Untangling the complex food webs of tropical rainforest streams

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Conflict of Interest

The authors declare no conflict of interest.

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Statement on Inclusion

Our study brings together authors from Brazil and the UK, led by a scientist based in the country where the study was carried out. All authors were engaged early on with the research and study design to ensure that the diverse sets of perspectives they represent was considered from the onset. This study includes undergraduate, graduate and established researchers from the region where the study was carried out.

Data availability statement

Data will be publicly available in a repository after publication.

UNTANGLING THE COMPLEX FOOD WEBS OF TROPICAL RAINFOREST STREAMS

ABSTRACT

1. Describing food webs with precision and detail is critical for understanding the functioning of ecosystems. Streams in the Brazilian Atlantic Rainforest stand out as one of the most threatened ecosystems worldwide and yet, little is known about whether these systems house stable food webs and what are the trophic properties associated with that.
2. In this study, we applied a Bayesian Hierarchical approach unifying three sources of information (gut content analysis, literature compilation, stable isotope data) to derive a well-resolved meta food web and five individual food webs from an Atlantic Forest catchment in Brazil. Together with data on population-level biomass and individually measured body mass, we applied a bioenergetic model and assessed their dynamical stability using a Lotka-Volterra approach. From the meta food web, we simulated individual population extinctions and their impacts on the food web stability. Finally, we calculated network dissimilarity indices in order to estimate the relative importance of regional processes for the maintenance of the meta food web.
3. The hierarchical model resulted in a meta food web with 307 probable links among the 65 components, from basal resources to consumers (aquatic insects, shrimps, and fish). At the regional scale, a surprisingly large proportion of total energy flow (70%) occurred through a set of ten invertebrates with large variation in body masses, from chironomids to shrimps. The remaining 30% of total energy flow relied on 51 different taxa supporting a significant dependency on a diverse community.

4. We found that these food webs were robust against disturbances, with dynamic resilience of the bioenergetic models. The simulations of population extinctions resulted in scenarios with stable food webs indicating that these systems do not rely strongly on keystone species for their resilience. Finally, approximately 40% of the interactions changed among local food webs mostly due the absence of predators in individual streams.
5. At the local scale, stability was likely maintained due to the high diversity of organisms with different body-sizes buffering against size specific disturbances. At the regional scale, high total diversity should entail the colonization of redundant taxa at a high rate, while high beta diversity of predators should enhance spatial asynchrony and regional stability. Here we integrated for the first time a hierarchical model to rebuild a detailed food web with methods for estimating energy fluxes within it. This innovative approach provides a baseline for the structure and functioning of tropical rainforest streams, setting the ground for future studies on how these ecosystems may respond to environmental change.

Key words: Food Webs, Metabolic Theory, Dynamic Stability, Macroinvertebrates, Stable Isotopes.

RESUMO

1. Descrever as teias alimentares com precisão e detalhes é crucial para compreender o funcionamento dos ecossistemas. Os riachos na Mata Atlântica brasileira destacam-se como um dos ecossistemas mais ameaçados em todo o

mundo e, no entanto, pouco se sabe sobre se esses sistemas abrigam teias alimentares estáveis e quais são as propriedades tróficas associadas a isso.

2. Neste estudo, aplicamos uma abordagem Bayesiana Hierárquica unificando três fontes de informação (análise de conteúdo estomacal, compilação de literatura, dados de isótopos estáveis) para derivar uma teia alimentar meta bem resolvida e cinco teias alimentares individuais de uma bacia da Mata Atlântica no Brasil. Juntamente com dados de biomassa em nível populacional e massa corporal medida individualmente, aplicamos um modelo bioenergético e avaliamos sua estabilidade dinâmica usando uma abordagem de Lotka-Volterra. A partir da teia alimentar meta, simulamos extinções individuais de populações e seus impactos na estabilidade da teia alimentar. Finalmente, calculamos índices de dissimilaridade de rede para estimar a importância relativa dos processos regionais na manutenção da teia alimentar meta.

3. O modelo hierárquico resultou em uma teia alimentar meta com 307 links prováveis entre os 65 componentes, desde recursos basais até consumidores (insetos aquáticos, camarões e peixes). Em escala regional, uma proporção surpreendentemente grande do fluxo total de energia (70%) ocorreu por meio de um conjunto de dez invertebrados com grande variação em massas corporais, desde quironomídeos até camarões. No entanto, 30% consideráveis do fluxo total de energia dependiam de 51 táxons diferentes, indicando uma dependência significativa de uma comunidade diversificada.

4. Descobrimos que essas teias alimentares eram robustas contra perturbações, com resiliência dinâmica dos modelos bioenergéticos. As simulações de extinções populacionais resultaram em cenários com teias alimentares estáveis, indicando que esses sistemas não dependem fortemente de espécies-chave para sua resiliência. Finalmente, aproximadamente 40% das interações mudaram entre as teias alimentares, principalmente devido à ausência de predadores em riachos individuais.

5. Em escala local, a estabilidade provavelmente foi mantida devido à alta diversidade de organismos com diferentes tamanhos corporais, amortecendo contra perturbações específicas de tamanho. Em escala regional, a alta diversidade total deve implicar a colonização de táxons redundantes em uma taxa elevada, enquanto a alta diversidade beta de predadores deve aumentar a assincronia espacial e a estabilidade regional. Aqui, integramos pela primeira vez um modelo hierárquico para reconstruir uma teia alimentar detalhada com métodos para estimar fluxos de energia dentro dela. Essa abordagem inovadora fornece uma linha de base para a estrutura e funcionamento de riachos na floresta tropical, estabelecendo as bases para estudos futuros sobre como esses ecossistemas podem responder a mudanças ambientais.

Palavras-chave: Teias alimentares, Teoria Metabólica, Estabilidade Dinâmica, Macroinvertebrados, Isótopos Estáveis.

INTRODUCTION

Food webs depict the tangled web of trophic interactions associated with the transfer of energy within an ecosystem. These ecological networks offer mechanistic insights into energy flow, nutrient cycling, and population dynamics, highlighting critical links that influence the stability and resilience of natural ecosystems. Consequently, food webs provide a vital tool for conservation efforts and ecosystem management, allowing better-informed decisions about how to protect and preserve the functioning of natural systems (Giakoumi et al., 2015). Therefore, characterizing food webs with precision and detail is pivotal to help us understand ecosystem functioning in face of the current environmental crisis (Giakoumi et al. 2015).

Surprisingly, the underlying patterns of energy fluxes are poorly described in many ecosystems worldwide. Despite the dozens of methods developed to characterize food webs in nature, from observations in the field to feeding trials in the laboratory, the use of individual methods to estimate trophic interactions commonly result in incomplete or simplified characterizations (Layman et al., 2012). Fortunately, the combination of recent theoretical and methodological advances gives us new tools to describe patterns of energy fluxes at the population level with high precision. First, a new method unifying multiple sources of information within a singular analytical Bayesian framework provides an excellent opportunity to untangle undescribed food webs, while overcoming problems of individual sources of information (Hernvann et al., 2022). This method integrates information taken from the literature, from direct observations (e.g. through gut contents analysis) and extracted from stable isotope analyses to infer the most likely diet proportion of each consumer and the likelihood of these interactions. Second, advances in metabolic scaling theory allow us to infer the energy requirements of populations based on their individual body masses and their total population sizes (Brown et al., 2004). Together with information about diet proportions, we can now estimate the required energy potentially flowing from prey to predators, quantifying the food web in a general ecological currency (Gauzens et al., 2019).

Describing food webs in streams and rivers is particularly challenging due to the inherent complexity and dynamics of these ecosystems (Winemiller, 1990). The continuous flow of water combined with the spatio-temporal heterogeneity of resources create a constantly changing environment, making it difficult to capture the food web in full detail. Tropical streams particularly pose a challenge as they are highly diverse and knowledge of trophic interactions is rare for many taxonomic groups (Motta & Uieda, 2005; Reboredo Segovia et al., 2020), resulting in few isolated food web descriptions (Ceneviva-Bastos et al., 2012; Motta & Uieda, 2005). Among these understudied ecosystems, streams of Brazilian Atlantic Rainforest are highly threatened through human land-use intensification and the substitution of forests to pasture and monoculture plantations (Siqueira et al., 2015). Since these ecosystems provide a myriad of services to human society, from fresh water and recreation to nutrient cycling (Meyer, 1997; Palmer et al., 2014), describing their food webs in well preserved regions is crucial to define the baseline in face of human impacts (Collyer et al., 2023).

Importantly, tropical stream ecosystems are likely to differ in their structure and functioning compared to their better-studied temperate counterparts (Saito, Perkins, et al., 2021). In terms of food webs, warmer tropical streams should be composed of species with fast life cycles, accelerated biomass turnover and decreased local densities (Saito, Stoppa, et al., 2021), resulting in highly variable composition (Siqueira et al.,

2020) and species interactions (Saito, Perkins, et al., 2021). A recent study hypothesized that such highly dynamic food webs, embedded in a high regional diversity, should be stable due to the weak interaction effects among multiple interacting species (Collyer et al., 2023; Kratina et al., 2012), where the functioning of a diverse, generalist community would be buffered against disturbances by interchanges of trophic interactions (Rooney & McCann, 2012). This could occur, for instance, if predator-prey interactions are weakly size-structured, with organisms feeding constantly on large and small prey due to their high metabolic requirement, thus deviating from optimal predator-prey mass ratios (Collyer et al., 2023; Kratina et al., 2012).

In addition, local food webs are always exchanging energy and matter within a regional meta food web (Winemiller, 1990). As such, the realization of potential trophic interactions are constrained by dispersal processes of predators and prey (Rooney & McCann, 2012; Winemiller, 1990). In general, mobile predators are important components that can stabilize communities and metacommunities by buffering variability originating at the base of food webs both among patches and among communities, respectively (Rooney et al., 2008; Siqueira et al. 2023). This should increase spatial asynchrony among communities entailing high food web dissimilarity in space, mostly due to changes in predator frequency. Considering the expected high local dynamism of Atlantic Forest stream communities due to the accelerated metabolism, one can expect a high dissimilarity of food webs in space stabilizing the regional meta food web along time. In the current era of biodiversity loss and ecosystem degradation, understanding how stable and safeguarded our interconnected ecosystems are is key to a sustainable use of resources (Seddon et al., 2016).

Leveraging a suite of advances in food web ecology, we evaluate how stable the pristine ecosystems of the Brazilian Atlantic Rainforest should be in face of disturbances. We characterize a well resolved meta food web of streams from Atlantic Forest through integration of node properties (body mass and abundance) and various sources of feeding link information: gut contents from predators, carbon and nitrogen stable isotopes of their body tissues and a compiled list of interactions from the literature. By applying the recent methods for calculating the energy necessary to sustain predators in dynamic equilibria, we defined the interaction coefficients between predators and prey and assessed the stability of the food web to disturbances within an energetic framework (Gauzens et al., 2019; Moore & de Ruiter, 2012). We hypothesized that 1) due to the elevated metabolic demands in warm conditions, invertebrates and fish assemblages would be weakly size-structured, characterized by predators feeding at multiple trophic levels, with no systematic and proportional size differences between predators and their prey. 2) Given the high species diversity in tropical streams, we anticipated that energy flow within the food webs would involve a multitude of links. Predators would engage in a range of non-specialized interactions with prey resulting in bioenergetically stable food webs (Kratina et al., 2012). 3) We simulated loss of individual food web nodes (taxa), hypothesizing that the initial removal of individual taxa from a complex tropical food web would not significantly disrupt its stability, as it would not heavily rely on key strong interactions. 4) Finally, we expected that the spatial dissimilarity of local food webs would be associated with the absence of large predators, indicating that mobile predators enhance spatial asynchrony among communities entailing regionally stable meta food webs (Rooney et al., 2008).

METHODS

We sampled food web components (basal resources, invertebrate and vertebrate consumers) in five relatively pristine streams in Southeast Brazil. We then applied a Bayesian framework to describe a regional meta food web and five individual food webs integrating data from gut contents analysis, published studies and stable isotope analysis of consumers and resources (Hernvann et al., 2022). Finally, we applied an energetic model using body masses, energy efficiencies estimates and the network topology to describe the amount of energy fluxes and the food web stability (Gauzens et al., 2019).

Field and laboratory protocols

We sampled five (2nd to 3rd orders) stream stretches within the Cananeia catchment (state of São Paulo, Brazil) to collect high-resolution community data for food web reconstruction. We sampled three streams (S1, S6 & S8) which were less than 3 km apart, and in one stream (S8) we sampled three different stretches along an altitudinal gradient (with 24, 45 and 88 meters of altitude). The vegetation at the studied sites is predominantly formed by Ombrophilous Dense Atlantic Forest, mostly well-preserved stretches and few sections of secondary forest (Schaeffer-Novelli et al., 1990). The climate is humid subtropical (Alvares et al., 2013), with mean annual rainfall above 2,200 mm (Schaeffer-Novelli et al., 1990). There are two main seasons: summers (November - April) have mean air temperature of approximately 27° C, whereas mild winters (May - October) have a mean temperature of approximately 22° C. The five stream sites were sampled in October 2019 and had a mean water temperature of 22°C

(SD=0.68), pH of 5.7 (SD=0.66) and oxidation-reduction potential (mv) of 381 (SD=111) (see Table S1).

The field protocol encompassed standard sampling techniques for surveying stream food webs. First, we performed 3-pass quantitative depletion electrofishing at each site (up to 100 m) using a Smith-Root LR-24 backpack electrofisher with the boundary of survey reaches enclosed by stop nets. We identified most captured fish to species level and measured (fork length) in the field (SISBIO ethical approval number 72482-1). We sampled macroinvertebrates (>250 µm) using 10 replicate Surber samples (30 cm X 30 cm) randomly positioned in the stream benthos. We sorted these samples live at the host laboratory using a transilluminated tray and identified them to the lowest possible taxonomic level without preparing slices (e.g. Chironomidae were identified to family level). We took measurements of individual linear dimensions (e.g. head-capsule width or body length) with a calibrated ocular micrometer allowing individual dry mass to be calculated from published regression equations (Collyer et al., 2023). We sacrificed a subsample of all captured fish (n = 142) and fixed them in 99,3% Isopropyl alcohol for subsequent gut content analysis in the laboratory. We extracted guts and examined under a stereomicroscope (Leica EZ4, 40x maximum magnification). We identified and measured all diet items found (n = 502) to the lowest possible taxonomic level (28 categories).

During fieldwork we also collected material for subsequent stable isotope analysis (SIA). We obtained fish tissue from fin clips for a subset of individuals of each species caught during electrofishing surveys. We performed kick net sampling qualitatively in all microhabitats (mostly gravel and boulder riffles and some leaf litter pools) to collect macroinvertebrates which were processed whole for stable isotope analysis. We sampled basal resources from each stream by collecting allochthonous leaf packs, mosses attached to the substrate and scrapes of biofilm from stones and boulders. We froze all samples in the host laboratory in liquid nitrogen (at -80 °C) in individually labeled tubes until subsequent analysis. Back at the UFSCar laboratory, we stored all samples at 60° C in an oven until total dry mass was achieved. We grinded the tissues to a fine powder. Subsequently, 1mg +/- 0.2mg of fish and macroinvertebrate samples, and 2.5mg ±0.2mg of basal resources were weighed into tin capsules (Elemental Microanalysis® 8×5mm) prior to isotopic analysis. For small taxa invertebrates we pooled 6-20 individuals of similar size together to reach the minimum weight expected for each sample (e.g. Chironomidae). We analyzed samples of macroinvertebrates (n=139), fishes (n=160) and basal resources (n=30) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios at an analytical laboratory at Queen Mary University of London, UK using a continuous flow isotope ratio mass spectrometer (SerCon Integra 2, Stable Isotope Analyser, Crewe, UK). Protein IRMS Standard (Elemental Microanalysis® OAS/Isotope 5g) encapsulated like the other samples was used as a standard and inserted in each run after every ten samples. We then applied an ANOVA comparing the $\delta^{15}N$ of basal resources (biofilm, moss and leaves) among stretches and found that they were not distinct (ANOVA, $F = 0.35$, $P = 0.84$) indicating that $\delta^{15}N$ baselines among stream sites were comparable.

Food web reconstruction

We applied the Ecodiet approach to derive a well-resolved meta food web (Hernvann et al., 2022). This Bayesian hierarchical model jointly considers three sources of information: direct observations (e.g. gut content analysis) including the potential proportion of different diet items, literature information describing putative feeding links based on interactions described in other sites, and data from stable isotope analysis, derived from a Bayesian mixing model to estimate the likelihood of the interactions and the diet proportions inferred from the two first sources of information. Here, we integrated data from the five stream sites including 329 $\delta^{15}N$ and $\delta^{13}C$ values of basal resources, macroinvertebrates and fish; 502 diet items identified from fish gut content analysis and; 226 potential feeding interactions for Neotropical stream communities derived from a literature search including bibliometric analysis of 52 references and 530 potential trophic links (see Supplementary Material). This hierarchical model generated the probabilities of trophic links and provided the proportions of diet items for each taxon. The key strengths of this framework included the quantification of i) the reliability of individual published datasets (i.e. different values range between 0 and 1 depending on the methods used in a published study for each interaction) and ii) the relative importance of gut vs. literature data to define the food web topology to be investigated in the mixing model. For this last comparison, a parameter was set to define how equivalent literature and gut content data are (e.g. setting the parameter value to 50 indicates that literature data are equivalent to analyses of 50 new stomach contents). In our case, all studies in the literature search were set to 1 (reliable) and the literature data were given equal weight compared to gut content data. This method was implemented using the Ecodiet package in R (Hernvann et al., 2022).

Using the estimated food web topology and the diet proportions, we inferred the amount of energy flowing through each population considering a steady-state system (Gauzens et al., 2019). This method was applied to both the meta food web and each of the five individual food webs. The approach requires the average species body mass and population density information to estimate the total biomass of each population and their energetic requirements.

The fluxweb method considers a top-down perspective where the energetic requirement of each consumer species is calculated from the mean body mass of that species, and its population density, balanced against the population biomasses of their prey. In this balanced system, gains G_i are balanced with losses L_i which are calculated as

$$L_i = X_i + \sum_j F_{ij}, \qquad \text{(eq. 1)}$$

where X_i defines species losses (e.g. metabolic costs or death rates) and F_{ij} is the flux of energy from species *i* to predator *j*. In this case, gain G_i is defined as

$$G_i = \sum_j F_{ji} e_{ij}, \qquad \text{(eq. 2)}$$

where F_{ji} defines the influx coming from other species depending on a diet proportion (estimated with Ecodiet in our study) and e_{ij} denotes the efficiency in energy uptake given the prey identity (see below). In our study, the metabolic cost X_i was defined from the allometric equations (Brown et al. 2004)

$$X_i = X_0 M_i^b, \qquad \text{(eq. 3)}$$

where X_0 and *b* are constants related to organismal physiologies and M_i is a given body mass. We defined X_0 equal to 18.18 for fishes, 17.17 for invertebrates, and *b* equal -0.29 for all organisms following Brown et al. (2004). Efficiencies were defined at prey level and set to 0.906, 0.545 and 0.158 for animals, plants and detritus, respectively (i.e. consumers feeding on animals have higher efficiencies per unit of mass consumed) (Gauzens et al., 2019).

Data analysis

Hypothesis 1

To test our first hypothesis and to determine the relationship between body mass and trophic position, we related $\delta^{15}N$ tissue values (a proxy for trophic position) and body mass by applying linear mixed effects models for the isotopic values of fish and invertebrate consumers. Log-transformed mean consumer body mass (mg of dry mass) was treated as a fixed effect while taxonomic identity was treated as random intercept and random slope, separately. We could not apply a single model with both random intercept and slope due to the large number of samples and taxa in our dataset (lack of sufficient degrees of freedom). Thus the models were applied to macroinvertebrates and fish separately.

We then determined size-density relationships regressing species mean body mass and population density on double-log axes. These relationships depict how energy is shared among species with different body masses, providing information about species that have higher or lower densities than expected based on allometric scaling principles (White et al., 2007). We applied this method for the meta food web and each of the five individual food webs.

Hypothesis 2

First, we characterized the meta food web and each individual food web by calculating the number of nodes (number of taxa in each food web), number of links (number of trophic interactions), link density (mean number of links per node), connectance (the ratio between the number of links and all possible links), compartmentalization (measures the degree of connectedness of subsystems within a network, with higher values of connectance indicating more discrete subsystems), mean trophic position (mean trophic position of nodes within a food web with basal resources set to 1) and omnivory (the mean degree of variation in the trophic position of consumed resources) (Kones et al., 2009; Kratina et al., 2012; Latham, 2006; Pimm & Lawton, 1980). Indices were calculated using the package *NetIndices* in R (Kones et al., 2009).

Then, we tested our second hypothesis by using the estimated energy fluxes from the fluxweb analysis and calculating the dynamic stability dynamic of food webs (stability of their Jacobian matrices) considering a Lotka-Volterra model of consumers and resources (Moore & de Ruiter, 2012). The stability of a food web can be measured using a Jacobian interaction matrix concerning the partial derivatives of the equations for each species with respect to all species in the food web near equilibrium (May, 1973; Neutel et al., 2002). A food web is therefore considered stable when the Jacobian matrix has negative real parts eigenvalues for every interaction - i.e., all consumers can be sustained based on the biomasses of their resources (see Supplementary Material from Gauzens et al. 2019).

Hypothesis 3

To test the third hypothesis, we simulated multiple species extinction scenarios to determine the consequences of individual species loss for the stability of the meta food web. Here, in each scenario we individually removed one species at a time, recalculating energy fluxes and the dynamic stability using fluxweb in R (Gauzens et al., 2019). We did this species removal for all species in our meta food web. With the resulting values of stability as our response variable, we applied a multiple regression using the number of interactions from the extinct species, its average body mass, population biomass and total energy fluxes from the meta food web as explanatory variables.

Hypothesis 4

We calculated compositional and network dissimilarity using the Bray-Curtis index (Legendre, 2014). Whereas the compositional dissimilarity is based on differences in the relative species abundances, the network dissimilarity also accounts for the presence or absence of species interactions following species addition - e.g. one species can add multiple interactions to a food web, while another species can add only one interaction. In order to test our hypothesis 4, we decomposed the network beta diversity into the components of ‘changes caused by the absence of predator’, ‘changes caused by the absence of prey’, or ‘changes caused by mutual absences’ (Novotny, 2009). We did not calculate the components of network dissimilarity associated with rewiring and turnover, as our interactions are defined at the meta food web level, preventing our assessment of rewiring - i.e. species always interact with the same species once they are present. Beta diversity indices were calculated using the bipartite package (Dormann et al. 2008).

RESULTS

Food web components

We identified 1352 invertebrates from 54 different taxonomic groups. From this, 313 individuals were *Macrobrachium* (Paleomonidae, Decapoda) collected through both Surber sampling and electrofishing, while the others mostly included insects with a small proportion (<1%) of Oligochaeta, Platyhelminthes and Gastropoda collected by Surber sampling (0.9 m^2 in total). The insects with the highest abundances were *Macrostemum* (Hydropsychidae, Trichoptera, n = 170), *Gripopteryx* (Gripopterygidae, Plecoptera, 118), *Simulium* (Simuliidae, Diptera, 95), Chironomidae (Diptera, 92), *Chimarra* (Philopotamidae, Trichoptera, 79), *Smicridea* (Hydropsychidae, Trichoptera, 60), *Helicopsyche* (Helichopsychidae, Trichoptera, 46), *Farrodes* (Leptophlebiidae, Ephemeroptera, 30), Baetidae (Ephemeroptera, 26) and Belostomatidae (Hemiptera, 20). We also captured 332 fish from 12 species (413,5 m^2 sampled). Two fish species were found across all sites: catfish *Acentronichthys leptos* Eigenmann & Eigenmann, 1889 (66), and knifefish *Gymninotus pantherinus* Steindachner, 1908 (32). Other abundant (but less widespread) fish species included the lambari *Deuterodon iguape* Eigenmann, 1907 (71) and the characids *Hollandichthys multifasciatus* Eigenmann & Norris, 1900 (61), *Characidium lanei* Travassos, 1967 (34) and *Mimagoniates microlepis* Steindachner, 1877 (34).

Relationship between trophic position and body mass

We analyzed 329 samples for stable isotopes that were categorized into 42 groups, including basal resources, macroinvertebrates and fish. We found a generally positive relationship between $\delta^{15}N$ and $\delta^{13}C$, ranging from smaller values for moss and leaves, to biofilm and macroinvertebrates, and finally fishes (Figure 1, left). Fish generally has the highest $\delta^{15}N$ values (i.e. occupied the highest trophic positions), but there was a considerable overlap with many macroinvertebrates and shrimps. Among basal resources, biofilm had a wide overlap in δ^{15}N values with macroinvertebrates, likely an indicative that biofilm was composed of both meiofauna and periphyton, while moss and leaves had distinctly lower δ^{15}N and δ^{13}C values and largely overlapped with each other. The relationship between δ^{15}N and individual body masses suggested that fishes are more strongly size structured with higher δ^{15}N values for larger animals and also with a clearer bi-dimensional separation between taxa than macroinvertebrates, partly supporting our first hypothesis (Figure 1C). For example, the catfish *Rhamdia quelen* Quoy & Gaimard, 1824 included relatively large individuals with high δ^{15}N values, which did not overlap with any pleco fish species (*Schizolecis guentheri* Miranda Ribeiro, 1918 and *Phalloceros* sp., the only ones without a positive association between δ^{15}N and body mass). Linear mixed effects models supported these inferences with strong effects of body mass in both models with species as random intercept (body mass fixed effect estimates = 0.40, SE = 0.07, t-value = 5.13) and random slope (body mass fixed effect estimates = 0.60, SE = 0.09, t-value = 6.44). Macroinvertebrates had high isotopic variability with large overlap in bi-dimensional space between species with different body masses (Figure 1B), although some taxa were clearly distinct (e.g. *Macrobrachium* and damselflies with high δ^{15}N values and grazers *Psephenus* and *Gripopteryx* with the lowest δ^{15}N values). The mixed effects models did not show a clear effect of macroinvertebrate body mass on their trophic position with species as random intercept (body mass fixed effect estimates = 0.11, SE = 0.07, t-value = 1.55) and slope (body mass fixed effect estimates = 0.10, SE = 0.07, t-value = 1.30). The patterns in community-level isotopic composition for individual sites were largely similar to those observed for the regional metaweb (Figure S1).

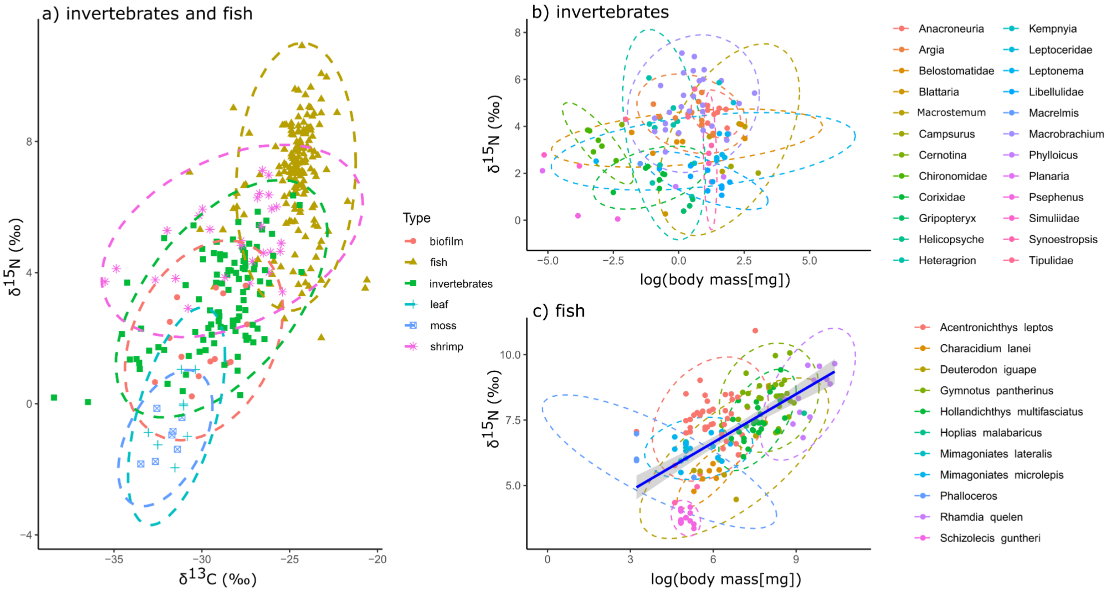

Figure 1. Isotopic composition of fish, macroinvertebrates and basal resources from Atlantic Forest streams (Cananeia, Brazil). a) Scatterplot of δ13C and δ15N values for all organisms. Association between mean body mass (mg) and δ^{15}N values (relative trophic position) of organisms (some isotopic samples encompass multiple individuals, e.g. Chironomidae) for macroinvertebrates (b) and fish (c). Ellipses correspond to 95% confidence level for a multivariate t-distribution. The line in the fish panel indicates the fitted linear mixed effects model with body mass as fixed effect and species as random intercept (body mass fixed effect estimates = 0.40, SE = 0.07, t-value = 5.13).

Food web construction

The EcoDiet model identified a high link probability (>90%) for most of the analyzed interactions. Only 54 links had Gelman-Rubin diagnostic higher than 1.1, indicating they are not reliable interactions to be considered in the food web (Hernvann et al. 2022). The final meta food web contained 307 links, including the likelihood of diet proportion for each consumer. The number of nodes in each site ranged between 28 and 41 while the number of interactions ranged between 73 and 171, with a positive trend between the two characteristics - more diverse sites had more links and also occurred in wider and shallower stretches of streams (e.g. S1 and

S8C). Consequently, connectance was similar between stretches with a mean of 10% of all possible links actually realized (see Table S1 for other network metrics).

Energy fluxes

For the meta food web, the 10 populations with highest energetic demands were composed of six Trichoptera genera *Macrostemum*, *Leptonema*, *Synoestropsis*, *Helicopsyche*, *Cernotina* and *Phylloicus*, one Ephemeroptera (*Campsurus*), one Diptera (Chironomidae), one Plecoptera (*Gripopteryx*) and one Paleomonidae (*Macrobrachium*). In sum, these species account for 70% of total energy flux in these ecosystems. Yet, a considerable 30% of the energy flow is shared between the remaining 51 consumers, which is partially in agreement with the second hypothesis. The biofilm was the main basal resource that mostly contributed to food web energy flows, followed by moss and leaves (Figure 2 and Figure S3). These resources were predominately consumed by Trichoptera, Ephemeroptera and Diptera followed by the Chironomidae (the consumer with the highest vulnerability in the network). Several of these intermediate consumers (e.g. Chironomidae and Trichoptera) were also important resources for apex predators, highlighting how populations with large biomasses both consume large amounts of resources and are important conduits of energy flow to higher trophic levels.

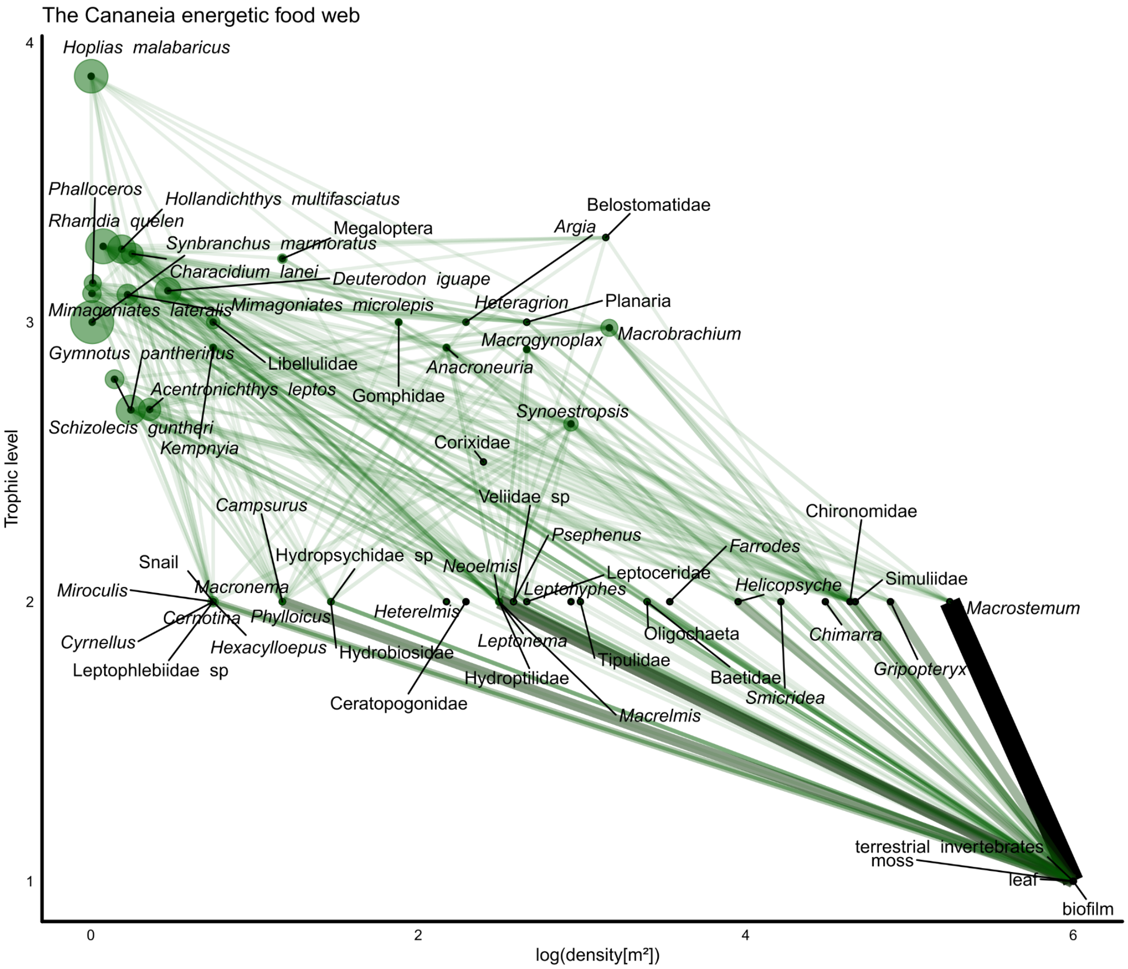

Figure 2. Regional meta food web with energy fluxes from Atlantic Forest streams (Cananeia, São Paulo, Brazil). Width of links are proportional to differences in the amount of energy flowing (J/year/m²) at the population level estimated by the *fluxweb* energetic model. Position along the y axis represents the trophic position of each taxon. Node sizes are proportional to species average body masses (mg).

Many small invertebrates (e.g. *Macrostemum*, *Leptonema*, *Helicopsyche*, Chironomidae) contributed disproportionately to the flow of energy given their mean body mass (Fig 3b). The relationship between body mass and population density showed that these taxa tended to be regionally more abundant than expected from their mean body masses (i.e. they were above the regression model log[density]~log[average body masses], Figure 3). This pattern was also evident at the local scale, but the species above the regression model varied from site to site (Figure S2).

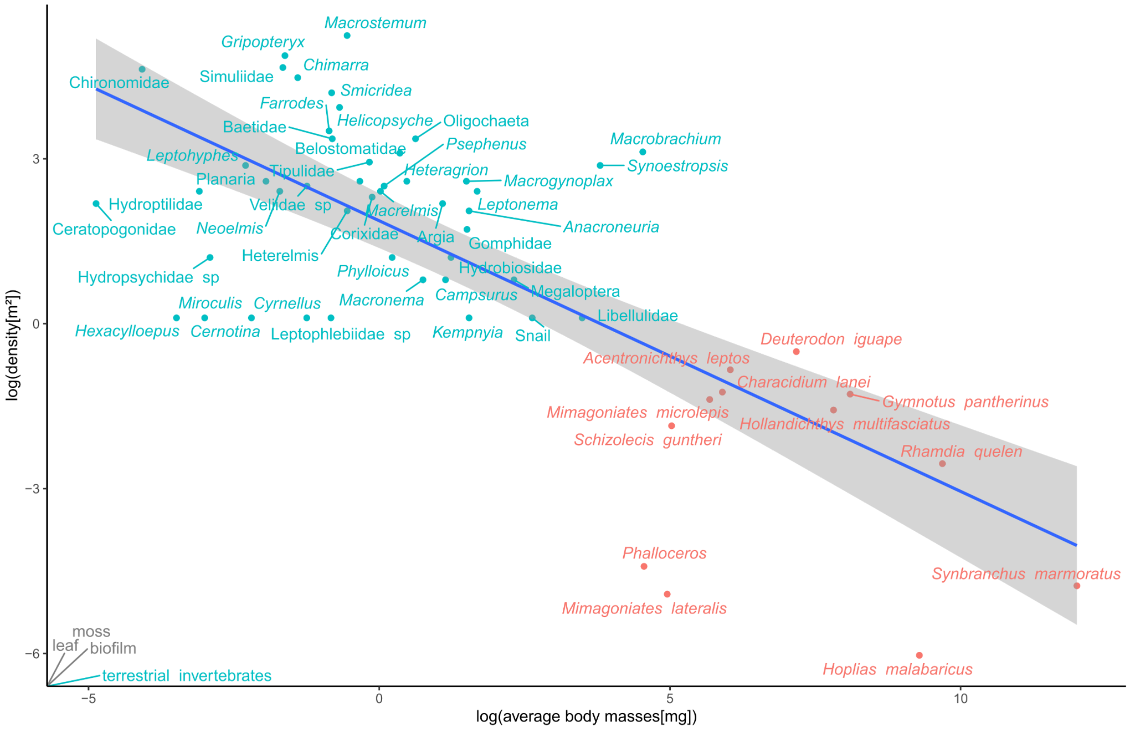

Figure 3. Species size-density relationship for Cananeia streams (density relationship for the regional meta-web). Log-transformed density of individuals in the regional species pool is regressed against log-transformed mean body masses of each taxonomic group ($R^2 = 0.25$, $P < 0.001$). Species above or below the regression line indicate species with more or less individuals than expected according to allometric scaling theory.

For individual stream sites, some species were important contributors across all sites while some of them were only important for some specific ones (Figure 4). For instance, *Macrostemum*, Chironomidae, *Gripopteryx* and *Macrobrachium* were widespread and abundant in the regional species pool and contributed strongly to the energy flux in all five stream food webs. In contrast, *Campsurus*, *Farrodes*, *Deuterodon* *iguape*, *Helicopsyche*, *Macrelmis* and *Synoestropsis* were only important in one or two sites (Figure 4).

Besides these differences among individual food webs, supporting our second hypothesis, all food webs and the meta food web were stable, i.e. had negative values for the maximum eigenvalue of the Lotka Volterra Jacobian matrices. This analysis considered that stream food webs are in a stable state if the real parts of eigenvalues from the Jacobian are all negative (between -4.9 and -13.5). This means that the population energy demands are consistent with the amount of energy available at lower trophic levels, meaning that there is no population too large that cannot be sustained by the amount of energy from prey. We then explored the associations between food web properties using Pearson's correlations. The stability responses to small perturbations (Jacobian matrices eigenvalues) were not related to the total amount of energy fluxes in each food web (r = 0.02, P = 0.97), the number of nodes (r = -16, P = 0.79), nor the number of links (r = -0.35, P = 0.55). Yet, the total energy flux tended to increase with the number of nodes (r = 0.78, P = 0.11) and links (r = 0.86, P = 0.05).

In agreement with our third hypothesis, the simulation extinction resulted in food webs that remained dynamically stable after the removal of individual species each at a time (negative Jacobian eigenvalues ranging between -14.60 and -7.41). The multiple regression model associating food web stability (one value for each species extinction scenario) and species properties in the meta food web (number of links, mean body mass, total population biomass, and total energy fluxes) had no explanatory power (multiple regression F-statistic: 0.16, P = 0.95, 4 and 52 degrees of freedom). Therefore, there were no specific species, whose extinction would significantly destabilize the meta food web.

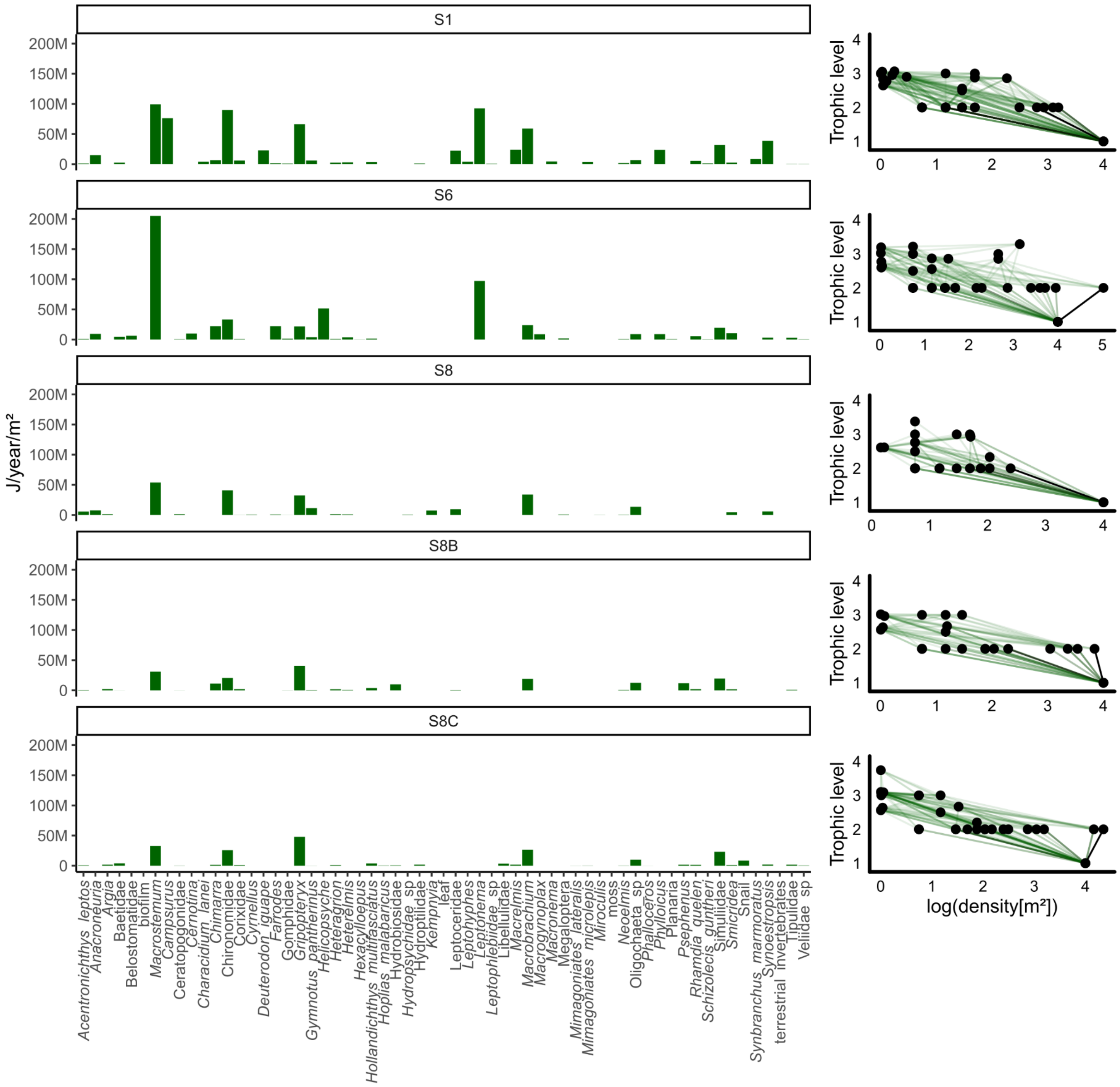

Figure 4. Energy fluxes (J/year/m²) from individual nodes and the five food webs from Atlantic Forest streams from Cananeia (São Paulo, Brazil). Position along the y axis in the individual food webs represent the trophic position of each taxon. Darker link colors represent interactions of higher energy fluxes.

Food web dissimilarity

As predicted by the fourth hypothesis, the dissimilarity of the network was higher than the compositional dissimilarity (Figure 5). Approximately 40% of interactions changed from one food web to another, while pure compositional dissimilarities among stream communities were approximately 25%. These compositional changes were strongly associated with the absence of predators when prey taxa were present, with a much smaller contribution of the component of prey absence and mutual absence (less than 10%, Figure 5).

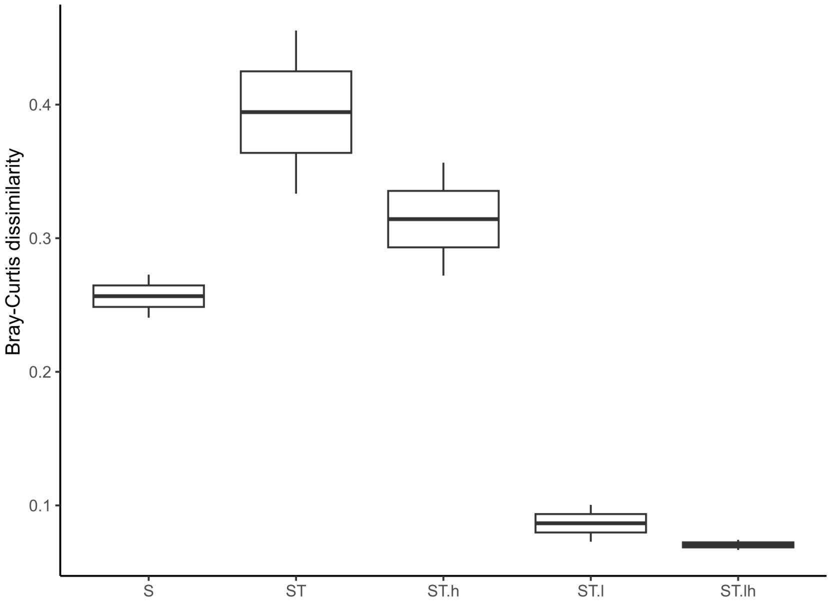

Figure 5. Compositional and network dissimilarity among local streams from the Cananeia watershed (São Paulo, Brazil). S = compositional beta diversity based on Bray-Curtis index, ST = network beta diversity based on Bray-Curtis index, ST.h = network dissimilarity caused by the absence of predator, ST.l = network dissimilarity caused by the absence of prey, St.lh = network dissimilarity caused by the absence of both predators and prey.

DISCUSSION

Untangling a network of trophic interactions through integration of energetic principles has been proposed as a key new approach to understand ecosystem functioning under a comparable currency (Barnes et al., 2014). Here, we combined recently advanced methods and theories (Gauzens et al., 2019; Hernvann et al., 2022) to derive a well-resolved food web from tropical rainforest streams with a metabolic model of energy fluxes for the first time. By doing so, we found locally and regionally stable networks where energy was channelized through a taxonomically diverse set of organisms, such as chironomids and stoneflies, and from small to large taxa (e.g. from chironomids to paleomonid shrimps). Invertebrate consumers had a weak association between their body mass and trophic position, with weak isotopic niche differentiation, all indicative of widespread omnivory and generalistic feeding interactions. These characteristics potentially govern food web stability through weakening interaction in diverse ecosystems (Kratina et al. 2012; Collyer et al., 2023). Below, we explain these new insights for understanding the dynamics of tropical stream ecosystems and how important are these baseline mechanisms for anticipating the impacts of the current environmental crisis.

The relationship between organism body mass and their trophic position provides a measure of food web size structure (i.e. the extent to which larger consumers feed on small prey and whether this is at a constant ratio) (Perkins et al. 2021). Partially in contrast to our first hypothesis, there was a stronger association between δ^{15}N values and body mass for fish than for invertebrates, both within and across species, indicating that larger fish are commonly higher up the food chain. This suggests that fish predators interact within a well-defined prey size spectrum to fulfill their energetic demands, a pattern expected in simple food web compartments comprising only a limited number of functional groups (Keppeler et al., 2020). However, this pattern did not hold for invertebrates where there was a large overlap in the isotopic niche space among taxa, more in agreement with our second hypothesis. This weak association indicates omnivorous and generalist behavior meaning that invertebrate consumers feed plastically up and down the food web to meet their metabolic requirements (i.e. feeding on larger or smaller prey than expected based on their optimum predator-prey mass ratio, (Potapov et al., 2021). It has been suggested that tropical streams have a greater prevalence of omnivorous and generalist feeding than in their temperate counterparts that have proportionally more taxa from delimited trophic groups like shredders and obligatory predators (Boyero et al., 2011). This generalist feeding strategy decreases energy transfer efficiency along the food chain due to a decrease in net gains when feeding upon proportionally small or large prey (Brose et al., 2005; Stephens & Krebs, 2019). Our findings add mounting evidence that generalist macroinvertebrates at intermediate trophic positions are likely inefficient, but represent diverse conduits of energy that potentially enhances the stability of these complex systems (Collyer et al., 2023).

For macroinvertebrates, 70% of the total energy flux depended on a subset of ten taxa spread over orders of magnitude in body masses (from chironomids with an average 0.2 mg per individual to large shrimps with an average of 1060 mg per individual). This may have consequences for the stability of the food web as different body masses are associated with differences in life cycles and environmental responses such as size-selective predation, size-related risks of dislodgement by hydraulic forces, and use of size-related refugia in streambeds to counter these risks (Woodward et al., 2005). Indirectly, body mass is closely related to other traits, with smaller species often exhibiting population resilience with short life cycles, rapid growth, high reproductive rates and high number of dispersal events in time (Brown et al., 2004; Saito et al., 2015). Moreover, competitive status can be positively correlated with size, whereas possessing an aquatic imago stage, ovoviviparity, and vulnerability to environmental contaminants are all negatively correlated with body mass (Statzner et al., 2004). We hypothesize that having a set of invertebrates with a wide range of body masses contributing strongly to energy flow could enhance resilience to local perturbations in tropical streams. Environmental changes negatively affecting specific size classes could be compensated by organisms with different body masses, which could stabilize the energy flows through the food webs.

Despite the subset of macroinvertebrates that were important for energy flows in all five streams and agreeing with our second hypothesis, some taxa were only important in one or two sites. This diverse set of taxa summed approximately 30% of the energy flow, regionally. Moreover, two key results highlight the importance of the local and regional diversity for the functioning of tropical stream food webs, where energy flows do not rely strongly on specific trophic interactions. 1) We found that simulating individual extinctions did not destabilize the meta food web. 2) Local food webs were approximately 40% dissimilar among each other. A high regional diversity coupled with high spatial variation can assure that multiple species can contribute a little to the energy flow, but also that a diverse pool of potential colonizers would be able to compensate for the eventual loss of species. Indeed, we found that several size classes were regionally composed by multiple taxa that could potentially compensate for species losses. For instance, *Macrostemum* (a net-spinning caddisfly) is the main contributor to energy fluxes, that regionally have at least three other genera from the same family with fairly similar sizes co-occurring in the watershed on different densities (*Leptonema*, *Macronema*, *Smicridea*). Intriguingly, *Leptonema* and *Smicridea* were found to be the most abundant taxa in the presence of *Macrostemum* in other well preserved Atlantic Forest streams (Saito, Stoppa, et al., 2021; Siqueira et al., 2020). Tropical stream communities have been suggested to be more stochastic in terms of colonizations and demography in comparison to temperate systems due to an accelerated pace of life that leads to more dispersal events and smaller population sizes (Saito, Perkins, et al., 2021; Siqueira et al., 2020). The greater contribution of stochastic processes should render spatial and temporal variation in the relative abundance of these taxa but with potentially weak impacts for patterns of energy flow. Therefore, having a diverse regional portfolio of taxa that could compensate for eventual losses may be key to sustain food webs in tropical ecosystems.

In summary, we found that all five tropical stream sites have energetic food webs that are stable to small perturbations. Despite having only five highly-resoluted food webs, we observed an association between diversity and the total energy flowing up to the apex predators. This was potentially due to two ecological mechanisms: (i) the diversity of body masses buffering against size-dependent disturbances and (ii) high regional taxonomic diversity buffering against local stochastic losses of species. These two mechanisms critically depend on the maintenance of local and regional biodiversity in tropical streams, which is known to be strongly affected by human land use intensifications from forests to monoculture plantations in different ways (Neves et al., 2023; Siqueira et al., 2015). Therefore, an urgent challenge is to understand how the systematic loss of diversity jeopardizes the stability of stream food webs under human impacts and what could be the consequences for a sustainable use of resources.

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Untangling the complex food webs of tropical rainforest streams

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Supporting Information

Table S1. Physical and chemical water variables in 5 stream stretches in Cananeia (São Paulo, Brazil).

site	replicate	Temperature C°	pH	ORP mv	mS/cm	mg/L OD	% OD	TPS
S8A	1	23.06	4.79	454		14.61	174	0.023
S8A	2	22.63	5.79	430		12.3	136	0.033
S8A	3	21.34	5.09	454	0.037	14.15	165.4	0.024
S1	1	23.35	6.64	372	0.035	13.97	167.6	0.023
S1	2	23.12	6.58	392	0.034	13.06	156.1	0.022
S1	3	23.08	6.54	401	0.035	12.36	147.6	0.023
S6	1	21.65	6.3	396	0.034	14.68	171	0.022
S6	2	22.63	6.36	416	0.034	13.54	157.7	0.022
S6	3	22.59	6.27	426	0.034	15.2	176.9	0.022
S8C	1	21.72	5.71	352	0.035	14.19	165.5	0.023
S8C	2	21.65	5.62	359	0.034	13.91	162	0.022
S8C	3	21.66	5.55	355	0.035	13.27	154.6	0.022
S8B	1	21.92	5.11	459	0.024	10.59	123.9	0.016
S8B	2	21.73	5	456	0.036	13.07	152.7	0.024
S8B	3	21.68	4.91	4.58	0.036	10.89	126.9	0.024

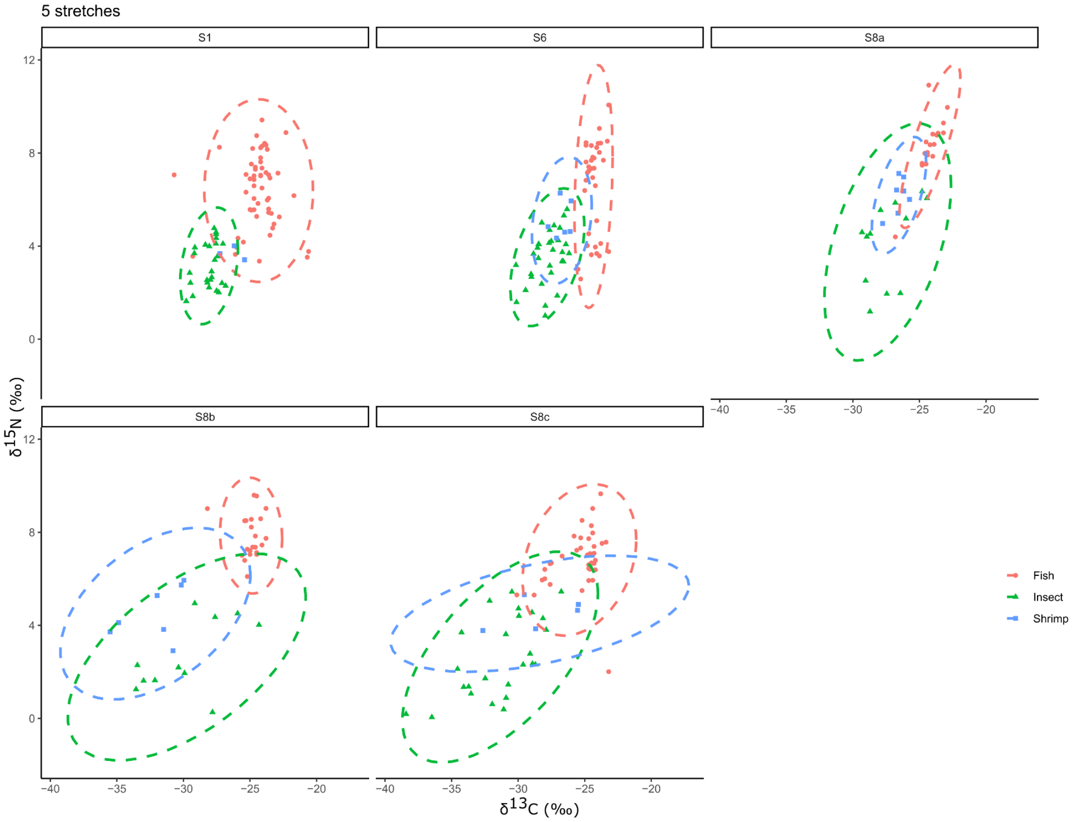

Figure S1. Isotopic signature of consumers for δ13C and δ15N for five stretches from the Cananeia watershed.

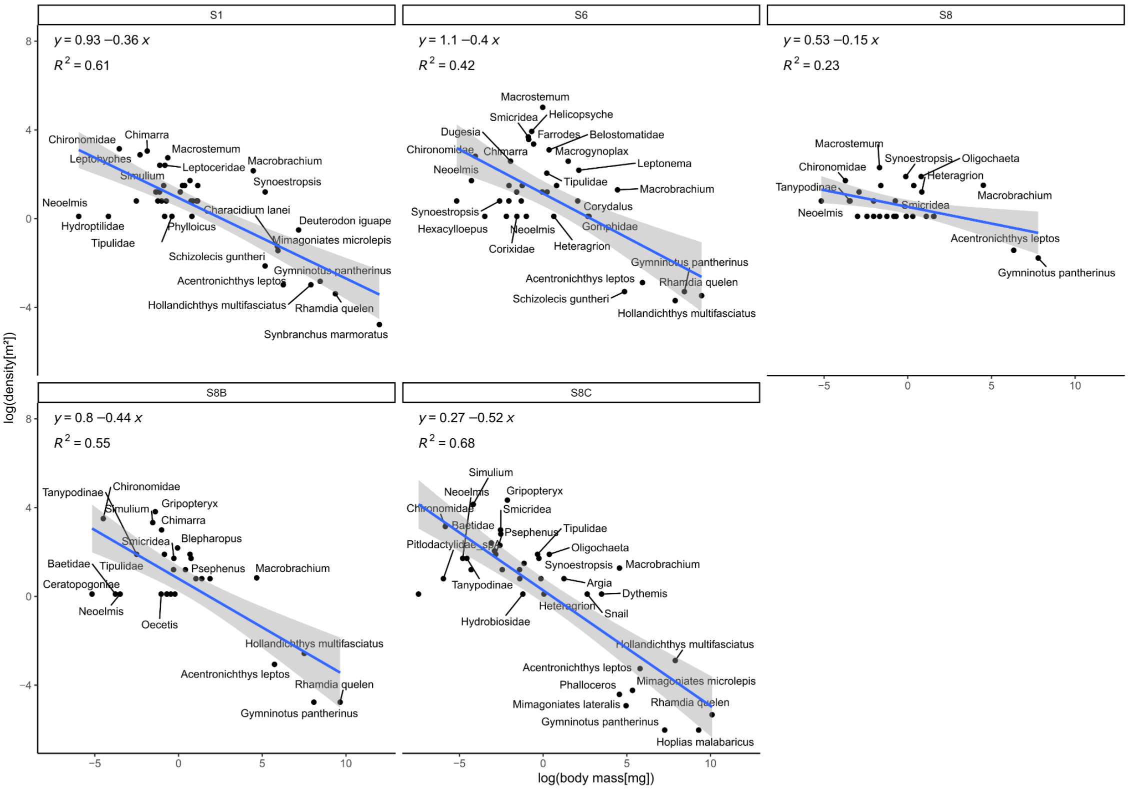

Figure S2. Local size density plot for stretches at the Cananeia watershed. Average body-masses are regressed against their summed abundances. Equations and R² are associated to linear regressions.

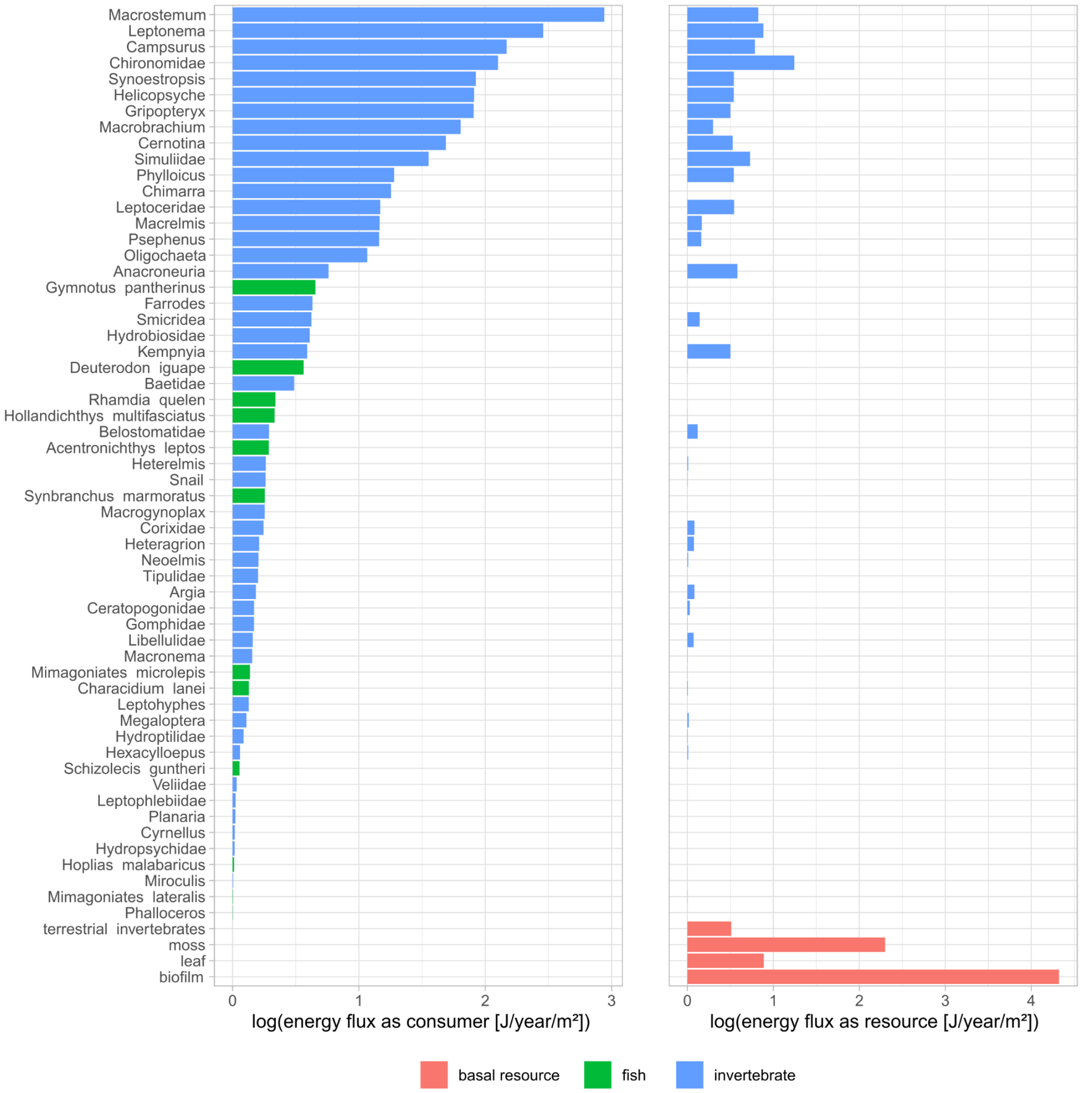

Figure S3. Proportion of energy flowing throught each node in the stream meta food web from Cananeia (Brazil). Left: Energy flowing as consumers. Right: Energy flowing as resources.

Table S1. Network indices for the meta food web and individuals food webs from the Cananeia watershed. See methods for the description of each index.

	Meta food web	S1	S6	S8	S8B	S8C
N nodes	65	41	38	28	28	35
N links	307	171	129	73	78	132
Link density	5,032787	4,170732	3,394737	2,607143	2,785714	3,771429
Connectance	0,08388	0,104268	0,09175	0,096561	0,103175	0,110924
Average link weight	0,836826	0,798652	0,888517	0,825698	0,798346	0,820007
Compartmentalization	0,180084	0,20211	0,186202	0,226761	0,235359	0,200116
Mean trophic level	2,35	2,21	2,24	2,17	2,11	2,24
Mean omnivory	0,12	0,11	0,11	0,10	0,09	0,15