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Anthropogenic Factors Drive the Depletion of Fish Functional B Diversity Within and Outside Two National Reserves in the Peruvian Amazon

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ABSTRACT

Understanding the impact of human activities on fish communities is critical for the effective conservation of Amazonian ecosystems. In this study, we sampled environmental DNA (eDNA) from 17 sites (lakes and rivers) in two Peruvian national reserves, Pacaya-Samiria and Allpahuayo-Mishana, using a metabarcoding approach with four genetic markers (2 COI and 2 12S). We hypothesized that fish community composition and functional diversity in Amazonian freshwater systems could be impacted by anthropogenic factors, such as deforestation or a set of variables included in the Human Modification Index (SEDAC). Our analyses identified 319 species in Pacaya-Samiria and 207 species in Allpahuayo-Mishana, demonstrating the efficacy of eDNA for generating robust biodiversity inventories. While human disturbances did not significantly reduce species richness, they altered species composition and impacted functional diversity. Specifically, deforestation reduced functional richness, indicating the loss of ecological functions, while increasing functional dispersion, reflecting shifts towards more generalist species. These findings underline the vulnerability of functional diversity to anthropogenic pressures, even in high-biodiversity systems, and emphasize the need for conservation strategies that incorporate functional diversity alongside taxonomic diversity. In practical terms, management actions should prioritize the maintenance of structurally complex and forested riparian habitats, which support functionally diverse fish assemblages, and monitor functional traits as early indicators of ecosystem degradation. Approaches such as functional vulnerability analysis can help identify key ecological functions at risk, while targeted habitat restoration in deforested buffer zones may mitigate human impacts. Strengthening community-based management and integrating eDNA monitoring into long-term conservation plans offer a proactive, adaptable framework for safeguarding Amazonian freshwater ecosystems.

1 | Introduction

The Amazon covers approximately 7,000,000 km² and contains 40% of the planet's remaining tropical forest, much of which, especially in the west, remains relatively preserved (Hubbell et al. 2008). This region hosts the highest global diversity of amphibians, butterflies, birds and many other taxa and is estimated to harbour 10% of all known species

worldwide (Jenkins et al. 2013). The Amazon Basin also contains the largest river network on Earth, accounting for around 16% of the world's freshwater (Latrubesse et al. 2017) and contributing approximately 20% to global freshwater flows into the oceans (Molinier et al., 1995). It is also a highly complex system featuring a mosaic of ecosystems, including floodplain ecosystems that shape the river network seasonally by creating wetlands, channels, lakes and alluvial deposits (Park and

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Latrubesse 2017). These dynamic interactions between main watercourses and adjacent floodplain habitats partly explain the high biodiversity of these ecosystems, providing access to food and spawning grounds in new habitats (Couto et al. 2018). Thus, the Amazon basin is home to the greatest diversity of freshwater fish in the world, with 2406 described native species, representing 15% of all known freshwater fish (Jézéquel, Tedesco, Bigorne, et al. 2020), as well as great functional (Su et al. 2022) and genetic (Manel et al. 2020) diversity. It is all the more important to preserve this vast diversity since more than 50% of these Amazonian fish species are endemic (Jézéquel, Tedesco, Darwall, et al. 2020).

Once relatively preserved due to low exploitation, the Amazon Basin now faces increasing threats from expanding human activities and climate change. These threats have been grouped into the following five categories, which interact with one another (Dudgeon et al. 2006): (i) overexploitation of resources (Castello et al. 2015; Kirkland et al. 2020), (ii) water pollution (Beltran-Pedrerros et al. 2011; Castello et al. 2013), (iii) flow modification (Almeida et al. 2019; Duponchelle et al. 2021), (iv) habitat degradation (Soares-Filho et al. 2006) and (v) invasive species (Doria et al. 2021). As an example, in 2017, the Amazon Basin had already lost 13.4% (813,944 km²) of its original forest cover due to deforestation, and Peru 6.9% (49,852 km²) (Smith et al. 2021). Given the numerous threats and the complexity of their impacts on biodiversity, whether specific or functional, it is crucial to accurately quantify these effects. However, this task is often challenging due to the difficulty and time required to conduct exhaustive species inventories. Furthermore, Amazonian ecosystems are vast and often difficult to access, making biodiversity surveys logistically difficult. However, a relatively established technique, environmental DNA (eDNA), offers a promising solution to these challenges. The eDNA sampling involves collecting and analyzing genetic traces present in the environment without requiring the direct collection of living organisms. This method allows monitoring biodiversity in a much shorter time frame than traditional inventories and is often more efficient for the detection of rare or elusive species (Deiner et al. 2017; Doi et al. 2021). These DNA fragments, originating from cells or excretions (Balasingham et al. 2018; Beng and Corlett 2020), enable the identification of species within an ecosystem from environmental samples such as water (Ficetola et al. 2008), soil (Echeverría-Beirute et al. 2021) or air (Gusareva et al. 2019). This noninvasive method serves as a valuable tool for monitoring biodiversity and assessing the health of natural habitats.

The eDNA-based studies aiming at investigating species diversity are growing rapidly in the Amazon (Cilleros et al. 2019; De Santana et al. 2021; Jackman et al. 2021; Mariac et al. 2022). These studies highlight the incompleteness of databases, which supports a recent analysis of global gaps in reference databases, revealing that only 13% of the more than 33,000 known species of teleost fish have been sequenced for the 12S gene (Marques et al. 2020). These eDNA studies conducted on Amazonian ichthyofauna use two different target genes, namely, 12S (Cilleros et al. 2019; De Santana et al. 2021; Jackman et al. 2021) and COI (Mariac et al. 2022), with varying degrees of success. Indeed, Jackman et al. (2021) identified 84 molecular operational taxonomic units (MOTUs), of which only 4 (1.6%) were identified at the species level out of the 258 fish species detected using

traditional sampling methods in Brazilian Amazonia. De Santana et al. (2021) identified 58 species (13.1%) out of the 443 fish species detected using traditional sampling methods in the Javari River, western Brazil. In contrast, Cilleros et al. (2019) identified 132 species (65%) out of the 203 fish species detected using traditional sampling methods in several rivers of French Guiana, and Mariac et al. (2022) detected 207 species (47%) out of the 444 species identified in the Beni Basin, after sampling only 10% of the basin's area. Although studying the patterns of taxonomic biodiversity has been the main focus of aquatic surveys using eDNA metabarcoding, the functional roles of component species (diversity of ecological roles/functional diversity) are also essential to understand how ecosystems respond to natural and anthropogenic perturbations (D'agata et al. 2014; Aglieri et al. 2021). Moreover, phylogenetic diversity and functional diversity appear more responsive to anthropogenic influence than species richness (alpha diversity) (Flynn et al. 2009; D'agata et al. 2014). Several studies have demonstrated the suitability of eDNA surveys for studying the functional diversity of terrestrial and aquatic ecosystems (Donald et al. 2021; Marques et al. 2021; Seymour et al., 2021; Mathon et al. 2022). However, very few have been carried out in the Amazon basin (Cantera et al., 2022; Coutant et al. 2023).

The Amazon Basin includes a vast network of protected areas and indigenous territories, covering approximately 4.1 million km², or about 47.2% of the region, making it one of the most significant global conservation efforts (RAISG, 2020). These areas play a crucial role in reducing deforestation and preserving biodiversity. For instance, Nepstad et al. (2006) demonstrated that protected areas effectively mitigate deforestation, acting as buffers against agricultural expansion and other land-use changes. Similarly, Soares-Filho et al. (2010) emphasized the critical role of protected areas in preserving forest cover, which contributes to reducing deforestation rates and mitigating climate change through carbon sequestration. However, despite their importance, several studies reveal that these reserves remain vulnerable to anthropogenic pressures such as illegal logging, mining and overfishing (Barlow et al. 2016; Kirkland et al. 2020). These pressures are particularly concerning for aquatic ecosystems, where ichthyofauna, a key component of biodiversity and local livelihoods, is often overlooked in the design and management of reserves (Azevedo-Santos et al. 2019; Couto et al. 2024), except for a few that would explicitly aim to protect freshwater ecosystems, such as the Pacaya-Samiria Reserve (Castello et al. 2013). Fish communities are frequently affected by factors such as habitat modification, overexploitation and pollution, even within areas considered protected (Winemiller et al. 2016). This lack of focus on the specific and functional diversity of fish in conservation efforts raises questions about the resilience of aquatic ecosystems (Villéger et al. 2017). It is therefore essential to explore how anthropogenic pressures influence biodiversity, particularly within and around natural reserves.

This study investigates whether anthropogenic impact affects the species and/or functional diversity of Amazonian fish in and around natural reserves. To access Amazonian diversity, an eDNA sampling campaign was conducted in two reserves and their surroundings: the Pacaya-Samiria and Allpahuayo-Mishana National Reserves. Both are located in Peru near the city of Iquitos, offering remarkable and diverse ecosystems.

The extensive and prestigious Pacaya-Samiria National Reserve is the second largest in Peru and the second largest in the entire Amazon. These two reserves are administratively structured with a monitored protection zone (the reserve itself) and a buffer zone (peripheral area around the reserve with intermediate use restriction between the reserve and outside). Additionally, Allpahuayo-Mishana reserve is located close to the city of Iquitos and Pacaya-Samiria close to the city of Nauta. In both reserves, the chosen sampling sites present a gradient of distance from these cities, hence supposedly a gradient of anthropization. This term describes the progressive alteration of natural ecosystems resulting from human activities such as, in our case deforestation, infrastructure development and land-use change. The hypotheses we are testing are that anthropization could lead to discernible changes in the composition of Amazonian fish species that could alter the ecological functions of species assemblages. To test these hypotheses, we (1) analyzed the species composition of the study sites and their alpha diversity in relation to anthropization

variables and (2) identified life traits linked to the ecology of each species in order to conduct a study of functional diversity at each site.

2 | Methods

2.1 | Sampling and Analysis Process of eDNA

2.1.1 | Study Area and Sampled Sites

This study was conducted in two national natural reserves, Pacaya-Samiria (created in 1972) and Allpahuayo-Mishana (created in 2004), located in the Loreto department in the northeastern Peruvian Amazon (Figure 1). The Pacaya-Samiria national reserve covers an area of 2,080,000 ha and is bordered by two major tributaries of the Amazon River, the Ucayali and Marañón rivers, encompassing the watersheds of the Pacaya, Samiria and Yanayacu rivers. The

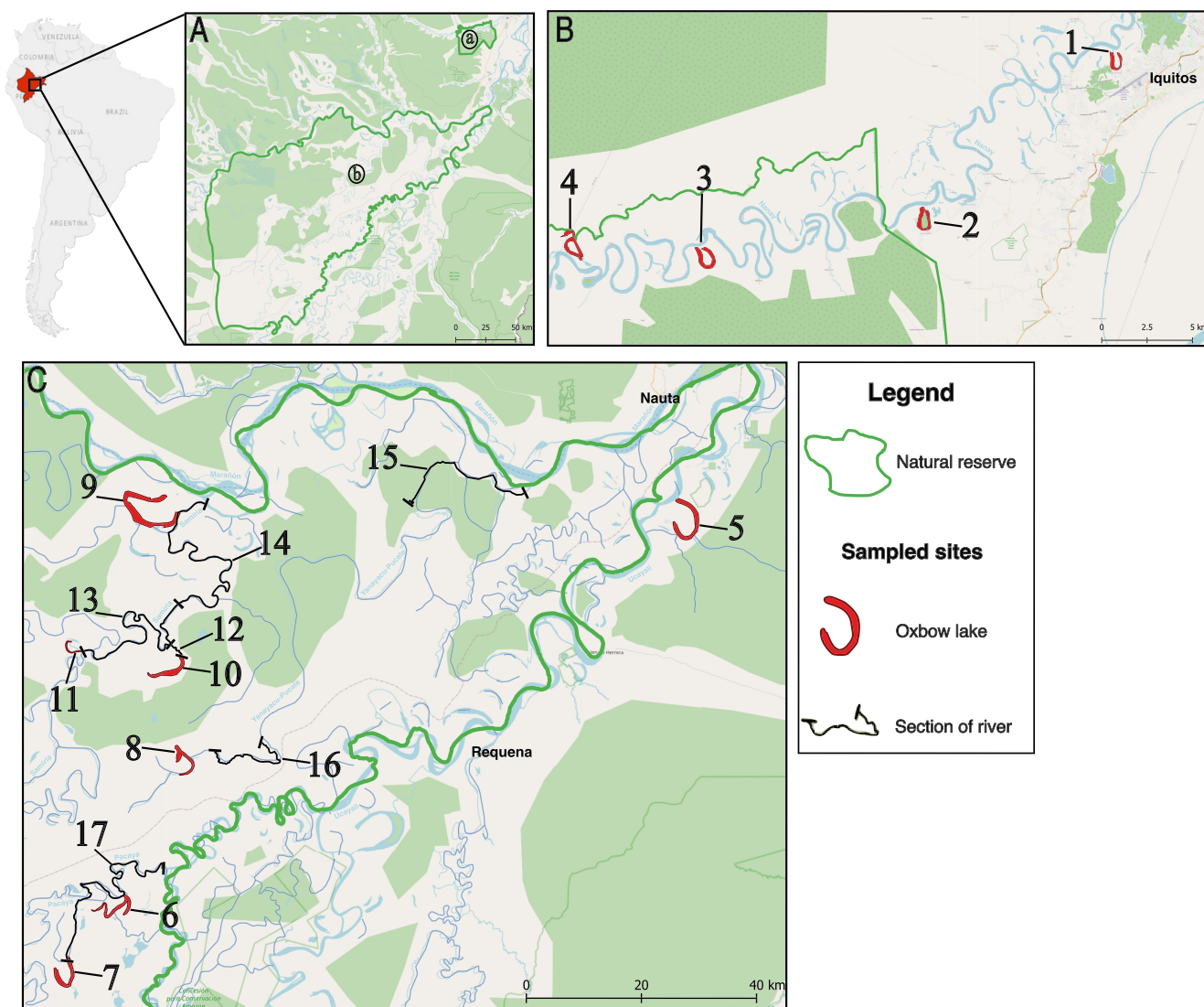


FIGURE 1 | Map of sampling sites. (A) Location of the two national reserves, Allpahuayo-Mishana (a) and Pacaya-Samiria (b), in the Loreto region. (B) Sampling sites along the Nanay River and within the Allpahuayo-Mishana national reserve. (C) Sampling sites within the Pacaya-Samiria national reserve. Oxbow lakes are numbered from 1 to 11, river sections are numbered from 12 to 17, and information on each site is provided in Table S1. The maps were created using QGIS v3.34 with the ESRI Standard base map.

Allpahuayo-Mishana reserve is situated about 20 km south-west of the city of Iquitos in the Nanay River watershed. These two reserves are characterized by significant hydrological variations, alternating between rising water periods from October to May and descending water periods from June to September (Reis et al. 2019; Wittmann et al., 2022). In total, 17 sites were sampled as part of this study. Among these 17 sites (Table S1), four oxbow lakes were sampled in the Nanay Basin: two within the Allpahuayo-Mishana Reserve and two outside the reserve with distances to the city of Iquitos ranging from 1 km for the closest to 32 km for the farthest (Figure 1B). Additionally, the remaining 13 sites were sampled in the Pacaya-Samiria Reserve (Figure 1C). Among these sites, eight are oxbow lakes and five correspond to river sections, distributed between the core of the reserve, its border and one located outside of it.

2.1.2 | Sampling Method and Laboratory Analysis of eDNA

The eDNA samples were collected from rivers and oxbow lakes at the end of the low-water periods in 2021 (September and October, 7 sites) and 2022 (September to November, 10 sites). At this time the connectivity between lakes and rivers is broken, ensuring that the DNA collected in the lakes corresponds to species present in the lakes and not to DNA transported by rivers entering the lakes. Additionally, it is important to highlight that the lakes sampled are permanent lakes, and their surfaces range from 29 to 1308 ha. For each site, approximately 30 L of water were collected from sampling points evenly distributed across the surface along three transects (left bank, right bank and middle) for the lakes. For the rivers, a single transect was conducted in the middle. The total number of sampling points per site ranged from 45 to 120 (see Table S2 for more details about sampling points). For each site, an average volume of 27.4 L was filtered using Envirocheck HV cartridges with a 1- μ m pore size. Only one cartridge was used per site, except for the Llanhama site (Table S1) where two cartridges were used because of premature filter clogging. The sampling kit includes a drill driver (AEG GmbH—BSB12cli202c) and a peristaltic pump (Watson-Marlow 313-DW). The marpene suction hose is connected upstream to a 5- to 6-m polyethylene suction hose and downstream to a polypropylene connector, which ensures the connection to the filter cartridge. To ensure ballasting and prevent the entry of large particles into the cartridge, lead sinkers and a suction strainer (2-mm mesh) have been added to the end of the suction pipe. The equipment, consumables and conservation buffer (Longmire et al. 1997) used are those described in Mariac et al. (2022). Strict precautions were taken to limit and control contamination, including disinfecting equipment between sites and performing field blanks. Throughout the collection process and until storage in the laboratory, the cartridges were kept cool (~4°C in coolers with blocks of ice). In total, 23 cartridges were processed, including five field blanks (on-site filtration of 20-L mineral water). For each of the 23 collected cartridges, 10 eDNA extractions were performed (splitting the 150 mL of buffer contained in each cartridge into 10 extraction subsamples), totalling 230 extractions, plus 15 negative extraction controls. DNA extraction and subsequent library preparation

were carried out at ADNid laboratories (Montferrier-sur-Lez, France; <http://www.adnid.fr>). For library construction, the 245 extracted DNA samples, plus three positive controls (namely, IRD, bulk1 and bulk2) previously described in Mariac et al. (2022), and four negative PCR controls, were amplified using four different barcodes to maximize the number of identified species. The barcodes used included two nested cytochrome oxidase I (COI) barcodes and two 12S barcodes (12S MiFish and 12S vertebrates (Riaz et al. 2011; Miya et al., 2015). In total, 1008 libraries (4 barcodes $\times$ [245 extractions + 3 three positive controls + 4 negative PCR controls]) were prepared with dual combinatorial indexing and sequenced (paired-end, 150 bp) using a Novaseq 6000 Illumina platform at the Novogene Co. Ltd. facilities.

2.1.3 | Bioinformatics Analysis of Sequences

The amplicon sequences were analyzed using the FROGS pipeline (Escudié et al. 2018) following standard operating procedures. Amplicons were split according to their primer sequences and size and clustered into operational taxonomic units (OTUs) using Swarm (aggregation distance: $d=1$). After chimera removal, OTUs were kept if present in at least one library and with a minimum abundance of three sequences (parameters used and command lines are reported in Table S3). The taxonomic identification of OTUs was carried out by alignment using Blastn (version 2.8.1) against a custom COI database (Table S4a), three public databases for 12S (Table S4b, obtained at https://mare-image.weebly.com/12sdb_all-sequences.html, <https://ucla.app.box.com/s/45akkrjxba2nkt3a7c2bzlapazg6fw06> and <https://ucedna.com/reference-databases-for-metabarcoding>) and one public database for MiFish (Table S4c; obtained at <https://mitofish.aori.u-tokyo.ac.jp>). Currently, the COI and 12S databases used cover 39.4% and 55.0% of the 2406 described Amazonian species, respectively, and 1026 of these species have neither of these two barcodes available. The Blastn results were processed with the MEGAN software version 6.24.11 (Huson et al. 2016) to assign taxonomy using parameters shown in Table S3. Finally, the process includes two filtering steps to remove contaminant reads and rare OTUs. Negative controls, including field, extraction and PCR controls, were employed to detect and eliminate contaminant reads in the sample libraries using the R package Microdecon (McKnight et al. 2019). Taxa (OTUs assigned at either family, genus or species levels) with less than five reads were excluded and species identified as marine were removed (See Table S5). In the final table used for analysis (Table S6), only OTUs assigned to species level were kept.

3 | Data Analysis

3.1 | Comparison of the Markers Used

In this study, four different markers were used to analyse specific diversity. To compare the effectiveness of each marker, we examined the number of taxa (of order- to species-level) detected by each one, as well as the number of species detected. Two Venn diagrams using the ggven function, R-package 'ggven 0.1.10' were created to illustrate these results. In order to assess the accuracy and specificity of each marker in species identification,

the taxonomic resolution of each marker was calculated by dividing the number of species detected by the number of taxa detected for each marker considering their respective database.

For the following analyses, only the OTUs identified at the species level were retained.

3.2 | Assessment of Sequencing and Sampling Effort

In order to assess whether the sequencing effort was sufficient, rarefaction curves were established for each library. The results are available in Figure S2.

To assess the sampling effort in each of the two reserves, the second-order jackknife estimator (Jack2) was calculated by performing a series of successive site draws within each reserve, using the vegan package 2.6-4 (Burnham and Overton 1979). Based on the number of singleton and doubleton, Jack2 performs an extrapolation to provide a better estimate of the true species richness. It was calculated for each reserve, as was the percentage coverage, equal to the number of species observed divided by the number of species expected (Jack2 value). It is important to note that the Jack2 estimator only partially represents the actual diversity of the study area due to the incompleteness of the COI and 12S databases. Additionally, sampling conducted only during the dry season overlooks species that are present only during the flood season, making our species richness estimate partial.

3.2.1 | Selection of Environmental Variables Related to Anthropogenic Impacts

Areas around the sampled sites were defined, and environmental variables were collected for these areas. The QGIS v3.34 tool was used for this purpose. The creation of these areas was done arbitrarily, but differently depending on the site type, whether it was an oxbow lake or a river segment. For lakes, the areas were created to cover 10 times the surface area of the lagoon, while for river segments, the areas were established by defining a 1-km zone around the river. First, a Human Modification Index variable (Center for International Earth Science Information Network—CIESIN—Columbia University, Kennedy et al. 2020) was extracted from Socioeconomic Data and Applications Center (SEDAC), and then QGIS v3.34 was used to extract an average index from the raster file for each previously defined area at each site. This index reflects the proportion of modified landscape, based on modelling the physical extents of 13 anthropogenic stressors and their estimated impacts (population density, built-up areas, croplands, livestock, major roads, secondary roads, tracks, railways, mining/industrial zones, oil wells, wind turbines, power lines and night-time lights). Next, the polygon file for each previously defined area on QGIS v3.34 was imported into Global Forest Watch to retrieve several environmental variables related to forest cover for each of these areas. Global Forest Watch applies tree cover thresholds to determine what is considered 'forest.' For this study, we chose a parameter of 30% tree cover. For each area, the following environmental variables were retrieved: surface area (in ha), population density

(number/ha), forest cover (in %), total forest cover loss (deforestation and fire) between 2001 and 2023 (in %), forest cover loss due solely to deforestation between 2001 and 2023 (in %) and the average net greenhouse gas flux per year between 2001 and 2023 (in tCO₂/year). All these variables account for the difference in area between zones, except for the last variable (average net greenhouse gas emissions), which was normalized to enable comparison from one site to another.

To avoid issues of multicollinearity between variables, a variable selection process was carried out. Pearson correlation coefficients were calculated between all pairs of variables (using the `cor` function, R package 'stats 4.3.2'). To select variables, any correlation with an absolute coefficient greater than 0.8 was considered a strong correlation. Consequently, because the Human Modification Index is strongly positively correlated with population density and negatively correlated with forest cover (Figure S1), only the Human Modification Index was retained for further analysis. It is also noteworthy that the average net greenhouse gas flux is strongly positively correlated with total forest cover loss and loss due to deforestation (Figure S1), therefore, only forest cover loss due to deforestation was retained. In summary, three variables were chosen for further analysis: the forest cover loss due to deforestation, the Human Modification Index and the surface area.

3.2.2 | Analysis of Species Diversity

Data formatting, calculation of species richness (α diversity) and species composition of different sites (β diversity) were performed using the R packages `phyloseq` 1.46.0 and `vegan` 2.6-4.

The α diversity was calculated for each site (estimate-richness function, R package: 'vegan 2.6-4'). Subsequently, Pearson correlation models were calculated for the Pacaya-Samiria Reserve (`cor.test` function, R package 'stats 4.3.2') to examine any potential relationship between alpha diversity and anthropogenic activity, using three previously selected anthropogenic variables: forest cover loss due to deforestation, the Human Modification Index and the surface of sample sites. The correlation models were not calculated for Allpahuayo-Mishana Reserve because of the low number of sites (4).

Species composition variation (β diversity) was calculated for each pair of sites using the binary Jaccard distance. Given that eDNA does not allow a direct estimate of species abundance through the number of reads, the use of the Jaccard distance is more appropriate as it only considers the presence or absence of species. The β diversity was graphically represented using a nonmetric multidimensional scaling analysis (NMDS, ordinate function, R package 'phyloseq 1.46.0'). To determine any potential differences in species composition between the two reserves a Permutation-based multivariate analysis of variance model, known as PERMANOVA (`adonis2` function, R package 'vegan 2.6-4') was performed, as it allows for a statistical test to determine the potential existence of variation in species composition between reserves. Another PERMANOVA was carried out to evaluate the potential existence of variation in species composition between the Nanay, Ucayali and Marañón watersheds, as well as between lakes and rivers. A PERMANOVA was also

conducted on the Pacaya-Samiria Reserve to measure the link between three environmental variables (site area, forest cover loss due to deforestation and a Human Modification Index) and the variation in species composition within Pacaya-Samiria Reserve.

3.3 | Analysis of Functional Diversity

Functional diversity analyses were carried out at the specific level excluding species without formal identification, for example, sp., confer and affinis using the R mFD 1.0.7 package, according to the method described by Magneville et al. (2022).

Three life traits (maximum size, trophic category and migration type) were selected as biological descriptors. Migration type was assigned using Duponchelle et al. (2021) and Herrera-R et al. (2024). Maximum size and trophic category were determined from max length and diet information (trophic levels, diet type and/or prey type) available in the Fishbase database (Froese & Pauly, 2024). The trophic category variable was constructed from four variables extracted from FishBase (Herbivory2, FeedingType, FoodTroph and AddRems) and supplemented with information collected from the literature (Mérona and Rankin-de-Mérona 2004; Mourão et al. 2014; Arantes et al. 2019), and defined based on their main diet (data in Table S7).

In order to calculate functional richness (FRic) and functional dispersion (FDis) indices, the read abundance table was converted into a presence-absence table. These indices were calculated with the R package 'mFD' version 1.0.7. to quantify the functional diversity of fish species communities, taking into account the distribution of their functional traits.

The calculation of the FRic index is based on the volume occupied by the species in functional space, thus reflecting the range of ecological functions present in a community. The FDis index, meanwhile, measures the dispersion of species relative to the mean traits of all species in the community in the functional space defined by FRic. This index thus evaluates the variability of functional traits within the community, indicating how dispersed the traits of different species are around this mean.

To understand how environmental variables influence functional diversity, the relationships between three environmental variables (site area, forest cover loss due to deforestation, the

Human Modification Index) and the FRic and FDis indices for each site were assessed. Statistical analyses were performed using Pearson correlation models (cor.test function, R package 'stats 4.3.2'), allowing for the detection of potential linear relationships between environmental variables and functional diversity indices. The correlation models were exclusively conducted with the sites from the Pacaya-Samiria Reserve.

Functional β diversity was calculated and compared between sites (beta.fd.multidim function, R package 'mFD 1.0.7'). The functional diversity index can be divided into two primary components: functional turnover and functional nesting. The first one represents the variation in functional diversity due to the replacement of species from one site to another; it then measures how much the functional traits of species change between sites, indicating a substitution of ecological functions. Functional nestedness reflects the variation in functional diversity due to the loss or gain of species without a change in the dominant functional traits; it then indicates to what extent the functional trait composition of one site is a subset of that of another site. The R package 'pheatmap' version 1.0.12 was used to produce heatmaps of the functional β diversity and its two components.

4 | Results

4.1 | Sequencing Results and Comparison of the Markers Used

The average number of filtered reads (paired end assembled, with 5' and 3' primer, with expected length and without N) per library for each barcode is 732,769 (see Table S8 for details). Rarefaction curves generated at both the library and site levels (Figure S2) suggest, based on the shape of the curves approaching the asymptote, that the sequencing effort was sufficient for all libraries.

Of the 344 species identified, the two COI markers (MK1 and MK2) and the two 12S markers (MiFish and 12S vertebrates) identified 217 and 219 species, respectively, with only 91 common species, showing the complementarity of the barcodes (Figure S3).

COI markers have a 10% higher taxonomic resolution (number of detected species/numbers of detected taxa, Table 1). Given

TABLE 1 | The number of fish taxa and species detected is reported for each barcode used in this study. The taxonomic resolution of each marker and each gene was calculated as the ratio of species detected to the number of taxa. To compare the resolution of the COI and 12S barcodes, the number of species with a barcode available in their respective databases is reported (number of detectable species).

Gene	Marker	Number of taxa detected *		Number of species detected		Taxonomic resolution		Number of Amazonian species in the databases
COI	MK1	206	267	165	217	0.8	0.81	949
	MK2	201		171		0.85		
12S	MiFish	251	308	177	219	0.71	0.71	1314
	12S vertebrates	122		91		0.75		

*Taxa detected either at the family, genus or species level.

that 94% of the species in the COI database are also present in the 12S database, and assuming the databases have no geographic representation bias, the MK COI markers allow for the identification of a significantly (bilateral proportion comparison: $p < 0.001$) higher proportion of species (22.9%) compared to the 12S markers (16.7%) (Table 1 for details).

4.2 | Species Biodiversity

4.2.1 | Alpha Diversity

Across all the sites in the two reserves, a total of 344 species were identified (Table S6), of which 33 are unnamed species (noted as sp., *aff.* or *cf.*). Among the remaining 311 species, all were present in South America (Ortega and Hidalgo 2008; Jézéquel, Tedesco, Bigorne, et al. 2020; GBIF), but one (*Ancistrus trinitatis*), which was therefore removed from the dataset. Additionally, of the 310 species present in South America, 281 have already been observed in the Amazon (Ortega and Hidalgo 2008; Jézéquel, Tedesco, Bigorne, et al. 2020; GBIF), and 199 of them are also present in Peru (Ortega and Hidalgo 2008). Of the 311 identified species, 30 had not previously been recorded in the Amazon. Although each showed a molecular identity rate above 98%, these species

were classified as *affinis* because they had not been identified in the Amazon basin until now. The 344 species are distributed across 11 orders, 38 families and 174 genera. The orders Siluriformes and Characiformes are the most represented, with 39.8% and 39.2% of the species, respectively, followed by Cichliformes and Gymnotiformes, which represent 9% and 7.6% of the species. The remaining 4.4% of species are distributed among the orders Clupeiformes, Cyprinodontiformes, Gobiformes, Myliobatiformes, Osteoglossiformes, Perciformes and Synbranchiformes.

In the four sites of the Allpahuayo-Mishana National Reserve (4 sites), 210 species have been identified, representing 79.2% of the estimated richness (JackK2=265 species) (Figure 2). For the Pacaya-Samiria National Reserve (13 sites), 308 species have been identified, corresponding to 84.6% of the estimated richness (JackK2=364 species) (Figure 2).

The distribution of the observed richness for all libraries at each site is presented in Figure S4. The number of species per site ranges from 99 at Tipishca to 199 at Rio Dorado Yanayacu (Figure 3). River sections, referred to here as 'rios,' have higher alpha diversity (Kruskal-Wallis $\chi^2 = 9.38$, $p = 0.002$). In total, 23.3% of the species (82) were identified at only one or two sites (for more details, see Figure S5), while only 3.5% of the

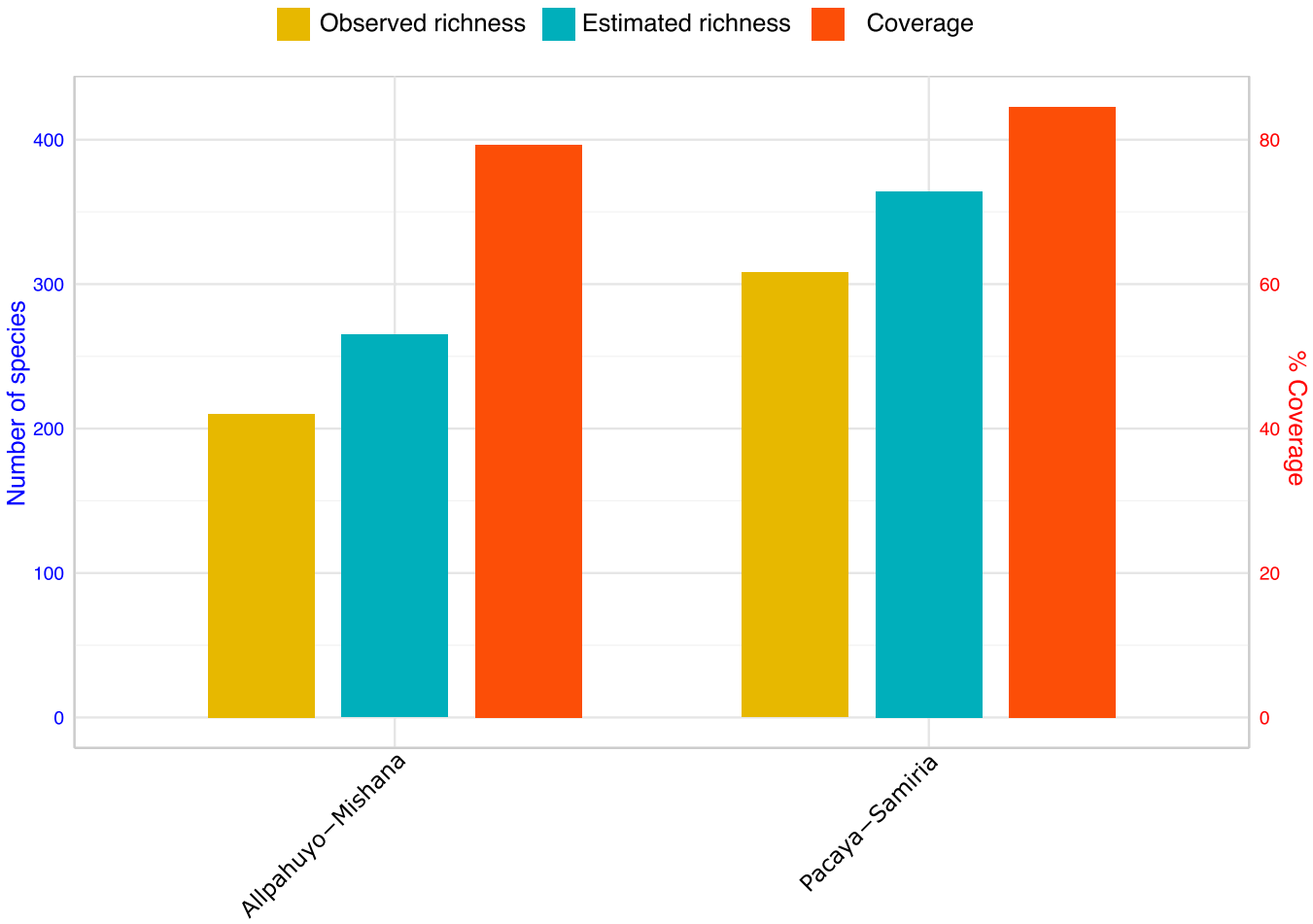


FIGURE 2 | Number of observed species (yellow bars) and estimated richness using jack2 index (blue bars) for Allpahuayo-Mishana and Pacaya-Samiria national reserve. The orange bars represent the coverage percentage (number of observed richness divided by to the estimated richness $\times$ 100).

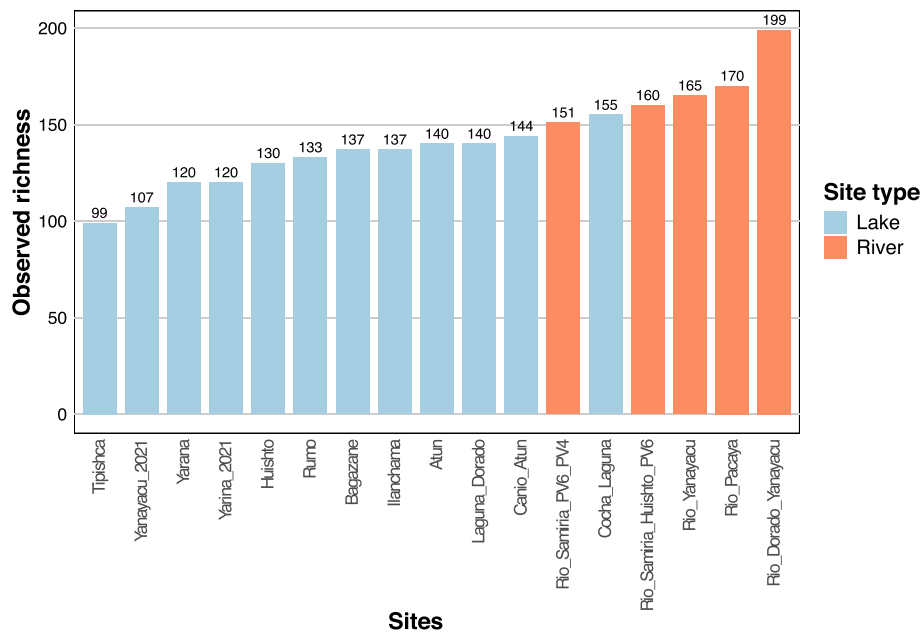


FIGURE 3 | Species richness: Plot report alpha diversity for each sampling site. Colour coding distinguishes lake sites from river sites.

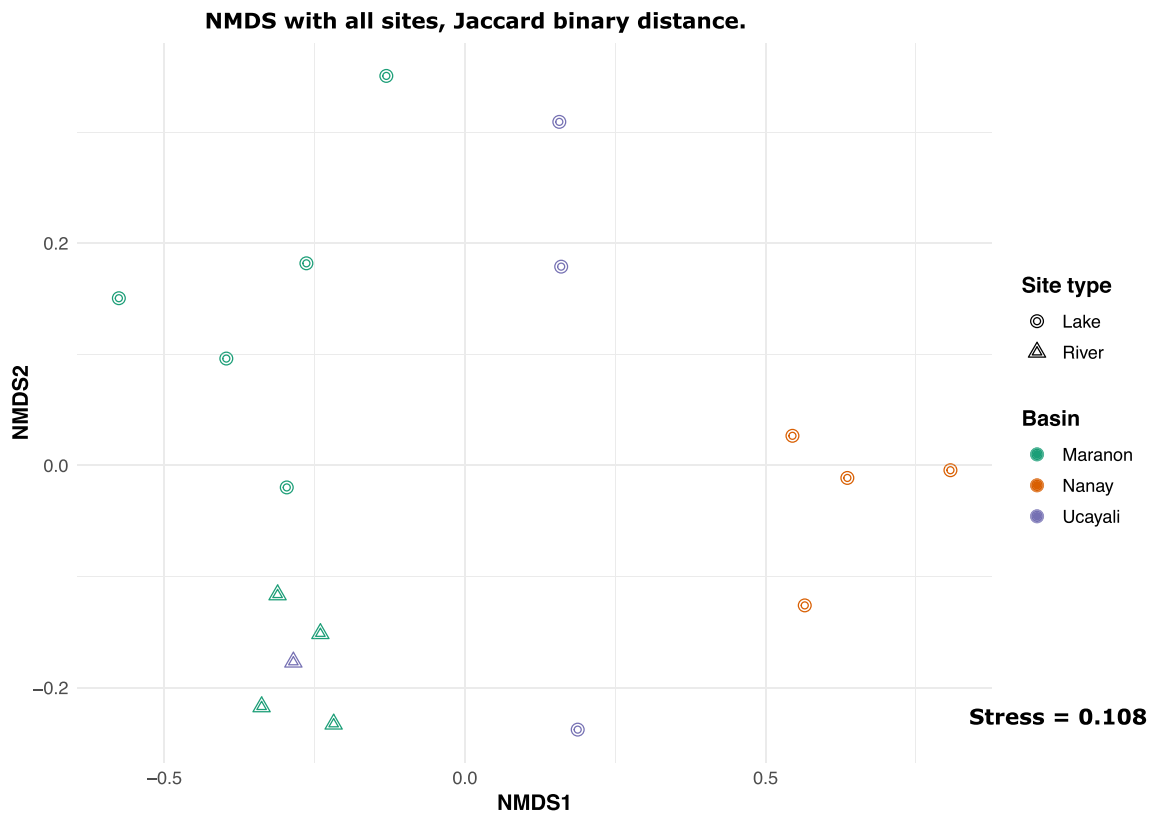


FIGURE 4 | Graphical representation of the NMDS analysis for all sampled sites based on their species composition. This representation plot the sites based on their species assemblages. Colour codes distinguish basins (Marañon, Nanay and Ucayali) and shape codes distinguish lakes from rivers. The Marañon and Ucayali basins are located in the Pacaya-Samiria national reserve and the Nanay basin in the Allpahuayo-Mishana national reserve.

species (*Heros efasciatus*, *Hoplias malabaricus*, *Plagioscion squamosissimus*, *Potamorhina latior*, *Prochilodus aff. costatus*, *Prochilodus aff. harttii*, *Prochilodus lineatus*, *Prochilodus nigricans*, *Psectrogaster amazonica*, *Pygocentrus nattereri*, *Pygocentrus piraya* and *Steindachnerina elegans*) were observed at all sites.

4.2.2 | Beta Diversity

The NMDS revealed a strong dissimilarity in species composition between the two national reserves studied (Figure 4), which was supported by the PERMANOVA analysis ($p = 0.002$).

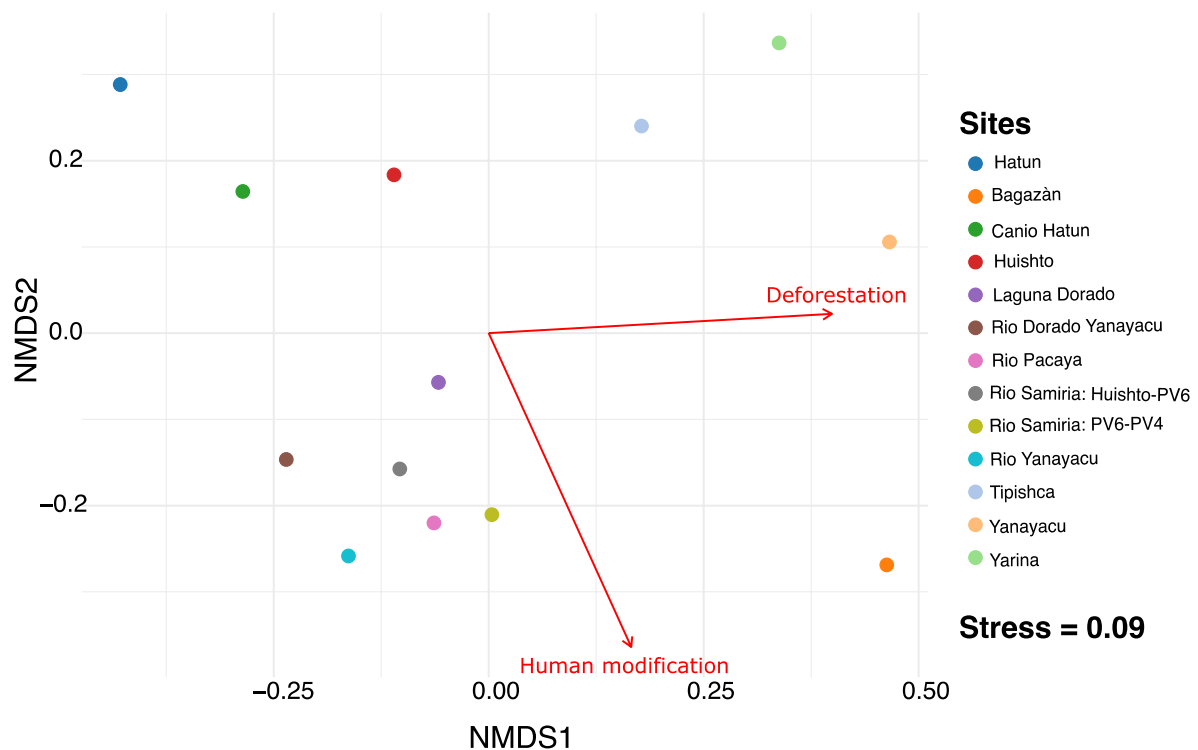


FIGURE 5 | Graphical representation of the NMDS analysis of sites in the Pacaya-Samiria National Reserve based on their species composition. Colours indicate the different sites. The red arrows show the contribution of the variables with significant contributions, and their length is proportional to the strength of this contribution.

The NMDS ordination performed on all sites visually illustrates a difference in species composition between the three basins (Nanay, Marañón and Ucayali) as well as between the types of sites: lakes or rivers (Figure 4). This graphical structure is confirmed by the PERMANOVA analysis, which reveals a statistically significant difference in species composition between basins ($p=0.001$) and between the types of sites ($p=0.016$). Pairwise tests between basins also show significant differences: Marañón–Ucayali ($p=0.012$), Marañón–Nanay ($p=0.005$) and Nanay–Ucayali ($p=0.028$).

In the Pacaya-Samiria national reserve, a distinct composition is also observed between rivers and lakes ($p=0.004$).

4.3 | Impact of Human Activity on Species Biodiversity

Correlation models indicate that there was no significant correlation between alpha diversity and our tested environmental parameters including the Human Modification Index, deforestation or the area of sampled sites ($p>0.05$) (Figure S6).

The PERMANOVA conducted exclusively on the sites within the Pacaya-Samiria National Reserve to assess the contribution of selected variables (site area, deforestation and human modification index), revealed a statistically significant contribution of deforestation ($p=0.001$) and Human Modification Index ($p=0.022$) to the variation in species composition (β diversity). In contrast, site area does not have a significant influence on the variation in species composition (Figure 5). This figure also

shows the direction and strength of each variable's contribution with a red arrow, indicating that the sites Bagazàn, Tipishca, Yanayacu, Yarina and the two sections of the Samiria River are the most impacted by anthropogenic factors (deforestation and human modification).

4.4 | Functional Biodiversity

4.4.1 | Functional Diversity Indices

Regarding functional diversity indices, FRic ranges from 0.610 at Bagazàn to 0.988 at Cocha-Laguna, while FDis ranged from 0.546 at Hatun to 0.619 at Yarina_2021. Correlation models show that there is no significant correlation between FRic and the Human Modification Index ($p=0.123$). However, there is a significant negative correlation ($R=-0.63$, $p=0.021$) between FRic and deforestation. For FDis, there is no correlation with the Human Modification Index ($p=0.559$), but a significant positive correlation ($R=0.64$, $p=0.019$) is observed with deforestation (see Figure S6).

4.4.2 | Functional β Diversity

The representation of functional β diversity in the heatmap (Figure 6A) shows that it ranges from 0 to 0.18 for each pair of sites, indicating that all sites have a similar or nearly similar functional space. However, two sites are exceptions: Rumo Cocha and Bagazàn. These sites exhibit a higher functional β diversity (between 0.31 and 0.39) with all other sites, but a very low β diversity between themselves (0.08) (Figure 6A). This means

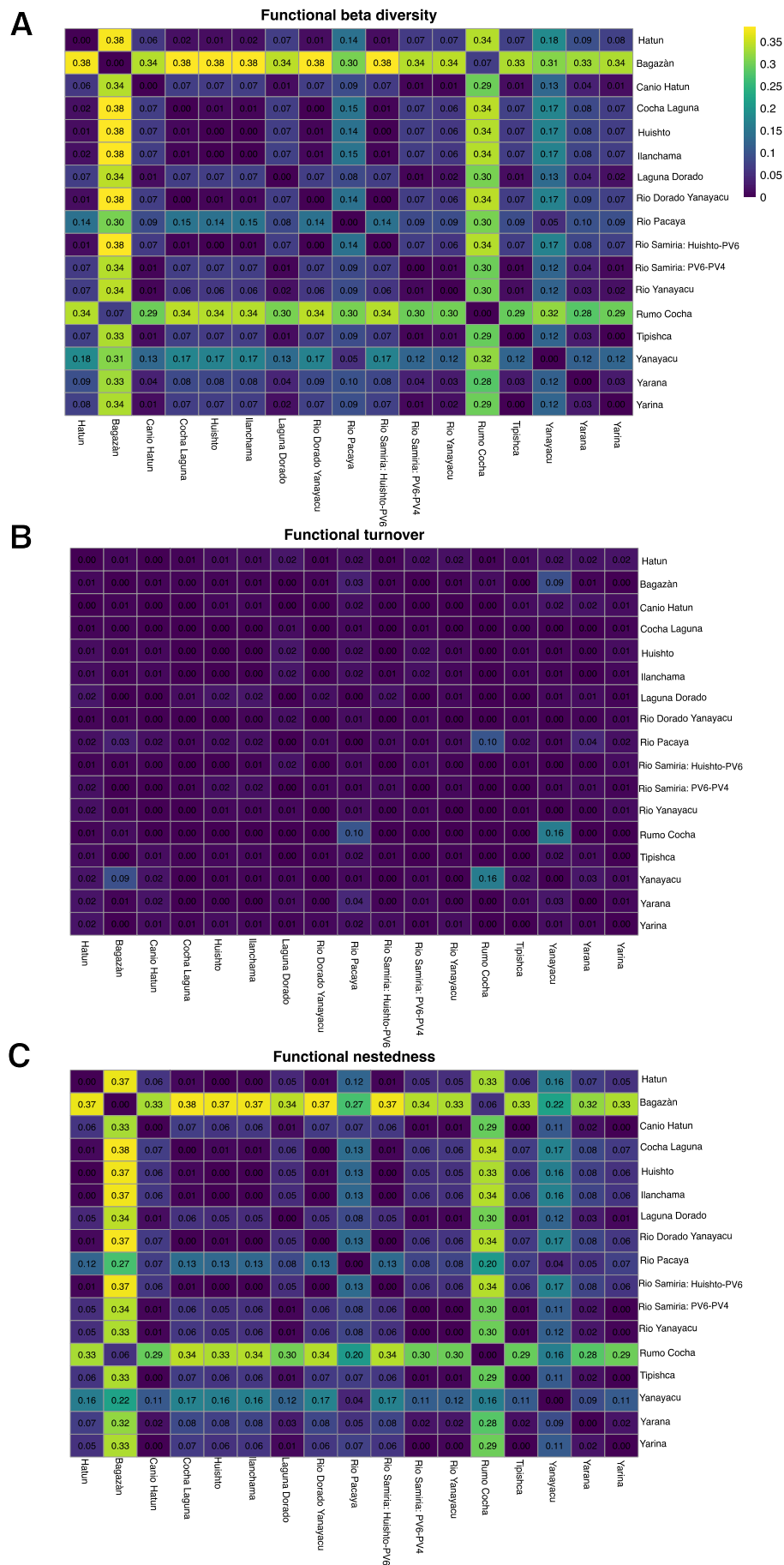


FIGURE 6 | Legend on next page.

FIGURE 6 | Heatmaps representing for all sampled sites the functional β diversity indice (A) and its decomposition into its two subcomponents: functional turnover (B) and functional nestedness (C) Each heatmap uses a similar colour gradient with the same value scale, which is displayed at the top right of the first heatmap.

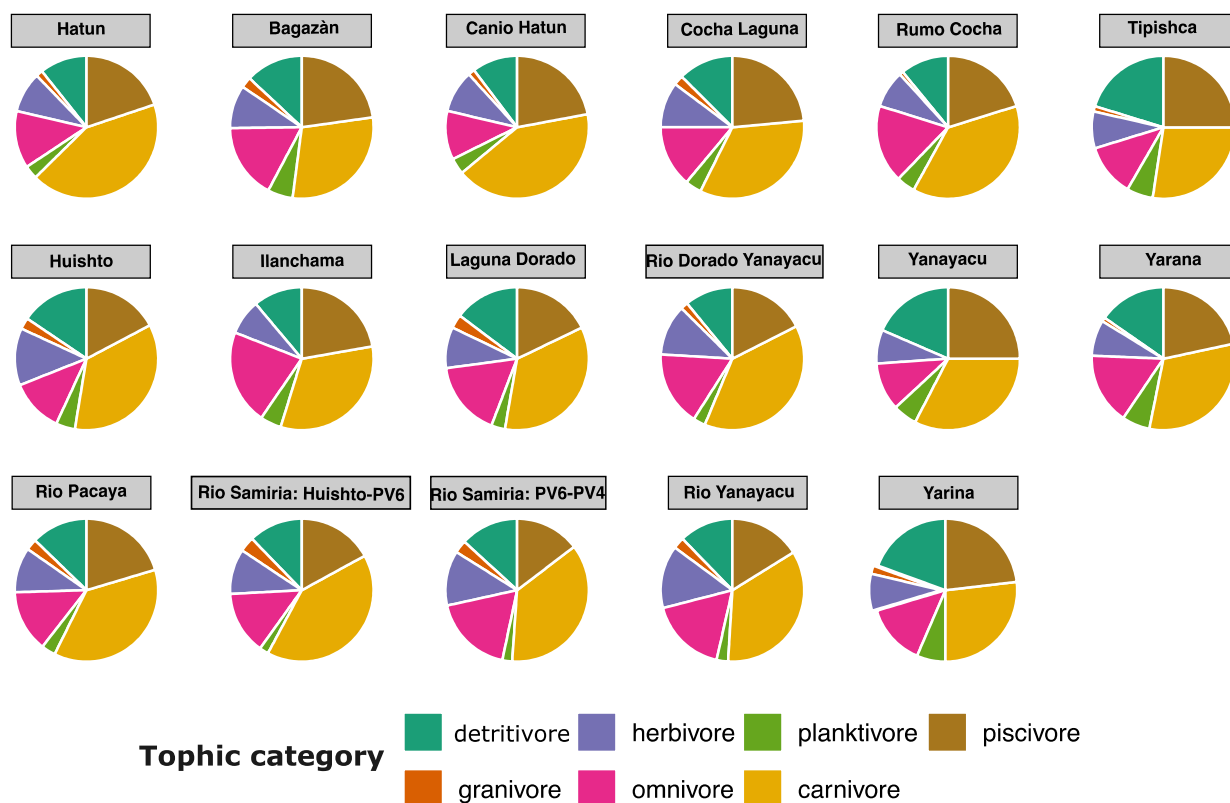


FIGURE 7 | Proportion of species by trophic category for all sampled sites.

that Rumo Cocha and Bagazàn have nearly identical functional spaces between them, but different from those of the other sites.

The heatmap representing functional turnover (Figure 6B) highlights the absence of significant functional turnover between most sites, with the exception of Rumo Cocha and Yanayacu_2021, where a slight turnover is observed (0.15).

Two sites, Rumo Cocha and Bagazàn, show functional nestedness with all other sites (Figure 6C), similar to the functional β diversity observed in figure 6A. All other sites show little or no functional nestedness. Thus, their functional spaces are nested within those of the other sites (Figure S5). The functions absent in Rumo Cocha and Bagazàn are primarily associated with large piscivores, some carnivores and, notably, large migratory species (Figures S7-S8).

4.5 | Change in the Trophic Structure of Communities Related to Anthropization

The pie charts (Figure 7) show that the fish communities in this section of the Amazonian hydrographic network are dominated by carnivorous species, as they represent about one-third of the species at almost all sites. Granivorous and planktivorous species are the least abundant. Graphically, it appears that there

is little variation in the proportions of the trophic categories. This is supported by correlation models, showing no significant correlation between the variation in the proportions of species in each trophic category and the anthropization variables. The only exception is the proportion of piscivorous species, which is positively and significantly correlated with deforestation ($R = 0.68$, $p = 0.01$).

5 | Discussion

5.1 | eDNA: An Efficient Tool for Species Inventory

The vast and remote expanses of the Amazon Basin pose significant logistical challenges for biodiversity assessments. In this context, eDNA methods have gained prominence as a cost-effective, noninvasive and less destructive alternative to traditional sampling techniques, especially in such difficult-to-access regions. These methods have demonstrated high potential for species identification, as evidenced by Mariac et al. (2022), who identified 47% of the 444 species identified in the Beni Basin using eDNA. However, contrasting results from other studies, such as Jackman et al. (2021), where only 1.6% of fish species were identified at the species level, highlight the challenges posed by incomplete genetic reference databases, the choice of genetic markers, sampling strategies (number of sampling

points per site, volume of water filtered ...) and varying ecological or hydrological conditions. These discrepancies mainly stem from sampling design and methodology. Jackman et al. (2021) filtered only 4.5 L per river (three points per transect—left bank, midchannel, right bank at 0, 25 and 50 m; 3 × 500 mL), which likely constrained species detection in hyperdiverse settings. By contrast, Mariac et al. (2022) and our study used the same cross-channel scheme but replicated across 45–120 points per site with substantially larger volumes (~27–30 L per site). Moreover, sampling at the end of the low-water period not only reduced DNA transport among connected water bodies—yielding a more local signal—but also likely increased eDNA concentration via lower dilution. These choices of spatial coverage, filtered volume and timing explain the higher detection rates and underscore the critical importance of sampling design for reliable eDNA inventories. Furthermore, regarding the current study, the higher taxonomy resolution obtained can also be attributed to the use of the multilocus approach. By sampling only four sites along the Nanay river (Allpahuayo-Mishana), we identified 207 species out of the 643 distributed over the entire Nanay basin (Jézéquel, Tedesco, Bigorne, et al. 2020). Similarly, in the Pacaya-Samiria National Reserve, sampling 13 sites enabled the detection of 319 species out of the 464 known species (Jézéquel, Tedesco, Bigorne, et al. 2020). These results underscore the power of eDNA for generating robust species inventories, even in regions with vast biodiversity and logistical constraints. Indeed, compared to traditional methods, eDNA has consistently shown its ability to identify more species, more efficiently and with less ecological disturbance (Garlapati et al. 2019; Polanco Fernández et al. 2021). Regarding sampling effort, recent recommendations suggest that a single 34-L sample can identify 87% of the ichthyofauna in tropical small streams and 67% in rivers (Cantera et al. 2019). In our case, approximately 30 L of water per site were filtered, which provided sufficient DNA to cover 79.2% and 84.6% of the estimated number of species for the studied sites in the Allpahuayo-Mishana and Pacaya-Samiria national reserves, respectively. However, it is important to note that the potential completeness of the census also depends on the completeness of the reference sequence databases, which account in our case study for only 39.4% (COI) and 54.6% (12S) of the 2406 described Amazonian fish species (see Table 1 for more details). With more complete and accurate reference databases, the number of OTUs assigned to species would be higher, further increasing the recovered diversity and ultimately resulting in even more accurate inventories. This would also result in a more refined assessment of the anthropogenic impacts on species functional diversity.

Despite a less complete reference database for COI (28% less Amazonian species than the 12S database), COI markers (MK1 and MK2) were more effective than 12S markers (MiFish, 12S vertebrates) as they detected as many species. Moreover, COI markers also have a taxonomic resolution 10% higher than 12S markers. This difference in performance can be attributed to several factors. Firstly, the length of the barcoding regions differs between COI and 12S markers. COI barcodes tend to be longer (180 and 285 bp), which can provide more genetic information and allow for finer taxonomic resolution. In contrast, 12S regions are shorter (150 and 175 bp), which, while advantageous for degraded DNA, may limit their discriminatory power (Hänfling et al. 2016; Thomsen et al. 2016). Indeed, a positive relationship exists between the length of the amplified barcode

and its taxonomic resolution power (Meusnier et al. 2008; Coissac et al. 2012). Secondly, the intrinsic qualities of the loci play a role. The mitochondrial COI gene is highly conserved in its function as part of the electron transport chain but exhibits greater variability at the nucleotide level compared to 12S rRNA genes. This variability in COI often makes it more suitable for distinguishing closely related species (Hebert et al. 2003; Hajibabaei et al. 2007; Ward 2009). However, the conserved nature of 12S genes might explain their greater reliability for higher taxonomic levels but reduced resolution at the species level. It is important to emphasize that a high taxonomic resolution, as achieved in this study using COI markers, is particularly valuable in hyperdiverse ecosystems such as the Amazon. The ability to accurately distinguish closely related species allows for the assessment of fine-scale patterns in community composition and functional diversity. Such resolution is crucial, as species with only subtle genetic differences may possess distinct functional traits (Muschick et al. 2012; Mouillot et al. 2014), thereby enhancing the accuracy and ecological relevance of functional diversity assessments. Furthermore, the performance of both markers is influenced by the completeness of reference databases, which remains a limitation for COI in Amazonian ichthyofauna but is improving over time. These findings underscore the need for continued refinement of reference databases and optimization of marker choice depending on study objectives. For broad biodiversity inventories, combining multiple markers may offer complementary advantages, enhancing both resolution and detection rates (Olds et al. 2016; Evans et al. 2017; Li et al. 2018).

Among the 244 detected species, 12 were found across all sampled sites, representing 3.5% of identified the following species: *Heros efasciatus*, *Hoplias malabaricus*, *Plagioscion squamosissimus*, *Potamorhina latior*, *Prochilodus aff. costatus*, *Prochilodus aff. harttii*, *Prochilodus lineatus*, *Prochilodus nigricans*, *Psectrogaster amazonica*, *Pygocentrus nattereri*, *Pygocentrus piraya* and *Steindachnerina elegans*. Notably, *Prochilodus costatus* and *Prochilodus harttii* are non-Amazonian. These observations may stem from multiple factors. First, non-Amazonian species might have been intentionally or accidentally introduced, explaining their detection; this potential explanation, however, fails to account for their systematic detection at all sites. Second, and more likely, this issue stems from taxonomic assignment challenges due to the incompleteness of the reference database. This includes erroneous matches potentially caused by the very low mitochondrial divergence among species within the *Prochilodus* genus (Melo et al. 2018). These findings emphasize the need to refine reference databases to enhance taxonomic accuracy. While eDNA is a powerful tool for biodiversity inventories, methodological and taxonomic limitations must be addressed for accurate result interpretation.

5.2 | Impact of Anthropogenic Activity on Species and Functional Diversity

One of the objectives of this study was to understand how species diversity was impacted by anthropogenic activity. Our analyses reveal significant variations in species composition between the two national reserves. These differences were also observed within the Pacaya-Samiria National Reserve between

watersheds and between rivers and lakes, likely related to the ecological and/or hydrological variables specific to each watershed. This observation is consistent with previous studies showing that hydrological variables, such as water type (black, clear or white-water), strongly influence fish community composition (Saint-Paul et al. 2000; Siqueira-Souza et al. 2021). Within the Pacaya-Samiria National Reserve, the site area does not seem to influence the variation in species composition. In contrast, anthropogenic variables such as human modification and deforestation significantly contribute to this variation. This suggests that anthropogenic disturbances alter natural habitats by decreasing habitat diversity, for example (Larentis et al. 2022), leading to changes in fish communities (Cantera et al., 2022). However, it is interesting to note that alpha diversity is not significantly correlated with the modification index or with deforestation. This lack of correlation may indicate a degree of resilience in some species to small-scale anthropogenic disturbances. The combination of these results indicates that in the Pacaya-Samiria National Reserve, anthropogenic activity modifies species composition without necessarily affecting species richness, reflecting a potential change in ecological functions, as already observed in several studies (Su et al. 2022; Cantera et al., 2022; Coutant et al. 2023).

Analyses of functional diversity indices (FRic and FDis) reveal a decrease in FRic associated with increasing deforestation, accompanied by an increase in FDis. The decline in FRic reflects a reduction in the diversity of functional traits within the ecosystem, indicating the loss of species occupying specific ecological roles and, consequently, a diminution of functional space, leading to a functional simplification where fewer traits are represented, limiting the ecosystem's capacity to maintain diverse ecological functions (Mouillot et al. 2014; Oliveira et al. 2018). Conversely, the rise in FDis suggests that the remaining species exhibit more divergent traits, indicating increased fragmentation of ecological niches and a reduction in functional continuity (Mason et al. 2005; Legras et al. 2020). This pattern can be explained by the gradual elimination of specialist species, which are often positioned at the margins of functional space and adapted to narrow ecological niches, while generalist species, capable of tolerating a wider range of environmental conditions, tend to occupy more diverse niches in disturbed ecosystems (Villéger et al. 2010). This phenomenon, characteristic of degraded habitats, results from pressures caused by deforestation, including habitat fragmentation and structural simplification (Leal et al. 2016). These processes reduce habitat complexity, limit the availability of specific ecological niches for specialist species and favour generalist species, thereby intensifying competition for already limited resources. Indeed, while specialists are more competitive when occupying preferred habitats, generalists tend to be more competitive on average across multiple habitats (Marvier et al. 2004).

Functional β diversity analyses highlighted functional homogeneity among most sites within the two reserves. However, the lakes Rumo Cocha and Bagazán contrast because they share identical functional spaces but differ from those of other sites. Rumo Cocha and Bagazán are the closest lakes to towns or villages. Indeed, Rumo Cocha is located in the city of Iquitos, and Bagazán is on the edge of the Pacaya-Samiria National Reserve, bordered by several villages and near the town of Nauta. It is

therefore interesting to understand how the functional space of these two sites differs from that of other sites. This would provide better insights into how functional space evolves in response to anthropogenic pressure in this region. Our analyses of functional renewal and overlap reveal that the functional space of these two sites does not differ from that of other sites by the presence of different ecological functions. On the contrary, their functional space is nested within the functional space of other sites (Figure S7). Thus, it is reasonable to think that these two lakes have experienced erosion of their functional diversity, likely due to significant anthropogenic pressure. The absence of certain functions in Rumo Cocha and Bagazán is primarily associated with the default of large piscivores, carnivores and large migratory species (Figures S7–S8). These species, particularly the large piscivores (such as *Arapaima gigas* and *Brachyplatystoma* spp.), are important components of the ecosystem and face significant fishing pressure due to their commercial value (García Vásquez et al. 2009; Ardura et al. 2010). Thus, the increase in the proportion of piscivorous species observed in connection with deforestation could partially be explained by the process of trophic cascade (Ripple et al. 2016). The disappearance of top predators in heavily anthropized lakes reduces predation pressure on smaller piscivores, allowing these species greater access to resources. This dynamic can potentially increase the populations of existing species and enable the establishment of additional species.

5.3 | The Role of Protected Areas and the Use of eDNA In Conservation Management

In our study, significant differences in species β diversity and functional diversity between sites within the reserve and those near villages or towns suggest that the proximity to human activities plays a crucial role in shaping biodiversity. The Rumo Cocha and Bagazán lakes, located near urban centres, show significantly reduced functional diversity, likely due to increased anthropogenic pressures (deforestation and human modification), despite Bagazán being within the reserve boundaries. This highlights a key aspect of conservation management in the Amazon: The challenge of accessibility, rather than simply the designation of an area as a protected zone, appears to be a more critical factor for preserving ecological functions, as previously suggested by Bruner et al. (2001) and Curran et al. (2004). Our results align with other studies that emphasize the variability in the 'health' of protected areas, with approximately half functioning effectively or adequately, while the other half experiences significant biodiversity loss, both taxonomically and functionally (Laurance et al. 2012). Additionally, our study, along with other research, clearly shows that the landscapes and habitats surrounding natural reserves are often subject to pressing threats (DeFries et al. 2005; Hansen and DeFries 2007; Frederico et al. 2018). It is therefore imperative to recognize that environmental changes occurring immediately outside reserves can be just as impactful as those occurring within them (Laurance et al. 2012). Indeed, external ecological conditions can significantly affect the viability of reserves and their biodiversity, for instance, by increasing their isolation or exacerbating edge and surface effects (Terborgh et al. 2001; Hansen and DeFries 2007; Laurance et al. 2011; Laurance et al. 2012).

Furthermore, it is essential to integrate functional diversity alongside taxonomic diversity into conservation policies, particularly in the Pacaya-Samiria and Allpahuayo reserves. Although our results indicate that anthropogenic activities in these areas have not drastically reduced taxonomic richness, they have significantly impacted functional diversity. In the Pacaya-Samiria and Allpahuayo reserves, we observed a decrease in FRic and an increase in FDis, reflecting the loss of specialized ecological roles, particularly those of large piscivores, carnivores and migratory species. These changes highlight the loss of rare species with unique roles in ecosystems, potentially compromising ecosystem stability and resilience (Mouillot, Bellwood, et al. 2013). Such disruptions could threaten ecosystem services like nutrient cycling, trophic regulation and habitat structuring, all of which are vital for the sustainability of freshwater systems in the Amazon (Tilman et al. 1997). For example, in the Loreto region, which includes the Pacaya-Samiria reserve, fishing pressure has disproportionately targeted large piscivores over several years, leading to their decline (Garcia et al. 2009). Similarly, in the Allpahuayo reserve, deforestation and habitat degradation may explain the reduction of species fulfilling critical ecological roles. The recurrence of these patterns across these two independent reserves underscores the pervasiveness of such pressures and emphasizes the urgency of addressing them in conservation strategies.

To improve conservation in these reserves, targeted habitat restoration, such as key spawning and feeding grounds in Allpahuayo and Pacaya-Samiria, could maintain or restore critical ecological functions. Additionally, incorporating functional diversity into reserve management plans offers a more holistic conservation approach. By ensuring the protection of habitats necessary to sustain diverse functional traits, these strategies could enhance ecosystem resilience to environmental changes (Mouillot, Graham, et al. 2013; McLean et al. 2019). As demonstrated by Mouillot et al. (2014) for tropical reefs and applicable to the Amazon Basin, functional diversity remains highly vulnerable to species loss, even in ecosystems with high biodiversity. In these systems, certain functions or functional entities are supported by only a few species, or even a single one. This highlights the importance of integrating functional diversity into conservation strategies, using approaches such as functional vulnerability analysis, which assesses how many species sustain each functional entity (Auber et al. 2022). Moreover, utilizing tools such as eDNA to monitor taxonomic and functional diversity in fish communities could refine conservation policies, making them more proactive and adaptable (Thomsen and Willerslev 2015; Levi et al. 2019; Beng and Corlett 2020; Marques et al. 2021). In the case of Pacaya-Samiria and Allpahuayo, this approach could serve as a model for other reserves, underscoring the importance of preserving both biodiversity and ecological functions in the Amazon. Finally, it is crucial to focus on mitigating anthropogenic pressures such as overfishing and habitat destruction. Establishing no-fishing zones, regulating fishing activities and promoting sustainable livelihood alternatives could help reduce the erosion of functional traits. Typically, promoting community-based management fisheries, which provide excellent results in the Amazon (Almeida et al. 2009; Anderson et al. 2009; Castello et al. 2011; Arantes and Freitas 2016; Campos-Silva et al. 2019), would likely help reduce the erosion of the functional traits associated with the loss of large piscivores.

Author Contributions

C.G.D., C.M., F.D. and J.-F.R. conceived this study. C.A., C.M., G.E., F.D. and J.-F.R. collected and processed the samples. C.M. and J.-F.R. carried out laboratory analyses. B.P., C.M., F.D. and J.-F.R. analysed the data and wrote the manuscript with revision by all authors.

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Ethics

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Proyecto eDNA1.

Título de la subvención = Adquisición de un analizador genético de 24 capilares AB3500XL para fortalecimiento de las actividades de caracterización molecular de los vertebrados acuáticos como base para la implementación del ADN ambiental (eDNA) en la Amazonía Peruana.

Contract N°: 017-2018-FONDECYT-BM.

Financiador: Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (Fondecyt).

Proyecto eDNA 2.

Título de la subvención = Efectos antropogénicos sobre la biodiversidad en la Amazonia peruana evaluados mediante el uso del ADN ambiental (eDNA).

Contract N°: 074-2021-FONDECYT.

Financiador: Programa Nacional de Investigación Científica y Estudios Avanzados (PROCIENCIA).

Autorization Numbers.

Numero de permisos de colectas 2021 y 2022 Para Pacaya samiria:

Numero de permisos de colectas 2022 para Nanay:

Numero de permisos de exportaciones 2021 y 2022.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Sequence Read Archive at <https://www.ncbi.nlm.nih.gov/sra>, reference number BioProject: PRJNA1189465, Accessions: SRR31442580-SRR3144316.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1: Heatmap of correlation coefficients between pairs of variables.** Values close to 1 indicate a positive correlation, values close to –1 indicate a negative correlation and values close to 0 indicate a weak correlation. **Figure S2: Rarefaction curves.** Rarefied taxa accumulation by read count per site. **Figure S3:** Venn diagram of the different markers used showing the number of taxa at the families, genera and species level (A) and at only the species level (B) identified by each marker. **Figure S4:** Distribution of the observed richness for all libraries at each site. Each box plot represents the distribution of the observed richness, indicating the median (the central line) as well as the first (Q1) and the third quartiles (Q3), which allows for visualizing the interquartile range of the data. The individual point within each box correspond to the observed richness for each of the libraries at that site. **Figure S5: Frequency distribution of species based on the number of occupied sites.** This figure shows the proportion of species present exclusively at a single site, only at two sites, three sites and so on. In our case, approximately 12% of the species are found exclusively at a single site. We observe that this proportion decreases as the number of occupied sites increases, reflecting the rarity of ubiquitous species. **Figure S6: Graphical representation of the correlation models established for each index (alpha diversity, Fdis and Fric) with the variables (Modification Index, deforestation and site area).** (A) Alpha diversity. (B) Functional dispersion (Fdis). (C) Functional richness (Fric). These models were created using only sites from the Pacaya-Samiria Reserve. For each correlation model, the correlation coefficient and *p* value are shown directly above each

graph. **Figure S7: Graphical comparison of the functional space between Laguna Dorado, Bagazán and Rumo Cocha lakes.** This graph presents the functional spaces in the 3-first dimensional space of the PCA. Each colour indicates the functional space of one of the two sites, and the white panel shows the total functional space calculated with the species from all sampled sites. Only the functional space of Laguna Dorado is represented because the functional space of all other sites is nearly similar to that of Laguna Dorado. **Figure S8: Correlation between functional traits and NMDS axes.** This graph illustrates the correlations between NMDS axes and functional traits. Each row represents one NMDS axis (PC1, PC2 and PC3), while each column corresponds to a trait such as body size (Length), migration pattern and trophic category (Trophic guild). The strength and direction of the correlations are indicated by the data point and the accompanying tables, revealing how each trait contributes to structuring the functional space along the NMDS dimensions. This helps identify key ecological gradients driving the functional diversity of fish communities. In summary, the three axes (PC1, PC2 and PC3) are influenced by distinct functional traits. Specifically, PC1 and PC3 are primarily shaped by migration pattern, whereas PC2 is mainly influenced by both body size (Length) and trophic guild. **Table S1: Information on sites.** For each site, the table provides details on the year, water type, basin, number of collections, volume of water filtered, surface area for lakes and length for rivers. **Table S2: Information on sites and each point of collect per site.** The date, localisation, volume of water collected, sampling depth, date of collect and geographical coordinates are reported for each sampling point. **Table S3: Frogs parameters.** **Table S5: Abundance table before filtering.** This table includes all taxa detected with the markers, even those that are not fish, along with the number of reads per library. **Table S6: Abundance table after filtering.** This table presents the 344 species remaining after filtering, along with their previous identification locations (AMAZ = Amazon, ORTEGA = Peru and AS = South America) and the number of reads per species in each library. **Table S7: Table of traits.** **Table S8: Summary of filtered reads per library and barcode.** This table provides details on the average number of filtered reads per library and barcode after processing steps, including paired-end assembly, retention of reads with both 5' and 3' primers, expected length and exclusion of reads containing 'N.' **Table S4a: COI, MIFISH and 12S fish database used.** **Table S4b:** Supplementary Information. **Table S4c:** Supplementary Information.