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Aquatic and Terrestrial Invertebrates Support Fish Assemblages of Amazonian Streams

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ABSTRACT

1. The Amazonian stream network is remarkable not only for its extent but also for its rich fish diversity. These streams are interconnected with the surrounding forests. However, key ecological aspects of Amazonian freshwater ecosystems, such as energy flows and carbon sources sustaining fish assemblages, remain poorly understood.
2. In this study, we used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to evaluate, for the first time, the trophic structure of Amazonian stream fish and to identify the food resources sustaining their assemblages. We sampled fish and a broad array of potential food resources, including macrophytes, riparian vegetation, filamentous algae, aquatic and terrestrial invertebrates, and periphyton. We calculated diversity metrics, trophic niches, trophic positions, and assimilated resource proportions.
3. Fish biomass was primarily supported by terrestrial invertebrates (31%–55%) and benthic invertebrates (22%–40%). Shrimps were the third most important resource contributing to fish biomass (9%–22%). Collectively, invertebrates accounted for 79%–90% of the total fish biomass across the studied streams. Trophic diversity metrics showed little variation among stream sites, with most pairwise Bayesian comparisons indicating substantial overlap in posterior distributions.
4. Our findings underscore the critical role of both aquatic and terrestrial invertebrates in supporting the trophic dynamics of stream fish in the Amazon, demonstrating the strong ecological linkages between streams and their surrounding landscapes. Moreover, the results highlight the importance of riparian forests in providing essential food resources for fish assemblages of Amazonian aquatic ecosystems.
5. Our study provides novel insights into the carbon flow and isotopic ecology in small Amazonian streams, contributing to understanding ecosystem functioning and informing conservation strategies for one of the most species-rich freshwater systems on Earth.

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1 | Introduction

Headwater streams are fundamentally connected to their surrounding landscapes. Terrestrial–freshwater exchanges of organic and inorganic matter, as well as nutrients, are key to their ecological integrity and energy flow (Baxter et al. 2005; Esteves et al. 2021). These energy flows are also reflected in large rivers downstream, as streams supply them with a continuous input of energy and matter, maintaining ecological connectivity across the basin (Vannote et al. 1980). In the Amazon, small *terra firme* streams run through forests and are shaded by riparian vegetation, resulting in low primary productivity (McClain and Elsenbeer 2001). Therefore, riparian forests provide essential food resources and carbon sources for fish (Lowe-McConnell 1999), shaping their trophic ecology.

The complex forest-stream linkages are also reflected in the variety of microhabitats available for the diverse Amazonian fish fauna (Vieira et al. 2018). As a result, these fish species exhibit a wide range of ecological roles, habitat use, and foraging strategies (Brejão et al. 2013). In these systems, fish exploit various energy sources, including autochthonous food resources and allochthonous resources supplied by the riparian forest (Cardoso and Couceiro 2017). Temporary pools formed in the adjacent stream margins during rainfall periods are also a source of detritus and terrestrial invertebrates for fish (Espírito-Santo et al. 2009). Altogether, this ecological diversity reflects a wide range of trophic strategies that sustain complex food webs and energy flows in these systems (Saito et al. 2024).

Stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a powerful tool for investigating energy flow and trophic ecology in aquatic ecosystems (Pereira and Benedito 2007; Albrecht et al. 2021). The isotopic composition of fish tissue reflects the array of resources assimilated by them, enabling an understanding of carbon flow across ecosystems (Fry and Sherr 1984; Newsome et al. 2007; Albrecht et al. 2021). $\delta^{13}C$ stable isotopes exhibit minimal trophic fractionation along the food web, with an average increase of approximately 0.4‰ ± 1.3‰ per trophic level (Post 2002), enabling the identification of different basal resources that sustain the food web of an ecosystem. In contrast, $\delta^{15}N$ stable isotopes are consistently fractionated along the trophic web, with a typical trophic fractionation of about 3.4‰ ± 0.98‰ per trophic level (Post 2002). Therefore, $\delta^{15}N$ stable isotopes are particularly useful for estimating the trophic position of consumers within a food web (DeNiro and Epstein 1981; Fry 2006; Perkins et al. 2014; Alonso et al. 2020).

In the Amazon basin, one of the first studies using stable isotopes was conducted in the mid-1980s, when Araujo-Lima et al. (1986) analysed the main carbon sources for detritivorous fish in the Amazon River. Later, other studies applied this tool to investigate fish trophic ecology, but mainly with a focus on species from large rivers (Forsberg et al. 1993; Leite et al. 2002; Mortillaro et al. 2015; Zuluaga-Gómez et al. 2016; Seabra et al. 2025), which exhibit an energetic dynamic and overall ecological functioning markedly different from small headwater streams. The isotopic ecology of Amazonian stream fish remains poorly understood, despite the anticipated importance of riparian vegetation in sustaining aquatic communities, particularly through allochthonous inputs such as terrestrial invertebrates and plant organic matter (Vannote et al. 1980).

To address this knowledge gap, we conducted the first study using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes to elucidate the trophic structure and carbon assimilation of fish assemblages in Amazonian streams. Specifically, we seek to: (a) Determine the main carbon sources sustaining fish assemblages, estimating the percentual assimilation of different food resources across species and the corresponding biomass supported by these inputs; and (b) Evaluate the trophic structure of fish assemblages by estimating trophic niches, trophic positions, and community-wide metrics of trophic diversity. We tested the following hypotheses: (1) Given the limited light availability due to dense riparian canopy cover, fish assemblages are predominantly sustained by allochthonous resources, with invertivorous species comprising the dominant trophic guild; and (2) Because the studied streams share similar environmental conditions, we anticipate minimal variation in trophic position, trophic diversity, and isotopic niche structure among assemblages. By disentangling fish diets and energy pathways, this study provides critical insight into the ecological linkages between aquatic and terrestrial environments. The resulting patterns reflect the intricate processes that maintain biodiversity and ecosystem functioning in Amazonian streams and highlight the fundamental role of riparian forest integrity in sustaining tropical freshwater food webs.

2 | Methods

2.1 | Study Area

The study was conducted in five streams (2nd to 3rd Strahler order) within the Curuá-Una River basin, a tributary of the Amazon River, Pará, Brazil (Figure 1). The region is characterized by a mosaic of land uses and cover, including mechanized agriculture, cattle ranching, silviculture, smallholder agricultural settlements, secondary forests, and undisturbed primary forests, the latter mostly located in the Tapajós National Forest protected area (Gardner et al. 2013). The stream sites are located outside protected areas but within landscapes characterized by low anthropogenic disturbance and similar land-use cover.

This study was carried out under the Sustainable Amazon Network (RAS) framework sampling design (Gardner et al. 2013). Each stream site consisted of a 150 m stretch where we measured the physical habitat and sampled fish and resources, similarly to other studies in the Amazon (Leal et al. 2017; Leitão et al. 2018; Montag et al. 2019). This level of sampling effort is considered sufficient to sample fish species richness in megadiverse tropical regions (Pompeu et al. 2021). Land-use cover was estimated using MapBiomas (MapBiomas 2024). We calculated the percentage of mature forest (i.e., primary forest + old secondary forest) at the catchment scale upstream of each stream site (Table 1). Old secondary forest was defined as secondary forest that is at least 10 years old. Non-forest areas consisted of pasture, grassland, soybean and other

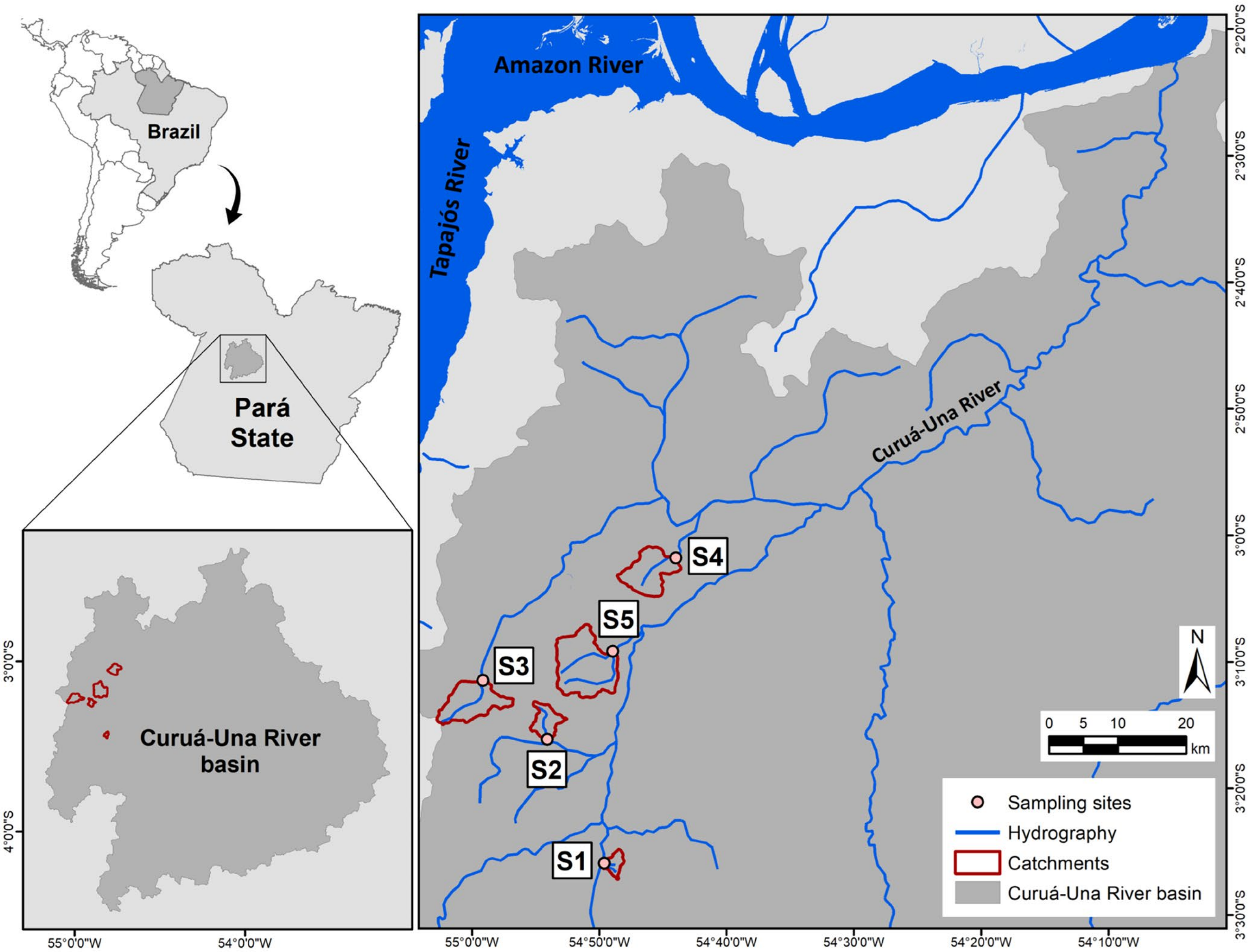

FIGURE 1 | Stream sites in the Curuá-Una River basin, Pará, Brazil. (Some streams were omitted for better visualization of sampling sites)

TABLE 1 | Land-use cover and physical habitat characteristics of the stream sites sampled in July/August 2023 in the Curuá-Una River basin, Amazon basin.

Stream sites	Strahler order	Geographic coordinates	Mean depth (m)	Mean width (m)	Fine substrates (%)	Channel shading (%)	Mature forest (%)	Other land uses (%)
S1	3°	−54°49′34.42″ −3°25′44.88″	0.55	2.71	43.80	92.91	82.83	17.17
S2	2°	−54°54′0.77″ −3°15′57.02″	0.39	2.68	26.31	88.50	62.59	37.41
S3	3°	−54°59′2.68″ −3°11′17.72″	0.76	12.94	16.19	87.96	78.51	21.49
S4	3°	−54°43′52.15″ −3°1′39.14″	0.83	3.92	39.04	86.89	56.47	43.53
S5	3°	−54°48′51.04″ −3°9′1.53″	1.02	5.96	70.47	94.25	80.89	19.11

temporary crops. To characterize the physical habitat of streams we measured depth, width, substrate type and channel shading, following the field protocol proposed by Kaufmann et al. (1999) and Peck et al. (2006). Fieldwork was conducted once during the dry season (July–August 2023), when logistics and sampling conditions are more suitable (Leitão et al. 2018).

2.2 | Fish Sampling

For fish sampling, we subdivided the 150-m stream site into 10 continuous 15-m sections. Each section was sampled by two people for 12 min, totaling 120 min of sampling effort (Leal et al. 2017; Leitão et al. 2018). Fish were collected using semicircular sieves (80 cm in diameter, 1-mm mesh) and seine nets (3 m long, 5-mm mesh) in all stream sites. All sampling procedures were authorized by ICMBio (SISBIO 87389-1/2023) and the Ethics Committee of the Federal University of Pará (UFPA) (8293020418/2018).

For the stable isotope analysis, we selected up to five individuals per species, whenever possible. We weighed, measured, and collected muscle samples from each large individual, while the small individuals (< 2 cm) were analysed whole, after removal of the digestive tract. Fish samples for isotopic analysis were frozen and later lyophilized for 48 h and ground into a fine homogeneous powder using a mortar and pestle. Voucher specimens were also weighed and measured and fixed in 10% formalin solution and then stored in 70% ethanol solution and deposited at the Fish Collection of the Federal University of Lavras (CI-UFLA), where the species identifications were confirmed using identification keys and by consulting specialists.

2.3 | Basal Resources Sampling

At each stream site, we collected up to five samples of each of the following basal resources, when available: periphyton (PE), filamentous algae (AL), macrophytes (MA), fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), riparian vegetation (RV), benthic invertebrates (BI), shrimps (SH), and terrestrial invertebrates (TI). PE was sampled by scraping and washing wood or rocks with a small brush and stored in distilled water. FPOM samples were collected by resuspending sediment. CPOM was manually collected from the streambed. MA and RV were also manually collected, and AL was collected using tweezers. BI were sampled using a D-frame kicking net (30 cm aperture, 500 μm mesh), while TI were manually and randomly collected in the riparian zone using tweezers. SH samples were collected using semicircular sieves (80 cm in diameter, 1-mm mesh). All samples were stored on ice until transferred to freezers. In the laboratory, PE and FPOM samples were filtered through quartz fibre filters (Whatman QMA quartz filters) using a filtration apparatus connected to a vacuum pump. The samples were then oven-dried at 40°C for 48 h and ground into a fine, homogeneous powder using a mortar and pestle. RV, CPOM, AL, BI, and TI were lyophilized for 48 h and subsequently ground into a fine, homogeneous powder using a ball mill (Retsch MM 400) or a mortar and pestle.

2.4 | Isotopic Analyses

Isotopic analyses were performed at the Stable Isotope Center at São Paulo State University (Unesp) in Botucatu, Brazil. We used an isotope ratio mass spectrometry system with elemental analyser EA-IRMS (Flash 2000—Delta V Advantage, Thermo Scientific, Germany). For each analysis, 0.5–3.0 mg of dry and homogenized samples were weighed in a tin capsule. The system simultaneously determined the isotopic ratios $R({}^iE/{}^jE)_{\text{sample}}$, which were expressed as a relative difference from the isotopic ratio δ^iE, in ‰ according to equations (Coplen 2011):

$$\delta^i E_{(‰)} = \frac{R\left({}^iE/{}^jE\right)_{\text{sample}}}{R({}^iE/{}^jE)_{\text{standard}}} - 1$$

where ${}^iE$ is ${}^{13}C$ or ${}^{15}N$, ${}^jE$ is ${}^{12}C$ or ${}^{14}N$, and $R({}^iE/{}^jE)_{\text{standard}}$ are the international standard ratios VPDB (Pee Dee Belemnite) for $R({}^{13}C/{}^{12}C)_{\text{VPDB}}$ and Air (atmospheric Nitrogen) for $R({}^{15}N/{}^{14}N)_{\text{Air}}$. The results were normalized from certified reference standards USGS61, USGS62, and USGS63 (Paul et al. 2007; Schimmelmann et al. 2016, 2020). The standard uncertainty of the analysis was estimated to be ±0.10‰ and ±0.15‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.5 | Trophic Structure and Carbon Sources

Fish were classified into trophic guilds according to Leal et al. (2017), who previously sampled the same streams and evaluated fish diets based on stomach content analysis. To assess the food web structure of the fish assemblages, individuals of all fish species were plotted in a biplot space using isotopic values of $\delta^{13}C$ (x-axis) and $\delta^{15}N$ (y-axis), providing a visual representation of the food web structure at each stream site. Mean $\delta^{13}C$ and $\delta^{15}N$ values were plotted for each fish species, while the food resources were represented by their mean and ± standard deviation values.

2.6 | Proportion of Food Resources Assimilated and Biomass

To evaluate trophic partitioning of food resources, we applied a Bayesian stable isotope mixing model implemented in the SIMMr package in R (Parnell et al. 2013; R Core Team 2024). A Markov Chain Monte Carlo (MCMC) analysis was conducted using four independent chains to assess convergence. Each chain was run for 50,000 iterations, of which the first 10,000 were discarded as burn-in to remove the influence of initial values. Samples were thinned by retaining every 10th iteration to reduce autocorrelation among posterior samples. For this analysis, the resources were selected based on the main food items identified through stomach content analysis for each guild (Leal et al. 2017): Piscivorous/Carnivorous (TI, BI, SH, and fish), Omnivorous (TI, BI, SH, CPOM, and AL), Invertivorous (TI and BI), Periphytivorous (PE, FPOM, and AL), and Detritivorous (CPOM, FPOM, and PE). Given that $\delta^{13}C$ signature of leaves can vary vertically along tree height (Domingues et al. 2005; Martinelli et al. 2009) and we only sampled leaves at head height, we decided to use CPOM as a proxy for the riparian vegetation. This is because CPOM should reflect the average $\delta^{13}C$ composition of all the leaves that fall into the stream. The fractionation values (mean ± standard deviation) considered for this analysis were 0.4‰ ± 1.3‰ for $\delta^{13}C$ and 3.4‰ ± 0.98‰ for $\delta^{15}N$ (Post 2002). To investigate the forest contribution to the benthic food web, we applied the mixing model to estimate the proportional contribution of food resources to the BI. For this analysis, we considered the basal resources that appeared to be more assimilated, as indicated by the biplot (i.e., CPOM, FPOM, and PE) and fractionation values (mean ± standard deviation) considered for this analysis were also 0.4‰ ± 1.3‰ for δ13C and 3.4‰ ± 0.98‰ for $\delta^{15}N$ (Post 2002).

To calculate the fish biomass supported by food resources in each stream site, we used the percentage contribution of each food resource consumed by each species, applying the formula from Pompeu and Godinho (2006):

$$\text{Biomass}\ (\%) = (C / S) \times F$$

where C is the total biomass of the fish assemblage (g), S is the species biomass (g), and F is the percentage contribution of each food source to species diet.

The relative contribution of a given food resource to the local assemblage biomass was determined by summing the contribution values of all species that used that resource, multiplied by each respective relative biomass. For this analysis, we considered the biomass of all fish species and individuals captured in each stream site.

2.7 | Trophic Niches and Trophic Diversity

We calculated the standard ellipse area (SEA) to quantify the isotopic niche of each fish assemblage using the SIBER package in R (Jackson et al. 2011; R Core Team 2024). Bayesian estimates of standard ellipse area (hereafter SEAb) were used to assess differences in isotopic niche width among assemblages by comparing the overlap of 95% credible intervals derived from the posterior distributions.

To compare the trophic structure across stream sites, we used the SIBER package to calculate six metrics originally proposed by Layman et al. (2007) and reformulated within a Bayesian framework by Jackson et al. (2011). The $\delta^{15}N$ range (NR) offers a vertical perspective of the food web structure, reflecting the distance between the most and least enriched $\delta^{15}N$ values across both resources and consumers. A broader $\delta^{15}N$ range typically indicates the presence of multiple trophic levels within a community. The $\delta^{13}C$ range (CR) represents the horizontal variation, indicating the diversity of resources used by the community. A wider $\delta^{13}C$ range is generally linked to food webs that use a diverse set of resources with distinct $\delta^{13}C$ signatures and/or exhibit greater variability in the $\delta^{13}C$ values of available resources. The mean distance to the centroid (CD) measures the average trophic diversity within the food web, calculated as the mean Euclidean distance of each species from the centroid. The mean nearest neighbour distance (MNND) reflects the average Euclidean distance to the closest neighbour for each species, providing an estimate of the community's overall trophic redundancy. Smaller MNND suggests a high degree of trophic redundancy, where many species share similar trophic niches. The standard deviation of the nearest neighbour distance (SDNND) gauges the evenness of spatial density and the packing of species within biplot space. Unlike MNND, SDNND is less affected by sample size. Lower SDNND values indicate a more uniform distribution of trophic niches across the community (Layman et al. 2007). Differences among stream sites were evaluated by comparing posterior distributions and by estimating the probability that a given metric at one site was greater than that at another stream site.

2.8 | Trophic Position

We estimated the trophic position (TP) of fish species using the Bayesian package tRophicPosition in R (Quezada-Romegialli et al. 2018; R Core Team 2024). This approach combines prior information with observed $\delta^{13}C$ and $\delta^{15}N$ values to generate posterior probability distributions of trophic position, thereby explicitly accounting for uncertainty in baseline isotopic values and trophic discrimination factors. We applied the two-baseline full Bayesian model, in which $\delta^{13}C$ isotopic values are used to estimate the relative contribution of two $\delta^{15}N$ baselines to consumers. Baseline 1 CPOM, representing terrestrial-derived organic material, and baseline 2 corresponded to FPOM, representing organic matter of mixed origin (Lamberti et al. 2017). Trophic discrimination factors (mean ± standard deviation) used in the model were 0.4‰ ± 1.3‰ for $\delta^{13}C$ and 3.4‰ ± 0.98‰ for $\delta^{15}N$ (Post 2002).

2.9 | Data Analysis

To test whether the $\delta^{13}C$ and $\delta^{15}N$ values of fish and food resources varied among stream sites, we first assessed normality and homoscedasticity. Then we applied either ANOVA or Kruskal–Wallis tests, followed by the appropriate post hoc tests (Tukey or pairwise comparisons). To evaluate trophic positions or whether the proportions of energy sources consumed varied among sites, the same procedure was applied.

3 | Results

We collected 770 individuals from 37 fish species across all stream sites (Table S1). For stable isotope analyses, we used 285 fish individuals and 268 food resource samples, totaling 556 samples for $\delta^{13}C$ and $\delta^{15}N$ analyses. We collected 30 samples for each resource type (BI, TI, CPOM, FPOM, RV, and PE), except for MA (20 samples), AL (14 samples), and SH (24 samples). Among the 37 fish species, only *Gymnorhamphichthys rondoni*, *Hyphessobrycon ericae, Moenkhausia conspicua*, and *Saxatilia* aff. *brasiliensis* were recorded in all stream sites.

3.1 | Trophic Structure and Carbon Sources

The $\delta^{13}C$ and $\delta^{15}N$ values of fish assemblages differed among stream sites ($\delta^{13}C$: $p = < 0.001$ and $\delta^{15}N$: $p = < 0.001$). For food resources, both BI ($\delta^{13}C$: $p = 0.443$; $\delta^{15}N$: $p = 0.118$) and TI ($\delta^{13}C$: $p = 0.291$; $\delta^{15}N$: $p = 0.110$) isotopic values were similar among stream sites (Figure 2). However, other resources, such as macrophytes, differed among sites (Table S2). All assemblages were dominated by invertivorous species, followed by carnivorous, omnivorous, and piscivorous species (Figure 3). Periphytivorous species were present at three stream sites, while detritivorous species were observed at two sites.

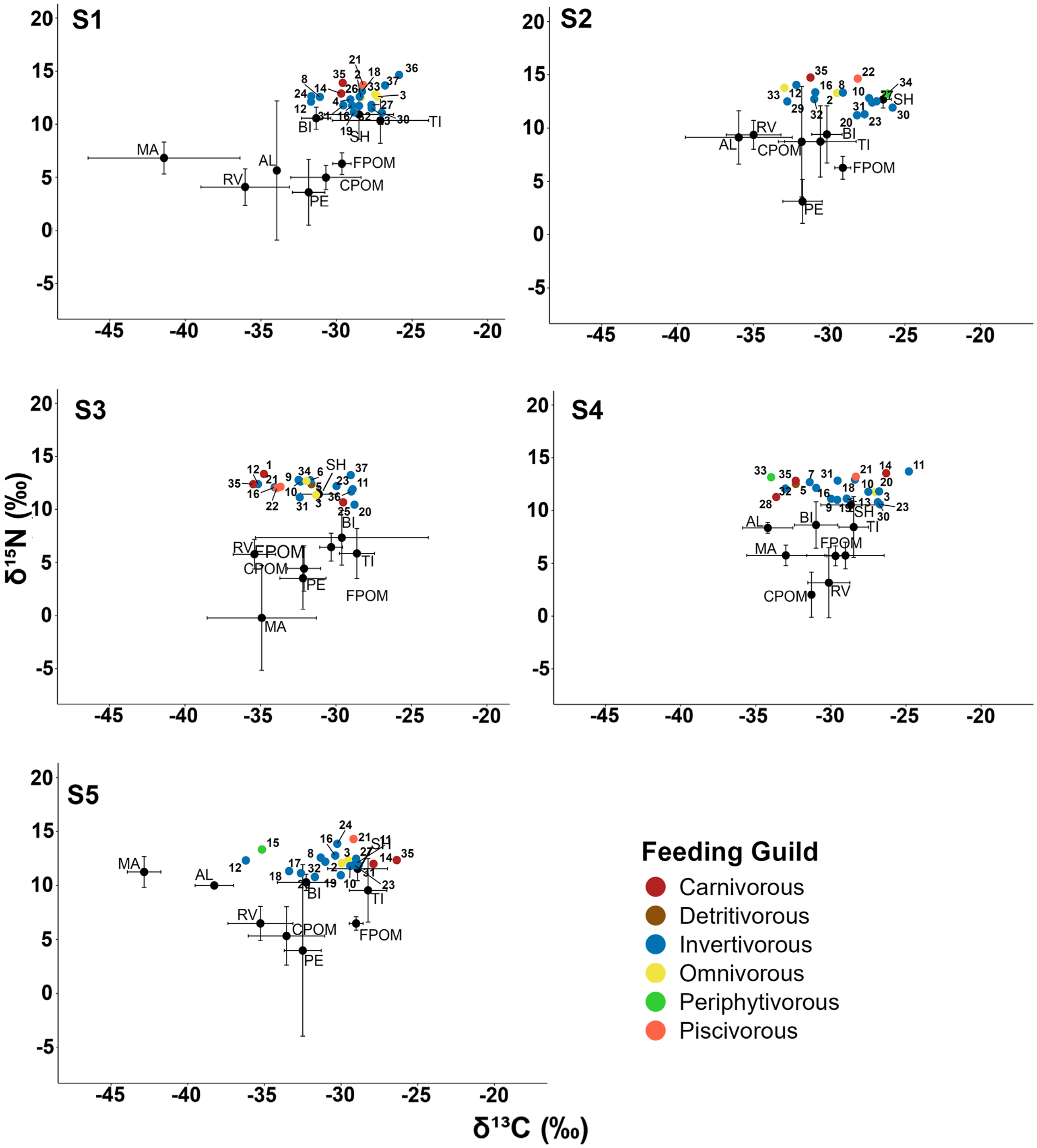

FIGURE 2 | Trophic structure of stream sites in Curuá-Una River basin considering the isotopic composition of fish (mean) and food resources (mean and standard deviation). Fish species: 1 (*Acanthodoras cataphractus*), 2 (*Aequidens epae*), 3 (*Aequidens tetramerus*), 4 (*Anablepsoides* cf. *urophthalmus*), 5 (*Apistogramma taeniata*), 6 (*Apteronotus albifrons*), 7 (*Batrochoglanis raninus*), 8 (*Brachyglanis microphthalmus*), 9 (*Brachyhypopomus* cf. *brevirostris*), 10 (*Bryconops* sp.), 11 (*Bryconops melanurus*), 12 (*Characidium* aff. *pteroides*), 13 (*Copella callolepsis*), 14 (*Erythrinus erythrinus*), 15 (*Farlowella* cf. *smith*), 16 (*Gymnorhamphichthys rondoni*), 17 (*Gymnotus* aff. *coatesi*), 18 (*Gymnotus coropinae*), 19 (*Helogenes marmoratus*), 20 (*Holopristis* cf. *neptunus*), 21 (*Hoplias curupira*), 22 (*Hoplias malabaricus*), 23 (*Hyphessobrycon ericae*), 24 (*Hypopygus lepturus*), 25 (*Hypselecara temporalis*), 26 (*Ituglanis amazonicus*), 27 (*Knodus savannensis*), 28 (*Lugubria strigata*), 29 (*Melanocharacidium dispilomma*), 30 (*Moenkhausia comma*), 31 (*Moenkhausia conspicua*), 32 (*Nannostomus marginatus*), 33 (*Rhamdia quelen*), 34 (*Rhinotocinclus hera*), 35 (*Saxatilia* aff. *brasiliensis*), 36 (*Synbranchus madeirae*), 37 (*Synbranchus* sp.).

3.2 | Proportion of Resources Assimilated and Biomass

The proportions of food resources assimilated by consumers across all stream sites were not different (Table S3). TI and BI were the most assimilated food resources across stream sites (Figure 4). TI were the main food source for *Synbranchus madeirae* (67%), *Hyphessobrycon ericae*, and *Helogenes marmoratus* (up to 55% and 58%, respectively). BI accounted for more than 70% of the carbon assimilated by *Characidium* aff. *pteroides*. AL were the main food source for *Rhinotocinclus hera* (80%) and *Farlowella* cf. *smithi* (65%). SH represented up to 45% of the

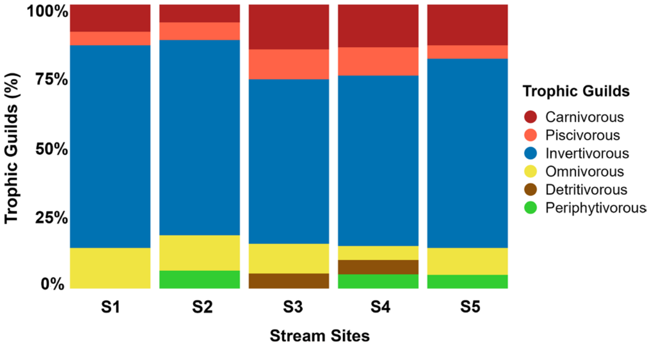

FIGURE 3 | Relative richness of fish trophic guilds (%) in streams of the Curuá-Una River basin. Trophic guilds were classified according Leal et al. (2017), based on stomach content analysis.

assimilated resources by *Rhamdia quelen*, while FH accounted for 52% of the assimilated resources for *Lugubria strigata*. The proportions of energy sources assimilated by BI across stream sites were FPOM (36%), CPOM (34%), and PE (30%) (Tables S4 and S5) (Figure S1).

Most of the fish biomass was supported by terrestrial invertebrates (TI; 31%–55%) and benthic invertebrates (BI; 22%–40%). Shrimps were the third most important food source, contributing 9%–22% to the biomass. Together, these three sources accounted for 79%–90% of the total biomass across all sites. In turn, FPOM and PE were the least important sources for biomass (Figure 4).

3.3 | Trophic Niches and Trophic Diversity

SEAb values varied among sites (Table 2), and credible intervals formed a continuous gradient with substantial overlap across all five stream sites (Figure 5). Among Layman metrics, CD showed the strongest variation among stream sites, with several pairwise comparisons showing posterior probabilities >0.9, indicating that CD is greater in specific sites compared to others. In contrast, CR, NR, and TA displayed only moderate variation, with posterior probabilities generally below 0.9, indicating weak support for directional differences in pairwise comparisons (i.e., low probability that one site consistently exhibits higher values than another). MNND and SDNND showed probabilities close to 0.5 in most comparisons, indicating no clear directional differences (i.e., neither $A>B$ nor $B>A$ is supported) and strong overlap among sites in posterior distributions (Tables S6 and S7).

3.4 | Trophic Position

Mean trophic position differed among stream sites (ANOVA $p<0.05$). Post hoc comparisons suggested that this result was largely influenced by higher trophic positions at site S4, whereas most other pairwise comparisons were not statistically significant (Tables S8 and S9). The mean trophic position of the four species common to all stream sites ranged from 2.90 to 3.65 for *Saxatilia* aff. *brasiliensis*, from 2.77 to 3.56 for *Gymnorhamphichthys rondoni*, from 2.31 to 2.74 for *Hyphessobrycon ericae*, and from 2.63 to 3.59 for *Moenkhausia conspicua* (Figure 6).

4 | Discussion

Our results support our first hypothesis that both aquatic and terrestrial invertebrates play a key role in supporting fish assemblages in Amazonian streams. Allochthonous resources were essential to the trophic dynamics of these streams, with terrestrial invertebrates and coarse particulate organic matter (CPOM) collectively comprising approximately 42% of the local biomass. Their importance could be even greater, since benthic invertebrates, which accounted for 33% of fish biomass, derive approximately one-third of their biomass from CPOM. Our second hypothesis, which predicted small variation in the trophic structure of fish assemblages under similar environmental conditions, was partially supported. Although mean trophic position differed among stream sites, trophic diversity metrics indicated small or weak differences among sites. Furthermore, the overlap of SEAb credible intervals indicates great congruence of the isotopic niche across stream sites.

We found both aquatic and terrestrial invertebrates to be the main energy sources for Amazonian fish, similar to previous findings (Sabino and Zuanon 1998; Lowe-McConnell 1999; Ibañez et al. 2007; Cardoso and Couceiro 2017). The importance of invertebrates evidenced in stomach content studies was confirmed through our stable isotope approach, which indicated high assimilation of these invertebrates. Unlike stomach content analyses, stable isotopes reveal the sources assimilated by fish over longer timescales, ranging from weeks to months (Nielsen et al. 2018), providing more robust and complementary information about carbon flow in the ecosystem.

We also highlighted the importance of certain often-overlooked resources, such as shrimps. They occupied a position in the biplot similar to that of invertivorous fish, suggesting they might feed on similar resources. In fact, Amazonian freshwater shrimps predominantly prey on arthropods, particularly aquatic insect larvae (Kensley and Walker 1982). Therefore, their substantial contribution to fish biomass indicates that shrimps constitute a relevant component of energy pathways in these systems.

The high consumption of invertebrates by fish represents a nutritious diet and has also been reported for assemblages in other preserved tropical ecosystems, including the Amazon (Correa and Winemiller 2018), Cerrado (de Carvalho et al. 2019), and Atlantic Forest (Paiva et al. 2024) biomes. The abundance of invertivorous fish may be indicative of good stream integrity (de Carvalho, de Castro, et al. 2017; de Carvalho, Leal, et al. 2017). Invertivorous species with high swimming abilities have been associated with greater environmental heterogeneity and higher catchment forest cover (Cantanhêde and de Assis Montag 2024). In fact, the links between forest cover and freshwater integrity are well established for Amazonian streams (Leal et al. 2016, 2017; Castello and Macedo 2015; Brito et al. 2024). Thus, disturbances affecting the terrestrial–aquatic interface, such as deforestation, have the potential to alter the availability of resources and nutrients essential for maintaining ecosystem functioning (Machado-Silva et al. 2022) and supporting aquatic assemblages (Zeni and Casatti 2014).

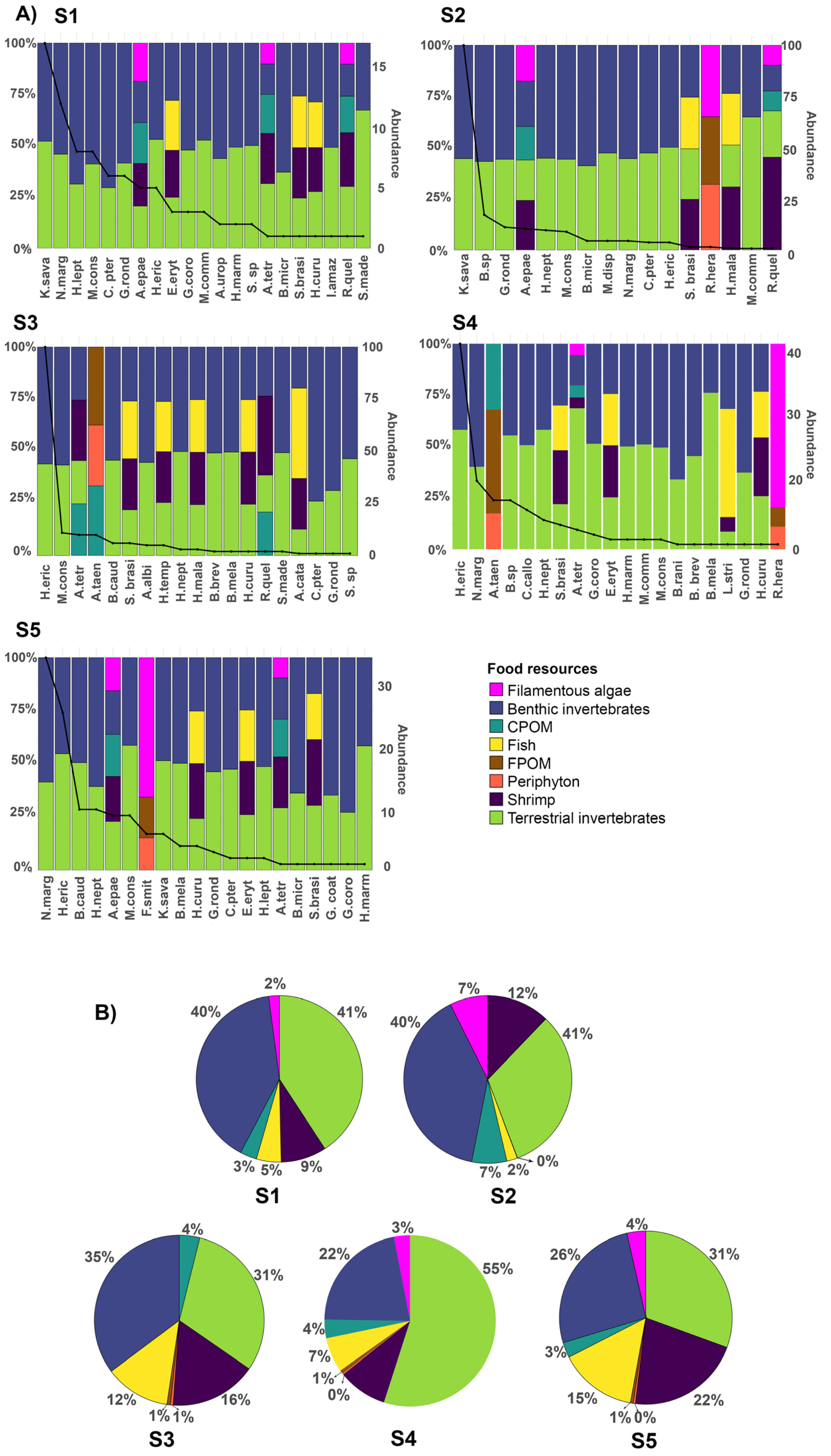

FIGURE 4 | Legend on next page.

Our stream sites exhibited small differences in trophic niche diversity, density, and evenness. This pattern may be associated with the similar proportions of assimilated resources among stream sites, suggesting that fish assemblages are supported by comparable basal energy sources, which may explain the small differentiation observed in isotopic niche metrics. Even in the

FIGURE 4 | (A) Fish abundance (black line), proportion of food resources assimilated by each fish species, and (B) contribution of food sources to the local assemblage biomass (pie) in stream sites in Curuá-Una River basin. Fish species: A.cata (*Acanthodoras cataphractus*), A.epae (*Aequidens epae*), A.tetr (*Aequidens tetramerus*), A.urop (*Anablepsoides* cf. *urophthalmus*), A.taen (*Apistogramma taeniata*), A.albi (*Apteronotus albifrons*), B.rani (*Batrochoglanis raninus*), B.micr (*Brachyglanis microphthalmus*), B.brev (*Brachyhypopomus* cf. *brevirostris*), B.sp (*Bryconops* sp), B.mela (*Bryconops melanurus*), C.pter (*Characidium* aff. *pteroides*), C.nigr (*Copella callolepis*), E.eryt (*Erythrinus erythrinus*), F.smit (*Farlowella* cf. *smithi*), Gym.rond (*Gymnorhamphichthys rondoni*), Gym. coat (*Gymnotus* aff. *coatesi*), Gym. coro (*Gymnotus coropinae*), H.marm (*Helogenes marmoratus*), H.nept (*Holopristis* cf. *neptunus*), H.curu (*Hoplias curupira*), H.mala (*Hoplias malabaricus*), H.eric (*Hyphessobrycon ericae*), H.lept (*Hypopygus lepturus*), H.temp (*Hypselecara temporalis*), I.amaz (*Ituglanis amazonicus*), K.sava (*Knodus savannensis*), L.stri (*Lugubria strigata*), M.disp (*Melanocharacidium dispilomma*), M.comm (*Moenkhausia comma*), M.cons (*Moenkhausia conspicua*), N.marg (*Nannostomus marginatus*), R.quel (*Rhamdia quelen*), R.her (*Rhinotocinclus hera*), S.bra (*Saxatilia brasiliensis*), S.made (*Synbranchus madeirae*), S.sp (*Synbranchus* sp.).

TABLE 2 | Layman trophic metrics of stream sites in the Curuá-Una River basin.

Site	NR	CR	TA	CD	MNND	SDNND	SEAb
S1	2.22	4.97	7.49	1.32	0.55	0.35	5.75
S2	2.84	5.89	10.01	1.82	0.69	0.55	8.08
S3	2.07	6.48	7.63	1.58	0.77	0.33	9.6
S4	2.47	6.29	9.12	2.15	0.66	0.38	12.15
S5	3.50	6.50	12.79	1.48	0.69	0.95	8.20

Abbreviations: CD, centroid distance; CR, carbon range; MNND, mean nearest neighbour distance; NR, nitrogen range; SEAb, standard ellipse area Bayesian; SNND, standard deviation of nearest neighbour distance; TA, total area.

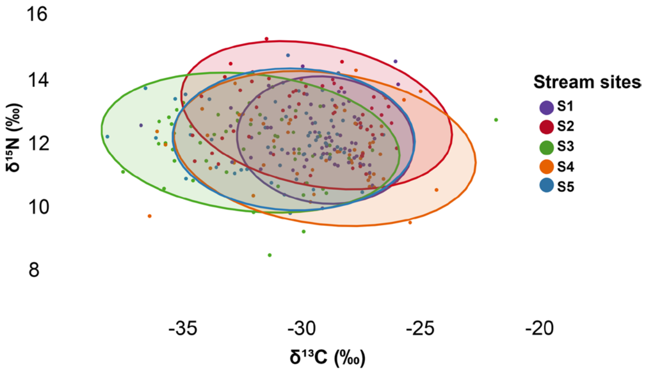

FIGURE 5 | Isotopic niche (Standard Ellipse Area, SEA, calculated using a 95% confidence interval) of fish assemblages from streams in the Curuá-Una River basin.

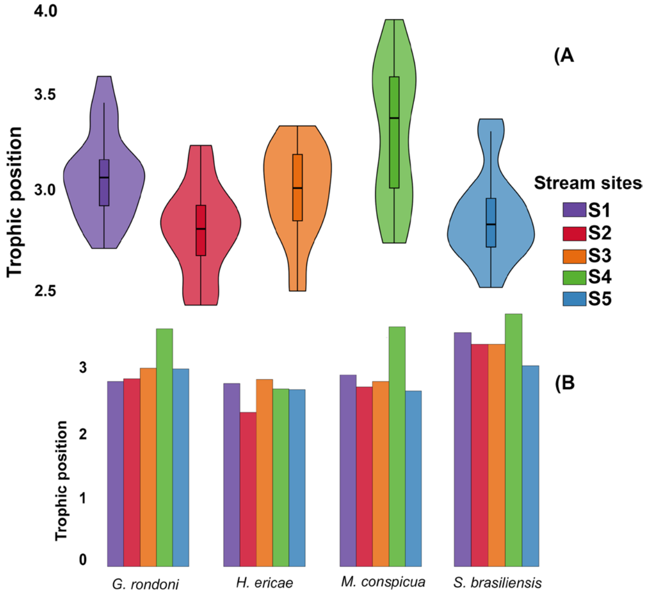

FIGURE 6 | (A) Food chain length (violin chart) represented by the mean species trophic positions in each site and (B) trophic position estimates for fish species common to all stream sites in the Curuá-Una River basin.

presence of site-specific variation in trophic positions, the underlying pathways of energy assimilation appear to be comparable across streams. This pattern suggests that streams with low anthropogenic disturbance tend to display comparable trophic structures. Despite this general pattern, mean trophic positions (TPs) differed among sites, with S4 displaying the highest TP values. This site also showed the widest trophic niche and the broadest δ^{13}C range, coinciding with the highest number of trophic guilds. This stream site also had the lowest riparian cover, which potentially enhanced primary production and increased the availability of food resources (de Carvalho et al. 2015; de Carvalho, de Castro, et al. 2017; de Carvalho, Leal, et al. 2017; Machado-Silva et al. 2022).

In our study, the use of δ^{13}C and δ^{15}N stable isotopes proved to be efficient in assessing the trophic structure and resources sustaining Amazonian streams fish. While this approach provides a comprehensive understanding of resource assimilation and trophic ecology (Pereira and Benedito 2007; Albrecht et al. 2021), it also presents certain challenges. For example, mixing models, which estimate the probability distributions of resource contributions, can sometimes lead to misinterpretations (Phillips et al. 2014). To avoid biases and inaccurate probability estimates, we incorporated stomach content data from Leal et al. (2017). This helped guide the selection of resources that appeared to be more assimilated by each guild. In the absence of this information in the literature, we recommend a thorough evaluation of the isotopic compositions of resources and consumers, using biplot graphs, for example, alongside species life history data, to help identify the most relevant resources to be included in the models. Another limitation of our study is that sampling was conducted only once and during the dry season. Although fish diets may vary during the rainy season, stable isotope values integrate dietary information over longer time scales and therefore provide a robust representation of trophic structure and isotopic niche patterns.

Despite these challenges, the use of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in trophic ecology studies has significantly advanced our understanding of complex processes in aquatic ecosystems (Zuluaga-Gómez et al. 2016; Ceneviva-Bastos et al. 2017; de Carvalho et al. 2020; Urbano et al. 2024). Our study is the first to assess the trophic ecology of fish assemblages from Amazonian streams through stable isotope analyses. We found that both aquatic and terrestrial invertebrates were the most important food resources for fish. Moreover, our results suggest great similarity in the trophic structure of streams with low anthropogenic disturbances. Our study also contributed to understanding the trophic ecology, including resource assimilation and trophic position, of 37 Amazonian fish species, many of which poorly studied. Although our streams remain relatively well preserved, they are located in the Santarém region, one of the agriculture frontiers in the Amazon (Gardner et al. 2013). The progressive loss and degradation of forest cover in such areas may shift the basal energy sources of stream food webs. As terrestrial subsidies decline, fish may increasingly rely on aquatic invertebrates and detrital material (Ferreira et al. 2011, 2012), potentially leading to food webs that are less complex and more detritus-based (Ceneviva-Bastos et al. 2017). Such shifts can have cascading effects, altering trophic interactions, reducing energy transfer efficiency, and jeopardizing the functional integrity of aquatic communities. In this context, stable isotope approaches provide not only a powerful diagnostic tool for detecting subtle changes in trophic pathways but also a critical foundation for predicting how freshwater biodiversity and ecosystem functioning may respond to environmental degradation. As land-use change intensifies in the Amazon, advancing our understanding of these processes is not only scientifically relevant, but also vital for guiding conservation strategies in one of the planet's most biodiverse and vulnerable regions.

Author Contributions

Conceptualization: P.H.S.B., C.G.L., D.R.C., P.S.P. Developing methods: P.H.S.B., C.G.L., D.R.C., P.S.P. Conducting the research: P.H.S.B., C.G.L., D.R.C., G.L.B., M.A.A.-F., D.H.C.D., G.O.F., G.S.F., R.P.L., V.E.C., L.F.A.M., G.N.S., J.V.A.S.S., E.C.S., P.S.P. Data analysis: P.H.S.B., V.E.C. Data interpretation: P.H.S.B., C.G.L., D.R.C., G.L.B., M.A.A.-F., D.H.C.D., G.O.F., G.S.F., R.P.L., L.F.A.M., G.N.S., J.V.A.S.S., E.C.S., P.S.P. Preparation of figures and tables: P.H.S.B. Writing: P.H.S.B., C.G.L., D.R.C., G.L.B., M.A.A.-F., D.H.C.D., G.O.F., G.S.F., R.P.L., V.E.C., L.F.A.M., G.N.S., J.V.A.S.S., E.C.S., P.S.P.

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Disclosure

We used ChatGPT (OpenAI; GPT-5.2) to support English language editing (clarity and grammar); all scientific content and interpretations remain the responsibility of the authors.

Ethics Statement

This study was approved by the Ethics Committee for Animal Use of the Federal University of Pará (UFPA), under protocol no. 8293020418, and authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio/SISBIO), permit no. 87389-1.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Number of fish samples collected at stream sites for Stable Isotope Analysis (SIA) and voucher specimens deposited in the Fish Collection of the Federal University of Lavras (CI-UFLA). Fish species were classified into trophic guilds following Leal et al. (2017). **Table S2:** (A) Results of statistical tests assessing differences in $\delta^{13}C$ and $\delta^{15}N$ values among stream sites for each food resource. ANOVA or Kruskal–Wallis tests were applied depending on data normality and homoscedasticity. MA (macrophytes), BI (benthic invertebrates), TI (terrestrial insects), AL (filamentous algae), CPOM (coarse particulate organic matter), RV (riparian vegetation), SH (shrimp), PE (periphyton), and FPOM (fine particulate organic matter). (B) Mean $\delta^{13}C$ and $\delta^{15}N$ values of fish assemblages and food resources, with their respective standard deviations (SD), across stream sites. AL (filamentous algae), BI (benthic invertebrates), SH (shrimp), CPOM (coarse particulate organic matter), FPOM (fine particulate organic matter), TI (terrestrial invertebrates), MA (macrophytes), PE (periphyton), RV (riparian vegetation), and FH (fish). **Table S3:** Results of statistical tests assessing whether the proportional contribution of assimilated energy sources differed among stream sites. ANOVA or Kruskal–Wallis tests were applied depending on data normality and homoscedasticity. TI (terrestrial invertebrates), BI (benthic invertebrates), SH (shrimp), FH (fish), CPOM (coarse particulate organic matter), AL (filamentous algae), PE (periphyton), and FPOM (fine particulate organic matter). **Table S4:** Mean proportional contribution and standard deviation (SD) of assimilated resources for each fish consumer across stream sites. TI (terrestrial invertebrates), BI (benthic invertebrates), SH (shrimp), FH (fish), CPOM (coarse particulate organic matter), FPOM (fine particulate organic matter), PE (periphyton), and AL (filamentous algae). Panels (A–E) correspond to stream sites: (A) S1, (B) S2, (C) S3, (D) S4, and (E) S5. **Table S5:** Mean proportional contribution and standard deviation (SD) of assimilated resources for benthic invertebrates across stream sites. FPOM (fine particulate organic matter), CPOM (coarse particulate organic matter), and PE (periphyton). **Table S6:** Standard Ellipse Area Bayesian (SEAb) estimates for each stream site, including mean, median, and 95% and 99% credible intervals (CI). **Table S7:** Pairwise Bayesian probabilities comparing Layman trophic metrics among stream sites. Values represent the posterior probability that metric A is greater than metric B. NR (nitrogen range), CR (carbon range), TA (total area), CD (centroid distance), MNND (mean nearest neighbour distance), and SDNND (standard deviation of nearest neighbour distance). **Table S8:** Trophic position of each fish species across stream sites. **Table S9:** Pairwise comparisons of trophic position among sampling sites, showing estimated differences, 95% credible intervals (CI), and adjusted *p*-values. **Figure S1:** Proportional contribution of assimilated resources by benthic invertebrates across stream sites in the Curuá-Una Basin, Pará, Brazil.