ECOLOGY

Ecological similarity governs non-native fish establishment while human pressure and native diversity shape invasion richness

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Biological invasions by non-native fish species pose a major threat to global freshwater ecosystems. However, our understanding of why invaders establish in some communities but not others (i.e., occurrence probability) and why some communities harbor more invaders than others (i.e., invasion richness) remains limited. Here, we combine species- and community-level analyses across taxonomic, phylogenetic, and functional dimensions to investigate both the occurrence probability and invasion richness of exotic and translocated fish species across nearly 3000 North American freshwater fish communities. We found that ecological similarity between non-native and native species primarily governed non-native fish establishment, with invaders more likely to succeed when closely related to native species. In contrast, invasion richness was largely promoted by human pressure, while native diversity reduced richness specifically for translocated species. Additionally, both the occurrence and richness of invaders increased toward lower latitudes. Together, these findings highlight the distinct yet complementary roles of environmental filtering, human activities, and biotic interactions in shaping freshwater fish invasions.

INTRODUCTION

In the Anthropocene, the widespread invasions of non-native species pose a substantial and escalating threat to global biodiversity, economies, and human well-being (*1*–*3*). Freshwater ecosystems, in particular, are highly susceptible to non-native fish invasions, experiencing biodiversity loss at rates exceeding those of other ecosystems (*4*–*6*). These invasions disrupt the community structure and functions (*7*, *8*), leading to substantial economic impacts worldwide (*9*, *10*). Understanding the key mechanisms driving the success of these invaders is crucial for developing effective strategies to manage and mitigate the impacts of freshwater invasions. Two of the most fundamental aspects of biological invasions—occurrence probability, which primarily reflects the establishment likelihood of non-native species, and invasion richness, which refers to the number of established non-native species and mainly indicates invasion extent—are well recognized (*11*–*13*). However, in freshwater ecosystems, why invaders are more likely to establish in some communities than others (i.e., higher occurrence probability) and why some communities harbor more invaders than others (i.e., higher invasion richness) remain poorly understood.

Traditionally, many studies have sought to understand the invasion outcomes of non-native fish species by investigating species invasiveness, which typically focuses on the functional traits of non-native species (*11*, *14*, *15*). While species invasiveness provides insights into why different invaders vary in success, it remains challenging to explain why the same invader experiences different levels of success across communities. Phylogenetic similarity, which measures the phylogenetic distance between invaders and native species, provides an insightful perspective on why certain invaders succeed in specific communities. Charles Darwin proposed two seemingly contradictory hypotheses to understand the relationship between phylogenetic similarity and invasion success. In his first hypothesis, Darwin proposed that non-native species closely related to native species tend to be more successful because they might favor similar environments to their native relatives, which is known as the preadaptation hypothesis (*16*). In his second hypothesis, Darwin postulated that non-native species phylogenetically distinct from native species are more likely to establish, because they might share fewer natural enemies and experience less competition with native species, which is referred to as Darwin's naturalization hypothesis (*17*). These two opposing hypotheses, which emphasize the dominant roles of environmental adaptation and biotic interactions in understanding the effect of phylogenetic similarity on invasion outcome, have been collectively referred to as Darwin's naturalization conundrum (*18*). Building on the assumption that closely related species share similar traits, functional similarity has been incorporated into Darwin's framework to provide a comprehensive understanding of the relationship between ecological

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similarity and biological invasions (*19*–*21*). Recent studies have shown that Darwin's framework can help explain fish invasions in freshwater ecosystems (*22*–*24*). However, how ecological similarity influences different aspects of fish invasions—specifically occurrence probability and invasion richness—remains unclear.

In addition to ecological similarity, biotic and abiotic characteristics of the native community also influence the invasion success of non-native fish species. In particular, the diversity of the native community can have opposing effects on invasion success based on a similar consideration to Darwin's naturalization conundrum, as described by the invasion paradox (*25*, *26*). On the one hand, higher native diversity may reduce invasions by limiting the niche space and resources available to non-native species (*27*, *28*). On the other hand, diverse communities may be more susceptible to invasions, as the favorable environmental conditions supporting rich native species can also benefit non-native species (*29*). Geographic attributes of native communities, such as latitude—which reflects large-scale climate conditions—and geographic area, have also been shown to correlate with non-native species richness (*30*, *31*). In addition, human activities, recognized as a major driver of propagule pressure and ecosystem disturbance, are also closely associated with biological invasions (*26*, *32*). However, how these factors influence the occurrence probability and invasion richness of non-native fish species remains underexplored, and their relative roles compared to ecological similarity in shaping both aspects of fish invasions are still unknown.

To fully understand the impacts of species-level similarity and community-level characteristics on fish invasions, several potential confounding factors must be explicitly considered. First, while phylogenetic and functional similarities may not always provide consistent insights into invasion outcomes (*22*), combining both aspects is crucial for a comprehensive understanding of similarity's effect. Similarly, species diversity alone may not fully capture the proportion of niches occupied within the native community (*33*). Therefore, integrating phylogenetic and functional diversity is essential for more accurately representing the roles of native diversity. Second, spatial scales are often cited as a key factor contributing to inconsistent results regarding the effects of ecological similarity (*34*) and native diversity (*35*) on invasion success. Accounting for variation in spatial scales is necessary to understand the processes driving the success of non-native fish species. Third, the origin of non-native fish species, whether introduced from another continent (exotic species) or translocated within a continent across different watersheds (translocated species), is crucial for understanding their success and ecological impacts (*7*, *23*, *36*). Distinguishing between these two types of non-native fish species and identifying the mechanisms driving their success would provide a more comprehensive understanding of fish invasions.

North America is one of the regions most severely affected by fish invasions, boasting the most comprehensive and systematic records of non-native fish species (*11*, *37*–*39*). This provides a unique opportunity to explore the key mechanisms driving their invasion success. By compiling occurrence and richness data, along with taxonomic, phylogenetic, and functional information for 921 fish species (including both native and non-native species) across 2993 North America watersheds (Fig. 1 and fig. S1), we investigated the factors influencing the occurrence likelihood and invasion richness for exotic and translocated fish species at local and regional scales. Our findings reveal that ecological similarity predominantly governs the occurrence of non-native fish species, while human pressure and native diversity primarily shape their richness, emphasizing how environmental filtering, anthropogenic influence, and biotic interactions differentially drive the establishment and richness of non-native species.

RESULTS

Effects of multiple factors on the occurrence probability of non-native fish species

Across North America, 334 non-native fish species have successfully established in 2309 of the 2993 watersheds (77.2%). Among these, 62 exotic fish species have established in 1624 watersheds (54.3%), while 272 translocated fishes have established in 2170 watersheds (72.5%) (Fig. 2). The occurrence probability of non-native fish species significantly decreased as their phylogenetic and functional distances from native species increased, indicating a higher likelihood of establishment for non-native species that were more similar to native species (Fig. 3 and tables S1 to S4). This relationship remained consistent whether these non-native species were introduced from other continents or translocated among watersheds within North America and held true regardless of whether dissimilarity was measured using phylogenetic or functional distances (Fig. 3, A, B, D, and E), with phylogenetic distance providing a slightly better fit to the data (tables S14 and S15). Furthermore, similarity emerged as the most important factor in predicting non-native fish occurrence (except in the case of translocated fish species, for which functional similarity was the second most important predictor), accounting for 23.7 to 46.3% of the explained variance (Fig. 3, C and F). These relationships remained robust even after statistically accounting for the phylogenetic nonindependence of samples (tables S5 to S8) and when further evaluated at the regional watershed scale (figs. S2 and S3). Native species richness positively predicted the occurrence probability of non-native fish species at the local watershed scale, but this effect was not evident at the regional scale (Fig. 3 and fig. S3). Higher latitude was associated with lower occurrence probability, while increased human pressure was associated with greater occurrence (Fig. 3 and figs. S3 and S4). Although these effects were significant and may interact with phylogenetic and functional similarities (figs. S5 and S6), they explained only a limited portion of the variation in occurrence probability compared to the stronger influence of similarity (Fig. 3 and fig. S3).

Effects of multiple factors on the invasion richness of non-native fish species

The invasion richness of non-native fish species significantly increased with increasing human pressure (Fig. 4 and tables S9 to S12). This relationship held true for both exotic and translocated fish species (Fig. 4, A, B, D, and E) and remained significant after considering the impact of spatial scales (fig. S7). Moreover, human pressure was the strongest predictor of exotic fish richness and the second most important for translocated species, accounting for 20.5 to 32.4% of the explained variance (Fig. 4, C and F). The influence of human pressure was even more pronounced at the regional watershed scale, accounting for 21.4 to 37.6% of the explained variance (fig. S7, C and F). In contrast, phylogenetic and functional similarities played a limited role in explaining the invasion richness of non-native fish species and their effects varied between exotic and translocated species (Fig. 4 and fig. S7). Native species richness

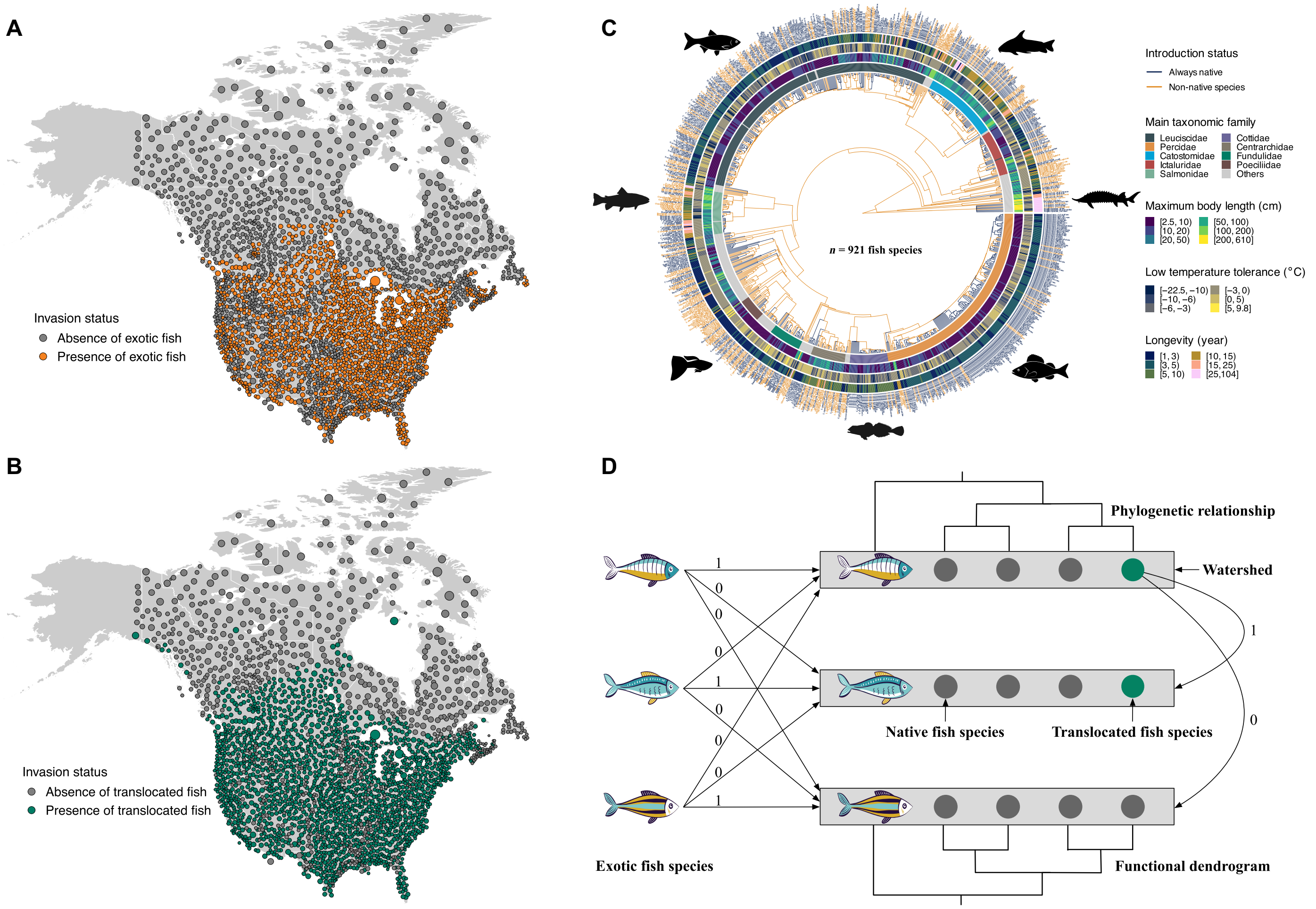


Fig. 1. Occurrence pattern, phylogenetic tree, and algorithm diagram of non-native freshwater fish species across North America. Occurrence pattern of exotic (those introduced from other continents) (**A**) and translocated (those translocated beyond their native ranges within North America) (**B**) fish species in 2993 watersheds. Watershed locations are depicted by their centroids, with point size indicating the respective watershed area. (**C**) Phylogenetic tree for 921 freshwater fish species occurring in these watersheds. It illustrates the evolutionary relationship of freshwater fish species in North America, with 322 species (represented by orange branches) being exotic or translocated. (**D**) Conceptual diagram illustrating the approach to associate non-native–native phylogenetic and functional distances with non-native fish occurrence. For each exotic fish species, its presence in one (or multiple) watershed of North America is defined as a success (1), while its absence in all the other watersheds is considered as a failure (0). Similarly, for each translocated fish species, its presence in one (or multiple) watershed of North America is denoted as a success (1), and its absence in all the other watersheds is considered as a failure (0). For each non-native fish species, its phylogenetic and functional distances with all native species in each watershed are calculated, regardless of whether the non-native species occurs in that watershed. This approach establishes the corresponding connection between non-native–native phylogenetic and functional distances and the conditions of non-native fish occurrence, enabling the examination of the relationship between phylogenetic and functional similarities and the likelihood of non-native fish occurrence. The fish silhouette illustrations in (C) and (D) are sourced from Freepik (www.freepik.com), following their license agreement.

significantly constrained non-native species richness, but this effect was only evident for translocated fish (Fig. 4 and figs. S4 and S7). Higher latitudes were always associated with lower invasion richness (Fig. 4 and figs. S4 and S7). Human pressure, native richness, and latitude may interact with phylogenetic and functional similarities (figs. S5 and S6), but they explained only a small fraction of invasion richness (Fig. 4 and fig. S7).

Direct and indirect effects of multiple factors on non-native fish invasions

Structural equation modeling revealed that comprehensive similarity had the strongest direct effects on the occurrence probability of both exotic and translocated fish species, with lower similarity to native species (i.e., greater phylogenetic and functional distance) reducing their establishment success (Fig. 5, A and B). In addition, higher native comprehensive diversity and human pressure were associated with increased occurrence probability, while higher latitude predicted lower occurrence (Fig. 5, A and B). Conversely, human pressure emerged as a relatively strong predictor of invasion richness, with higher levels of human activity linked to greater numbers of non-native species (Fig. 5, C and D). Lower similarity was associated with the greater invasion richness of both exotic and translocated fish species, although the effect was relatively weak. Higher latitude was associated with lower invasion richness, while native comprehensive diversity showed a limited and inconsistent effect across the two types of non-native species (Fig. 5, C and D).

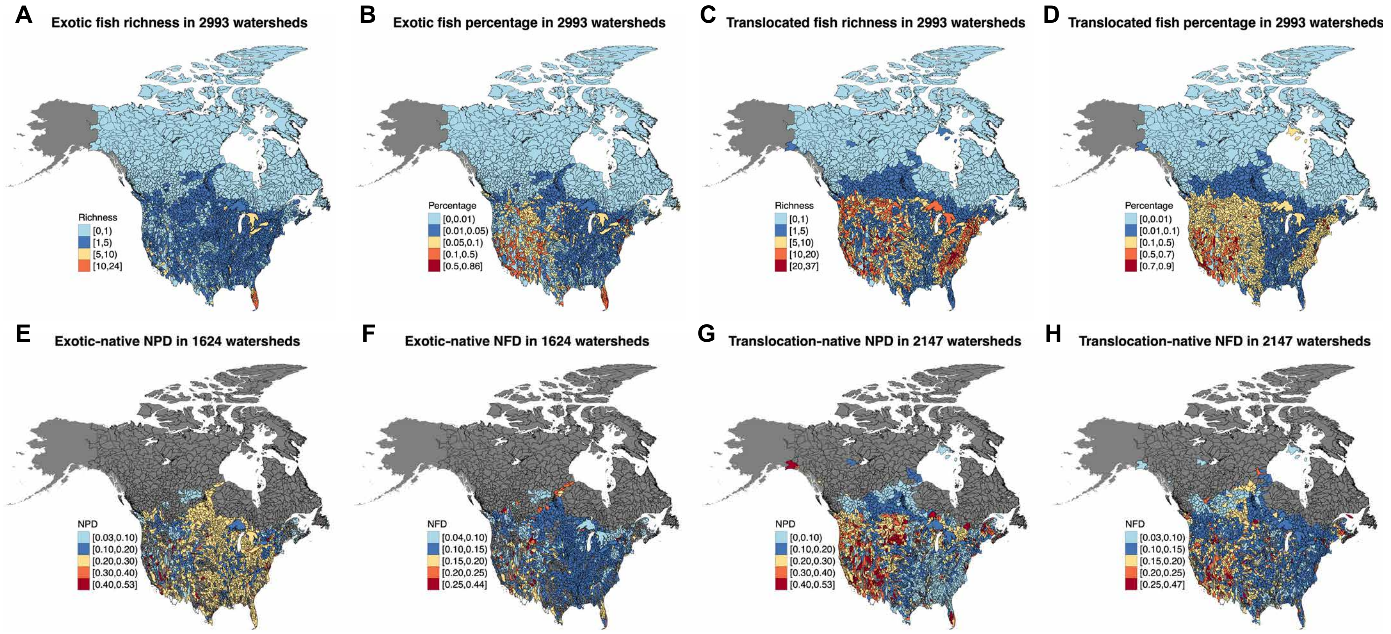


Fig. 2. Geographic distribution of richness and similarity for exotic and translocated freshwater fish species across North America. (**A**) Geographic pattern of exotic fish richness. (**B**) Geographic pattern of exotic fish percentage. (**C**) Geographic pattern of translocated fish richness. (**D**) Geographic pattern of translocated fish percentage. (**E**) NPD between exotic and native fish species. (**F**) NFD between exotic and native fish species. (**G**) NPD between translocated and native fish species. (**H**) NFD between translocated and native fish species. The percentage represents the ratio of non-native species richness to the total species richness in each watershed. The number of watersheds used for assessing these patterns is displayed at the top of each panel.

Native diversity could also indirectly influence occurrence and richness by adjusting the similarity between non-native and native species. Latitude and human pressure could also indirectly influence non-native fish invasions by affecting similarity and native diversity. Overall, these results demonstrate that ecological similarity to native species is key to predicting the occurrence of non-native fish species, whereas human pressure plays a comparatively more important role in determining their richness.

DISCUSSION

Using occurrence, richness, phylogenetic, and functional datasets for 921 freshwater fish species across nearly 3000 North American freshwater fish communities, our study revealed the distinct and complementary factors that influence the occurrence likelihood and invasion richness of non-native fish species. Ecological similarity to native species was identified as a key predictor of non-native fish occurrence, with invaders more likely to succeed when they were closely related to native species. In contrast, invasion richness was primarily shaped by human pressure and native diversity. Specifically, higher human pressure was linked to greater invasion richness, while native diversity acted to constrain invasion richness but only for translocated fish species. These findings were consistent across phylogenetic and functional dimensions at local and regional spatial scales while accounting for the effects of latitude and watershed area. These results suggest that freshwater fish invasions are shaped by multiple ecological and evolutionary processes, with environmental filtering primarily determining why an invader establishes in some communities, while human activities and biotic interactions govern why some communities harbor more invaders than others.

We integrate ecological similarity with native community characteristics, including native diversity, geographic features, and human pressure, to understand both the occurrence and richness of fish invasions. This combined perspective provides valuable insights into the drivers of biological invasions and contributes to reconciling several long-standing hypotheses, distinguishing our work from previous studies that rarely examined these aspects simultaneously. For example, several studies have explored the combined effects of some of these predictors on specific dimensions of biological invasions, such as occurrence (*22*, *23*), performance (*40*, *41*), abundance (*42*), or richness (*26*, *32*). However, the lack of analyses across different invasion dimensions makes it challenging to draw general conclusions about invasion outcomes and may have led to biased interpretations that favor only one side of several hypotheses, such as Darwin's naturalization conundrum (*18*), the invasion paradox (*25*), and the human activity hypothesis (*26*). Alternatively, some studies have explored the effects of similarity (*21*, *43*), diversity (*13*, *33*), or human pressure (*12*) across different invasion aspects. However, failing to examine these predictors simultaneously makes it difficult to assess their relative significance and actual roles in shaping biological invasions. Our comprehensive analysis of the effects of similarity, diversity, and human pressure on both aspects of fish invasions has allowed us to identify the dominant role of ecological similarity in predicting invader occurrence and the role of human activities and native diversity in shaping their invasion richness. These findings provide integrative insights into the multifaceted characteristics of fish invasions and their underlying drivers. We encourage future research to validate these findings across diverse taxonomic groups to gain a more comprehensive understanding of how multiple ecological and evolutionary mechanisms synergistically influence biological invasions.

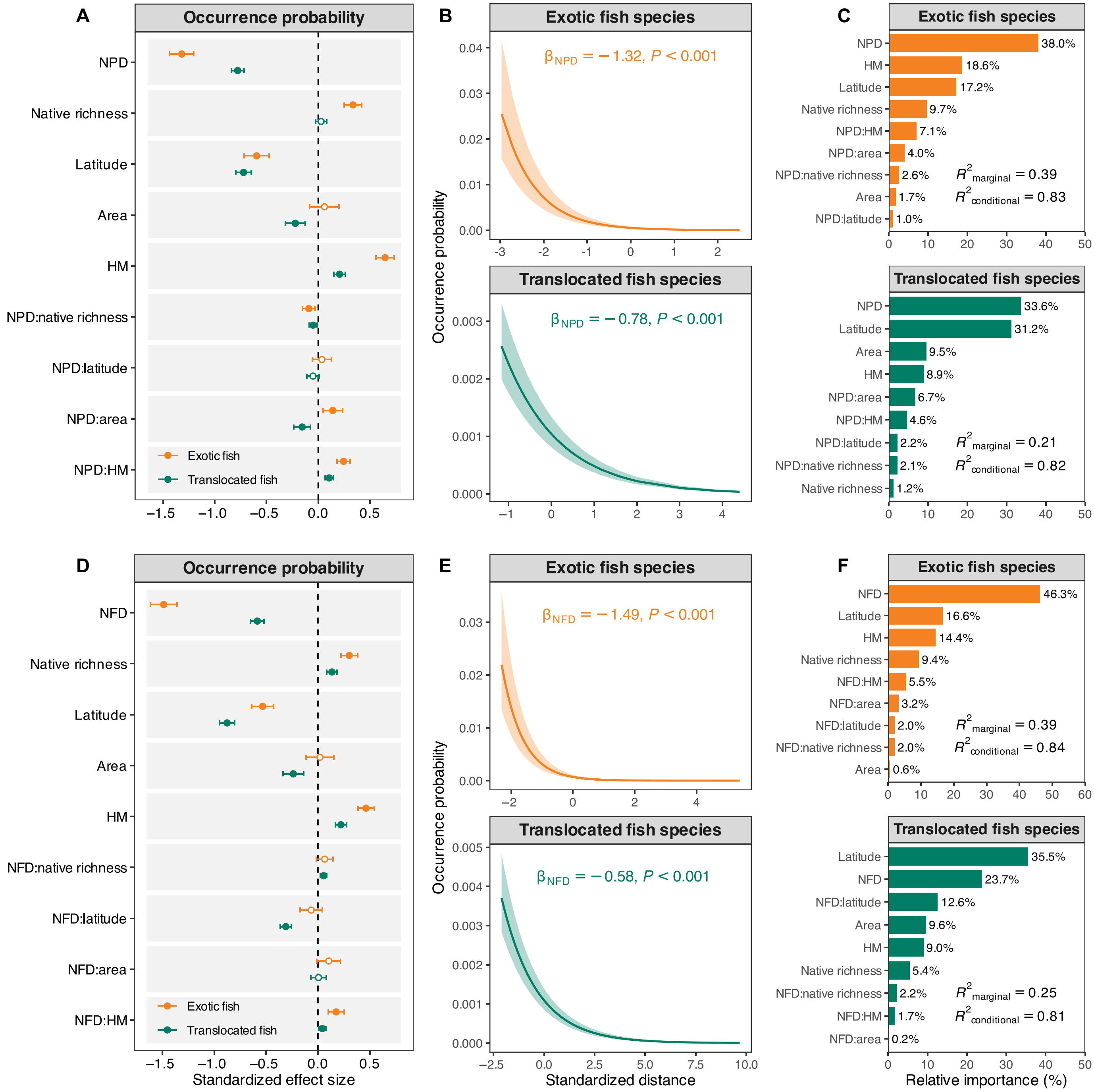


Fig. 3. Effects of similarity, native species richness, latitude, watershed area, human pressure, and their interactions on the occurrence probability of non-native fish species. The NPD (**A**) and NFD (**D**) were used to measure ecological similarity and predict occurrence likelihood. Variation in occurrence probability for both exotic and translocated fish species along standardized NPD (**B**) and standardized NFD (**E**). (**C** and **F**) Relative importance of predictive variables in explaining the occurrence likelihood of both exotic and translocated fish species. The standardized effect size (±95% confidence intervals) was derived from GLMMs with a binomial error distribution, with predictive variables standardized by subtracting the mean and dividing by the standard deviation. Positive effects (the 95% confidence interval does not include zero) indicate that the occurrence probability increases with increasing values of predictive variables, while the negative effects indicate the converse. Effect values and statistical significance (*P* values) for NPD and NFD are presented in the figure. The explained variances, including $R^2_{marginal}$ for the fixed effect and $R^2_{conditional}$ for both the fixed and random effects, are also reported.

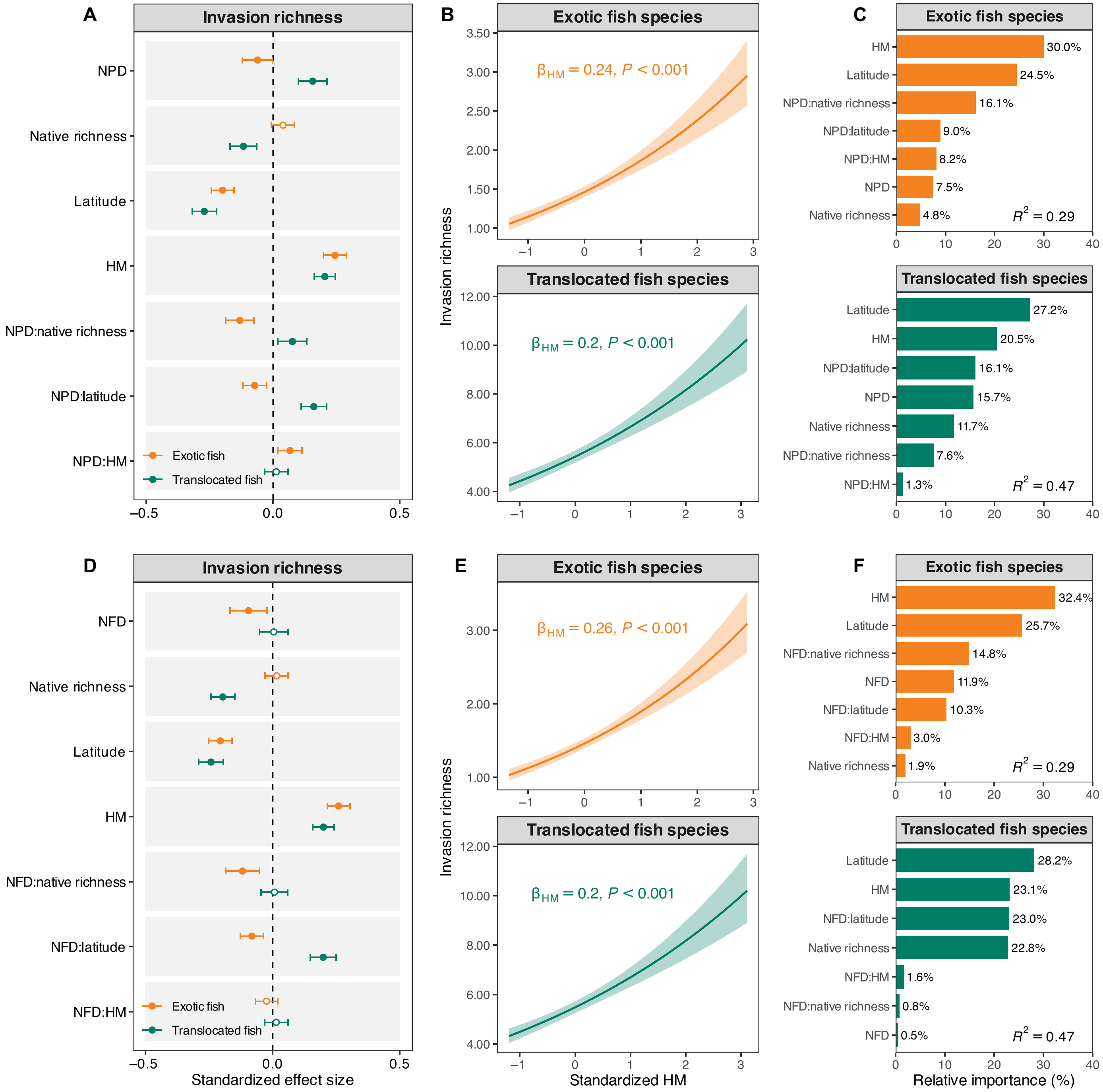
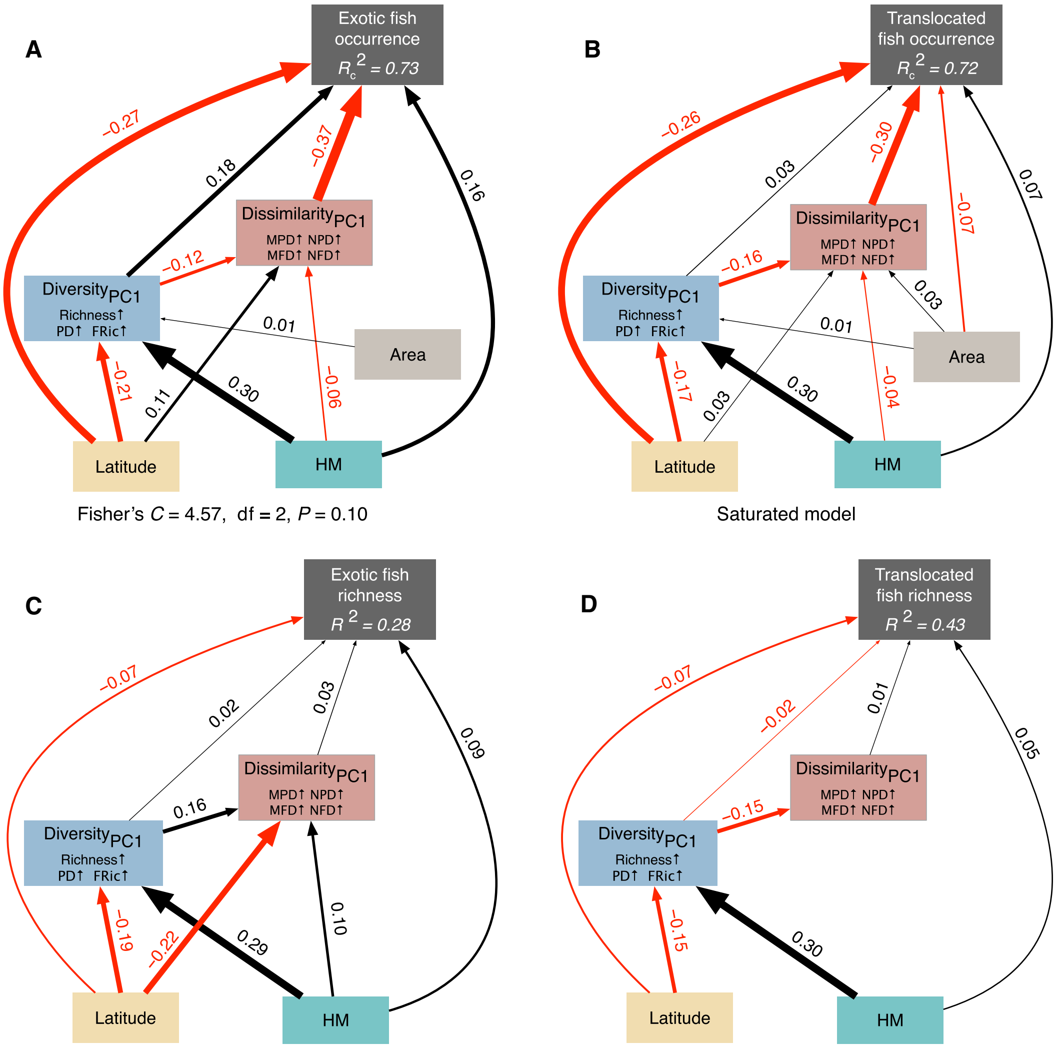


Fig. 4. Effects of similarity, native species richness, latitude, human pressure, and their interactions on non-native fish richness. The NPD (**A**) and NFD (**D**), calculated as the average value across all exotic or translocated species within a watershed, were used to measure ecological similarity and predict invasion richness. (**B** and **E**) Variation in invasion richness of both exotic and translocated fish species along standardized HM. (**C** and **F**) Relative importance of predictive variables in explaining the invasion richness of both exotic and translocated fish species. The standardized effect size (±95% confidence intervals) was derived from GLMs with a negative binomial error distribution, with predictive variables standardized by subtracting the mean and dividing by the standard deviation. Positive effects (the 95% confidence interval does not include zero) indicate that the invasion richness increases with increasing values of predictive variables, while the negative effects indicate the converse. Effect values and statistical significance (*P* values) for HM are presented in the figure. The explained variances of the predictors, namely pseudo R^2, are also reported.



Fisher's $C = 4.57$, df = 2, $P = 0.10$

Saturated model

Saturated model

Fisher's $C = 3.62$, df = 4, $P = 0.46$

Fig. 5. PSEM for evaluating direct and indirect effects on the occurrence and richness of non-native fish species. (**A**) Effects on the occurrence probability of exotic fish species. (**B**) Effects on the occurrence probability of translocated fish species. (**C**) Effects on the richness of exotic fish species. (**D**) Effects on the richness of translocated fish species. The dissimilarity was quantified as the first principal component (PC1) of MPD, NPD, MFD, and NFD between non-native and native species, with ↑ indicating positive correlations between the factors and the PC1. The native diversity was measured as the PC1 of native species richness, Faith's PD, and FRic, with ↑ indicating positive correlations between the factors and the PC1. Boxes represent the variables, highlighted with distinct colors, while arrows represent relationships among variables. Black and red arrows respectively denote positive and negative effects, with the arrow width proportional to the strength of the relationship. The numbers adjacent to the arrows denote standardized path coefficients. For clarity, only the significant paths ($P < 0.05$) are shown in the figure. The conditional R_c^2 (based upon both fixed and random effects) for occurrence and pseudo-R^2 for richness are reported in the corresponding boxes. Shipley's d-sep test was used to evaluate the fit of the PSEM, with results shown below each panel ($P > 0.05$ indicates a good fit of the model to the data). The goodness of fit cannot be evaluated for saturated models.

We found that non-native fish species were more likely to occur when they were more phylogenetically or functionally similar to native species at watershed scales across North America. This finding is consistent with previous results at river-basin scales globally (*23*). This narrower watershed scale better reflects natural conditions where non-native and native fish species coexist and interact with each other (*11*). At this scale, biotic interactions are typically assumed to play a critical role, with non-native species similar to native species being less likely to establish because of strong competition (*18*, *34*). Nevertheless, our results showed that closer phylogenetic or functional similarity to native species still predicts a higher probability of non-native fish occurrence, supporting the preadaptation hypothesis. Furthermore, phylogenetic and functional similarities emerged as the most important predictor of occurrence probability compared to other factors, aligning with previous findings in freshwater lakes (*22*). In addition, we also observed that including either phylogenetic or functional similarity in the models provided very similar explanatory power (R^2) (Fig. 3 and fig. S3), although model comparisons indicate that phylogenetic similarity offered a slightly better fit to the data compared to functional similarity (see tables S14 and S15). This suggests that phylogenetic distances largely capture the functional trait similarities considered in our study, yielding consistent effects in predicting fish invasions. These consistent patterns suggest that freshwater fish species generally follow a preadaptation strategy during the establishment stage, highlighting the importance of adaptability to novel environments for the initial success of non-native fish species. Conversely, our structural equation modeling revealed that when non-native species were similar to native species, their richness significantly decreased, albeit with a weaker effect compared to human pressure (Fig. 5, C and D). This result, derived from the comprehensive similarity index, supports Darwin's naturalization hypothesis, indicating that competition with native species or predation pressure constrains the richness of non-native fish invasion after their successful establishment. Together, these findings highlight the dominant role of ecological similarity in shaping non-native fish establishment. They also offer insight into Darwin's naturalization conundrum (*18*), suggesting that the influence of ecological similarity on invasion outcomes may depend on the specific invasion dimension examined. While non-native species similar to natives may establish more easily by better adapting to novel environments, they may simultaneously face stronger biotic interactions that limit their richness in a community.

We also observed that higher native diversity predicted a greater likelihood of non-native fish occurrence, although its effect was weaker than that of ecological similarity (Fig. 5, A and B). This finding supports the biotic acceptance hypothesis, suggesting that environmental filtering plays an important role in the establishment of non-native fish species. In contrast, we found that native diversity significantly reduced the richness of translocated fish species, albeit with a relatively weak effect compared to human pressure, consistent with recent studies supporting the biotic resistance hypothesis (*11*, *44*). This suggests that resource competition may play a notable role in limiting non-native fish invasions. Alternatively, higher native diversity could support a greater presence of resident predators, which may also contribute to restricting fish invasion (*45*). Regardless of the specific competitive or consumptive mechanisms involved, our results highlight the importance of biotic interactions in mitigating invasion richness in freshwater ecosystems. However, we did not observe clear evidence of biotic resistance for exotic fish richness; instead, our structural equation modeling even indicated a weak positive effect of native comprehensive diversity. This may reflect the importance of habitat or ecological similarity in shaping biotic resistance. Translocated species often share similar habitats or have greater ecological and evolutionary history with native species, leading to stronger niche overlap and, thus, stronger biotic resistance. In contrast, exotic species from distant regions may exploit novel niches, allowing them to avoid local competition or predation. This possibility is partially supported by the significant interaction effect between native richness and ecological similarity on invasion richness (Fig. 4 and fig. S7), where greater phylogenetic and functional distances between non-native and native species generally reduced the effect of native richness. Another important consideration is that the larger number of translocated fish species, compared to the relatively few exotic species (Fig. 2, A and C), may have increased our statistical power to detect biotic resistance against them. Collectively, the contrasting effects of native diversity on the occurrence likelihood versus invasion richness, and on exotic versus translocated species, underscore the need to consider multiple invasion dimensions and incorporate ecological similarity to better understand the role of native diversity. This also sheds light on the long-standing invasion paradox (*25*), suggesting that the effects of native diversity may vary across different invasion dimensions. While native diversity may facilitate non-native species establishment by indicating favorable environments, it can simultaneously limit invasion richness through biotic resistance.

We consistently found positive correlations between human pressure and both the occurrence and richness of non-native fish species. Moreover, human pressure emerged as a more prominent predictor of invasion richness than ecological similarity and native diversity. In addition, human pressure may indirectly influence fish invasions by adjusting similarity and diversity. These results highlight the critical role of anthropogenetic factors, such as propagule pressure, habitat disturbance, and infrastructure development, in facilitating fish invasions. Although these factors are widely recognized as key drivers of biological invasions (*26*, *32*, *42*), their influence is often examined in relation to specific aspects of invasion [but see (*12*)] and is not often compared quantitatively to other ecological factors [but see (*32*)]. Our results underscore the need to assess the relative contributions of human and natural drivers across multiple invasion dimensions. Doing so will enhance our understanding of how human pressure shapes biological invasions and provide insights into the human activity hypothesis (*26*). We also consistently observed negative associations between latitude and both the occurrence and richness of exotic and translocated fish species. The latitudinal distribution of non-native fish species in North America mirrors that of naturalized plant species (*46*), peaking around 30°N and decreasing as latitude increases. This likely indicates that large-scale climate conditions, such as temperature and precipitation, substantially influence the invasion success of fish species in North America (*38*).

Several limitations of our study should be acknowledged. First, we focused on the occurrence and richness of non-native species to characterize invasion outcomes, primarily due to the limited availability of data on other facets, such as abundance, biomass, or spatial spread, for non-native fish in North America. Although occurrence and richness provide valuable insights into invasion patterns, they capture only certain aspects of the invasion process and may constrain the generalizability of our findings. Future studies that incorporate additional measures related to spread and ecological impact will be crucial for achieving a more comprehensive understanding of fish invasions and their underlying drivers. Second, introduction preferences for non-native fish species may obscure the observed effects of ecological similarity and native diversity on invasion outcomes. For example, species intentionally introduced for aquaculture or recreational purposes may exhibit artificially elevated establishment success, whereas regions lacking fish introductions might lead to underestimations (*16*). These introduction biases could potentially confound interpretations centered on resource competition and environmental filtering. To address these issues, future research should more explicitly evaluate the roles of similarity and diversity across different invasion stages, including introduction, establishment, and impact.

In summary, by investigating two critical dimensions of biological invasions, occurrence likelihood and invasion richness, we uncovered distinct and complementary roles of ecological similarity, native diversity, and human pressure in shaping freshwater fish invasions. These contrasting effects highlight different mechanisms driving the establishment and accumulation of non-native fish species. Specifically, successful establishment is primarily governed by environmental adaptation, as indicated by phylogenetic and functional similarities to native species. In contrast, the accumulation of established non-native species is more strongly influenced by human activities and biotic interactions, shaped by the contextual characteristics of native communities. Our findings indicate that non-native fish species highly similar to native taxa are more likely to establish, whereas low-diversity native fish communities exposed to intense human activity tend to harbor greater numbers of non-native species. These results underscore the multidimensional nature of freshwater fish invasions and highlight the importance of jointly considering ecological, evolutionary, and anthropogenic drivers when assessing and predicting invasion outcomes. Extending this integrative framework to other taxonomic groups and across multiple invasion stages in the future will substantially contribute to a more comprehensive understanding of biological invasions in the Anthropocene.

MATERIALS AND METHODS

Occurrence data for North American freshwater fish species

We used an extensive database of native and non-native fish occurrences in 2993 watersheds across North America (*38*). For the continental United States, native species occurrence records in 2073 eight-digit Hydrologic Unit Code (HUC8) watersheds were obtained from NatureServe (www.natureserve.org) and non-native species records were obtained from the Non-indigenous Aquatic species database of the US Geological Survey (https://nas.er.usgs.gov). For Canada, native and non-native occurrence records in 988 tertiary watersheds (comparable to HUC8 watershed scale in the US) were compiled from multiple sources (*38*). Both HUC8-scale hydrologic units and their Canadian equivalent (referred to here as watersheds) have been widely used to study the diversity and distribution of freshwater fish species in North America (*11*, *37*–*39*). However, it is important to note that these hydrologic units are not entirely equivalent to natural watersheds, as they also encompass areas draining to stream segments, remnant zones, noncontributing regions, which could potentially affect hydrological interpretations (*47*). Non-native occurrence data only considered the species that have successfully established self-sustaining populations. We categorized non-native species by geographic origin into exotic species (those non-natives originating outside North America) and translocated species (those non-natives introduced to watersheds outside their historical range within North America) (Fig. 1, A and B). Overall, this database contains occurrence data for 949 fish species across 2993 HUC8-scale watersheds, including 62 exotic species and 272 translocated species, representing all fish species inhabiting North America's freshwater ecosystems. For each watershed, geographic coordinates of its centroid and the surface area were also provided.

Native species richness and human pressure for each watershed in North America

We calculated native species richness for each watershed by summing the number of native species present to understand the effects of native species diversity on non-native fish invasions. To assess the influence of human activities, we extracted the human modification index (HM) for each watershed from the human pressure database (*48*) using the geographic coordinates of the centroid of each watershed. The HM comprehensively quantifies human-driven changes in the global landscape, including settlement, agriculture, transport, energy production, and electrical infrastructure, and is highly correlated with human population density (*48*). This index has been widely used to evaluate the impact of anthropogenic stressors on terrestrial and freshwater ecosystems (*49*, *50*).

Phylogenetic relationship for North American freshwater fish species

We constructed a phylogenetic tree for North American freshwater fish species using the FishPhyloMaker R package (*51*). This package builds a phylogenetic tree for fish species by incorporating and pruning species from a backbone phylogenetic tree (*52*). First, we used the FishTaxaMaker function to generate 944 valid species names, excluding five duplicated names from the initial list of 949 species names. These valid names were then used as the input for the FishPhyloMaker function, generating a phylogenetic tree that included 921 fish species, with 23 species names automatically excluded as unidentifiable. The final phylogenetic tree of 921 species was used to represent the evolutionary relationships among freshwater fish species in North America, of which 61 have been introduced as exotic species and 267 as translocated species (Fig. 1C). Using this phylogeny, we calculated the pairwise phylogenetic distances among all species using the cophenetic function of the ape R package (*53*). In the subsequent analyses, we used a cleaned occurrence dataset, including 921 species across 2993 watersheds, to quantify non-native–native similarity and native diversity.

Non-native–native phylogenetic similarity and native phylogenetic diversity

On the basis of the computed pairwise distances, we calculated two widely used phylogenetic distance metrics to represent the phylogenetic similarity between non-native and native fish species. The non-native–native mean pairwise phylogenetic distance (MPD), which measures the mean distance between a non-native fish species and all native fish species within a specific watershed. With this measure, we assume that each native fish species within a watershed equally contributes to the occurrence of non-native fish species. The non-native–native nearest phylogenetic distance (NPD) measures the distance between a non-native fish species and its closest native relative in a watershed. This metric, in contrast, assumes that the presence or absence of a non-native fish species in a watershed is primarily influenced by its proximity to its closest native relative. In addition, we calculated Faith's phylogenetic diversity (PD) for native fish species in each watershed using the pd function of the picante R package (*54*). Faith's PD measures the sum of branch lengths in the phylogenetic tree for these native species, representing the phylogenetic richness of the native fish community (*55*). We chose PD over phylogenetic divergence metrics because it effectively captures niche occupancy similarly to species richness, while divergence indexes might convey the opposite meaning when assessing biotic resistance (*33*).

Functional traits for North American freshwater fish species

We compiled data on morphological, tolerance, and life-history traits to represent the functional space of 921 fish species, which have been shown to highly correlate with fish invasions and are commonly used in prior studies (*11*, *56*). Specifically, we obtained 10 morphological traits related to fish locomotion and food acquisition from the FISHMORPH database (*57*): maximum body length, body elongation, relative eye size, oral gape position, relative maxillary length, vertical eye position, body lateral shape, pectoral fin vertical position, pectoral fin size, and caudal peduncle throttling. These morphological traits are relative measures expressed as unitless ratios, with the exception of maximum body length. We also compiled four tolerance traits (euryhalinity, minimum temperature, maximum temperature, and temperature range) and four life-history traits (diet breadth, mature age, longevity, and fecundity) from the Fish Traits database for North American freshwater fishes (*58*) and FishBase (*59*). Most of these traits are continuous variables, except for euryhalinity (binary yes or no) and diet breadth (ranges from 1 to 9). See table S13 for details on the 18 functional traits.

Because of insufficient information for some species, some values were missing in the raw data of functional traits. Overall, 16.3% of the morphological traits, 22.4% of the tolerance traits, and 22.4% of the life-history traits were missing in the raw trait dataset of 921 fish species. We first imputed the data of missing continuous traits using the mean values of congeneric species for the species with congeneric species in the dataset. Then, we statistically imputed other missing values using a machine-learning algorithm with the missForest R package (*60*). This method uses a random forest trained on the observed values of a data matrix to predict the missing values and automatically calibrates the filling values by a set of iterations. In the imputation process, after each iteration, the difference between the previous and new imputed data matrices is assessed for the continuous and categorical parts, and the algorithm stops once both differences become larger (*60*). This method can impute continuous and/or categorical data and has been proven to perform better compared to other methods (*11*, *61*).

Non-native–native functional similarity and native functional diversity

We integrated all these three types of traits and calculated the pairwise Gower distance among all species using the gowdis function of the FD R package (*54*). This method accommodates mixed data types and automatically standardizes trait values on the basis of their range, eliminating the need for prior *z*-score standardization or principal components analysis (*62*). Therefore, we calculated two commonly used functional distance metrics to represent the functional similarities between non-native and native fish species. The non-native–native mean pairwise functional distance (MFD) measures the mean functional distance between a non-native fish species and all native fish species within a specific watershed. The non-native–native nearest functional distance (NFD) quantifies the functional distance between a non-native fish species and its closest native relative in a watershed. We also calculated functional richness (FRic) for native fish species in each watershed using the dbFD function from the FD R package (*54*). FRic measures the convex hull volume of functional traits, representing the trait space for the native fish community (*63*). We selected FRic over other functional diversity metrics because it better characterizes a filled niche space, aligning with phylogenetic and species richness when examining biotic resistance (*64*).

Occurrence likelihood and invasion richness of non-native fish species

We quantified the occurrence likelihood of each non-native species by evaluating their presence or absence in each watershed, following the approach used in our previous study (*23*). Specifically, for each exotic fish species introduced into North America, the presence in a watershed was coded as a success (1) and the absence as a failure (0). We then calculated its MPD, NPD, MFD, and NFD relative to all native fish species in each watershed, regardless of whether the exotic fish was present there. Similarly, for each translocated fish species within North America, we coded its presence or absence in each watershed using the same binary approach. We also calculated its MPD, NPD, MFD, and NFD relative to all native fish species in each watershed, except those from which the translocated fish originated (Fig. 1D). This approach enabled us to examine how species-level phylogenetic and functional similarities predict the occurrence of exotic (n = 182,451 pairwise comparisons) and translocated fish species (n = 723,068 pairwise comparisons).

Invasion richness was defined as the number of non-native species present in watersheds that contained at least one non-native species. For each such watershed, we averaged the MPD, NPD, MFD, and NFD values across all exotic or translocated fish species present to represent overall phylogenetic and functional similarities with native communities. This approach allowed us to evaluate how community-level phylogenetic and functional similarities influence the invasion richness of exotic (n = 1624) and translocated fish species (n = 2147). By further incorporating native diversity (richness, PD, and FRic), geographic variables (latitude and watershed area), and human pressure at the watershed level, we statistically assessed how ecological similarity and these additional factors jointly shape both the occurrence likelihood and invasion richness of non-native fish species.

Statistical analysis

We modeled the occurrence (presence or absence) of non-native species using generalized linear mixed models (GLMMs) with a binomial error distribution and logit link function. For binomial response variables with notably more zeros than ones, we opted for the clog-log link function, as recommended by Zuur *et al.* (*65*). We included non-native–native similarity, native species richness, latitude, watershed area, and HM as fixed predictors, with species-specific intercept (1|species) and watershed-specific intercept and slope (1 + similarity|watershed) treated as random effects. The random intercept effects were used to account for the statistical non-independence of multiple presence/absence records of a specific non-native species and multiple records within a specific watershed. The random slope of similarity was used to account for random variations in similarity effects across different watersheds. We also considered interaction effects between similarity and diversity, latitude, watershed area, and HM as including them better explained the occurrence likelihood (tables S14 and S15). All fixed predictors were standardized using *z* scores (subtracting the mean and dividing by the standard deviation) to make parameter estimates of the predictors comparable. Multicollinearity issues were checked using variance inflation factors, ensuring that they were below three (*66*). The explained variance by the fixed effect (R^2_{marginal}) and both the fixed and random effects ($R^2_{\text{conditional}}$) were computed using the r^2 function of the performance R package (*67*), following Nakagawa and Schielzeth's methodology (*68*).

We addressed the phylogenetic nonindependence among non-native fish species by using Bayesian phylogenetic mixed models, incorporating an additional phylogenetic covariance matrix into the occurrence analysis. We constructed the phylogenetic covariance matrix using the vcv function of the ape R package (*53*) and extracted submatrices for exotic and translocated species. These models were fitted using the INLA R package (*69*) using integrated nested

Laplace approximation for Bayesian inference. This method rapidly approximates Bayesian posterior distributions and accommodates complex layered random effects, including autocorrelation terms (*70*). Because results from the Bayesian phylogenetic mixed models were similar to those from GLMMs, we present them in the Supplementary Materials (tables S5 to S8).

We modeled invasion richness (the number of non-native species) using generalized linear models (GLMs) with a negative binomial error distribution and log link function. The same standardized predictors used in the GLMMs were applied, while watershed area (log transformed) was included as an offset to control for the influence of watershed size. Compared to using area as a direct predictor, the offset approach is more commonly used in modeling count data as it effectively models invasion richness per unit area, making the results more interpretable and comparable (*71*, *72*). Random variations across species and watersheds could not be estimated like the previous GLMMs because the response variable was measured at the watershed level. Nagelkerke's pseudo-R^2 for these GLMs were computed using the r^2 function of the performance R package (*67*) on the basis of Nagelkerke's theoretical method (*68*, *73*).

We quantified the relative importance of each predictor in the GLMMs and GLMs by calculating the ratio between the absolute value of the parameter estimate for each predictor and the sum of all parameter estimates. The ratio represents the relative strength of predictors in influencing occurrence probability and invasion richness, which is similar to variance partitioning analysis and commonly used (*74*, *75*).

All of these GLMMs and GLMs were applied to both exotic and translocated fish species and to phylogenetic and functional dimensions and executed using the glmmTMB R package (*76*). Given the superior performance of the nearest similarity metrics (NPD and NFD) over the mean metrics (MPD and MFD) in most cases (tables S14 and S15), we reported only the model results on the basis of the former. For clarity, we also included only native species richness to represent diversity in these models.

To evaluate the direct and indirect effects of comprehensive ecological similarity (incorporating both mean and nearest similarity measures) and native comprehensive diversity (including taxonomic, phylogenetic, and functional components) on the occurrence and richness of non-native fish species and to assess their relative importance, we further conducted piecewise structural equation modeling (PSEM) using the piecewiseSEM R package (*77*). PSEM can integrate mixed component models, accommodate non-Gaussian error distributions, and assess multiple relationships simultaneously, making it suitable for our data structure (*77*). Specifically, we ran four PSEM approaches to separately analyze the occurrence and richness of exotic and translocated fish species. Each PSEM included six variables: non-native fish occurrence/richness, non-native–native dissimilarity, native diversity, latitude, watershed area, and HM, with watershed area (log transformed) used as an offset to analyze invasion richness. For the generalized linear mixed component model fitting the occurrence probability, we assumed a binomial error distribution with a logit link function and incorporated species and watershed as random effects. For the generalized linear component model fitting invasion richness, we assumed a Poisson error distribution with a log link function. The dissimilarity between non-native and native species was represented by the first principal component (PC1) of standardized MPD, NPD, MFD, and NFD, providing a comprehensive measure of phylogenetic and functional distances (explaining 52.12, 49.66, 66.37, and 51.72% of the variance in dissimilarity across the four PSEM approaches, respectively). Native diversity was represented by the PC1 of standardized species richness, PD, and FRic, indicating a comprehensive measure of taxonomic, phylogenetic, and functional diversity (explaining 89.84, 89.75, 89.43, and 89.58% of the variance in diversity across the four PSEM approaches, respectively). These comprehensive measures allowed us to better capture the overall effects of ecological similarity and native diversity. We verified that including these multiple factors in the PSEM approaches did not lead to multicollinearity issues, as there were low correlations among them and variance inflation factors for all metrics remained below three. We began with a full model including all reasonable paths and iteratively removed nonsignificant paths until only significant paths remained and/or the Akaike information criterion was minimized. Overall PSEM fits were evaluated using Fisher's *C* statistic based on Shipley's d-sep test (*78*). Standardized path coefficients and R^2 for the endogenous variables were calculated.

To explore the potential influence of spatial scales, we also used a regional HUC4 occurrence database, which is upscaled from the local HUC8 database (*37*). This regional database encompasses 954 species across 381 watersheds, including 62 exotic species and 232 translocated species. It has been previously used to explore the taxonomic and phylogenetic patterns of freshwater fish assemblages in North America (*37*, *79*). We recalculated the occurrence likelihood, invasion richness, and all predictor variables on the basis of these HUC4-scale watersheds and refitted all GLMMs and GLMs. Given the high consistency between our results at the regional HUC4 scale and the local HUC8 scale, we have included the regional results in the Supplementary Materials. All statistical analyses were performed in R version 4.3.0 (*80*).

Supplementary Materials

This PDF file includes:
Figs. S1 to S7
Tables S1 to S15

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