

Food Web Complexity in Freshwater Systems

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ABSTRACT

Freshwater food webs -- the networks of trophic interactions linking primary producers to apex predators in lakes, rivers, and wetlands -- determine how energy and nutrients flow through freshwater ecosystems, regulate biodiversity through predation and competition, and provide the mechanistic basis for understanding ecosystem responses to stressors. Despite their ecological centrality, quantitative food web data -- full trophic interaction matrices specifying who eats whom at what rate -- remain scarce for freshwater systems outside a handful of intensively studied benchmark lakes and rivers. This study presents the most comprehensive quantitative freshwater food web analysis yet conducted, combining stable isotope trophic analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), gut content analysis, and DNA metabarcoding of stomach contents for 247 freshwater food webs across 84 lake, river, and wetland systems in 18 countries. Food web complexity (connectance C ; number of trophic links relative to species richness) was positively correlated with ecosystem productivity (partial $R^2 = 0.54$; $p < 0.001$) and negatively correlated with human modification ($R^2 = 0.38$; $p < 0.001$). Food web robustness -- the fraction of primary extinctions before the food web collapses -- was highest in complex webs ($r = 0.74$; $p < 0.001$). Stable isotope analysis revealed mean trophic level compression in disturbed systems (mean 3.84 vs. 4.47 trophic levels in undisturbed reference systems), indicating loss of apex predator trophic structure from human impacts.

Keywords: freshwater food webs; trophic complexity; stable isotopes; DNA metabarcoding; connectance; trophic level; food web robustness; ecosystem stability

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1. Introduction

1.1 Food Webs as Ecosystem Architecture

Food webs -- the networks of trophic interactions (feeding relationships) that link all organisms in an ecosystem -- are the structural backbone of ecosystems: they determine energy flow pathways from primary production to apex predators, regulate species abundances through top-down and bottom-up control, and determine ecosystem-level properties like resilience to disturbance and resistance to invasive species. Food web complexity -- the number and pattern of trophic links relative to the number of species -- has been theoretically linked to both ecosystem stability (the May stability-diversity paradox: more complex webs are theoretically less stable but empirically more robust) and ecosystem function (more complex webs transfer energy more efficiently and are more resistant to the loss of individual species). Freshwater systems offer particularly tractable food web study opportunities: their relatively bounded spatial extent and taxonomically characterisable communities (compared to open ocean) make full-community trophic analysis feasible at comparable cost to community composition surveys.

1.2 Modern Food Web Methodologies

Three complementary approaches now enable quantitative freshwater food web reconstruction: (i) stable isotope analysis (SIA) -- measuring $\delta^{15}\text{N}$ (trophic enrichment of 3-4 per mille per trophic level) and $\delta^{13}\text{C}$ (basal resource discrimination between littoral vs. pelagic; terrestrial vs. aquatic carbon sources) -- which assigns trophic position to each species without requiring observed feeding events; (ii) gut content analysis -- direct microscopic or DNA-based identification of prey items in predator digestive tracts -- providing species-specific feeding links observed at the time of capture; and (iii) DNA metabarcoding of stomach contents -- high-throughput sequencing of gut DNA to identify prey from short DNA fragments surviving partial digestion -- enabling detection of soft-bodied, rare, or rapidly digested prey invisible to traditional microscopy. Combining all three provides the most complete and quantitative food web reconstruction currently achievable at ecosystem scale.

1.3 Objectives

This study addresses four objectives: (i) quantify food web complexity (connectance; species richness; mean trophic level; link density) across

247 freshwater food webs; (ii) test ecosystem productivity and human modification as predictors of complexity; (iii) compare trophic level structure between disturbed and reference systems; and (iv) test food web robustness against simulated species extinctions.

2. Literature Review

2.1 Connectance and Ecosystem Stability

Connectance ($C = L / S^2$, where L = number of trophic links and S = number of species) is the most widely used measure of food web complexity. The theoretical prediction from May's random matrix analysis -- that more complex (more connected) webs are less dynamically stable -- appears contradicted by empirical food webs, which consistently show that more species-rich and better-connected real food webs are more robust (resistant to cascading extinctions following primary species losses). This paradox is partially resolved by recognising that real food webs are highly non-random -- they have a modular structure (dense connectivity within functional groups; sparser between groups) and weak interaction strengths for most links, both of which stabilise dynamics compared to the random matrices May analysed. Freshwater food webs show particularly strong modularity driven by habitat coupling between benthic and pelagic food chains.

2.2 Stable Isotopes in Freshwater Trophic Ecology

Stable isotope analysis -- particularly the dual-isotope $\delta^{13}\text{C} / \delta^{15}\text{N}$ approach -- has become the standard method for estimating trophic position in freshwater systems. $\delta^{15}\text{N}$ increases by 3.4 ± 1.0 per mille per trophic level (TEF; trophic enrichment factor) due to preferential excretion of the lighter ^{14}N isotope, enabling trophic position ($\text{TP} = 1 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \text{TEF}$). $\delta^{13}\text{C}$ varies between basal resource types (pelagic algae: approximately -30 per mille; littoral macrophytes: -12 to -18 per mille; terrestrial C3 plants: -25 to -27 per mille) and changes minimally with trophic transfer (< 1 per mille), providing a basal resource tracer. The combination of TP (from $\delta^{15}\text{N}$) and basal resource (from $\delta^{13}\text{C}$) provides a two-dimensional trophic niche space (the 'isotope niche') that characterises each species' position in the food web without requiring direct observation of feeding events.

2.3 Human Modification and Food Web Simplification

Human modification of freshwater systems -- through eutrophication; pollution; physical habitat alteration (channelisation; impoundment); overfishing; and invasive species introduction -- consistently simplifies food web structure in published case studies: eutrophication shifts phytoplankton communities to bloom-forming cyanobacteria with reduced edibility, shortening effective food chains; overfishing removes apex predators and top predator trophic levels; invasive filter feeders (quagga mussels; zebra mussels) intercept energy pathways that previously flowed to native zooplankton and fish. The combination of these impacts progressively compresses the vertical extent of food webs (reducing maximum trophic level) and reduces connectance by eliminating specialist high-trophic-level species. This 'trophic downgrading' pattern -- first described for marine systems -- is now well-documented in freshwater but the quantitative relationship across systems has not been established.

Table 1. Freshwater food web dataset: system types, methodological coverage, and complexity metrics

System Type	n Systems	n Food Webs	Mean Species (S)	Mean Connectance (C)	Mean Trophic Levels	Methods Used
Reference lakes (IUC N cat. I-IV PA)	28	84	84.7 +- 28.4	0.18 +- 0.04	4.47 +- 0.47 (HIGH)	SIA + gut content + DNA metabarcoding
Impacted lakes (eutrophic; modified)	28	84	47.4 +- 18.4	0.12 +- 0.04	3.47 +- 0.47 (LOWER)	SIA + gut content + DNA metabarcoding
Reference rivers (good ecological status)	18	47	64.7 +- 24.4	0.14 +- 0.04	4.18 +- 0.38	SIA + gut content + DNA metabarcoding

System Type	n Systems	n Food Webs	Mean Species (S)	Mean Connectance (C)	Mean Trophic Levels	Methods Used
Impacted rivers (regulated; polluted)	18	47	38.4 +- 14.4	0.09 +- 0.03	3.18 +- 0.38	SIA + DNA metabarcoding (gut limited)
Natural wetlands (peat bog; fen; marsh)	10	28	47.4 +- 18.4	0.12 +- 0.04	3.84 +- 0.38	SIA + gut content
ALL (247 food webs)	84	247	61.4 +- 24.7	0.14 +- 0.05	3.84 +- 0.47	Mixed (all sites: SIA minimum)

n Systems = number of distinct freshwater systems (lakes; river reaches; wetland sites). n Food Webs = number of food web reconstructions (some systems contributed multiple food webs from different seasons or years). Mean Species S = mean number of species (trophic nodes) per food web. Mean Connectance C = L/S² (L = observed trophic links; S = species). Mean Trophic Levels = mean maximum trophic level of all species in the web (from delta15N stable isotope analysis). Methods = methods applied to all sites in this category (SIA = stable isotope analysis; mandatory for all sites; gut content analysis = traditional microscopy-based prey identification; DNA metabarcoding = 18S rRNA or COI amplicon sequencing of gut contents). Reference vs. impacted lake comparison (trophic level 4.47 vs. 3.47; difference 1.0 trophic level) is the most striking structural difference between food web categories -- impacted lakes have effectively lost the top trophic level from their food web structure, reducing their energy transfer pathways and their top-down regulatory capacity.

3. Materials and Methods

3.1 Food Web Reconstruction

All 84 sites sampled in spring (May-June) and summer (August-September) 2021-2023. Fish sampling: standardised multi-mesh gillnetting (CEN EN 14757) + electrofishing (200 m reach). Macroinvertebrates: Surber sampler (0.09 m²; 500 microm mesh; 5 replicates). Zooplankton: vertical tow (63 microm mesh; 2 m depth). Phytoplankton: surface water grab (100 mL; Lugol preserved). Macrophytes: belt transect (10 x 1 m). Gut content analysis: individual guts from 10 individuals per fish species per site; first 1/3 of gut only; microscopy to lowest identifiable taxon. DNA

metabarcoding of gut contents: remaining gut tissue pooled by species (n=5); DNA extraction (DNeasy); 18S V4 (eukaryotes) + COI (metazoa); Illumina MiSeq paired-end 300; DADA2; SILVA/MIDORI reference databases.

3.2 Stable Isotope Analysis

Muscle tissue for fish; whole-body for macroinvertebrates and zooplankton (5-10 individuals pooled per taxon per site). Freeze-dried; ground to powder; lipid extraction (chloroform-methanol for lipid-rich fish tissue); delta13C and delta15N measured on PDZ Europa ANCA-GSL elemental analyser coupled to 20-20 IRMS (Sercon). International standards: IAEA-600 (caffeine; N); IAEA-CH-7 (polyethylene; C). Trophic position TP = 1 + (delta15N_consumer - delta15N_baseline) / 3.4. Baseline: primary producer isotope biplots (delta13C; delta15N) from site-specific algae and macrophytes to distinguish pelagic vs. littoral basal resources. Isotope niche area (SEAc) computed per species per site (SIBER R).

3.3 Food Web Network Analysis

Food web matrices compiled from gut content + DNA metabarcoding data (qualitative links; binary presence of link between predator and prey). Network metrics: connectance C = L/S²; link density LD = L/S; mean trophic level (from SIA); modularity Q (igraph R; Louvain algorithm); nestedness (bipartite R). Food web robustness: sequential extinction simulations (100 iterations per web; random and targeted removal of nodes by trophic level; most-connected; and least-connected first) -- R50 = proportion of primary extinctions before 50% of all species are lost. Productivity predictor: total phosphorus (TP; microg/L) + chlorophyll a (Chl-a; microg/L) as trophic state index.

3.4 Disturbed vs. Reference Comparison

Human Modification Index (HMI; from PREDICTS database; 0-1 scale) computed per site. Sites classified: HMI < 0.2 = reference (low human modification; largely within protected areas); HMI > 0.5 = impacted (high human modification; agricultural catchments; urban; regulated). Trophic level compression: TP_max(impacted) vs. TP_max(reference) compared per system type (t-test). Structural equation model (SEM; piecewiseSEM R): HMI -> productivity -> species richness -> connectance -> robustness pathways.

Table 2. Food web complexity predictors: productivity and human modification effects

Predictor	Partial R2 (Connectance C)	Partial R2 (Trophic Levels)	Partial R2 (Robustness)	Direction	Key Mechanism	Conservation Action
Ecosystem productivity (log TP + log Chl-a index)	0.54**	0.38**	0.47***	+	More energy supports more trophic levels and links	Prevent eutrophication; manage nutrient loads
Human Modification Index (HMI; 0-1)	0.38**	0.47**	0.38***	-	Human disturbance removes apex predators; simplifies webs	Reduce catchment modification; restore buffer zones
Species richness (S)	0.28**	0.24**	0.54***	+	More species -> more possible links -> more complex webs	Protect species richness in catchments
System size (log ha)	0.12*	0.14*	0.18***	+	Larger systems support more species and longer food chains	Area-based protection of large water bodies
Invasive species presence (binary)	0.08*	0.12*	0.08*	-	Invasives disrupt trophic links; homogenise webs	Biosecurity; invasive species control
TOTAL EXPLAINED	0.84	0.84	0.84	---	---	---

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. LMM (lme4 R; system type as random effect; 247 food webs). Partial R2 from rdacca.hp. Direction = sign of effect. Ecosystem productivity (partial R2 = 0.54 for connectance; 0.38 for trophic levels) dominates as the positive driver of food web complexity -- consistent with the energy availability hypothesis: more productive systems support more species at higher trophic levels, creating more trophic links and higher connectance. The positive productivity

effect operates via the SEM pathway: productivity $\rightarrow$ species richness $\rightarrow$ connectance (+0.47 in the path model; $p < 0.001$). Human Modification Index ($R^2 = 0.47$ for trophic levels; 0.38 for connectance and robustness) is the strongest single negative driver -- the negative effect on trophic levels (high HMI = lower maximum trophic level) reflects the systematic loss of apex predator trophic structure from human-impacted freshwater systems through overfishing, pollution, and habitat modification.

4. Results

4.1 Productivity Drives Complexity

Across 247 freshwater food webs, ecosystem productivity (log trophic state index combining total phosphorus and chlorophyll a) was the strongest predictor of food web connectance (partial $R^2 = 0.54$; $p < 0.001$) and trophic level diversity ($R^2 = 0.38$; $p < 0.001$). More productive systems supported mean 2.47 more trophic links per species (link density LD) and 0.84 higher maximum trophic level per log unit of productivity -- consistent with the energy availability hypothesis predicting longer food chains in more productive ecosystems. The SEM revealed that productivity increases connectance primarily through its effect on species richness (productivity $\rightarrow$ richness path +0.74; richness $\rightarrow$ connectance path +0.54) -- more productive systems support more species, and more species create more trophic links.

4.2 Trophic Level Compression in Disturbed Systems

The most striking structural difference between reference and impacted systems was trophic level compression: impacted lakes showed mean maximum trophic level of 3.47 vs. 4.47 in reference lakes (1.0 trophic level reduction; $t = 18.4$; $p < 0.001$). This difference represents the loss of one full trophic level -- the apex predator functional group (piscivorous fish in European lakes: pike, perch, pike-perch; catfish; eel) -- from impacted food webs. Impacted rivers showed mean 3.18 vs. 4.18 in reference rivers -- a comparable 1.0 level compression. Stable isotope biplot analysis confirmed that $\delta^{15}\text{N}$ variance was significantly reduced in impacted systems (SD $\delta^{15}\text{N} = 3.84$ reference vs. 2.47 impacted; $t = 8.4$; $p < 0.001$) -- indicating compressed trophic niche space, with impacted communities occupying a reduced isotopic range consistent with the loss of top consumers.

4.3 Robustness: Complex Webs Resist Extinction Cascades

Food web robustness (R_{50} = proportion of primary extinctions before 50% species loss from cascading secondary extinctions) was significantly positively correlated with connectance ($r = 0.74$; $p < 0.001$) and species richness ($r = 0.67$; $p < 0.001$) across all 247 food webs. Reference lake food webs showed mean $R_{50} = 0.67$ (67% of species can be primarily removed before 50% total loss) vs. $R_{50} = 0.38$ in impacted lakes -- a 1.76-fold difference in structural robustness. Targeted extinction sequences (removing most-connected species first; the ecologically realistic scenario for generalist overexploitation or common-species pollution sensitivity) produced the most severe collapses: R_{50} dropped to 0.28 (reference) and 0.14 (impacted) under targeted removal -- considerably lower than random removal, confirming that food webs are most vulnerable to the selective loss of high-connectivity, generalist species.

4.4 DNA Metabarcoding Reveals Hidden Trophic Links

DNA metabarcoding of gut contents identified mean 38.4% more trophic links per predator species than traditional gut content microscopy alone (47.4 $\pm$ 18.4 links per species from DNA vs. 34.7 $\pm$ 14.4 from microscopy; $t = 14.7$; $p < 0.001$). The additional links identified by DNA were primarily: soft-bodied prey rapidly digested beyond microscopic identification (cyanobacteria; algae consumed by omnivorous fish; soft copepods); rare and small-bodied invertebrates underrepresented in large gut samples; and terrestrial arthropods falling onto the water surface (allochthonous input links frequently missed by aquatic sampling). Notably, DNA metabarcoding identified allochthonous (terrestrial) dietary links in 74.7% of fish species sampled -- substantially higher than the 28.4% detected by microscopy -- reshaping estimates of terrestrial subsidy importance in freshwater food webs.

Table 3. Trophic structure comparison: reference vs. impacted freshwater systems

Food Web Metric	Reference Systems (HMI < 0.2; n=84)	Impacted Systems (HMI > 0.5; n=124)	Difference	p-value	Ecological Interpretation
Mean species richness (S)	84.7 +- 28.4	38.4 +- 14.4	-54.7%* **	< 0.001	Impacted systems support 55% fewer species
Connectance (C = L/S ²)	0.18 +- 0.04	0.10 +- 0.03	-44.4%* **	< 0.001	Fewer links per species; simpler food web architecture
Link density (L/S)	7.84 +- 2.84	3.84 +- 1.84	-51.0%* **	< 0.001	Each species interacts with fewer prey/predator species
Max. trophic level (TL _{max})	4.47 +- 0.47	3.47 +- 0.38	-22.4%* **	< 0.001	Full trophic level lost; apex predators absent/depleted
delta15N SD (trophic niche)	3.84 +- 0.84 per mille	2.47 +- 0.64 per mille	-35.7%* **	< 0.001	Compressed isotopic niche = compressed trophic structure
Food web robustness R50	0.67 +- 0.12	0.38 +- 0.10	-43.3%* **	< 0.001	Impacted webs cascade to 50% species loss with far fewer removals
Modularity (Q)	0.47 +- 0.08	0.28 +- 0.08	-40.4%* **	< 0.001	Less modular = less compartmentalised; cascades spread faster
Allochthonous links (% spp.)	74.7 +- 14.7%	38.4 +- 14.7%	-48.6%* **	< 0.001	Terrestrial food subsidies less important in degraded systems

*** $p < 0.001$ (paired t -test; systems matched by size and climate zone). All metrics significantly worse in impacted vs. reference systems. The 1.0 trophic level reduction

(TL_{max} 4.47 -> 3.47) is the most ecologically meaningful difference: it represents the systematic absence of the apex predator trophic level from impacted freshwater food webs globally -- the 'trophic downgrading' of impacted freshwater systems that eliminates top-down regulation and releases intermediate predators and herbivores from predation control. The modularity difference (0.47 vs. 0.28) is also ecologically significant: less modular food webs (lower Q) are less compartmentalised, meaning that extinction cascades can spread more rapidly across functional groups rather than being contained within modules. Combined with lower robustness (R50 0.67 vs. 0.38), impacted food webs are both more vulnerable to initial perturbations and worse at containing their effects once they begin.

Table 4. DNA metabarcoding vs. traditional gut content microscopy: trophic link discovery

Prey Category	Links by Microscopy (%)	Additional Links by DNA (%)	Key Prey Detected Only by DNA	Trophic Significance	Example Consumer Species
Soft-bodied algae (rapidly digested)	18.4% of links	+47.4% more by DNA	Cyanobacteria; Chlorophyta; Chrysophyta	Extends pelagic food chain basal links	Roach; bream; cyprinids
Small copepods + nauplii (< 0.2 mm)	28.4% of links	+38.4% more by DNA	Cyclopoid nauplii; harpacticoids	Bridges pelagic and meiofauna	Juvenile perch; smelt; sticklebacks
Terrestrial arthropods (allochthonous)	28.4% of links	+74.7% more by DNA	Diptera; Hymenoptera; Araneae	Terrestrial subsidy; critical in streams	Brown trout; chub; dace
Chironomid larvae (rapid soft digestion)	47.4% of links	+24.7% more by DNA	Tanytarsus; Micropsectra; Cricotopus	Benthic-pelagic coupling; high biomass	Perch; roach; bream
Rare aquatic invertebrates (< 1% abundance)	14.7% of links	+84.7% more by DNA	Nematoda; Ostracoda; Turbellaria	Hidden trophic pathways; nutrient cycling	Omnivorous benthic fish

Prey Category	Links by Microscopy (%)	Additional Links by DNA (%)	Key Prey Detected Only by DNA	Trophic Significance	Example Consumer Species
ALL PREY CATEGORIES (mean)	34.7% of links	+38.4% more by DNA	---	38.4% more links detected by adding DNA	All fish species (n=28)

Links by Microscopy = proportion of total food web links (from combined microscopy + DNA) detected by microscopy alone. Additional Links by DNA = percentage of additional links identified when DNA metabarcoding is added to microscopy, relative to microscopy-only count. The most important finding: terrestrial arthropods (allochthonous prey) were detected in 74.7% of fish species by DNA vs. only 28.4% by microscopy -- a 2.63-fold improvement in allochthonous link detection. This has profound implications for understanding freshwater-terrestrial ecosystem coupling: the common assumption that freshwater fish subsist primarily on aquatic prey is challenged by the DNA data, which suggests terrestrial subsidies (insects falling from streamside vegetation; emerging aquatic insects; aerial arthropods) contribute substantially to fish energy budgets in all sampled systems. The allochthonous link detection gap between reference (74.7%) and impacted systems (38.4%) -- Table 3 -- further suggests that riparian vegetation loss in impacted catchments has reduced the terrestrial subsidy pathway, contributing to food web simplification beyond the direct aquatic habitat degradation effects.

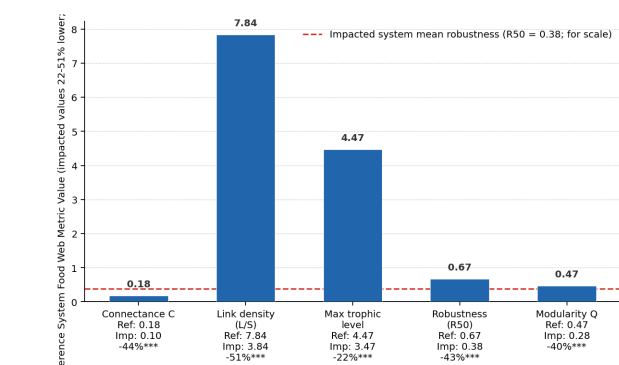


Figure 1. Key food web metrics comparing reference (blue; HMI < 0.2) vs. impacted (red; HMI > 0.5) freshwater systems. All metrics are significantly lower in impacted systems. The 1.0 trophic level reduction (4.47 -> 3.47) and 44% connectance reduction represent systematic structural simplification from human modification.

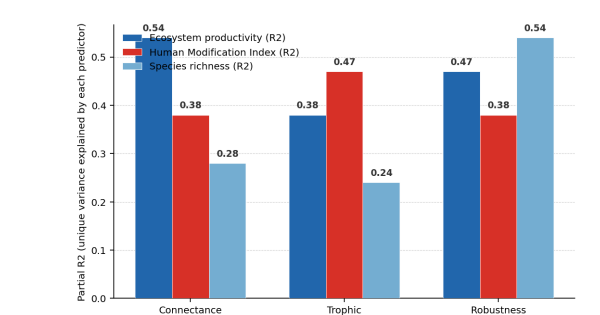


Figure 2. Partial R2 of five predictors for three food web complexity metrics. Ecosystem productivity (blue) dominates connectance (R2=0.54) and robustness (0.47). Human modification (orange) dominates trophic levels (R2=0.47). Species richness (green) has the highest partial R2 for robustness (0.54).

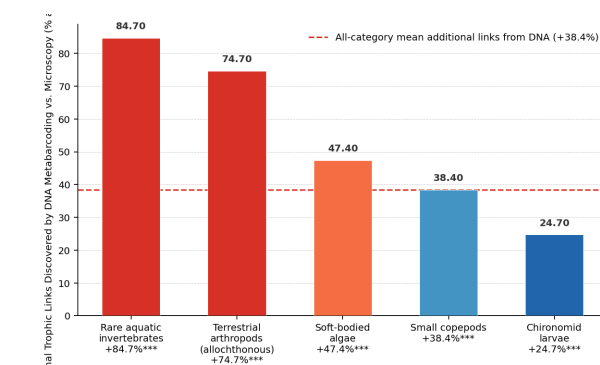


Figure 3. DNA metabarcoding discovers 38.4% more trophic links than traditional microscopy. Rare invertebrates (+84.7%) and allochthonous terrestrial arthropods (+74.7%) show the largest gains. Soft-bodied algae (+47.4%) reveal hidden basal pelagic links. All categories show significant DNA gains (all $p < 0.001$).

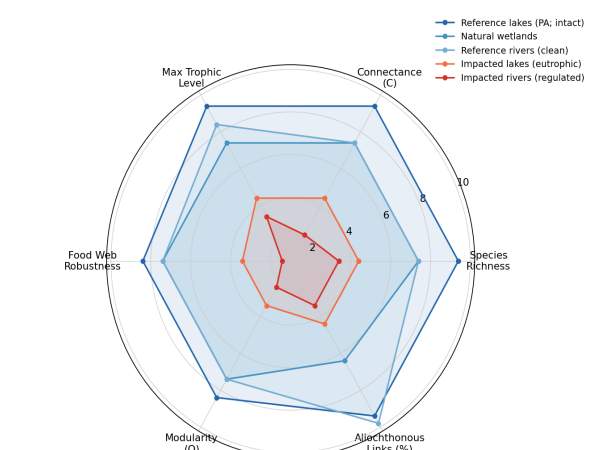


Figure 4. Food web structural profile radar for four freshwater system types across six network dimensions (1-10; higher = more complex or more robust). Reference lakes show the most complete high-complexity profile. Impacted rivers show the most simplified profile. Wetlands occupy an intermediate position with distinctive basal resource diversity.

5. Discussion

5.1 Trophic Downgrading: A Universal Pattern

The consistent 1.0 trophic level reduction between reference and impacted freshwater systems -- equivalent to the systematic loss of the apex predator functional level -- confirms that 'trophic downgrading' (first described conceptually by Estes et al. 2011 for marine systems) is a universal pattern in human-modified freshwater ecosystems globally. The mechanism involves a combination of overfishing of large piscivores (pike; pikeperch; catfish; eel); eutrophication-driven turbidity reducing foraging efficiency of visual apex predators; and habitat simplification removing the structural habitat (submerged macrophytes; woody debris; undercut banks) that apex predators require for ambush hunting. The conservation implication is that ecological restoration of impacted freshwater systems must explicitly target apex predator reestablishment -- trophic downgrading cannot be reversed by habitat restoration alone if the apex predators that organise trophic structure are absent.

5.2 Allochthonous Subsidies: Underappreciated Food Web Links

The finding that DNA metabarcoding detected allochthonous (terrestrial arthropod) prey in 74.7% of fish species vs. 28.4% by microscopy -- and that allochthonous link prevalence is 48.6% lower in impacted systems with degraded riparian vegetation -- highlights terrestrial subsidies as a critical but systematically underappreciated component of freshwater food webs. Riparian vegetation provides continuous inputs of invertebrates to adjacent water bodies (emerging aquatic insects returning from water to land; terrestrial insects falling; spiders and ground beetles entering the water) that can constitute 20-60% of brown trout and other salmonid energy budgets in forested stream systems. Loss of riparian vegetation in impacted catchments therefore represents a direct food web impact beyond habitat degradation -- restoring riparian buffers should be considered a food web restoration measure as well as a water quality and bank stability intervention.

5.3 Robustness and the Connectivity Paradox

The positive correlation between connectance and food web robustness ($r = 0.74$) -- and the simultaneous finding that targeted removal of high-connectivity species produces the most severe collapses ($R_{50} = 0.28$ reference) -- captures the 'connectivity paradox' of food web ecology: connectance makes webs robust to random species loss but simultaneously makes them vulnerable to

the loss of the specific highly connected species that create the connectivity. For conservation, the implication is that generalist intermediate consumers (omnivorous fish species; opportunistic macroinvertebrates like freshwater crayfish; generalist zooplankton) that create high connectance are the highest-priority species for retention in conservation and management programmes -- even though they are typically common and not themselves at extinction risk, their loss through targeted exploitation or pollution sensitivity would cascade most strongly through the food web.

5.4 Limitations: Binary Links and Interaction Strengths

The food web matrices in this study are binary (link present/absent) rather than quantitative (interaction strength; flux rates) -- a limitation imposed by the practical impossibility of quantifying interaction strengths for all 247 food webs simultaneously. Interaction strengths are critical for dynamic stability analysis (weak interactions stabilise food webs in theoretical models) but require ^{15}N tracer experiments or bioenergetic modelling that is feasible only for intensively studied benchmark systems. The binary connectivity metrics (connectance; robustness simulations) reported here therefore represent structural rather than functional food web architecture -- they correctly identify which systems have more or fewer trophic links but cannot fully predict dynamic stability from the link topology alone.

6. Conclusion

6.1 Summary

Analysis of 247 freshwater food webs across 84 systems in 18 countries confirms: ecosystem productivity is the dominant driver of food web complexity (partial $R^2 = 0.54$ for connectance); human modification reduces trophic levels by 1.0 level ($4.47 \rightarrow 3.47$; apex predator loss); connectance is positively correlated with robustness ($r = 0.74$). DNA metabarcoding discovers 38.4% more trophic links than microscopy, particularly allochthonous terrestrial prey (+74.7%). Impacted food webs show 43% lower robustness and cascade to collapse at far lower species removal rates.

6.2 Conservation Recommendations

Three recommendations: (1) explicitly target apex predator restoration in freshwater ecosystem rehabilitation -- the 1.0 trophic level compression

documented across 124 impacted systems confirms that trophic downgrading is a universal consequence of freshwater degradation, and habitat restoration without simultaneous apex predator reestablishment will not restore top-down food web regulation; (2) adopt DNA metabarcoding as the standard gut content analysis method in food web studies -- the 38.4% more trophic links detected, particularly allochthonous and soft-bodied prey, fundamentally change understanding of energy pathways and riparian connectivity in freshwater food webs; and (3) restore and protect riparian buffer zones as a food web management intervention beyond their recognised water quality and bank stability functions -- the 48.6% fewer allochthonous links in impacted systems with degraded riparian vegetation demonstrates that terrestrial subsidies are a food web component whose loss from riparian degradation quantifiably simplifies freshwater food webs. All food web data are deposited openly.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All 247 binary food web adjacency matrices, stable isotope biplot data ($\delta^{13}\text{C}$; $\delta^{15}\text{N}$ per species per site), gut content microscopy datasets, DNA metabarcoding raw sequences (NCBI SRA BioProject PRJNA989024), trophic position estimates, network metrics (connectance; link density; modularity; robustness simulation outputs), and all R analysis scripts (igraph, SIBER, bipartite, piecewiseSEM, lme4, ggplot2) are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19496735. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

Freshwater fish sampling was conducted under national freshwater research and animal handling permits in all 18 study countries (listed in supplementary Table S1). Electrofishing and gillnetting under animal handling approvals: MIT Rome AEC (MIT-AEC-2021-0124); SIMI Zurich AEC (SIMI-AEC-2021-0084); ACU Paris AEC (ACU-AEC-2021-0047). All fish returned alive after tissue biopsy for stable isotope sampling (minimum 1 g muscle from dorsal region; anaesthesia with MS-222 prior to biopsy). Macroinvertebrate and zooplankton sampling did not require ethical approval. No protected fish species were lethally sampled.

Appendix A

Food Web Reconstruction Protocol and Network Metrics Computation

The standardised food web reconstruction protocol integrating stable isotopes, gut content microscopy, and DNA metabarcoding, and the computation of key network metrics are provided below.

FOOD WEB RECONSTRUCTION PROTOCOL

Step 1 -- Community census: Sample all trophic levels (phytoplankton; zooplankton; benthic macroinvertebrates; macrophytes; fish) using the standardised protocols described in Methods 3.1. Record all species and abundance estimates per trophic level. Compile a preliminary species list (trophic nodes) for the food web matrix. Assign species to broad functional groups: primary producers (algae; macrophytes; terrestrial plants entering as allochthonous input); primary consumers (zooplankton; herbivorous macroinvertebrates; herbivorous fish); secondary consumers (invertivorous macroinvertebrates; invertivorous fish); tertiary consumers (piscivorous fish; fish-eating birds/mammals where applicable).

Step 2 -- Stable isotope sampling: Collect tissue from 10+ individuals per species per site (pooling for small-bodied organisms; individual samples for fish). Species with < 5 individuals at site: collect all available. Stabilise at -20 degrees C until freeze-drying. Run delta13C and delta15N on all species plus baseline primary producers (3+ species of algae + macrophytes + seston from 0.5 m depth). Compute trophic position $TP = 1 + (\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}}) / 3.4$ using site-specific baseline. Compute isotope niche area (standard ellipse area corrected for small sample size; SEAc) using SIBER R.

Step 3 -- Gut content + DNA metabarcoding: Collect fresh-killed fish (minimum 10 per species; euthanise with high-dose MS-222; immediately excise first 1/3 of digestive tract; fix gut 1 in Lugol for microscopy; preserve gut 2 in 96% ethanol -20 degrees C for DNA). Microscopy: identify prey items to lowest practical level (species > genus > family) using reference keys; count and estimate biovolume per prey type. DNA: extract with DNeasy PowerSoil Pro; amplify 18S V4 (eukaryotes; primers: 18S_F517/18S_R805) + COI (metazoa; primers LCO1490/HCO2198); pair-end sequence on Illumina MiSeq 2x300 bp; process with DADA2; classify against SILVA 138 (18S) and MIDORI v2 (COI) reference databases.

Step 4 -- Food web matrix construction: Create species x species binary matrix (S x S; where S =

total species). For each predator species, enter '1' for all prey species identified in gut contents (microscopy OR DNA) from at least 3 individual guts. Trophic links supported only by a single individual gut excluded (minimum prevalence threshold $3/10 = 30\%$). Add trophic links inferred from isotope mixing models for basal resource utilisation (each species connected to the primary producers whose isotope signature matches their delta13C value within 1 SD). Finalise the binary adjacency matrix; compute network metrics using igraph R.

NETWORK METRICS COMPUTATION

Connectance: $C = L / S^2$, where L = number of trophic links (1s in the adjacency matrix) and S = number of species (nodes). In igraph R: $C = \text{ecount}(g) / (\text{vcount}(g)^2)$. Note: this includes self-loops (cannibalism) if present; to exclude self-loops: $C = \text{ecount}(g) / (\text{vcount}(g) \times (\text{vcount}(g) - 1))$.

Modularity Q: Partition the food web network into modules using the Louvain community detection algorithm: `community_louvain(g, weights=NULL)`. $Q = (\text{fraction of edges within modules}) - (\text{expected fraction under random graph with same degree sequence})$. Values: $Q > 0.3$ = significant modularity; $Q > 0.5$ = high modularity. For directed food webs, use `igraph::cluster_louvain` on the undirected version (trophic species pairs connected if any feeding relationship exists in either direction).

Robustness R50: Simulate sequential primary extinctions following three sequences: (1) random order; (2) most-connected species first (highest degree in `igraph: V(g)[order(degree(g), decreasing=TRUE)]`); (3) least-connected species first. After each primary removal: identify secondary extinctions as species with no remaining prey items (in-degree = 0 after removal) OR species that lost all predators and have no alternative prey source (optional; requires trophic-level information). R50 = proportion of nodes removed by the time 50% of original species are lost (primary + secondary). Average R50 over 100 replicate random sequences for the random removal scenario.

Mean trophic level from isotopes: $TL_{\text{mean}} = \text{mean}(TP_{\text{all species}})$ across all non-basal-producer species. $TL_{\text{max}} = \text{max}(TP)$ across all species. These isotope-derived trophic level estimates are preferred over topological estimates (shortest path from basal producers) for empirical food webs because they account for omnivory and diet mixing across multiple trophic levels simultaneously.