales of analysis (Bueno et al., 2013; Chaves et al., 2015; Genes and Dirzo, 2022). Most previous studies have approached these questions at broad landscape scales, evaluating presence-absence data across different fragments; whereas our study assessed the impact of a key-frugivore density on a finer scale, within a single and highly diverse fragment, where other frugivores are also present (CENIBRA, 2023b; CENIBRA, Personal communication; Corrêa, 2023; Table S2). Detecting ecological signals in such fine-scale, noisy conditions is particularly challenging. Remarkably, even under these conditions, we found a weak but suggestive signal that *C. blumenbachii* presence may contribute to plant recruitment patterns. However, longer-term monitoring and complementary approaches are likely needed to better quantify the ecological consequences of frugivore reintroduction in complex tropical forest systems.

Although we expected a slight increase in dissimilarity between canopy and sub-canopy with greater *C. blumenbachii* density (Figure 5a), this pattern was not observed (Figure 5b). One possible explanation is that some sub-canopy plants recruited prior to *C. blumenbachii* reintroduction –given seed bank persistence, with particularly pioneers species exhibiting long-term viability, variable growth rates, and prolonged retention in lower strata (Table S5) (Clark and Clark, 2001; Dalling and Brown, 2009)– causing the observed pattern to be opposite of our expectations and non-significant (P = 0.466). Conversely, we expected no effect on dissimilarity between sub-canopy and understory due to the small variability in plant size. This hypothesis was confirmed, as no significant relationship was found (P = 0.726), although a slight decreasing trend was unexpected (Figure 5). The lack of significant results in both comparisons is likely also due to the short time frame of data collection (2021–2024). When sub-canopy plants recruited, *C. blumenbachii* density may have been lower, given the increase in reintroduced birds over the past decade (Table S1). Thus, current density estimates may not capture historical conditions, potentially underestimating its influence on floristic composition.

4.3. Seed species in *Crax blumenbachii*'s diet and understory responses

Since understory species composition showed greater dissimilarity in areas with higher *C. blumenbachii* density, we expected to find support for our hypothesis by zooming in on the floristic composition of this strata. However, this association was not accompanied by significant changes in two key metrics expected to respond to seed dispersal (Costa et al., 2022; Herrera et al., 1994; Trolliet et al., 2017): the abundance of seedlings belonging to species identified in the diet of *C. blumenbachii* (Figure 6a), and the overall richness of understory seedlings (Figure 6b). Both metrics showed no significant relationship with *C. blumenbachii* density, suggesting that, despite a spatial signal in vertical dissimilarity, the reintroduction has not yet produced detectable shifts in understory composition attributable to specific dispersed plant species.

One possible explanation for the absence of significant effects on these seedling-level metrics lies in the limitations of our dietary data. Although we included all plant species identified in *C. blumenbachii*'s faeces that were also recorded in the understory inventory, these species were not necessarily the most frequently consumed. In fact, five of the eleven species were detected in fecal samples only once (Table 1) (Chapter 4). This likely reflects collection bias, as nearly half of the faeces (113 out of 219) were gathered near the head office, where they are easier to spot due to sparse ground cover. Such spatial bias could distort diet representation, especially considering that seed dispersal is often spatially structured within frugivore home ranges (Sasal and Morales, 2013; Wenny and Levey, 1998), with certain plant species more likely to be deposited in specific microhabitats (Garcia et al., 2010; Santamaría et al., 2007). Additionally, despite extensive sampling, the species accumulation curve (Rufino, in prep) suggests that diet characterization remains incomplete, indicating that additional consumed species would likely be recorded with further effort. Given that *C. blumenbachii* is a generalist frugivore and the study area is highly diverse, fully capturing its dietary breadth is inherently challenging (Carlo and Yang, 2011; Santamaría and Franco, 2000).

A second possible explanation relates to the ecological characteristics of the species most frequently identified on *C. blumenbachii* diet, which were predominantly pioneer tree species (Rufino, in prep). This is likely because, in secondary forests such as our study site, trees that have reached reproductive age are mainly early successional species (Oliveira et al., 2011; Selaya and Anten, 2010; van Breugel et al., 2006). Therefore, these pioneer species are less likely to germinate and establish in the understory due to low light levels (Martínez-Ramos et al., 2021; Matsuo et al., 2021). As a result, their seedlings may be underrepresented in our plots, which were intentionally away from the forest borders to avoid edge effects. Given that *C. blumenbachii* faeces were commonly found at these edges, in areas where light levels are higher and pioneer tree species are more likely to establish, its actual influence on recruitment may be more pronounced in those areas. Consequently, our sampling design may have underestimated its effects on plant communities. Additionally, plot size and placement influence floristic composition assessments (Zeng and Wiens, 2021), representing a third factor affecting our findings. Increasing the number of subplots and including plots along edges and canopy gaps could provide a more comprehensive understanding of *C. blumenbachii*'s role in forest regeneration.

4.4. Outlook and implications

Our study highlights the potential, but still incipient, role that reintroduced frugivorous species can play in shaping forest composition within the Brazilian Atlantic forest and provides partial support for our hypothesis that areas with higher density of *C. blumenbachii* exhibit greater dissimilarity in species composition between the understory and the canopy. Although the observed effect was modest and marginally significant, it does suggest that *C. blumenbachii*'s seed dispersal activities may be beginning to influence vertical structuring of plant communities. Over time, this process could enhance forest resilience and support multitrophic interactions (Haas et al., 2011; Rottstock et al., 2014; Scherber et al., 2010), even in megadiverse ecosystems where niche complementarity and redundancy are high (Bueno et al., 2013; Lehouck et al., 2009; Rother et al., 2016). However, our findings must be interpreted with caution. Despite it being more than three decades since the beginning of the reintroduction project, the population of *C. blumenbachii* remains small in most of the forest fragment, which likely constrains its ecological influence. Moreover, no significant relationship was found between *C. blumenbachii* density and more direct indicators of its seed dispersal, such as the abundance of seedlings from consumed species or overall seedling richness. This reinforces the idea that detecting vegetation-level responses, especially in structurally complex and species-rich systems, is inherently difficult (Carlo et al., 2007; Ferger et al., 2016; Rigacci et al., 2021).

Understanding the consequences of defaunation, and the effectiveness of restoration efforts, remains a major scientific challenge, particularly in tropical forests where ecological interactions are diverse, diffuse, and influenced by multiple drivers. While most prior studies have focused on presence–absence comparisons across forest fragments (Chaves et al., 2015; Effiom et al., 2013; Gibbs et al., 2008; Mittelman et al., 2020; Terborgh et al., 2008), our study assessed the ecological signals of a reintroduced frugivore at a fine scale, within a single forest fragment still inhabited by other frugivores. By combining plant community data with species-level diet characterization and frugivorous birds' distribution, we offer a more integrative strategy for understanding plant-animal interactions, and how they may recover after the reintroduction of frugivorous species. Following this approach, our analysis also revealed complexities that warrant further investigation. The absence of significant effects on the abundance of specific plant species consumed by *C. blumenbachii* underscores the difficulty in capturing the entirety of its ecological role, particularly given the (i) the limitations of our dietary analysis due to potential biases in fecal sample collection, incomplete diet characterization, and difficulties in species identification; and (ii) the presence of other frugivorous species, which likely engage in complementary ecological roles. Although our analysis indicates that their spatial distribution does not mirror that of *C. blumenbachii*, functional overlap and niche complementarity among frugivores may dilute or obscure the specific effects of this reintroduced species (Bai et al., 2017; Lehouck et al., 2009; Rother et al., 2016; Schlautmann et al., 2021). To improve diet characterization, additional methodologies could be incorporated, such as direct feeding observations and the expansion of reference collections for species identification (Matthews et al., 2020). Furthermore, evaluating the extent of functional redundancy among frugivores could provide insights into how multiple species contribute collectively to seed dispersal processes in tropical forests (Chaves et al., 2015).

The full effects of *C. blumenbachii* on forest dynamics may take longer to manifest, particularly in the sub-canopy strata, due to historical variations in its density across the fragment. This aligns with recent findings showing that the functional recovery of seed-dispersal interactions can take decades, even after the partial reestablishment of frugivore and plant communities (Landim et al., 2025) Therefore, long-term monitoring is crucial to better understand the cascading effects of *C. blumenbachii* on forest composition (Fernandez et al., 2017). Future studies on frugivores species should also consider the temporal variations in frugivore activity and other variables that could affect plant recruitment (e.g., microclimate, light availability).

In summary, our results underscore the complexity of restoring lost ecological interactions. Even in the absence of strong vegetation-level responses, the effort to reintroduce key species like *C. blumenbachii* remains valuable. It illustrates the ecological potential of rewilding in tropical forests, while also exposing the need for long-term strategies to monitor and support these processes. Conservation actions must therefore go beyond species reintroductions and incorporate sustained, integrative monitoring to evaluate ecosystem recovery and resilience. Understanding these dynamics will be essential for informing conservation strategies that aim to restore and maintain the ecological integrity of the Brazilian Atlantic forest.

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Supporting Information

Table S1. Number of *Crax blumenbachii* individuals reintroduced per year in Mutum Project in the Fazenda Macedônia, Brazil.

Year	***Crax blumenbachii* individuals reintroduced**
1990	30
1991	37
2007	5
2010	6
2015	60
2017	40
2018	41
Total	**219**

Table S2. List of frugivorous bird and mammal species recorded by camera traps in the High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil.

Bird species	**Mammal species**
Aburria jacutinga	*Callithrix* sp.
Aramides sp.	*Cuniculus paca*
Cacicus haemorrhous	*Dasyprocta azarae*
Crypturellus noctivagus	*Didelphis aurita*
Penelope obscura	*Didelphis* sp.
Penelope superciliaris	*Eira barbara*
Tinamus solitarius	*Guerlinguetus ingrami*
	Marmosops incanus
	Marmosops sp.
	Nasua nasua
	Sapajus nigritus
	Tapirus terrestris

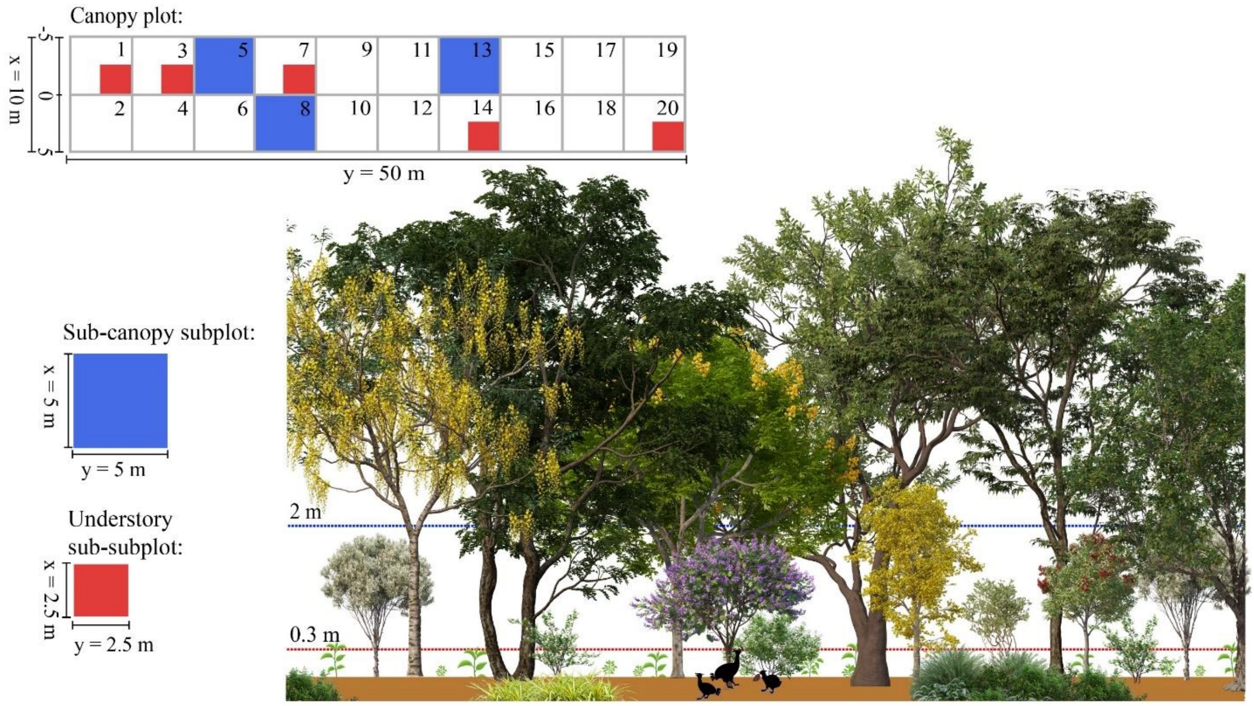

Figure S1. Sketch of subplot sectorization showing plots (white), subplots (blue), and sub-subplots (red) in the High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil.

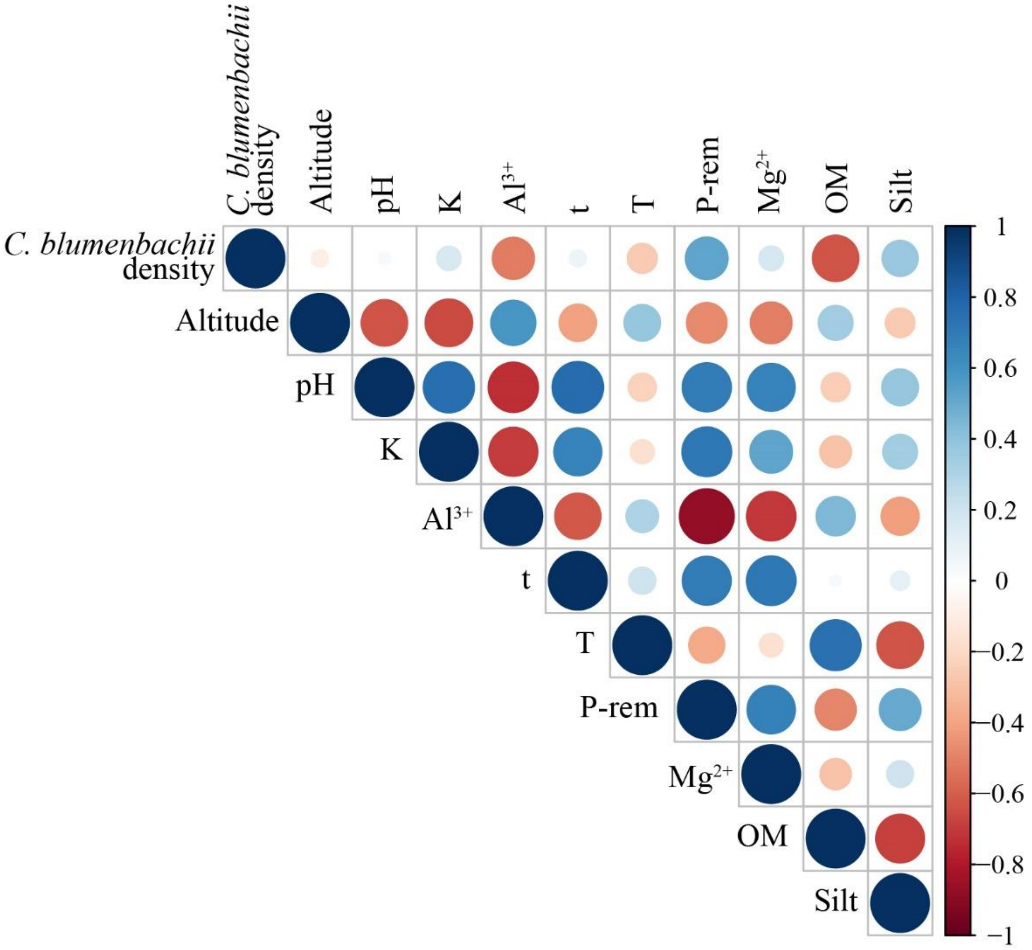

Figure S2. Pearson's correlation matrix among environmental variables and *Crax blumenbachii* density. Dark blue circles indicate positive correlations, while dark red circles indicate negative correlations; both the size of the circles and the color intensity represent the strength of the correlation. The matrix includes the environmental variables previously identified by Rufino et al. (2023) as drivers of floristic composition in the study area: altitude, pH, potassium (K), aluminum (Al^{3+}), effective cation exchange capacity (t), total cation exchange capacity (T), remaining phosphorus (P-rem), magnesium (Mg^{2+}), organic matter (OM), and silt fraction (Silt). The density of *C. blumenbachii* was subsequently added to this set to evaluate its potential covariation with environmental factors.

Table S3. Characterization of forest inventory plots in the 753-ha Atlantic forest fragment designated as a High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil. The table provides the total number of species (across all forest strata) per plot. Additionally, it provides data on number of living trees, number of dead trees, maximum height (Hmax), quadratic mean diameter (q, cm), and basal area (B, m^2) based on the canopy strata (sampled in 10x50 m plots). Number of species and density of living stems are then provided for each strata. For experimental design, see Figure S1.

Plot	**Total number species**	**Living trees (per m^2)**	**Dead trees (per m^2)**	**Hmax (m)**	**q (cm)**	**B (m^2)**	**Canopy**		**Sub-canopy**		**Understory**	
							Species	**Living stems (per m^2)**	**Species**	**Living stems (per m^2)**	**Species**	**Living stems (per m^2)**
1	43	0.13	0.00	13.8	12.3	0.82	32	0.14	12	0.28	9	1.08
2	66	0.16	0.01	15.4	11.4	0.96	45	0.19	18	0.63	22	2.20
3	39	0.14	0.01	15.2	11.1	0.77	29	0.16	7	0.09	13	1.40
4	29	0.11	0.00	16.8	15.1	1.37	21	0.15	9	0.19	11	1.20
5	48	0.14	0.01	15.7	13.1	1.09	39	0.16	12	0.24	14	0.64
6	28	0.1	0.01	21.6	17.2	1.47	21	0.13	5	0.13	13	0.88
7	42	0.1	0.01	26.6	16.5	1.13	33	0.11	6	0.08	11	0.68
8	33	0.13	0.01	15.6	14.6	1.15	23	0.14	11	0.21	11	0.72
9	35	0.10	0.01	15.7	14.1	0.87	25	0.11	10	0.2	11	0.72
10	30	0.11	0.01	14.9	12.9	0.89	21	0.14	4	0.08	13	1.52
11	64	0.12	0.00	27.6	17.7	1.47	34	0.12	12	0.28	28	1.92
12	29	0.10	0.00	27.7	14.8	0.87	25	0.1	11	0.24	4	0.40
13	38	0.12	0.01	15.9	10.6	0.60	27	0.14	16	0.49	16	1.12
14	14	0.09	0.01	18.7	9.7	0.36	13	0.1	3	0.04	2	0.08
15	47	0.09	0.01	21.5	20.2	1.73	29	0.11	12	0.21	15	1.36
16	51	0.09	0.01	23.0	12.9	0.62	31	0.1	15	0.25	22	1.52
17	38	0.12	0.01	25.4	15.2	1.13	29	0.12	10	0.19	7	1.08
18	36	0.09	0.01	14.3	13.7	0.75	25	0.1	7	0.11	9	1.16

Plot	Total number species	Living trees (per m^2)	Dead trees (per m^2)	Hmax (m)	q (cm)	B (m^2)	Canopy		Sub-canopy		Understory	
							Species	Living stems (per m^2)	Species	Living stems (per m^2)	Species	Living stems (per m^2)
19	40	0.10	0.01	17.7	12.7	0.69	28	0.11	10	0.33	12	1.60
20	41	0.07	0.01	26.7	19.3	1.26	26	0.09	5	0.16	16	2.48
21	44	0.10	0.00	17.8	15.7	1.24	14	0.13	15	0.43	30	3.28
22	40	0.13	0.00	16.7	13.6	1.06	22	0.15	9	0.19	23	2.56
23	29	0.07	0.04	18.6	21.7	1.30	20	0.07	11	0.21	12	1.20

Table S4: Sample completeness (SC) values estimated for each forest strata (canopy, sub-canopy, understory) within each of the 23 plots. SC was calculated using the coverage-based approach for order q=1 (Shannon diversity) as proposed by Chao et al. (2020), using the iNEXT.4steps package in R. These values represent the proportion of the total community that is represented in the sample and were used to assess the adequacy and consistency of sampling across the forest strata and along the *Crax blumenbachii* density gradient.

Plot	**Strata**	**SC**	**Plot**	**Strata**	**SC**	**Plot**	**Strata**	**SC**
1	Canopy	0.75	9	Canopy	0.70	17	Canopy	0.71
	Sub-canopy	0.62		Sub-canopy	0.55		Sub-canopy	0.44
	Understory	0.82		Understory	0.69		Understory	0.86
2	Canopy	0.67	10	Canopy	0.83	18	Canopy	0.66
	Sub-canopy	0.79		Sub-canopy	0.58		Sub-canopy	0.28
	Understory	0.76		Understory	0.85		Understory	0.87
3	Canopy	0.79	11	Canopy	0.65	19	Canopy	0.71
	Sub-canopy	0.05		Sub-canopy	0.66		Sub-canopy	0.77
	Understory	0.83		Understory	0.63		Understory	0.85
4	Canopy	0.80	12	Canopy	0.71	20	Canopy	0.49
	Sub-canopy	0.51		Sub-canopy	0.69		Sub-canopy	0.66
	Understory	0.81		Understory	0.84		Understory	0.82
5	Canopy	0.70	13	Canopy	0.75	21	Canopy	0.84
	Sub-canopy	0.57		Sub-canopy	0.79		Sub-canopy	0.82
	Understory	0.27		Understory	0.73		Understory	0.81
6	Canopy	0.78	14	Canopy	0.83	22	Canopy	0.79
	Sub-canopy	0.94		Sub-canopy	0.33		Sub-canopy	0.67
	Understory	0.60		Understory	0.67		Understory	0.91
7	Canopy	0.54	15	Canopy	0.62	23	Canopy	0.68
	Sub-canopy	0.07		Sub-canopy	0.38		Sub-canopy	0.51
	Understory	0.54		Understory	0.74		Understory	0.77
8	Canopy	0.86	16	Canopy	0.48			
	Sub-canopy	0.66		Sub-canopy	0.44			
	Understory	0.57		Understory	0.58			

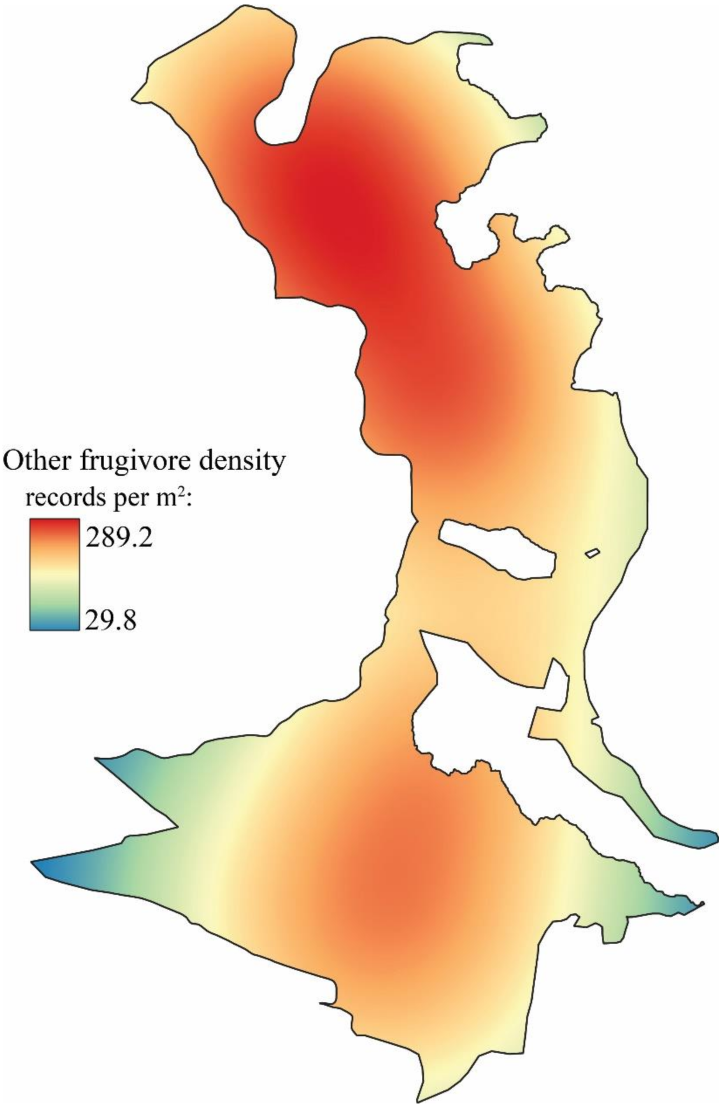

Figure S3. Density heat map, in records per square meter, of other frugivorous birds and mammals in the 753-ha Atlantic forest fragment designated as a High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil. Warm colors indicate the highest density of frugivore records, while cool colors indicate lower density. The squares represent the location of forest inventory plots. The density values represent the kernel-smoothed estimate of records per unit area, using a search radius of 2 km. These values should not be interpreted as absolute counts within a single square meter, but rather as an index of frugivore activity across the landscape. The analysis was based exclusively on camera trap data (2021 and 2024), and includes all bird and mammal species identified as frugivores (Table S2).

Table S5. Summary of floristic composition per plot and forest strata within the 753-ha Atlantic forest fragment designated as a High Conservation Value Area (HCVA), located at the Fazenda Macedônia, Brazil. The table presents the number of distinct families, genera, and species recorded in each plot for the canopy, sub-canopy, and understory strata. These values represent taxonomic richness (i.e., number of distinct taxa), not the number of individuals. Species richness is further categorized by successional group (SG): Early (early successional species), Late (late successional species), and Unclassified (species that were either not identified to species level or for which no successional group was available in the literature). Sampling area per strata: canopy = 500 m², sub-canopy = 75 m², and understory = 31.25 m².

	Strata	**SG**	**Plot**																						
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Families	Canopy	-	18	25	19	16	23	16	17	14	18	13	24	16	17	11	19	17	19	15	15	16	11	14	15
	Sub-canopy		9	13	7	8	11	5	5	10	7	4	9	10	11	3	11	10	8	7	9	5	8	9	8
	Understory		9	17	12	9	10	11	6	9	9	10	17	4	15	2	12	16	6	8	8	12	16	16	11
Genera	Canopy	-	31	38	28	20	34	21	27	21	23	18	28	23	25	13	27	30	26	23	27	26	14	19	19
	Sub-canopy		11	16	7	9	12	5	6	11	10	4	11	11	16	3	12	15	9	7	10	5	13	9	11
	Understory		9	21	13	11	13	12	11	11	10	12	21	4	15	2	15	21	7	8	12	16	25	22	12
Species	Canopy	Early	17	33	20	15	25	14	27	14	16	16	18	16	18	8	17	23	17	18	22	20	9	15	14
		Late	13	10	9	6	14	7	4	9	9	5	16	8	9	5	12	8	11	7	5	6	4	7	6
		Unclassified	2	2	-	-	-	-	2	-	-	-	-	1	-	-	-	-	1	-	1	-	1	-	-
	Sub-canopy	Early	6	14	6	7	7	3	4	7	7	3	7	8	11	3	7	13	5	3	6	4	13	6	9
		Late	6	4	1	2	5	2	2	4	3	1	5	3	5	-	5	2	5	4	4	1	2	3	2
		Unclassified	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Understory	Early	5	15	9	7	8	10	5	7	7	9	12	4	9	1	7	11	4	4	7	12	22	16	9
		Late	4	6	3	4	6	3	5	4	4	4	12	-	7	1	7	8	3	5	5	3	6	7	3
		Unclassified	-	1	1	-	-	-	1	-	-	-	1	-	-	-	1	3	-	-	-	1	2	-	-

We conducted separate NMDS ordinations for animal-dispersed (Figure S4a) and nonanimal-dispersed plant species (Figure S4b). In both cases, *C. blumenbachii* density was significantly correlated with the NMDS ordination (animal-dispersed: $P = 0.027$, $R^2 = 0.10$; non-animal-dispersed: $P = 0.002$, $R^2 = 0.21$), indicating that its presence is associated with community composition regardless of dispersal syndrome. Other significant variables included remaining phosphorus (animal-dispersed: $P = 0.001$, $R^2 = 0.60$; non-animal-dispersed: $P = 0.001$, $R^2 = 0.52$), silt content (animal-dispersed: $P = 0.006$, $R^2 = 0.14$; non-animal-dispersed: $P = 0.001$, $R^2 = 0.34$), and altitude (animal-dispersed: $P = 0.001$, $R^2 = 0.19$; non-animal-dispersed: $P = 0.002$, $R^2 = 0.17$). Strata were not significantly associated in either case (animal-dispersed: $P = 0.444$, $R^2 = 0.02$; non-animal-dispersed: $P = 0.417$, $R^2 = 0.03$). It is important to note, however, that the number of species differed between groups (190 animal-dispersed vs. 77 non-animal-dispersed), as expected in tropical forests, which affects the NMDS configuration and stress values (0.229 vs. 0.142, respectively), and therefore limits direct comparisons between them. Twelve unidentified species were excluded from both analyses, as their dispersal syndromes could not be determined. To improve visualization and account for zero-species subplots, a few outliers were excluded from each analysis (see Figure S4 caption for details).

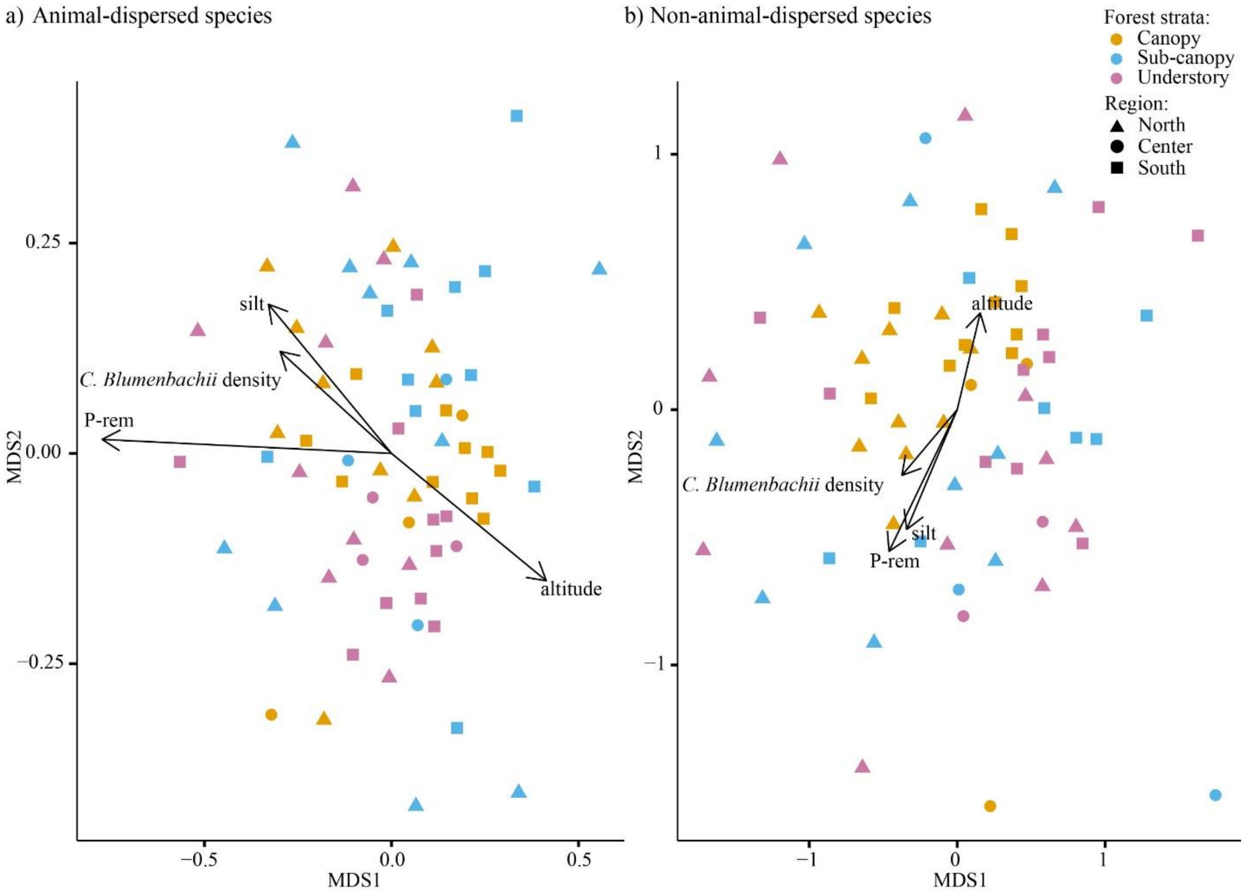

Figure S4. NMDS ordination of plant species composition by dispersal syndrome across understory, sub-canopy and canopy subplots. (a) Animal-dispersed species; subplot of understory plot 14 was removed to improve visualization, as it contained only one species. (b) Non-animal-dispersed species; subplots from sub-canopy plots 3, 7, 10, and 11 were removed due to absence of species. Significant variables ($p < 0.1$) fitted to the ordination include altitude, P-rem (remaining phosphorus), silt (soil silt fraction), and *C. blumenbachii* density. Strata was not plotted as it was not significant in either ordination. Arrows indicate the direction and strength of correlation between environmental variables and species composition. Stress values: (a) = 0.22; (b) = 0.14. Twelve unidentified species were excluded from the analysis due to the inability to assign a dispersal syndrome.

CONCLUSÕES E RECOMENDAÇÕES

A reintroduções de grandes frugívoros têm potencial para restaurar processos ecológicos perdidos, mas esse potencial nem sempre se traduz em mudanças rápidas ou facilmente detectáveis na estrutura da vegetação. No caso de *C. blumenbachii*, a reintrodução ocorre em uma paisagem marcada por fragmentação e presença de habitats antrópicos, e a integração funcional da população reintroduzida depende da configuração da paisagem, de legados comportamentais do cativeiro e do contexto socioambiental local. Mesmo diante de sinais fracos na comunidade de plântulas, a espécie provavelmente exerce funções difíceis de serem substituídas por frugívoros menores, especialmente na dispersão de sementes grandes. Isso aponta para a necessidade de que programas de reintrodução incorporem, além de métricas demográficas, indicadores explícitos de processos ecológicos, como dispersão, recrutamento e estabilidade de interações mutualísticas, monitorados em longo prazo e em múltiplas escalas.

À luz desses resultados, recomenda-se que ações de manejo da fauna na área de estudo considerem estratégias voltadas à facilitação do deslocamento dos indivíduos para porções da paisagem com maior conectividade florestal, particularmente em direção a região sul. Intervenções dessa natureza devem ser cuidadosamente planejadas, de forma a respeitar o comportamento natural da espécie e minimizar potenciais riscos associados ao manejo direto.

Os resultados também sustentam a recomendação de que o manejo da área incorpore ações voltadas ao aumento da conectividade da paisagem, com foco no estabelecimento e na consolidação de corredores funcionais de vegetação. A ampliação da conectividade pode aumentar a permeabilidade da matriz, favorecer o deslocamento de grandes frugívoros e potencializar processos ecológicos associados à dispersão de sementes em escalas espaciais mais amplas.

Por fim, recomenda-se que futuras análises adotem uma abordagem de modelagem em escala de paisagem mais abrangente, integrando informações sobre uso e cobertura da terra, relevo e configuração dos fragmentos florestais, com o objetivo de identificar áreas prioritárias para restauração e para o estabelecimento de corredores viáveis. Esse tipo de abordagem pode subsidiar decisões de manejo mais estratégicas, alinhando a recuperação populacional de *C. blumenbachii* à restauração de fluxos ecológicos e à funcionalidade dos ecossistemas florestais.

Em uma perspectiva mais ampla, esta tese reforça que reintroduções podem ir além da restauração de interações ecológicas e contribuir para resultados mais amplos de conservação. Projetos como o “Projeto Mutum” funcionam como plataformas para pesquisa aplicada, para o desenho e a avaliação de políticas públicas voltadas à recuperação de espécies ameaçadas e para ações de educação ambiental e engajamento social. Uma espécie carismática pode atuar como símbolo de biodiversidade, aproximando comunidades locais, instituições e tomadores de decisão da agenda de conservação. Esforços de refaunação que incluam grandes frugívoros, quando planejados e avaliados de forma cuidadosa, têm potencial para fortalecer a resiliência dos ecossistemas, conservar diversidade genética e reconstruir laços entre sociedade e natureza. No entanto, aproximar recuperação populacional de restauração de ecossistemas exigirá compromisso de longo prazo, integração entre ciência, gestão e participação social e uma visão abrangente sobre o que significa restaurar a natureza e as funções que a sustentam.