

**PROJECT REPORT** 

# Ecoacoustic Biodiversity Monitoring in Restoration Wildlife Corridors of Pontal do Paranapanema, Brazil

In partnership with: WeForest, Instituto de Pesquisas Ecológicas

## **Authors**

Dr. José Wagner Ribeiro, Quantitative Ecology Lead Dr. Thiago Bicudo, Quantitative Ecologist Dr. Gabriel Augusto Leite, Biodiversity Science Lead Dr. Tomaz Nascimento de Melo, Biodiversity Scientist Guilherme Melo, Biodiversity Scientist Dr. Nelson Buainain, Data Science Lead Dr. Claydson Bezerra Assis, Data Scientist Kris Harmon, Science Projects Strategist Dr. Marconi Campos-Cerqueira, Chief Scientist

## WildMon

261 Blue Jay Road, Dale TX 78616, USA <u>contact@wildmon.ai</u>

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# **Executive Summary**

WildMon, WeForest, and Instituto de Pesquisas Ecológicas have partnered on a passive acoustic monitoring project in Pontal do Paranapanema, Southeast Brazil, to assess how reconnecting forest fragments through wildlife corridor restoration influences regional biodiversity patterns. The study aims to document how birds, frogs, and mammals respond to restoration efforts that focus on the development and maintenance of wildlife corridors. Using acoustic species detection algorithms and soundscape analyses, we assessed wildlife presence, distribution, and the environmental factors influencing their occurrence. This report presents findings from three years of ecoacoustic monitoring.

We identified **219 species** in 120 survey sites, including **205 bird species**, **9 amphibians**, **3 mammals**, and **2 insects** across the three survey years. The picazuro pigeon was the most widely detected species, followed by the turquoise-fronted amazon, great kiskadee, and pauraque, each recorded at more than 90 sites. These species were consistently among the most prevalent across all three years of monitoring.

Three of the detected species—1 mammal and 2 birds—are endemic to Brazil. The presence of the Endangered black lion tamarin and 6 Near Threatened species (IUCN Red List; barethroated bellbird, solitary tinamou, turquoisefronted amazon, rusty-marginated guan, ornatehawk eagle, and black-horned capuchin) underscores the conservation importance of the area and the need for its protection Soundscape analyses revealed significant shifts in acoustic composition and increased acoustic space use (ASU) in restored areas, suggesting a **positive trajectory toward ecological recovery**. Over time, the soundscapes of restoration sites became more similar to those of native forests, indicating that **restoration areas are progressively supporting richer and more complex biological communities**. Notably, acoustic activity, particularly in the midand high-frequency ranges, showed a marked increase in 2023, further reinforcing the role of ecological restoration in enhancing biodiversity.

Occupancy modeling showed that bird species occupancy is strongly influenced by habitat quality. **Sites with higher connectivity, greater vegetation cover, and proximity to water had higher occupancy probabilities, while agricultural-dominated areas exhibited lower occupancy rates.** These results emphasize the role of restoration in improving habitat suitability for avian communities. Additionally, **species richness was consistently higher in restored and forested sites compared to agricultural areas**, with an overall increase observed in 2023. This trend suggests that restoration efforts are fostering richer bird assemblages over time.

This study provides valuable insights into species presence and distribution in Pontal do Paranapanema, demonstrating that ecological restoration plays a crucial role in sustaining and enhancing wildlife populations. By improving habitat connectivity, these initiatives contribute directly to the long-term conservation of the region's unique biodiversity, reinforcing the importance of wildlife corridors in landscapescale restoration efforts.

# Methods

## **Data Collection**

For the third consecutive year, we conducted passive acoustic monitoring (PAM) in Pontal do Paranapanema in Southeast Brazil. On-site partners deployed AudioMoth recorders (Open <u>Acoustic Devices</u>) from September 20 to October 25, 2023 across 120 sampling sites. These sites were distributed across three wildlife corridor blocks—East, West, and North—and stratified by land-use type, including 29 forest remnants (e.g., Morro do Diabo State Park, Mico Leão Preto Ecological Station), 28 conventional agricultural areas (e.g., pastures, sugar cane fields), and 63 restoration sites within WeForest project areas (Figure 1). This survey period coincides with the onset of the region's rainy season, which is expected to relate to the beginning of the peak of vocal activity for species such as birds, frogs, and insects. Aligning this period with the previous acoustic monitoring efforts (2021 and 2022) ensured temporal consistency across the study.

**Figure 1.** Map of the study area showing the distribution of 120 sampling sites across three habitat types: forest (29 sites, green circles), agricultural controls (28 sites, purple diamonds), and WeForest restoration areas (63 sites, yellow triangles).



In 2021, all 120 sampling sites were operational. However, five sites in 2022 (IPE04, IPE16, IPE58, IPE64, WF09) and three additional sites in 2023 (IPE07, IPE38, IPE89) did not yield suitable data for ecological analyses. Technical issues included timestamping errors, poor recording quality, and incomplete data uploads.

Audiomoths recorded a one-minute audio clip every five minutes (48kHz sample rate),

resulting in 288 recordings per site per day. Recorders collected data at each site for an average of 35.7 days in 2021 (range: 21-48), 29.5 days in 2022 (range: 24-36), and 28.1 in 2023 (range: 17-32, see Figure 2 for recording period in 2023). This resulted in a total of 1,599,770 oneminute recordings over the three years of acoustic monitoring.

**Figure 2.** Recording periods at 120 sampling sites during the survey in 2023. Each square represents a recording day. The first recording day was September 20, the last was October 25, 2023.



## **Environmental Variables**

To better understand how environmental variables influence species distribution and

occurrence, we extracted landscape-scale remote sensing data from GIS layers to use in our ecological models. We selected these environmental variables based on their public accessibility and potential to explain biodiversity patterns based on previous literature in the first year of the project (<u>Appendix A</u>). Some environmental variables tend to be collinear with each other, which can cause problems in ecological models. Therefore, to avoid such multicollinearity issues, we excluded highlycorrelated variables within the same models.

We utilized data from various geospatial data repositories:

- Farming and Forest: Forest cover and farming cover data were obtained from MapBiomas (<u>MapBiomas Collection 6</u>; accessed March 8, 2022).
- Distance to water: estimated from an adapted version of São Paulo State's regional waterways network (Secretaria do Meio Ambiente do Estado de São Paulo 2013).
- NDVI Mean and NDVI Standard Deviation (std): The Normalized Difference Vegetation Index (NDVI) was calculated using the Planet monthly reflectance basemap (Planet). For consistency, we used NDVI from October 2021, 2022, and 2023, as:
  - Mean NDVI: calculated as the average of all pixel values within the buffer across the three-year monitoring period. This metric provides an overall measure of vegetation cover and health around each sampling point.
  - NDVI Standard Deviation (std): standard deviation of NDVI calculated by first determining the standard deviation of NDVI values for each pixel across the three years (2021, 2022, and 2023) and then averaging these standard deviations within the buffer. This metric quantifies the

temporal variability in NDVI, reflecting changes in vegetation over time.

- dIIC: Integral Index of Connectivity was estimated using a forest cover layer, which represents habitat availability. The index was calculated considering a 500meter dispersion distance threshold between habitat patches, reflecting the typical dispersal capacity of bird species. Forest fragments smaller than 1 hectare were excluded from the analysis, as they did not meet our minimum size requirement to be considered suitable habitat for most species.
- **Year:** In addition to the landscape variables, the sampling year was included as a predictor variable to account for potential temporal variations in the data.

To incorporate these landscape variables in our model, we used ArcGIS Pro to create a 200meter radius buffer around each site (12.57hectare total area; this corresponds to the estimated detection distance of the recorder across species' calls). This buffer allows us to capture the site-specific environment to better understand how environmental variables influence species detection and occurrence. By using this approach, we can systematically and uniformly incorporate relevant environmental data into ecological analyses at a landscapescale, and effectively assess these variable's influence on species distribution and occurrence.

## Soundscape Analyses

A soundscape encompasses all sounds from a specific location and time, including biophony (sounds from living organisms), geophony (natural sounds like rain), and anthrophony (human-made sounds; <u>Pijanowski et al., 2011</u>). Soundscape analyses involve summarizing acoustic activity by site, aggregating the amount

of acoustic activity at each frequency during each hour of the day across all sampling days in each sampling period (year). This approach is a valuable tool for assessing spatial and temporal variations of acoustic patterns, unveiling patterns in the community of acoustically-active species (Figure 3). Soundscape analyses can also provide insights into how environmental factors, such as land cover and restoration, influence local biodiversity.

We applied two types of soundscape analyses: composition and Acoustic Space Use (ASU).

- Soundscape composition examines the recurrence of acoustic energy in specific time-frequency bins across recording days at a site, providing insights into the acoustic community structure.
- ASU provides a proxy for species richness by averaging the proportion of time-

frequency bins occupied across recording days per site, quantifying how much the soundscape from each location is used over time. Species-rich sites, particularly those with many insects, tend to generate more saturated soundscapes with a higher ASU (Aide et al., 2017; Campos-Cerqueira et al., 2019; Ramesh et al., 2023).

Both approaches can be valuable for understanding biodiversity patterns and ecological dynamics.

#### Soundscape Composition

We compared soundscape composition among the 120 sites over three years and tested whether any environmental variables influenced composition.

**Figure 3.** Soundscape visualization of acoustic activity throughout the day at two sites in 2023. The left panel (IPE11, id = 23488) represents a conventional agriculture site, while the right panel (IPE47, id = 23498) depicts a restoration site within the wildlife corridor project. The color gradient indicates soundscape saturation, with black representing no activity, red indicating low activity, and bright yellow signifying high activity within each time-frequency bin.



To summarize the variability and composition of acoustic activity in these soundscape maps we employed an ordination technique called nonmetric multidimensional scaling (NMDS). NMDS is a dimensionality reduction method that creates a visual representation of objects (in this case, study sites) based on their similarities of a certain variable (here, soundscapes; Figure 3). The resulting NMDS components were then used in further analyses to assess their relationship to environmental variables using linear models. We used a suite of follow-up tests (PERMANOVA, beta-dispersion) to confirm the robusticity of our model results.

#### **Acoustic Space Use**

We compared soundscape composition among the 120 sites and tested whether any environmental variables influenced the ASU.

To calculate each site's ASU, we divided each site's recordings into 1,656 time/frequency bins (24 hours x 69 frequency bins) and calculated the percentage of bins 'used' out of the 1,656 total. We considered a time/frequency bin 'used' if a sound with an amplitude >0.05 was detected.

We categorized soundscapes into three distinct frequency ranges:

- <2 kHz: low-frequency sounds typical of human noise/machinery, geophony (wind) and some animals
- **2-8 kHz:** mid-frequency sounds typical of terrestrial vertebrates (e.g., birds, amphibians, primates)
- >8 kHz: high-frequency sounds typical of insects (e.g., crickets, cicadas)

We conducted our analyses across the full frequency spectrum (0-24kHz) and individually across these frequency bins. This enables us to gain a more nuanced understanding as to which taxonomic groups/sound classes are influenced by which factors. Species can respond differently to various environmental factors, so analyzing ASU across distinct frequency bands gives us insights into these patterns.

We then used general linear models (using automated model selection/averaging) to

identify which environmental variables most influenced ASU across all frequencies as a whole, and also within each of the three frequency subsets. Additionally, we explored the relationship between ASU and species richness per site, to assess whether ASU can be used as a proxy for the number of bird species present in a given area. For all soundscape analyses, results were considered statistically significant for p-values <0.05.

## **Species Identification Pipeline**

Our pipeline involves a multi-step process to identify and validate species present in the recordings (Figure 4). First, we generate an initial list of potential species for the area. For this, we create a polygon with a radius of 10 to 30 km around the sampling points to define a spatial search area. Within this polygon, the algorithm uses the Global Biodiversity Information Facility (GBIF) database to search for any species groups that can typically be detected by sound, including birds, mammals, frogs, crickets, grasshoppers, and cicadas. This list serves as a starting point and is manually verified to ensure its accuracy.

Once the species list is finalized, it is processed through the BirdNET model, an AI-based sound recognition tool trained on an extensive library of animal vocalizations and human sounds (e.g. chainsaw). The BirdNET model analyzes the audio recordings from our sampling points, matching detected calls and songs to species from the list. The output provides a detailed report of potential matches, which we manually validate to confirm the presence of each species. This step helps us generate a refined list of species identified in the recordings (Appendix B).



Figure 4. Flowchart of the acoustic pipeline for species identification and validation.

To enhance the detection process, we also created a set of acoustic templates based on the species not identified by the BirdNET model, but that were present in the initial list of potential species in the area. This procedure extends detections to species missed by the BirdNET model and is especially important for identifying amphibian, mammal, and insect species not yet included in the model. Using these templates, we run an additional method based on Template Matching (TM) to detect these missing species. Our Template Matching tool allows us to select an example of a species' call or song (shown as a green box in Figure 5), which acts as the "template". These templates are carefully reviewed to ensure they represent high-guality, species-specific vocalizations. The tool then scans through recordings to automatically detect similar sounds that match the selected template (Figure 6).





**Figure 5.** Annotated spectrogram with species' calls from a 1-min recording. Green boxes represent the acoustic templates.

Finally, for species of particular interest to the project that were not detected through BirdNET, we conduct targeted searches using platforms such as Xeno-canto to find audio recordings of these species. These recordings are used to create external acoustic templates. These external templates are then used in TM, and the results are manually validated to further expand the list of identified species.

This iterative pipeline ensures a comprehensive inventory of species, combining the power of AI sound recognition, database queries, and expert manual validation to maximize detection accuracy and completeness.

The BirdNET model and Template Matching results were validated using two distinct filtering approaches. The first, Best per Location per Day, identifies the detection with the highest possible score for each sampling site and each day, ensuring that all species data used in analyses are verified. The second approach, All Detections, aims to increase the number of recorded presences, particularly for species with few detections, using the first filter to achieve a sufficient sample size to effectively train an AI model.



#### Figure 6. Template Matching visualization page from the WildMon Biodiversity Analytical Platform.

## **Ecological Analyses - Occupancy Models**

Occupancy modeling is a powerful method that can be used to predict the probability that a species "occupies" sites while accounting for imperfect detection. Further, occupancy modeling enables us to integrate environmental variables to assess how these influence species presence and distribution (<u>Dorazio & Royle,</u> <u>2005; Doser et al. 2022</u>).

We developed a multi-species, multi-season occupancy model (MSMSOM) for 86 bird species using the validated detections from TM models together with the aforementioned environmental variables. We focused on these species because they had validated data for the previous two years, ensuring data consistency and enabling comparisons of biodiversity patterns across all three years. The model incorporated temporal random effects for primary time period (years) to account for temporal autocorrelation across the three years of acoustic monitoring. The inclusion of temporal random effects helped address residual positive correlation in occurrence values from one year to the next.

Further information on all analyses (TM, soundscape, occupancy models, etc.), including support tables, candidate models, and figures, is available in the <u>supplementary files</u>.



# Results

## Soundscape Composition

The NMDS analysis captured a substantial portion of the original dissimilarity, with a nonmetric fit R<sup>2</sup> of 0.984 and a stress value of 0.13, indicating a robust representation of the data in reduced dimensions. All the variables tested (farming proportion, mean NDVI, standard deviation of NDVI, distance to lotic and connectivity index (dIIC), in addition to survey year and type site (forest, restoration, and farming)) were identified as significant factors influencing changes in soundscape composition across the sampling sites (<u>supplementary files</u>).

Our NMDS ordination results, combined with Envfit analysis, revealed significant differences in soundscape composition among site types

(farming, forest, and restoration) and across monitoring years (2021, 2022, and 2023; Figure 7). The biplot of the first two NMDS dimensions shows that restoration sites in 2021 were positioned between agricultural land and forest, reflecting an intermediate soundscape composition characteristic of areas transitioning from high-intensity farming to native forest remnants. By 2023, however, restoration sites shifted noticeably toward forest sites along the ordination axes, indicating a progression in soundscape composition toward conditions more typical of native forest fragments. This shift suggests that restoration efforts are gradually leading to ecological recovery, with soundscapes in restored areas becoming more akin to those of native forest forests over time.

**Figure 7.** NMDS ordination plot showing variation in soundscape composition from the 120 sampling sites over three years of acoustic monitoring.



To statistically validate these differences, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA), which confirmed significant variation in soundscape composition among site groups (F = 7.13, R<sup>2</sup> = 0.143, p = 0.0001). Post-hoc pairwise comparisons revealed that soundscape composition in agricultural areas remained stable across the three sampling years (there was no statistically significant change), reflecting a simplified and consistent acoustic landscape typical of anthropized environments. In contrast, both forest and restoration areas exhibited significant temporal variation in soundscape composition, likely due to the dynamic nature of these ecosystems and the variability of sound-emitting organisms. Additionally, pairwise comparisons supported the visual interpretation of the NMDS biplot, showing no significant difference between Farming 2021 and Restoration 2021. However, from 2022 onward, restoration sites became increasingly distinct from agricultural areas while remaining significantly different from forested areas across all three years. These findings suggest that while restoration areas are progressively diverging from agricultural soundscapes, they have not yet fully converged with the acoustic characteristics of forest fragments.

Multivariate homogeneity of group dispersion, assessed using Betadisper, indicated significant differences in variance among groups (F = 5.14, p < 0.001). This suggests that the observed differences in soundscape composition are partially driven by variability within groups, rather than solely by shifts in composition between groups.

There are several ecological and conservation implications around this finding, however. Firstly, the results reinforce the importance of long-term monitoring in restoration projects, as changes in biodiversity may not be immediately evident through traditional survey methods. Secondly, conservation efforts should prioritize maintaining or enhancing habitat complexity in restoration sites to support a diverse array of acoustically active species. Thirdly, the contrast between stable agricultural soundscapes and dynamic restoration and forest soundscapes underscores the importance of habitat connectivity and minimizing anthropogenic pressures on recovering ecosystems.

## Acoustic Space Use (ASU)

Our results revealed considerable variation in Acoustic Space Use (ASU) across sites, ranging from 5.9% to 98% in 2021 (mean = 70.7%, SD = 16.4%), from 26.5% to 93.9% in 2022 (mean = 72.9%, SD = 15.2), and from 35.3% to 97.6% in 2023 (mean = 79.1%, SD = 15.1). Notably, acoustic space use showed a gradual increase over the monitoring period. This trend suggests that more frequency bands and times of day were occupied by sound-emitting wildlife over time, reflecting an overall increase in acoustic activity and biodiversity in the studied landscape.

Our analysis of ASU across all frequencies (0–24 kHz) identified the most parsimonious model, which included site type, survey year, and the standard deviation of NDVI over the three survey years (AICc = -301.3, weight = 42.4%; <u>Appendix C1</u>). This model was only marginally better than the second-ranked model, which included site type, survey year, farming proportion, and distance to lotic systems (ΔAICc < 2; AICc = -300.1, weight = 21.9%; <u>Appendix C1</u>). These results suggest that while site type, survey year, and vegetation variability (ndvi\_std) are the primary drivers of ASU variation, farming intensity and proximity to water bodies may also play a role. In the top-ranked model, only the 2023 sampling year was statistically significant,

with ASU values significantly higher than those in 2021 (estimate = 0.084, SE = 0.02, p < 0.0001; Figure 8, left panel). This indicates a notable increase in ASU over the monitoring period, likely reflecting greater acoustic activity and biodiversity in the landscape. None of the other variables in the model (site type, 2022 sampling year, or ndvi\_std) were statistically significant, suggesting that their influence on ASU was limited within this dataset.

The analysis of ASU revealed notable differences in environmental and site-specific drivers across low, medium, and high-frequency soundscapes. Our analysis of Acoustic Space Use in the highfrequency range (ASU-high; >8 kHz), which primarily captures sounds from insects (e.g., crickets, cicadas), revealed results similar to those of the ASU-all model. The most parsimonious model for ASU-high included site type, survey year, farming proportion, and distance to lotic systems (distLotic; AICc = -142.7, weight = 38.2%; Appendix C2), closely followed by a model with site type, survey year, and the standard deviation of NDVI (ΔAICc < 2; AICc = -141.8, weight = 25.0%; Appendix C2). This pattern mirrors the ASU-all results, where the top two models also differed primarily in the inclusion of farming and distance to lotic water versus standard deviation in NDVI. In the topranked ASU-high model, only the 2023 sampling year was statistically significant, with ASU-high values significantly higher than those in 2021 (estimate = 0.105, SE = 0.025, p < 0.0001). This temporal increase aligns with the ASU-all results, where 2023 also showed a significant rise in acoustic activity (estimate = 0.084, SE = 0.02, p < 0.0001). Notably, none of the other variables in the ASU-high model (site type, 2022 sampling year, farming, or distLotic) were statistically significant (p > 0.05), similar to the ASU-all model, where only the 2023 sampling year stood out. This suggests that, like the overall acoustic activity, high-frequency

soundscapes—driven primarily by insects—are more influenced by temporal variation than by the spatial environmental gradients investigated here.

Analyses of ASU in the mid-frequency range (ASU-med; 2–8 kHz), which primarily captures sounds from terrestrial vertebrates (e.g., birds, amphibians, primates), revealed that the most parsimonious model included site type, survey year, proportion of agriculture, and distance to lotic systems (AICc = -484.9, weight = 30.6%; <u>Appendix C3</u>). This model was closely followed by others incorporating vegetation metrics (ndvi mean, ndvi std) and connectivity (dIIC), indicating that both land use and environmental factors influence mid-frequency soundscapes. Forest and restoration sites had significantly higher ASU-med values compared to agricultural sites (forest: estimate = 0.064, p = 0.045; restoration: estimate = 0.076, p = 0.002), suggesting that these habitats support greater acoustic activity from terrestrial vertebrates. Additionally, ASU-med increased significantly in both 2022 (estimate = 0.040, p = 0.011) and 2023 (estimate = 0.047, p = 0.003) compared to 2021, indicating a temporal increase in mid-frequency acoustic activity. However, neither agricultural intensity nor distance to lotic systems significantly influenced ASU-med (p > 0.05), highlighting that habitat type and temporal variation are the primary drivers of midfrequency soundscapes.

For the low-frequency range (ASU-low; <2 kHz), which includes sounds from many forestdwelling animals species as well as human noise, machinery, and geophony (e.g., wind), the most parsimonious model included all the variables tested except for the mean of NDVI (AICc = -634.23, weight = 41.4%; <u>Appendix C4</u>). This model was nearly equivalent to a simpler model without the standard deviation of NDVI ( $\Delta$ AICc < 2; AICc = -634.16, weight = 40.0%), suggesting that temporal variability in vegetation plays a minor role in low-frequency soundscapes. Forest and restoration sites had significantly higher ASU-low values compared to farming sites (forest: estimate = 0.115, p < 0.0001; restoration: estimate = 0.079, p < 0.0001), indicating that these habitats support greater low-frequency acoustic activity (Figure 8, right panel). ASU-low also increased significantly in 2023 compared to 2021 (estimate = 0.025, p = 0.048), though the change was less pronounced than in other frequency ranges. Notably, distance to lotic systems had a significant negative effect on ASU-low (estimate = -0.027, p < 0.0001), indicating that areas closer to water bodies (smaller distances) exhibited higher lowfrequency acoustic activity than areas farther away (larger distances). This suggests that proximity to water bodies may amplify lowfrequency sounds, potentially due to geophony (e.g., running water), human activities near water sources, or the presence of wildlife that vocalizes at low frequencies, such as some toads. Connectivity (dIIC) also had a small but significant negative effect (estimate = -0.020, p = 0.017), though further investigation is needed to clarify its role in shaping low-frequency soundscapes.





#### **Species Identification**

We identified a total of 219 species, including 205 birds, 3 mammals, 9 amphibians, and 2 insects (Appendix B). Among these, 7 species are classified as threatened on the IUCN Red List. The <u>black lion tamarin</u> is listed as Endangered, while six other species are categorized as Near Threatened, including the <u>bare-throated bellbird</u>, <u>solitary tinamou</u>, <u>turquoise-fronted amazon</u>, <u>rusty-marginated</u> guan, ornate-hawk eagle, and the <u>black-horned</u> <u>capuchin</u>. Validated species occurrences, sitelevel biodiversity patterns, and this report are available on the interactive <u>WildMon Dashboard</u> project page.

We detected three species endemic to Brazil, including one mammal (black lion tamarin) and two birds (greater crescent-chested puffbird and planalto slaty antshrike), along with nine bird species considered endemic to the Atlantic Forest biome. None of the detected species are invasive in Brazil (<u>Appendix B</u>).

The black lion tamarin is found only in the state of São Paulo and serves as a symbol of Atlantic Forest conservation. This species plays a crucial ecological role as a seed disperser and predator of small animals. The largest population of this species, with approximately 1,200 individuals, resides in <u>Morro do Diabo State Park</u>, where we detected its presence at four sites. Two of these sites are close together, likely within the territory of the same group. We also detected the species in two other forest fragments outside the state park. Although the species was not detected in restoration sites, continued monitoring is important to assess future habitat use and potential connections between isolated populations.

The bird species analyzed in this project exhibited diverse dietary habits, with invertivores (invertebrate eaters) making up 63% of the species (Figure 9a). Other common dietary groups included omnivores, frugivores (fruit eaters), and granivores. The presence of large seed dispersers, such as the <u>toco toucan</u> and rusty-marginated guan, is particularly valuable for <u>restoration projects</u>. Toucans, for example, play a key role by regurgitating seeds from various tree species while perching on trees, facilitating natural regeneration.



Figure 9. Treemap species plot, showing the diet (A) and habitat (B) preferences of species detected.

Among the 86 bird species included in ecological analyses, 48 species (56%) are forest-associated, primarily inhabiting tall tree vegetation with partially to fully closed canopies (Figure 9b). Other habitat specialists included woodland species (11 species), shrubland species (10 species), and grassland species (9 species). Notably, 7 species were associated with humanmodified environments, reflecting the historically transformed landscape of Pontal do Paranapanema, which has been significantly influenced by intensive agriculture.

The predominance of forest-associated birds is remarkable, considering that the Pontal do Paranapanema Hydrographic Basin has one of the lowest proportions of native forest cover among São Paulo's basins (12.6%; São Paulo (Estado) Secretaria de Infraestrutura e Meio Ambiente 2022 - Inventário da cobertura vegetal nativa do Estado de São Paulo). However, this pattern aligns with the study's design, which focused on restoring wildlife corridors connecting large blocks of protected native forest, such as Morro do Diabo State Park (>33,000 ha) and Mico Leão Preto Ecological Station (>6,500 ha), as well as other large fragments. Overall, these results underscore the importance of large native forest areas in maintaining forest-associated species within a landscape with intense agricultural activities. They also highlight the critical role of wildlife corridor restoration in connecting these areas, enabling forest-associated species to colonize new fragments, increasing their occurrence across the landscape, and supporting their conservation.

In the 2021 acoustic survey, the <u>picazuro pigeon</u> was the widely detected species, recorded at over 112 sampling sites (Figure 10A). Following the picazuro pigeon, the turquoise-fronted amazon, <u>great kiskadee</u>, and <u>pauraque</u> were each detected at more than 90 sites (Figure 10A). These generalist species occupy a wide variety of habitats and remained among the most widely distributed in 2022 and 2023, although their relative rankings varied across years (Figure 10B, C).

Although the turquoise-fronted amazon is one of the most abundant parrot species in South America, it is listed as Near Threatened due to heavy trapping for the cage-bird trade and small-scale studies suggesting population declines. Notably, our passive acoustic monitoring revealed that this species is widely distributed across the sampling sites, with detections in approximately 90% of sites each year. The proportion of sites where the species was detected remained consistently high across all three years, showing only minor fluctuations.



Figure 10. Multiplot showing bird species detected at the most sampling sites across all three survey years.

## **Occupancy Models - Species Detectability**

Overall, the Multi-Species Multi-Season Occupancy Models (MSMSOM) demonstrated good model convergence, with all communitylevel parameters showing satisfactory values for the Gelman-Rubin diagnostic (R-hat) and effective sample size (ESS).

Detection probabilities varied widely, with lows of 0.021 in 2021 and 2022, and 0.011 in 2023, and highs of 0.535 in 2021, 0.614 in 2022, and 0.569 in 2023 (mean = 0.173 in 2021; 0.172 in 2022; 0.183 in 2023). For the detection component of the MSMSOM, we included sample year (with 2021 as the intercept and 2022 and 2023 as comparisons), forest cover proportion, and the number of sampling days per site. Only the number of sampling days had a significant negative influence at the community level (estimate = -0.159; 95% credible interval (CRI) = -0.263 — -0.052). This likely reflects variability in species detectability throughout the sampling season due to changes in species activity (e.g., phenology, daily or seasonal patterns). Longer sampling periods may capture greater variability in species detection, leading to a negative effect of sampling effort on overall detection rates. Ideally, we would have used Julian days to account for temporal variability in species detectability, however, some 2021 sampling sites had dates outside the expected sampling period. To avoid discarding valuable data or introducing bias, we chose to use the number of sampling days as an indirect measure to quantify variation in species detectability over the sampling period.

Although sampling year and forest cover proportion did not have significant impact on detectability at the community level, they provide important insights at the species level. Most forest-associated bird species (32 of 48) showed a positive average response to the proportion of forest cover, with 19 of these relationships being statistically significant (Figure 11). This pattern reinforces that environments with greater native forest cover are more suitable habitats for this group of species, as they provide better environmental conditions, leading to increased individual activity due to factors such as higher vocalization rates and greater population density. In contrast, species associated with grasslands and human-modified landscapes (e.g., intensive agriculture, gardens) predominantly exhibited negative responses to forest cover, reflecting low vocal activity and or low population densities.

Regarding the sampling year, 2023 had a statistically significant positive influence on a considerably larger number of species (27 species) compared to those that responded negatively (17 species). Although a substantial number of species showed increased detectability in the third sampling year relative to the first, we found no clear pattern linking this trend to trophic guilds or habitat preferences. This suggests that the observed increase in detection rates from the first to the third year reflects idiosyncratic species responses rather than a broader ecological pattern (see <u>supplementary materials</u>)



**Figure 11.** Species-level detection probability in relation to the proportion of forest cover, with 95% credible intervals shown as shaded areas. Species exhibiting a significant negative relationship are displayed on the left (orange), while those with significant positive relationships are on the right (blue). Species with non-significant responses, where the 95% credible interval overlaps zero, are shown in black.



Wildmon

## **Occupancy Models - Species Occupancy**

We included survey year, farming proportion, mean NDVI, standard deviation of NDVI, distance to lotic systems, and the connectivity index (dIIC) as covariates to explain species occupancy in the MSMSOM. At the community level, there was no significant difference in occupancy between 2021 and 2022, indicating that, on average, species occupancy remained consistent during the first two years of the project—a pattern noted in the previous report. Notably, the 2023 survey year exhibited a significantly higher probability of occupancy at the community level compared to 2021 (estimate = 0.419; CRI = 0.227 to 0.609).

To further explore this increasing trend, we calculated the odds ratio (OR) using posterior samples of the latent occupancy probability. The odds ratio is a useful tool for comparing changes in occupancy probabilities between species and across studies, while accounting for differences in probability magnitudes. Our analysis revealed striking patterns, with 28 species (~33%) showing a significant increase in occupancy probability in 2023 compared to 2021 (credible intervals entirely above 1). In contrast, three species experienced a significant decrease in occupancy probability (credible intervals entirely below 1) (Figure 12).

Interestingly, the species with declining occupancy— grassland sparrow, white-browed meadowlark, and eared dove—are typically associated with grassland or human-modified environments (Figure 12). This pattern aligns with expectations, as ecological restoration efforts transition agricultural areas into forested conditions, reducing habitat suitability for these species. Conversely, 25% of forest-associated species (12 out of 48) showed a significant increase in occupancy probability. While our model does not directly attribute these increases to specific site types (restoration areas, forest fragments, or farming areas), the observed trends suggest that restoration efforts, including the creation of forest corridors, may be enhancing habitat connectivity and quality across the landscape. This improved connectivity could facilitate movement and colonization by forest-dependent species, even in existing native forest fragments and protected areas. Future studies with site-specific analyses could help determine the relative contributions of restoration areas versus other habitat types to these occupancy trends.

The model indicated that mean NDVI (estimate = 0.402; CRI = 0.232 to 0.585) and the connectivity index (estimate = 0.311; CRI = 0.119 to 0.505) positively influenced the probability of bird occupancy. In contrast, standard deviation of NDVI (estimate = -0.12; CRI = -0.220 to -0.027), farming proportion (estimate = -0.244; CRI = -0.411 to -0.089), and distance to water (estimate = -0.357; CRI = -0.477 to -0.239) were associated with lower occupancy probabilities (Figure 13).

These findings suggest that, on average, bird occupancy increases in habitats with lower temporal variability in vegetation, denser and greener native forests, higher connectivity, and closer proximity to water. Together, these results emphasize the importance of increasing tree cover and enhancing connectivity, particularly near watercourses, to create a more suitable landscape for improving bird community occupancy. These outcomes align closely with the conservation actions developed by WeForest in partnership with IPÊ, such as the implementation of wildlife corridors with native forest to restore and connect fragmented habitats.

Regarding the impact of distance to water, it is important to note that restoration projects in legal reserve areas are often concentrated in riparian forests close to water bodies. For example, the mean distance to restoration sites was 346 m (SD = 505), compared to 589 m (SD = 358) for farming areas and 968 m (SD = 900) for forest areas. This distribution creates a bias, as restored areas are inherently closer to water than farm areas. While the importance of proximity to water has been consistently demonstrated in other studies, this result

should be interpreted with caution. It's possible the observed pattern may primarily reflect the proximity of restored areas to water, rather than indicating the intrinsic importance of aquatic habitats for the occupancy of the studied bird species assemblage.

**Figure 12.** Odds ratio of species occupancy probabilities in 2023 relative to 2021, calculated from posterior samples of the latent occupancy probability. The 95% credible intervals (CRI) are shown as shaded areas. Species with a significant negative relationship (CRI < 1) are displayed on the left (orange), while those with a significant positive relationship (CRI > 1) are on the right (blue). Species with non-significant responses, where the 95% CRI overlaps 1, are shown in black.







Multi-species occupancy models provide valuable insights into how different species respond to environmental predictors (see species-specific responses for each variable in the supplementary materials). Among these predictors, mean NDVI emerged as a key factor

shaping species occupancy patterns, particularly for forest-associated species (Figure 14).

A strong positive response to mean NDVI was observed among forest-associated species, with 22 out of 48 species showing significant effects (credibility intervals did not overlap zero). This finding highlights the ecological importance of NDVI, a remote sensing-derived metric that reflects vegetation density and productivity.

By capturing habitat quality, NDVI serves as a valuable tool for monitoring the progression of restored areas and assessing the transition of open landscapes toward mature forests. Its predictive power in shaping bird distributions suggests that NDVI could be effectively used to anticipate the occurrence of forest-dependent species, enhancing its applicability in long-term biodiversity monitoring, especially in wildlife corridor restoration projects.

**Figure 14.** Species-level occupancy probability in relation to the mean NDVI, with 95% credible intervals (CRI) shown as shaded areas. Species exhibiting a significant negative relationship are displayed on the left (orange), while those with significant positive relationships are on the right (blue). Species with non-significant responses, where the 95% CRI overlaps zero, are shown in black.



#### **Predicted Species Richness**

We estimated the predicted bird species richness for each sampling site using the output of the multi-species, multi-season occupancy model. The results show a general trend of lower species richness in farm sites compared to forest and restoration sites, which exhibit similar levels of estimated richness. These findings highlight the importance of forest and restoration areas in supporting higher bird species richness. Additionally, bird species richness increased in 2023 compared to 2021 and 2022 across all three site types. This overall increase in 2023 may be attributed to several factors, including the progression of ecological succession in restored areas, favorable environmental conditions, or natural fluctuations in bird community dynamics. However, since 2023 was the final year of acoustic monitoring, it is difficult to determine whether this increase reflects a longterm trend or merely natural variation.

**Figure 15.** Mean estimated species richness at each site (colored circles) based on the multispecies model's Z matrix. The overall mean for site types is represented by colored diamonds, with credible intervals shown as vertical bars.



One of the key questions throughout the project was how restoration age influences biodiversity patterns. Although restoration age could not be directly included in the multi-species, multiseason occupancy model (MSMSOM)—since forest and farm areas do not have age data—we addressed this question by filtering the model output to include only restoration sites with known ages. We then estimated bird species richness for 2023, along with 95% credible intervals, and applied a generalized linear model (GLM) with a Poisson distribution. In this model, average bird richness was the response variable, and restoration age served as the predictor. The baseline year (2021) corresponds to restoration sites aged 1 year.

The results reveal considerable variation in species richness estimates within the youngest restoration ages (1 to 5 years; Figure 16). However, the general trend indicates an increase in species richness as restoration age increases, suggesting that restoration age is a significant driver of species diversity. These findings, combined with species richness estimates by site type and year, imply that the progression of ecological succession in restored areas may partially contribute to the overall increase in species richness across the landscape.

However, these results should be interpreted with caution, as the restoration age gradient is heavily concentrated in the early years. The limited sample size for middle to older restoration ages leads to less precise estimates, evidenced by the high uncertainty in the curve for older ages. This limitation may introduce bias into the analysis, highlighting the need for careful interpretation of these trends.

**Figure 16.** Estimated avian species richness across restoration age, with 95% credible intervals (shaded areas). Light green circles represent the mean estimated species richness at restoration sites, derived from the multispecies model's Z matrix, with vertical light green bars indicating credible intervals. The baseline year for age was 2021.



#### WildMon Dashboard

The WildMon Dashboard presents the main results from this project in a user-friendly, noncoding interface. The Dashboard allows partners and stakeholders to interact with the data, providing community-wide biodiversity metrics, visualizations, and species-specific information for all species detected during the project.

**Figure 17.** WildMon Dashboard: Wildlife Community tab for the WeForest Wildlife Corridors project in Pontal do Paranapanema, Brazil.





# Conclusions

## Current Limitations and Future Recommendations

While this project has provided valuable ecological insights, its three-year duration presents some limitations. We outline these below, along with recommendations to enhance future sampling and analysis.

The three-year duration of this study represents a significant improvement over the single-time point assessments common in many projects, allowing us to capture early trends in ecological recovery. The inclusion of the third year of acoustic monitoring has provided stronger evidence of increasing wildlife occupancy, highlighting the positive trajectory of restoration efforts. Determining whether restored areas will fully attain the ecological conditions of large native forest remnants, or how long this process may take, requires a longterm perspective, however.

To assess the sustained impact of restoration, particularly the effectiveness of wildlife corridors, we strongly recommend long-term monitoring. If annual sampling is not feasible, periodic reassessments every 3 to 5 years would allow for the tracking of biodiversity trends and an evaluation of the long-term success of these corridors in supporting species recovery and ecosystem functionality.

## Key Takeaways

1) Diverse Species Detection: Our passive acoustic monitoring across 120 sites in Pontal do Paranapanema detected 219 species overall, including the Endangered black lion tamarin and six Near Threatened species: bare-throated bellbird, solitary tinamou, turquoise-fronted amazon, rusty-marginated guan, ornate-hawk eagle, and black-horned capuchin. These findings underscore the critical conservation value of this region and highlight the importance of restoration initiatives in maintaining biodiversity.

2) Species Detection Rates: The picazuro pigeon, turquoise-fronted amazon, great kiskadee, and pauraque were among the most frequently detected bird species, each recorded at more than 90 sites. These generalist species, which adapt well to diverse habitats, remained consistently widespread throughout the monitoring period, demonstrating their ecological flexibility within the landscape.

Given their high detection rates and broad occupancy, these species may influence ecosystem processes, particularly through seed dispersal, with the exception of the pauraque, which is insectivorous. While the other species are known to consume fruits and seeds, further research is needed to evaluate their effectiveness as seed dispersers, including seed retention times, dispersal distances, and seed viability. Understanding their role in this process could provide valuable insights into their contribution to forest recovery and connectivity in fragmented landscapes.

**3)** Soundscape Analyses: Soundscape composition and acoustic space use analyses indicate that restored areas are progressively shifting toward acoustic patterns characteristic of native forests. Over time, restoration sites have increasingly resembled mature forests, particularly in the high- and mid-frequency bands, which are associated with richer biological communities. Additionally, lowfrequency ASU was significantly higher in forested and restored areas compared to agricultural sites, reinforcing the idea that these land covers support more complex and natural acoustic environments.

4) Bird Occurrence and Richness: Native vegetation cover, habitat connectivity, and water availability positively influenced species occupancy, while agricultural areas exhibited significantly lower bird occurrence. Additionally, bird species richness was higher in restored and forested areas than in farmed landscapes, with 2023 showing the highest richness levels observed during the study. This trend further reinforces the growing impact of restoration on avian biodiversity over time.

The results from the acoustic analyses, encompassing both soundscape dynamics and species occupancy, provide strong evidence that the wildlife corridors implemented through the partnership between WeForest and IPÊ are effective in supporting wildlife conservation in the region. These corridors not only help maintain local biodiversity but also likely enhance species distribution and diversity over time, reinforcing their role as a cornerstone of regional restoration strategies.



Olivaceous woodcreeper PC: Tomaz Melo



Black lion tamarin PC: Tomaz Melo



## **PROJECT REPORT**

# Ecoacoustic Biodiversity Monitoring in Restoration Wildlife Corridors of Pontal do Paranapanema, Brazil