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Habitat Preferences and Short-Term Dynamics of Alien Fishes Revealed by Environmental DNA in River Floodplains

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ABSTRACT

Floodplains are among the most diverse freshwater ecosystems, yet they are increasingly threatened by human-induced alterations. Recent research has shown that the ecological impacts of alien species in these systems can rival those of other anthropogenic disturbances. Effective management and mitigation strategies depend on accurate assessments of the distribution and temporal dynamics of alien species. However, traditional fish sampling methods are often inadequate in floodplains because their high spatial and temporal heterogeneity makes it impossible to apply a single traditional method effectively across different habitat types. In this study, we investigated how local habitat characteristics and regional-scale processes influence habitat use and the temporal dynamics of alien fish abundance inferred from environmental DNA in Danubian floodplain systems. By integrating community- and species-level analyses, we found that lateral hydrological connectivity is a key driver shaping alien fish community assembly, although the influence of other environmental factors, such as the local habitat structure, water chemistry, and land use, varied across taxa. We also revealed that the temporal dynamics of alien species were generally independent of local- and regional-scale drivers. This suggests that extrinsic factors like hydrology, connectivity, and resource availability may have only a limited influence on short-term eDNA-derived abundance fluctuations of alien fishes in floodplains. These findings have practical implications: management effectiveness is likely to vary across spatial scales and species, and control efforts may be more successful in areas where alien fish populations are less stable. Importantly, our study demonstrates that eDNA metabarcoding is a powerful tool for monitoring alien species in complex and dynamic environments such as floodplains. It enables efficient, non-invasive, and fine-scale detection of spatial and temporal patterns that would be challenging to capture through conventional sampling, thereby enhancing our ability to inform targeted and adaptive management strategies.

1 | Introduction

River-floodplain ecosystems are among the most species-rich environments worldwide (Tockner and Stanford 2002; Ward et al. 1999). These temporally dynamic systems include heterogeneous freshwater landscape elements—ranging from oxbow lakes and wetlands to main river channels—that provide a variety of habitats for organisms. Additionally, floodplains play a crucial role in supporting essential ecosystem services such as water purification, flood regulation and recreational

opportunities (Petsch et al. 2023; Tomscha et al. 2017). However, floodplains are among the world's most threatened ecosystems due to various human-mediated alterations, such as river regulation and damming, which degrade habitat quality and lead to fragmentation of the river-floodplain system (Agostinho et al. 2004; Nilsson et al. 2005; Ward and Stanford 1995).

The introduction and spread of alien species pose a major threat to native aquatic communities and overall ecosystem integrity (Vitule et al. 2009). Recent studies have increasingly emphasized that the impacts of invasive species on floodplain biota can be comparable to those of other anthropogenic disturbances (e.g., Ganassin et al. 2021; Ortmann-Ajkai et al. 2018; Rauber et al. 2025). Moreover, alterations to floodplain habitats can substantially increase the vulnerability of these systems to biological invasions by facilitating the spread of alien species, thereby creating a synergistic adverse impact on native organisms (e.g., Catford et al. 2011; Predick and Turner 2008; Wang et al. 2021).

In river-floodplain ecosystems, flood pulses and hydrological connectivity can play disproportionately important roles in shaping community structure compared to other factors (Erős et al. 2024; Larsen et al. 2019). For example, floods increase lateral connectivity, which is crucial for mobile aquatic organisms, such as fish, by enabling reproductive migrations, recruitment, gene flow, and access to food resources across habitat types (O'Mara et al. 2021; Stoffels et al. 2022). On the other hand, floods may also facilitate the dispersal and establishment of alien species by connecting habitat patches, substantially enhancing their success during the invasion process (De Amo et al. 2021; Thomaz 2022). Thus, an important first step toward the effective conservation and management of floodplain ecosystems is investigating how alien species are distributed within heterogeneous floodplain environments and how lateral connectivity, along with other factors, influences their occurrence and abundance patterns. However, disentangling the effects of local- and regional-scale processes on the habitat use and temporal dynamics of alien species in floodplains is challenging since local environmental conditions are strongly associated with flood dynamics (Zhang et al. 2024). Moreover, environmental variability has been shown to affect population growth and abundance of alien species in different ways, resulting in site- and species-specific differences in the invasion process (Thomaz 2022). By comparing the importance of regional and local factors on the establishment success and abundance of alien fishes in floodplains, Rayner et al. (2015), for example, emphasized the strong relationship between flood pulses and environmental conditions and highlighted that the timing of extensive floodplain inundations significantly influenced the reproduction and recruitment of alien fishes. At the species level, Espínola et al. (2014) found that the invasion of Peacock cichlid (*Cichla ocellaris* Bloch and Schneider 1801) coincided with a decrease in suspended sediment induced by changes in hydrology, while Jones and Stuart (2009) and Maiztegui et al. (2019) emphasized the overwhelming role of water level fluctuations during spring floods in the success of common carp (*Cyprinus carpio* Linnaeus 1758) invasion.

It is well-known that temporal fluctuations in species abundance are driven by spatial and temporal changes in habitat characteristics (Lande et al. 2003). Yet, while habitat use and spatial distribution of alien species have received considerable attention, the factors influencing their temporal dynamics remain comparatively understudied. Fauvergue et al. (2012), for example, suggested that population dynamics of alien species are influenced by the stage of invasion, with greater temporal fluctuations typically occurring during the initial stages. However, our understanding of how regional processes and local environmental conditions shape temporal variability in the abundance of alien species remains limited. These dynamics are likely to be highly species-specific, shaped by differences in ecological traits such as life-history, dispersal capacity, and environmental tolerance. Given the pronounced spatial and temporal heterogeneity of habitat characteristics in river-floodplains, these ecosystems offer an ideal natural setting for disentangling the respective roles of local and regional drivers in shaping the temporal dynamics of alien fishes.

Traditional fish sampling methods such as gillnets, fyke nets, and electrofishing can yield biased estimates of habitat use patterns for alien species, particularly in complex river-floodplain systems where habitat heterogeneity, both in size and structure, necessitates diverse gear types for representative sampling (De Leeuw et al. 2007; Knight and Bain 1996). To overcome these limitations, we employed environmental DNA (eDNA) metabarcoding, a non-invasive and highly sensitive approach that has consistently proven to be the most effective universal method for characterizing fish community structure across a wide range of freshwater habitats (e.g., Czeglédi et al. 2021; Hallam et al. 2021; Takahashi et al. 2023).

In this study, we investigated the influence of local habitat characteristics, such as physical habitat structure and water chemistry, and regional processes, such as land-use and hydrological connectivity, on the habitat use and temporal dynamics of alien fishes in Middle-Danubian floodplain systems using both community and species level analyses. We hypothesized that regional processes (primarily the level of flood-induced lateral connectivity) would have a dominant effect on the habitat use and eDNA-derived temporal abundance fluctuations of species (see Table 1 for species list), compared to local factors. However, we expected the importance and direction of these effects to be strongly species-specific, reflecting the varying habitat requirements and environmental tolerance of different species. We also predicted that alien species would exhibit lower temporal variability in abundance within their preferred habitats since they provide more favorable conditions for survival, reproduction, and recruitment. To our knowledge, this study is the first to quantify both the habitat use patterns and the spatial and temporal dynamics of alien fish species in such dynamic river-floodplain environments using eDNA.

2 | Material and Methods

2.1 | Study Area

Our study was conducted on two floodplain areas along the Danube River in Europe (Figure 1). Spanning a drainage basin of approximately 800,000 km^2 with an average river discharge rate of 6500 m^3/s at its mouth, the Danube crosses 19 countries, making it the most international catchment area in the world

TABLE 1 | Alien fish species detected by eDNA metabarcoding in the studied river-floodplains and their mean DNA copy number (±SD) across sampling sites and occasions.

Species scientific name	Species English name	Mean DNA copy number (±SD)
Ameiurus melas (Rafinesque 1820)	Black bullhead	310.1 (920.5)
Babka gymnotrachelus (Kessler 1857)	Racer goby	826.0 (1288.9)
Carassius gibelio (Bloch 1782)	Gibel carp	767.8 (1778.6)
Gasterosteus aculeatus (Linnaeus 1758)	Three-spined stickleback	0.6 (3.8)
Hypophthalmichthys molitrix (Valenciennes 1844)	Silver carp	287.4 (752.5)
Hypophthalmichthys nobilis (Richardson 1845)	Bighead carp	0.5 (6.8)
Lepomis gibbosus (Linnaeus 1758)	Pumpkinseed	490.1 (1453.6)
Micropterus salmoides (Lacepède 1802)	Largemouth black bass	2.2 (26.7)
Neogobius fluviatilis (Pallas 1814)	Monkey goby	14.7 (73.0)
Neogobius melanostomus (Pallas 1814)	Round goby	461.5 (1061.2)
Perccottus glenii (Dybowski 1877)	Chinese sleeper	27.7 (302.4)
Ponticola kessleri (Günther 1861)	Bighead goby	154.8 (537.3)
Proterorhinus semilunaris (Heckel 1837)	Western tubenose goby	532.4 (977.8)
Pseudorasbora parva (Temminck and Schlegel 1846)	Stone moroko	1265.7 (2552.0)

(ICPDR 2024). In the studied Danubian segment, the mean annual river discharge rate ranges between 1900 and 2400 m^3/s. One of the studied floodplains is located in the Donau-Auen National Park in Austria (AUT), while the other one in the Danube-Dráva National Park in Hungary (HUN). Both floodplains contain the full variety of river-floodplain functional habitat types, from side channels which are connected to the main channel at both ends to fully isolated oxbow lakes. Based on previous studies, the fish fauna (i.e., species pool) of the two studied systems is identical (Erős et al. 2017, 2024). Across 11 sampling occasions from 2021 to 2023 (11/08/2021; 23/09/2021; 10/03/2022; 04/05/2022; 24/06/2022; 19/08/2022; 20/10/2022; 30/03/2023; 14/06/2023; 30/08/2023; 20/09/2023), a total of 15–15 sites were investigated within the floodplains of AUT and HUN (Figure 1). These sites were chosen to represent the entire range of functional habitats, such as the main channel, permanently connected side arms, periodically connected side arms, floodplain lakes and the most isolated oxbow lakes.

2.2 | Sampling and Environmental Variables

Accurately determining spatio-temporal dynamics of species requires reliable estimates of abundance across multiple time periods. Several studies have shown that combining quantitative PCR (qPCR) with eDNA metabarcoding techniques can yield robust estimates of fish abundance in multiple habitat types (e.g., Doi et al. 2017; Pont et al. 2023; Spear et al. 2021). In this study, we thus used eDNA metabarcoding combined with qPCR to obtain a proxy of alien fish occurrence and abundance. eDNA samples were collected using in situ filtration with VigiDNA 0.45 μm capsules (SPYGEN), each filtering on average 18.5 L of water before preservation in CL1 buffer (SPYGEN). DNA extraction and amplification with teleo primers (Valentini et al. 2016), including 12 PCR replicates per sample, were performed following the protocol described in Pont et al. (2018). Sequencing was carried out on Illumina platforms. Bioinformatic processing with OBITools (Boyer et al. 2016) included read merging, demultiplexing, quality filtering, and taxonomic assignment using both public reference sequences and a local database (Pont et al. 2023). qPCR quantification was performed following Pont et al. (2023) using teleo primers. The quantity of teleo-DNA per sample was calculated from the ratio of taxa read counts over the total read count, multiplied by the teleo-DNA quantity extracted, and the final concentration of fish species DNA per liter was computed from the ratio of the quantity of DNA per taxon by the volume sampled for each sample. Extraction and amplification negative controls were processed in parallel. All standardized protocols are detailed in Appendix A.

Information on land-use within a 500 m wide buffer area around the water bodies was obtained from the CORINE Land Cover 2018 database (European Environmental Agency 2025, http://www.eea.europa.eu). The following five robust categories were used for the characterization of land-use: % agricultural, % forest and other seminatural habitats, % pasture, % urban areas, % water and wetland. Hydrological connectivity was defined as % mean number of days in a year a waterbody is connected to the main channel (i.e., Danube River). Local habitat structure variables were assessed following widely used and established survey methods (e.g., Gordon et al. 1992; Gorman and Karr 1978). Mean depth and mean current velocity were measured using a digital terrain model and a water velocity meter, respectively. Specifically, for mean depth elevation data were based on a digital terrain model (DTM) derived from a laser scan supplemented by bed elevation measurements from deeper side channels. Surface water levels in Austria were derived from the Austrian River Authority, and in Hungary from the National Water Authority. Within waterbodies, habitat structure was further characterized using visually estimated percentage composition of the following variables: emergent, submerged and floating vegetation, floating algae, open water habitat, woody debris. The bank structure was similarly characterized using the following

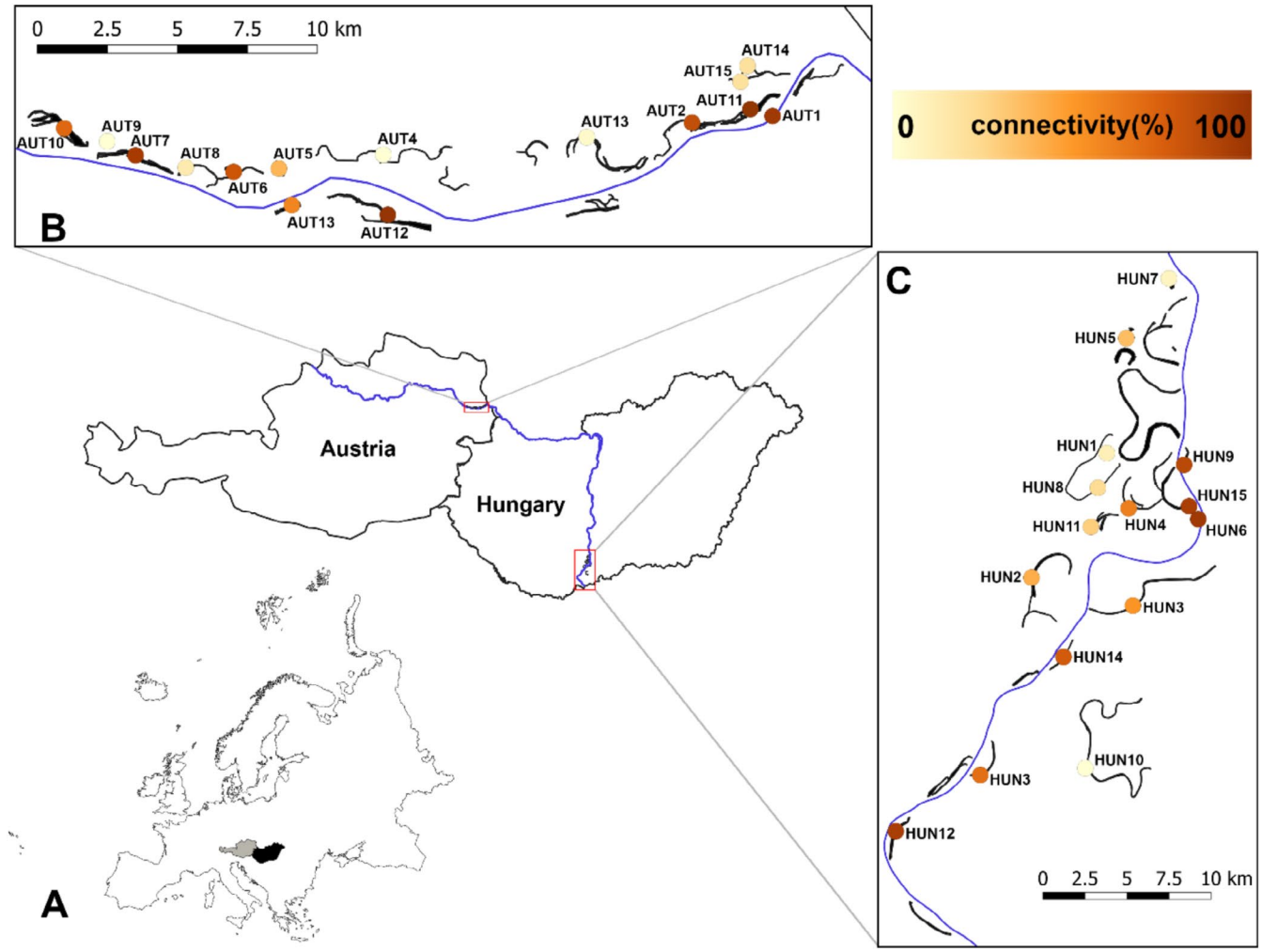

FIGURE 1 | Map of the study area (A) with the studied water bodies and sampling locations in Austria (B) and Hungary (C) (dots are colored based on their connectivity value).

variables: % woody (i.e., tree or large bushes), % herbaceous, % artificial (concrete, rip-rap). % canopy cover was also estimated. Substratum types were grouped into silt, sand, gravel and rock classes based on the particle size, and their percentage cover was estimated. Physicochemical variables were either measured in the field using portable sensors (pH, conductivity $\mu S\,cm^{-1}$, dissolved oxygen $mg\,L^{-1}$; Hach-Lange HQ40 sensor) or in the laboratory using water samples taken in the field (total suspended solids $mg\,L^{-1}$, particulate inorganic matter $mg\,L^{-1}$, total dissolved phosphorus $\mu g\,L^{-1}$, chlorophyll-a $\mu g\,L^{-1}$, CDOM—humic Pt color $mg\,L^{-1}$). To quantify chlorophyll-a 300–2000 mL of sample water was filtered through pre-combusted GF/C filters (Whatman). Chlorophyll-a was extracted in cold acetone (90%) and the absorption was measured using a spectrophotometer (Jeffrey and Humphrey 1975; Lorenzen 1967). Total P was digested with persulfate according to Eaton and Franson (2005) and analyzed complying with ÖNORM EN ISO 15681-2. To quantify total suspended solids (TSS) and particulate organic matter (POM) 300–2000 mL of sample water was filtered through pre-combusted GF/F filters (Whatman). For TSS the filters were dried at 80°C for 24 h. TSS was determined by weighing. POM was determined after ashing (450°C, 4 h). CDOM was measured following Cuthbert and Del Giorgio (1992) by filtering water with 0.45 μm Teflon membrane filters and measuring light absorbance at 440 nm.

2.3 | Data Analysis

All data analyses were performed in the R ver. 4.4.1 (R Core Team 2024). An alpha value of 0.05 was used to determine statistical significance of all tests. For describing general habitat characteristics and land-use, we carried out three separate principal component analyses (PCA) on the correlation matrix of the recorded physical and chemical environmental variables, as well as the land-use data using the package "factoextra" ver. 1.0.7 (Kassambara and Mundt 2016). PCA is a method that can reduce the dimensionality of complex datasets to a small number of largely independent (orthogonal) explanatory variables (here, physical, chemical and land-use gradients) (see e.g., Borcard et al. 2011; Czeglédi et al. 2025). Data from each sampling site and occasion (i.e., sampling period) were used for these analyses, except for those site–sampling period combinations where the given water body was dried up (10.4%). Altogether, 296 site–sampling period combinations were used. While several studies use multiple PCA axes

as predictors in further analyses, in our study the first axis of each PCA represented the main environmental and land-use gradients. These primary gradients are characteristic of this ecoregion and have been effectively used in previous research (e.g., Czeglédi et al. 2025; Feng et al. 2025). Thus, site coordinates along the first principal components (hereafter, gradients) were used as explanatory variables in subsequent analyses to ensure model parsimony and minimize the risk of overfitting. The importance of environmental and land-use variables in the distribution patterns of the sites along the first PCA axes was determined by calculating the Spearman's rank correlation coefficient between the component scores of the sites and the original variable values.

Temporal replication was treated differently across analytical sections to address distinct ecological questions. At the level of the alien fish community, we fitted a generalized linear mixed-effect model (GLMM) with a Poisson error distribution to explore the relationships between the number of alien fish species and local and regional drivers using the package "lme4" ver. 1.1.35.3 (Bates et al. 2015). We selected Poisson distribution because the response variable represents count data. We checked for overdispersion, and the ratio of residual deviance to residual degrees of freedom was <1, indicating no evidence of overdispersion (Gelman and Hill 2007). Prior to running the GLMM, multicollinearity among the predictors was assessed using Spearman's rank correlation (Shrestha 2020). Site ID and sampling occasion were included as random factors in the model to account for spatial and temporal clustering of observations. As fixed effects, we used the physical, chemical, and land-use gradients, as well as the hydrological connectivity values of the water bodies. Since desiccation tolerance of alien fishes may vary, a binary variable indicating whether a given water body is periodically subject to drying was also assigned to each site (hereafter, drying regime). Finally, to account for possible larger-scale spatial differences, we included the country identity (HUN vs. AUT) as an explanatory variable. This was also needed due to the differences in the history of species introductions between the two countries (Rabitsch and Essl 2006; Takács et al. 2017). We did not evaluate interaction effects in order to maintain model parsimony and to avoid overparameterization. Furthermore, given that most predictors were continuous, their interactions are challenging to interpret in a biologically meaningful way. Thus, in total, six predictors were used in the models. A separate GLMM with the same predictors was fitted on the total abundance of alien fish using the number of DNA copies per species as response variable. This metric, obtained by multiplying the species' relative abundance inferred from eDNA metabarcoding by the total number of fish eDNA copies quantified by qPCR and standardized by the volume of water collected, was used as a proxy for species-level fish abundance. Here, the Gaussian error distribution was used, because DNA copy number represents a continuous variable with sufficiently large range and resolution to be treated as approximately continuous. The response variable was $\ln(x+1)$ transformed to correct for violations of homoscedasticity and normality. After fitting the full models for both response variables, model selection was performed to select the final models based on the Akaike Information Criterion (AIC) using the package "MuMIn" ver. 1.48.4 and the function "dredge" (Bartoń 2024). This procedure performs full-subset selection, evaluating all possible combinations of the fixed effects in the global model, rather than stepwise (forward or backward) selection (Bartoń 2024).

We conducted redundancy analysis (RDA) on the $\ln(x+1)$-transformed eDNA-derived abundance data of alien fishes to explore general community-environment relationships, using the package "vegan" ver. 2.6-6.1 (Oksanen et al. 2013). The mean abundance of each species across sampling occasions was calculated prior to the analysis, and this data frame was used as the response matrix in the RDA. The mean component scores of the sites along the first axes of the physical, chemical, and land-use PCAs, as well as hydrological connectivity, drying regime, and country were used as predictors in the analysis.

At the species level, we fitted GLMMs with Gaussian error distribution to provide a more detailed understanding of species-environment interactions. We used the $\ln(x+1)$-transformed eDNA-derived abundance data of each alien species as response variables and the same predictors (fixed and random effects) and model selection method described in the previous GLMMs. Finally, generalized linear models (GLM) were used for characterizing the temporal dynamics of each species. For assessing temporal dynamics, we used the residuals from the linear regression of the logarithm of the sample variance against the logarithm of the sample mean across time periods (hereafter, stability values). This approach can serve as a measure of temporal stability, since it accounts for the systematic dependence of sample variance on sample mean (also known as Taylor's power law) (Döring et al. 2015; Döring and Reckling 2018). Thus, in contrast to the GLMMs, we did not retain temporal resolution or ordering here, as our objective was to quantify overall temporal variability in eDNA-derived abundance. Lower values of the index indicate higher stability over time. Here, we used the same predictors as in the RDA. After fitting the full models, a stepwise forward-backward selection was performed to select the final models based on the Akaike Information Criterion (AIC) for each species.

3 | Results

Across the 11 sampling occasions, a total of 14 alien fish species were detected by eDNA (Table 1). The occurrences of *Hypophthalmichthys nobilis* (Richardson 1845), *Micropterus salmoides* (Lacepède 1802), and *Perccottus glennii* (Dybowski 1877) were extremely sporadic (1.6%, 1.3%, 3.4% occurrence rates, respectively) in space and/or time; therefore, they were excluded from the RDA and species-specific GLMMs. *Neogobius fluviatilis* (Pallas 1814) and *Gasterosteus aculeatus* (Linnaeus 1758) were also rare, occurring only in 20% and 17% of the sites, respectively, which prevented the analysis of their stability values in GLMs.

The first axis of the PCA based on the physical habitat variables revealed a main gradient among the studied sites (explained variance: 26.4%) (Table 2). This gradient separated sites characterized by deeper water, higher velocity, coarse substrate, and more artificial riparian modifications (more negative values) from those with more natural riparian vegetation and canopy cover, denser macrophyte cover, more woody debris, and finer substrate (more positive values). PCA on chemical variables

TABLE 2 | Spearman's rank correlation coefficients between site component scores from PCAs conducted on physical, chemical, and land-use variables, and the original variable values.

Physical	PC1 (explained variance: 26.4%)
Depth (m)	−0.51
Current velocity ($cm\,s^{-1}$)	−0.80
Aquatic vegetation (emergent) (%)	0.44
Aquatic vegetation (submerged) (%)	0.36
Aquatic vegetation (floating leaved) (%)	0.53
Aquatic vegetation (surface algae) (%)	0.13
Open water (%)	−0.75
Woody debris (%)	0.53
Shoreline (woody) (%)	−0.02
Shoreline (herbaceous) (%)	0.11
Shoreline (artificial) (%)	−0.42
Canopy cover (%)	0.44
Substrate (rock) (%)	−0.30
Substrate (gravel) (%)	−0.60
Substrate (sand) (%)	−0.52
Substrate (silt) (%)	0.80
Chemical	**PC1 (explained variance: 27.5%)**
Dissolved oxygen ($mg\,L^{-1}$)	−0.37
Conductivity ($\mu S\,cm^{-1}$)	−0.05
pH	−0.52
Particulate inorganic matter ($mg\,L^{-1}$)	0.24
Total phosphorus ($\mu g\,L^{-1}$)	0.85
Chlorophyll-a ($g\,L^{-1}$)	0.69
CDOM—humic (Pt color $mg\,L^{-1}$)	0.81
Total suspended solids ($mg\,L^{-1}$)	0.50
Land-use	**PC1 (explained variance: 43.4%)**
Agricultural (%)	0.67
Forest and seminatural (%)	−0.97
Pasture (%)	0.47
Urban (%)	0.44
Water and wetland	0.57

Note: Black color indicates negative, while gray color indicates positive relationships.

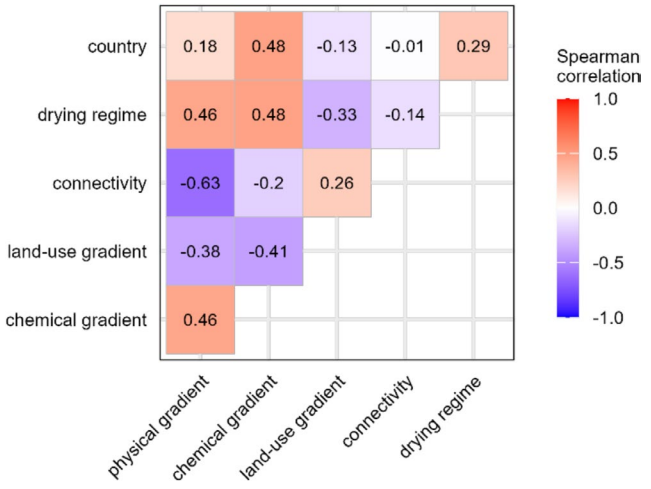

FIGURE 2 | Spearman's rank correlation coefficient between predictors used in data analyses.

separated sites with more alkaline and oxygen-rich water (more negative values) from more productive sites with higher concentrations of particulate inorganic material, total suspended solids, total phosphorus, chlorophyll-a, and CDOM (more positive values) along the first axis (explained variance: 27.5%) (Table 2). Finally, the first axis of PCA on land-use variables distinguished sites dominated by forested and seminatural areas (more negative values) from those with greater artificial modification (urban and agricultural land-use), as well as more pasture and wetland cover (more positive values) (explained variance: 43.4%) (Table 2).

Since the Spearman's rank correlation coefficient between all predictor pairs was below 0.7 (Figure 2), we retained all the six predictors in the GLMMs and further models (Dormann et al. 2013). According to GLMM, hydrological connectivity, country, and the chemical gradient were the significantly important predictors of alien species number in the studied floodplain systems, while the impact of the land-use gradient was marginally significant ($p = 0.0584$). Species number increased with connectivity (i.e., from isolated habitats to the main channel) and was higher in the Hungarian than in the Austrian floodplain sites (Appendix B). In addition, number of alien species decreased with increasing scores along the chemical gradients, while increasing scores along the land-use gradient were associated with higher alien species richness. In terms of total abundance, country was the only significant predictor in the model, with significantly higher alien species abundance in Hungary compared to Austria (Appendix B).

Predictors in the RDA explained a statistically significant (adjusted $R^2 = 0.60$, $p < 0.001$) amount of variation in eDNA-derived abundance-based community structure. All predictors, except for the land-use gradient ($p = 0.462$), proved to be significant in the model ($p < 0.001$). The physical gradient explained the highest proportion of variance, followed by country and hydrological connectivity (Figure 3, Appendix C). The chemical gradient and drying regime explained a smaller portion of the variance. Overall, the first RDA axis captured shifts in community structure and dominance patterns from highly connected sites to more isolated habitats and highlighted the influence of environmental gradients on community assembly. The second axis primarily distinguished Hungarian and Austrian sites based on differences in community structure.

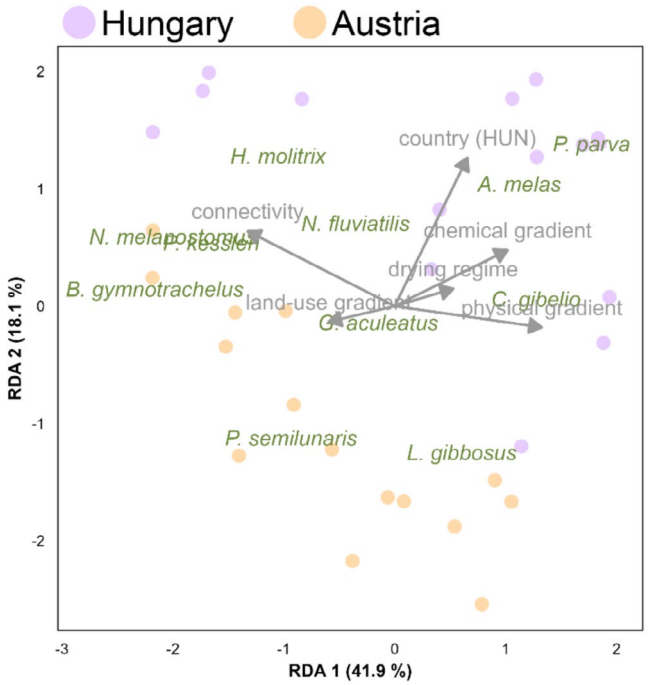

FIGURE 3 | Redundancy analysis of the studied sites based on the ln(x+1)-transformed abundance data of alien fishes and their associated predictor variables.

Species-specific GLMMs provided more detailed insights into the role of the predictors in determining eDNA-derived species abundances. Hydrological connectivity was retained in the final models of seven out of the 11 species (Appendix B). The models fitted for *Hypophthalmichthys molitrix* (Valenciennes 1844) and Ponto-Caspian gobies (except for *Proterorhinus semilunaris* Heckel 1837) identified hydrological connectivity as a significant predictor. The number of DNA copies of these species increased with higher connectivity values (Figure 4). This predictor was also significantly important in the final models for *Ameiurus melas* (Rafinesque 1820) and *Lepomis gibbosus* (Linnaeus 1758), but with an opposite effect: the number of DNA copies of these species decreased with increasing connectivity. Country was included in seven final models and emerged as a significant predictor of eDNA-derived abundance for *A. melas, Pseudorasbora parva* (*Temminck & Schlegel 1846*), *N. fluviatilis*, *P. semilunaris* and *G. aculeatus*. The first three species showed a higher number of DNA copies in Hungary (Figure 4), whereas the latter two exhibited higher DNA copy numbers in Austria. Drying regime was retained in five final models. The number of DNA copies of *Babka gymnotrachelus* (Kessler 1857), *L. gibbosus* and *P. semilunaris* was significantly higher in perennial water bodies, while *Carassius gibelio* (Bloch 1782) and *A. melas* had significantly higher values in intermittent sites. Although the chemical gradient was retained in four final models, it had a significant impact only on the DNA copy numbers of *A. melas*, *L. gibbosus*, and *P. semilunaris*. For the former species, copy number increased, while for the latter two, it decreased with increasing gradient values. The DNA copy number of *H. molitrix* also decreased along this gradient, but this relationship was only marginally significant ($p=0.0685$). The role of the physical gradient was less pronounced compared to the RDA results, as it was retained in only four final models. Nevertheless, in those models the gradient exhibited a significant effect. The number of DNA copies of *C. gibelio* and *P. parva* increased, while those of *Neogobius melanostomus* (Pallas 1814) and *Ponticola kessleri* (Günther 1861) decreased with higher gradient values. Of the four models that included land-use, this predictor had a significant influence only on the DNA copy numbers of *P. parva*, which decreased with increasing land-use gradient values.

GLMs fitted to stability values revealed variable patterns between the predictors and temporal dynamics across species; however, these relationships were generally not significant (Figure 4, Appendix D). The few exceptions included significantly more stable DNA copy numbers of *N. melanostomus* and *C. gibelio* in Hungary and the significantly more stable DNA copy number of *L. gibbosus* in Austria. Additionally, DNA copy number of *C. gibelio* was more stable in intermittent water bodies, while DNA copy number of *H. molitrix* were more stable at sites with higher physical gradient values. Some marginally significant relationships were also detected: the number of DNA copies of *B. gymnotrachelus* was temporally more stable at highly connected sites ($p=0.0671$), DNA copy number of *C. gibelio* was more stable at lower land-use gradient values (i.e., higher percentage of forested and seminatural area) ($p=0.0810$), and DNA copy number of *H. molitrix* was more stable in Austria compared to Hungary ($p=0.0766$).

4 | Discussion

Flood-induced lateral hydrological connectivity played a major role in alien fish community assembly. Our study was based on a combination of qPCR and eDNA metabarcoding approaches. Both eDNA metabarcoding proportions and qPCR estimates may be subject to method-specific biases and variance. This means that eDNA-derived abundance values may be over- or underestimated for some taxa or samples. However, as a standardized protocol was applied consistently across all sites and years, potential method-specific biases are likely to affect all samples in a similar way, making relative comparisons between samples and overall spatial and temporal trends robust. Indeed, our results support previous findings that highlighted the overarching impact of hydrological connectivity on the distribution patterns of floodplain fishes. However, earlier studies primarily focused on general community assembly patterns (e.g., Erős et al. 2024; Hurd et al. 2016), considered longitudinal connectivity as the main driver (e.g., Radinger and García-Berthou 2020) or examined single-species responses (e.g., De Amo et al. 2021). To our knowledge, this is the first study which provides a comprehensive assessment of how environmental gradients, lateral hydrological connectivity, and geographic context shape the distribution and temporal dynamics of alien fishes in river-floodplain systems.

Alien species richness was positively associated with hydrological connectivity, indicating that the main channel and highly connected sites provide heterogeneous environmental conditions conducive to supporting various species throughout at least part of their life cycle. Additionally, factors such as geographical location, historical biogeographic events, and the species–area relationship also contributed to the elevated species richness observed in the main channel (Oberdoff et al. 1995).

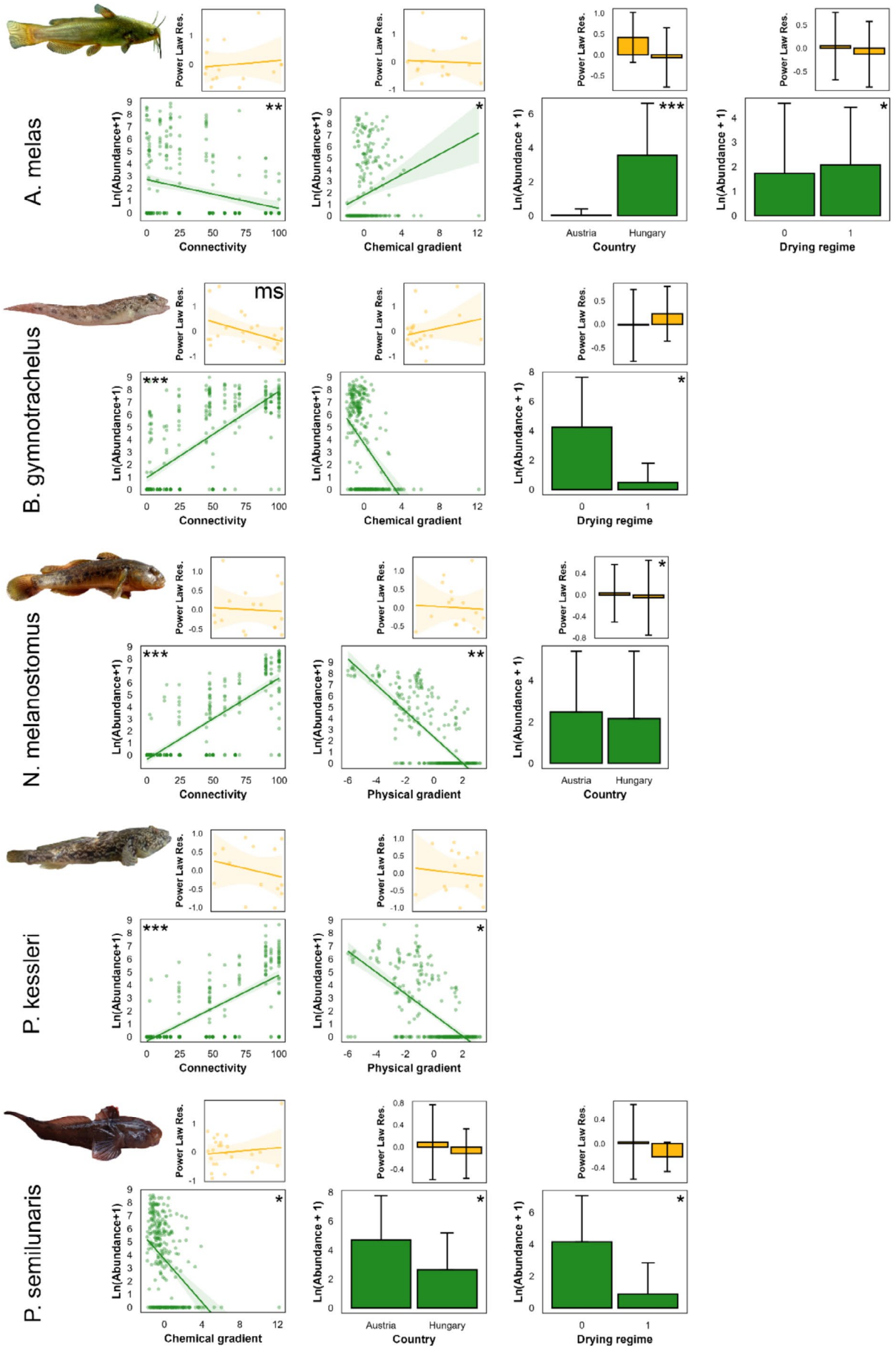

FIGURE 4 | Legend on next page.

FIGURE 4 | Relationships between the abundance and stability values (Power Law Residuals, see Section 2 for details) of alien species and predictors. Only significant associations are presented, where either abundance or stability values were significantly explained by a given predictor as determined by GLMM (for abundance) or GLM (for stability). Shaded areas represent 95% confidence intervals. *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$, ms: marginally significant. In bar plots with drying regime as the predictor, "0" indicates permanent and "1" indicates intermittent water bodies. Error bars on the boxplots indicate standard deviations. Please note that *Neogobius fluviatilis* and *Gasterosteus aculeatus* were rare, preventing the analysis of their stability values in GLMs. Photographs of fishes: Péter Takács.

Moreover, the Danube River is one of the main corridors for the spread of aquatic alien species in Europe (Paunović et al. 2015), facilitating the occurrence of both riverine and lentic species—at least periodically—at highly connected sites. Although the influence of connectivity was largely species-specific, it emerged as one of the most important explanatory variables in the RDA and was included in the majority of species-specific GLMs. These results highlight that connectivity plays a critical role not only at the community level but also at the species level—an insight particularly important for the management of alien species. For example, for controlling the populations of *A. melas* and *L. gibbosus*, species that preferred more isolated habitats, local-scale management actions (e.g., targeted removal) could be effective. These species find favorable conditions for establishment in these, primarily oxbow lakes, likely due to reduced predation pressure and the availability of suitable reproductive habitats (Penczak et al. 2004). In contrast, goby species and *H. molitrix*, whose eDNA-derived abundance was significantly higher in more connected water bodies, would require broader, regional-scale management strategies. These species are known to prefer main river channels and side channels, habitats that offer increased opportunities for dispersal, access to refugia, and resource availability for them (Harka and Bíró 2007; Prechtel et al. 2018).

Although only moderate to low correlations were found among the predictors, we assume that habitat preferences of the studied species are, to some extent, captured by the interrelationships among the predictors. For example, we found that *P. kessleri* and *N. melanostomus* preferred water bodies with deeper water, higher flow velocity, coarse substrate, and more artificial riparian modifications—habitats typically occurring in highly connected areas, primarily in the main channel of the Danube River and its most closely connected side channels. Similarly, *B. gymnotrachelus* favored highly connected sites, which are typically more oxygen rich, while *A. melas* preferred isolated oxbow lakes—habitats that are generally more productive, highlighting the link between connectivity and chemical gradient. Finally, regarding the continuous predictors, land use influenced the habitat use of *P. parva*, with higher abundances occurring in more natural landscapes (i.e., more forested areas). However, similarly to other species favoring slow-flowing or lentic waters, *P. parva* preferred still, macrophyte-rich habitats, which were mainly located in forested areas within the studied floodplains. Although several authors suggested that explanatory variables with correlation values below 0.7 (Dormann et al. 2013) or 0.8 (Shrestha 2020) could be retained in statistical modeling, our results indicate that distinguishing the impacts of predictors can be challenging even when correlations are moderate to low. Furthermore, the observed differences in the importance of the physical gradient between community- and species-level analyses highlight the need of integrating multiple ecological scales to more comprehensively understand alien fish distribution patterns and community assembly processes.

The total number of alien species DNA copies, as well as the eDNA-derived abundance of *A. melas*, *P. parva*, and *N. fluviatilis*, were significantly higher in Hungary than in Austria. This pattern can be—at least partially—attributed to historical factors. Hungary was part of the former socialist bloc prior to 1990 and during this period, several alien fish species were intentionally introduced into fishponds, from which they later escaped and established self-sustaining populations (Takács et al. 2017). *A. melas* was introduced deliberately for aquaculture purposes, whereas *P. parva* was accidentally introduced along with stocking material of commercial fish species, such as common carp (*Cyprinus carpio*) and Asian carps (*H. molitrix* and *H. nobilis*). However, eDNA-derived abundances of *P. semilunaris* and *G. aculeatus* were higher in Austria, which may also reflect differences in floodplain morphology or regional propagule pressure. For instance, intentional local releases of *G. aculeatus* by aquarists are plausible, given its popularity as an ornamental fish in the region (Makhrov et al. 2025).

A notable observation of our study is the higher mean DNA copy numbers of *A. melas* and *C. gibelio* in periodically drying water bodies compared to perennial ones, supporting previous findings that alien species—especially opportunistic taxa with broad environmental tolerance—can thrive under variable and stressful conditions (García-Berthou 2007; Marchetti et al. 2004). This is particularly concerning in the context of climate change coupled with increasing human demand for water, both of which are expected to increase the frequency and extent of drying events in many aquatic ecosystems in this region—conditions that may favor the establishment and spread of alien species while disadvantaging native ones (Bêche et al. 2009; Ramírez et al. 2018). In this context, future studies should focus on evaluating and comparing the tolerance thresholds and recolonization dynamics of native and alien fish species in intermittent floodplain habitats. Such insights would be essential for guiding hydrological restoration strategies (e.g., enhancing connectivity, maintaining permanent water levels) that prioritize the resilience and long-term conservation of native fish assemblages while mitigating the proliferation of alien taxa (Funk et al. 2023).

Contrary to our initial predictions, we found that the temporal dynamics of alien species were generally independent of local- and regional-scale drivers, at least as inferred from eDNA copy numbers used as proxies for true population size. This suggests that extrinsic predictors such as hydrological variability, connectivity, or resource availability may play only a limited role in regulating short-term fluctuations in the eDNA-derived abundance of alien fishes in floodplain systems. Several explanations may account for this pattern. First, temporal dynamics of alien

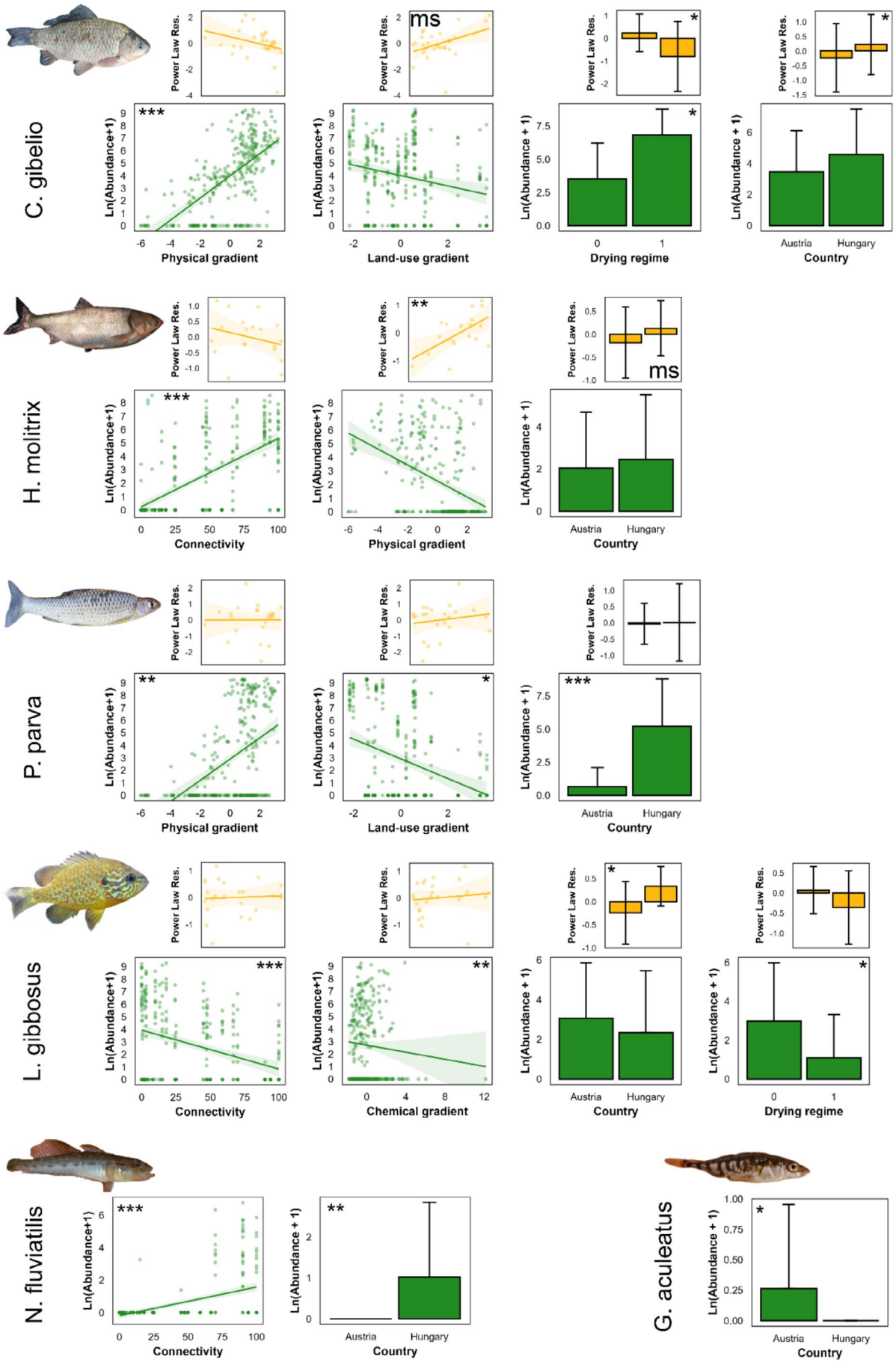

FIGURE 4 | (Continued)

species may be more strongly governed by intrinsic traits (e.g., life-history strategies, physiological tolerance), which are key drivers of alien fish establishment and spread (Olden et al. 2006; Ribeiro et al. 2008; Vila-Gispert et al. 2005), although their roles in abundance fluctuations remain poorly understood. Second, it is also possible that human-mediated factors, particularly propagule pressure, exert a stronger influence on these dynamics than the predictors considered in this study (Copp et al. 2007; Woodford et al. 2013). Third, the weak relationships observed may reflect the broad ecological tolerance and behavioral flexibility of alien species. Environmental fluctuation proved to be a crucial stressor in native fish population dynamics in riverine ecosystems (Magalhães et al. 2003; Schlosser 1995; Tonkin et al. 2019), with populations generally exhibiting greater temporal stability in less dynamic environments (Czeglédi et al. 2022). By contrast, we did not detect this pattern for alien species, even though our study encompassed habitats with varying degrees of environmental fluctuation induced by, for example, flooding. It is possible that the time scale, over which the study was conducted, was too short to detect such an impact of environmental factors. Long-term studies examining the temporal dynamics of freshwater fishes have primarily focused on native or even endangered species (e.g., Magalhães et al. 2003; Tonkin et al. 2019; Van Haverbeke et al. 2013). Future studies that simultaneously evaluate the long-term population trajectories of both native and alien species are required to disentangle their relative sensitivities to environmental change and to determine the temporal scales over which such responses occur (see e.g., Rocha et al. 2025).

The effectiveness of management actions targeting alien fish depends on multiple factors, including the stage of invasion, the abundance and density of alien species, habitat characteristics, and the eradication method applied (Donaldson and Cooke 2016; Rytwinski et al. 2019). Since temporally variable fish populations may be more sensitive to environmental and anthropogenic disturbances, we assume that eradication operations would require less effort in sites where populations are less stable. Although only a few relationships between temporal dynamics and predictors were detected, we suggest that targeting temporally variable populations may help prevent re-establishment, as these populations might have lower reproductive capacity or weaker resilience to additional stressors. However, future studies are needed to investigate how population dynamics influence the outcomes of alien species management efforts.

Five rare or sporadically detected alien species were excluded from species-level and stability analyses due to insufficient temporal replication. While this was necessary for statistical robustness, it may bias our interpretations toward well-established alien species and limit inference about early-stage or transient invasions. Therefore, our conclusions primarily reflect the temporal dynamics of consistently detected alien taxa rather than newly emerging invaders.

Finally, our findings reinforce previous research demonstrating the effectiveness of eDNA techniques in evaluating fish communities and species in freshwater ecosystems (e.g., Curto et al. 2025; Hallam et al. 2021; Sard et al. 2019). We successfully applied this method across various floodplain habitat types—where even multiple traditional sampling methods may yield biased community estimates (Czeglédi et al. 2021)—to uncover habitat use and temporal dynamics of alien fishes. Although several studies reported strong correlations of DNA copy numbers and true abundance or biomass using qPCR of eDNA (e.g., Benoit et al. 2023; Pont et al. 2023; Spear et al. 2021), it is important to note that eDNA-derived abundance estimates may not fully reflect actual population sizes due to the multiple factors affecting DNA concentration, degradation and drift in aquatic environments (Joseph et al. 2022; Pont 2024). However, despite the potential limitations, we suggest that, when applied at appropriate spatial and temporal scales, eDNA techniques can offer a highly effective tool for monitoring changes in fish communities, particularly for tracking the dynamics of alien species.

Author Contributions

Conceptualization: István Czeglédi and Tibor Erős. Data curation: István Czeglédi, Tibor Erős, Andrea Funk, Paul Meulenbroek, Bálint Preiszner, Didier Pont, Thomas Hein, Alice Valentini. Data analysis: István Czeglédi. Methodology: István Czeglédi, Tibor Erős, Andrea Funk, Paul Meulenbroek, Bálint Preiszner, Didier Pont, Thomas Hein, Alice Valentini. Visualization: István Czeglédi. Writing – original draft: István Czeglédi. Writing – review and editing: Tibor Erős, Andrea Funk, Paul Meulenbroek, Bálint Preiszner, Didier Pont, Thomas Hein, Alice Valentini.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in HUN-REN ARP Research Data Repository at https://researchdata.hu/en.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix A.** eDNA metabarcoding analysis. **Appendix B.** The results of the generalized linear mixed-effects models (GLMM) for total species number, total abundance, and the abundance of each species. **Appendix C.** The results of the redundancy analysis on the abundance data of fishes. **Appendix D.** The results of the generalized linear models (GLM) for the temporal population dynamics of each species.