Global Evidence for Biotic Differentiation Amid Contrasting Freshwater β-Diversity Responses to Human Impacts

Running title: Freshwater β-Diversity Change

Vitor M. B. Ferreira[1, 2*], Natália C. L. dos Santos[1], Míriam P. Albrecht[1†], Bruno E. Soares[1, 2†]

[1] Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

[2] Institute of Environmental Change & Society, University of Regina, Regina, Saskatchewan, Canada

*Corresponding author: Vitor M. B. Ferreira (vitormbf2@gmail.com)

†Míriam P. Albrecht and Bruno E. Soares should be considered as joint senior authors.

Keywords: biodiversity change | biotic differentiation | biotic homogenization | freshwater | human impacts | meta-analysis | β-diversity

ABSTRACT

Human pressures are reshaping freshwater biodiversity worldwide, yet the generality of biotic homogenization and the contexts in which differentiation instead emerges remain unresolved. These contrasting responses reflect decreases or increases in spatial β-diversity, respectively, as local assemblages within a region become more similar or more distinct in community composition. We conducted a global meta-analysis of 495 reference–impact contrasts to assess the direction and context dependence of freshwater β-diversity change across major biological groups and taxonomic, functional, and phylogenetic facets. We evaluated variation among impact types, study designs, and data types, as well as associations with five broad-scale factors: latitude, spatial extent, geographic distance, climatic heterogeneity, and temporal window. Overall, impacted assemblages had higher β-diversity than reference assemblages, indicating biotic differentiation across taxonomic and functional facets. Among impact categories, only land-use change showed a clear directional response, indicating differentiation. Across study designs, spatially interspersed and spatiotemporal comparisons also indicated differentiation, whereas spatially segregated comparisons showed no consistent direction. Global differentiation was observed despite broad variation in the five broad-scale factors, none of which showed detectable associations with β-diversity change. Presence–absence and quantitative data yielded opposite responses: occurrence-based differentiation and quantitative homogenization. Excluding the most-represented biological groups (fish and invertebrates) also reversed the pooled response from differentiation to homogenization. These findings further challenge the long-standing expectation that human impacts generally promote biotic homogenization, while highlighting that the inferred direction of freshwater β-diversity change depends on ecological context, taxonomic representation, and the type of data considered. Whether assemblages homogenize or differentiate may reflect the extent to which human pressures override local ecological differences, producing similar impacted states, or interact with local and historical contexts, generating distinct outcomes. Recognizing both ecological and methodological sources of context dependence is essential for understanding freshwater biodiversity reorganization under global change.

1 | Introduction

Earth's biodiversity is inevitably changing under the Anthropocene. When ecosystems are altered by human activities, local communities may undergo substantial reorganization, while their regional consequences are reflected in changes in β-diversity—the compositional variation among local assemblages (Whittaker 1960). Assemblages may therefore become increasingly similar or increasingly dissimilar from one another, resulting in biotic homogenization or differentiation, respectively (Taylor 2004). To understand the effects of human activities on biodiversity and inform conservation strategies, ecologists have increasingly sought to determine which trajectory biodiversity is following (Socolar et al. 2016). But this endeavor has proved challenging. The classical view predicts that human activities should promote homogenization by favoring a relatively small set of widespread "winners" while many species decline or are extirpated, termed "losers" (McKinney and Lockwood 1999). However, temporal syntheses have revealed an approximate balance between homogenization and differentiation across ecosystems (Blowes et al. 2024). Moreover, a recent global synthesis based on reference–impact comparisons found an overall tendency toward differentiation under human pressures (Keck et al. 2025). These findings highlight the idiosyncratic nature of biodiversity change, emphasizing the need to identify the conditions underlying variation around general patterns (Dornelas et al. 2023). Greater emphasis on context-dependent responses will therefore be necessary to understand why biodiversity changes in different directions and to guide more targeted conservation actions.

Biodiversity is inherently multifaceted; it can be measured not only by which species are present, but also by the ecological roles they play and their evolutionary relationships (Naeem et al. 2016). Because these facets capture complementary dimensions of community reorganization, the facet considered may influence the inferred direction of β-diversity change. Taxonomic β-diversity has been central to broad-scale syntheses, revealing whether local assemblages are becoming more or less similar in species composition (Blowes et al. 2024). Functional and phylogenetic β-diversity extend this perspective by revealing whether changes in species composition are accompanied by corresponding shifts in trait composition and evolutionary lineages. For example, freshwater assemblages may undergo taxonomic homogenization while simultaneously differentiating in functional (Campbell and Mandrak 2020) or phylogenetic composition (Jiang et al. 2019). Such contrasting responses highlight the importance of integrating multiple facets to capture dimensions of community reorganization that species identities alone may not reveal, with potential implications for understanding the consequences of biodiversity change for ecosystem functioning (Srivastava et al. 2012; Gross et al. 2017; Mori et al. 2018).

Many macroecological factors may shape β-diversity patterns. For instance, β-diversity can increase with spatial extent as larger regions encompass greater distances among sites (Soininen et al. 2018), while environmental heterogeneity can further increase compositional variation through differences in environmental filtering among localities

(Heino et al. 2015a). Latitudinal patterns may influence both β-diversity and its responses to human pressures, with lower β-diversity and greater biotic homogenization reported at higher latitudes (Soininen et al. 2018; Shen et al. 2026). Such natural variations should therefore be considered when evaluating anthropogenic effects, particularly given the strong scale dependence of β-diversity (Barton et al. 2013). Among these factors, geographic distance is particularly relevant because compositional similarity generally declines as environmental differences and dispersal limitation increase among more distant assemblages ('distance-decay relationship'; Nekola and White 1999).

The distance dependence becomes particularly relevant in impact assessments, where reference and impacted assemblages may be distributed in different spatial configurations. Comparisons involving spatially separated sets of sites may therefore be influenced by distance-decay, potentially confounding natural spatial variation with responses to human pressures. Such spatial separation can challenge the comparability of reference and impacted assemblages, highlighting the value of before–after control–impact (BACI) designs, which account for baseline differences when assessing impact-related change, but remain comparatively uncommon (Keck et al. 2025). Temporal assessments offer a complementary perspective by tracking changes in spatial β-diversity within the same region through time (Heino et al. 2024). Such approaches have long been considered particularly informative for detecting biotic homogenization (Rahel 2002; Olden and Rooney 2006). Study design and the spatial configuration of reference and impacted assemblages may therefore influence the inferred direction of β-diversity change under human pressures.

Freshwater ecosystems support high levels of biodiversity while facing multiple and widespread pressures associated with global environmental change (Sayer et al. 2025). Understanding how these pressures alter freshwater β-diversity is particularly challenging because community organization reflects complex interactions among environmental conditions, connectivity, flow regime, and dispersal processes, whose relative importance varies across ecosystem types, spatial scales, and organism groups (Heino et al. 2015b; Rolls et al. 2016; Lansac-Tôha et al. 2021; Rolls et al. 2023b). Evidence from freshwaters increasingly indicates that β-diversity change does not follow a consistent direction under human pressures, with homogenization and differentiation reported in response to land-use change, biological invasions, eutrophication, and hydrological alteration (Petsch et al. 2021, 2022; Rolls et al. 2021; Jiang et al. 2024; Ferreira et al. 2026; Shen et al. 2026). Yet, the extent to which these contrasting responses depend on ecological and methodological contexts remains poorly understood. To our knowledge, these sources of context dependence have not yet been jointly examined in a global meta-analysis focused on freshwater β-diversity change. The key question is therefore not whether freshwater assemblages homogenize or differentiate under human impacts, but rather: in which contexts does each trajectory emerge?

Here, we provide a global synthesis of β-diversity responses to human impacts in freshwater ecosystems. We compiled reference–impact comparisons across multiple biological groups and β-diversity facets to test whether impacted assemblages tend towards regional biotic homogenization or differentiation. Given the contrasting responses increasingly reported in the literature, we expected β-diversity change to be context-dependent rather than consistently directional. To examine this context dependence, we assessed how responses varied across impact categories, biological groups, β-diversity facets, and study designs, while also investigating the potential influence of spatial, geographic, environmental, and temporal factors. Because occurrence and quantitative data capture different aspects of community reorganization, we further examined whether data type influenced the inferred direction of β-diversity change. By integrating these ecological and methodological dimensions, we aim to assess the generality of biotic homogenization in freshwaters and identify the conditions under which human impacts may instead promote regional differentiation.

2 | Methods

2.1 | Literature Search and Data Extraction

We conducted an extensive literature search in the Web of Science and Scopus databases, completed in May 2026, using the following keywords: ("beta divers*" OR "beta-divers*" OR "β-divers*" OR "β divers*" OR "community dissimilar*" OR "spatial dissimilar*" OR "temporal dissimilar*" OR "compositional dissimilar*") AND ("taxonomic" OR "functional" OR "phylogenetic") AND (freshwater OR river* OR lake* OR stream* OR reservoir* OR "inland water*") AND (impact* OR disturb* OR "human activit*" OR "anthropogenic activit*" OR deforest* OR pollut* OR impound* OR "climate chang*" OR inva* OR connectivity OR nonnative OR non-native OR fragment* OR dam* OR tourism OR "biotic homogen*" OR homogen* OR "biotic heterogen*" OR heterogen* OR "biotic differentiation" OR differentiation OR "land us*" OR "land cover" OR warm* OR exotic OR "environmental chang*"). This initial search returned 1,849 peer-reviewed articles published in English (PRISMA flow diagram; Figure S1). After removing duplicates (n = 672), 1,177 articles remained for title and abstract screening. At this stage, we retained studies that, based on their title and abstract, appeared to assess freshwater biological communities or community-level ecological diversity and reported, or were likely to report, community-level data on major freshwater groups, such as fish, invertebrates, zooplankton, macrophytes, algae, or phytoplankton. This screening retained 520 articles for full-text assessment.

During full-text screening, we selected articles that reported extractable spatial β-diversity estimates for at least one β-diversity facet (taxonomic, functional, or phylogenetic) and assessed anthropogenic impacts through clearly defined comparisons between groups of reference and impacted sites (hereafter reference–impact contrasts). Reference sites represented non- or less-impacted conditions, whereas impacted sites represented impacted or more-impacted conditions. For articles reporting more than two

impact levels, only non-impacted or less-impacted groups of sites were classified as reference, whereas intermediate-impact levels were classified as impacted in the reference–impact contrasts.

Eligible articles had to evaluate spatial β-diversity using one of three study designs, which we classified according to the spatial and temporal arrangement of reference and impacted conditions. Drawing on the distinction between spatial interspersion and segregation of treatments in ecological experimental design (Hurlbert 1984), spatial comparisons were classified as either (i) interspersed, when reference and impacted sites occurred within the same sampled region and the areas covered by the two conditions overlapped, or (ii) segregated, when they occupied distinct, non-overlapping sampled areas. Segregated configurations included comparisons between different basins or watersheds; separate rivers, streams, or tributaries; and distinct reaches or areas within the same river. Both interspersed and segregated configurations were classified based on the descriptions, maps, and coordinates available in each article. For the subset of segregated configurations with sufficient geospatial data, the classification was additionally cross-checked by confirming non-overlap between the reference and impacted sampling areas delineated during spatial-extent estimation (see "Sensitivity analyses"). Moreover, comparisons were classified as (iii) spatiotemporal when spatial β-diversity was evaluated between different time points within the same sampled region, including classical before–after designs. Studies spanning an explicit temporal increase in impact intensity were also classified as spatiotemporal. In these cases, we retrieved data from the earliest and latest time points. After full-text screening, 66 articles met our inclusion criteria. Two articles analysed the same underlying fish assemblage dataset but assessed different β-diversity facets (Campbell and Mandrak 2019, 2020). We therefore retained estimates from both articles while treating them as a single source, yielding 65 independent article-level datasets.

The included articles yielded 103 study cases, which were used as the sampling units rather than articles to avoid combining distinct β-diversity estimates obtained for different biological groups, impact types, sampled regions, or sampling periods. Thus, a study case was defined as a set of β-diversity estimates reported within an article for a unique combination of these categories. Separate study cases were created only when β-diversity estimates for different combinations were reported independently. For example, an article investigating two biological groups under one impact type yielded two study cases, one for each group. An article investigating only one biological group under two different impact types also yielded two study cases, one for each impact type. Similarly, when an article investigated one biological group under one impact type but reported β-diversity estimates separately for different seasons, each season was treated as a separate study case. The 103 study cases comprised 327 observations, including 146 reference and 181 impacted observations. Each observation represented a reference or impacted group of sites. Study cases could contribute one or more reference or impacted observations, therefore yielding one or several reference–impact contrasts.

For each observation, we extracted all available dissimilarity-based estimates of spatial β-diversity from the text, tables, or figures. When β-diversity estimates were available only in figures, numerical data were retrieved using the *digitize* package in R (Poisot 2011). For boxplots, means were approximated from the median and lower and upper quartiles, and standard deviations were estimated when sample size (number of sites) was available (Wan et al. 2014). Extracted β-diversity estimates were mostly derived from presence–absence data, but some were based on quantitative data, including abundance, biomass, cover, and relative read abundance derived from metabarcoding. When an article reported β-diversity calculated from both presence-absence and quantitative data, we extracted both estimates. β-diversity was quantified using a range of indices that varied among facets. Taxonomic β-diversity was most commonly measured using Sørensen, Jaccard, and Bray–Curtis dissimilarities, but also Whittaker β-diversity and its standardized form (Harrison et al. 1992). When studies reported the unstandardized multiplicative Whittaker measure, calculated as $\beta_w = \gamma/\bar{\alpha}$, values were standardized between 0 and 1 as $(\beta_w - 1)/(N - 1)$, where γ is regional species richness, $\bar{\alpha}$ is mean local species richness, and N is the number of sampling sites (Harrison et al. 1992). We also extracted taxonomic data using Simpson dissimilarity, calculated as 1 − Simpson similarity, and Euclidean dissimilarity when abundance data were Hellinger-transformed. Functional β-diversity included Sørensen, Jaccard, Bray–Curtis, and Simpson dissimilarities, as well as trait-based measures derived from Gower distances, community-weighted means, convex hulls, functional spaces, and mean pairwise functional distances. Phylogenetic β-diversity was quantified using phylogenetic adaptations of the Jaccard and Sørensen indices.

2.1.1 | Classification of Human Impacts

The selected articles assessed a wide range of anthropogenic pressures. To enable comparisons among studies, we assigned each study case to one of five broad impact categories based on the pressure directly evaluated in the original study: (i) hydrological alteration, (ii) land use, (iii) pollution and nutrient enrichment, (iv) invasion, and (v) multiple impacts. Because one article contributed study cases to two impact categories (Simões et al. 2020), article counts across categories were not mutually exclusive. Hydrological alteration (14 articles) encompassed pressures that disrupted hydrological connectivity or modified freshwater flow regimes. This category included damming (11 articles), with comparisons involving reservoir, upstream, and downstream assemblages, and the anthropogenic lateral disconnection of rivers and floodplain lakes by hydraulic engineering structures, such as levees, dykes, and sluice gates (3 articles). Land-use impacts (19 articles) encompassed agriculture, mining, forest loss or logging, and urbanization. Agricultural comparisons included sites dominated by cropland, arable fields, farms, pasture, or intensively managed grassland, generally contrasted with forested or less intensively managed sites. Urban comparisons generally used rural sites as reference conditions. Pollution and nutrient enrichment (9 articles) included changes in water chemistry or trophic state associated with eutrophication, phosphorus

enrichment, salinization, and organic or nutrient inputs, including those originating from intensive aquaculture. This category frequently included pressure gradients, such as comparisons among oligotrophic, mesotrophic, eutrophic, and hypereutrophic conditions, or across increasing phosphorus concentrations or salinity levels. Intermediate conditions were classified as impacted, following the reference–impact framework described above. Invasion (8 articles), the least-represented category, included studies assessing community changes associated with the introduction or establishment of non-native species. Finally, multiple impacts (16 articles) included studies in which impacted conditions were exposed to two or more co-occurring anthropogenic pressures whose individual effects could not be separated.

2.2 | Effect Size Calculation

The log response ratio (lnRR) was calculated for each reference–impact contrast as the natural logarithm of the ratio between the β-diversity estimates for the impacted and reference observations:

$$lnRR = log\left(\frac{\bar{\beta}\ impacted}{\bar{\beta}\ reference}\right).$$

An lnRR of zero indicates no differences ($\bar{\beta}\ impacted = \bar{\beta}\ reference$), while positive values ($\bar{\beta}\ impacted > \bar{\beta}\ reference$) indicate biotic differentiation, and negative values ($\bar{\beta}\ impacted < \bar{\beta}\ reference$) correspond to biotic homogenization. Within each study case, every reference observation was paired with every impacted observation; no pairings were made across study cases. Within each observation pair, reference and impacted β-diversity estimates were matched by facet. Each facet-specific pairing constituted one reference–impact contrast and yielded one lnRR estimate. Thus, an observation pair could yield multiple contrasts when estimates were available for more than one β-diversity facet. Figure 1 illustrates these pairing rules across the three study designs. The number of lnRR estimates per study case ranged from 1 to 81, resulting in 495 estimates overall.

2.3 | Bootstrap Resampling Procedure

Because sampling variances could not be calculated for all effect sizes, inverse-variance weighting could not be applied to the full dataset (A = 65, SC = 103, lnRR = 495; number of articles, study cases, and lnRR estimates, respectively). We therefore used a bootstrap resampling procedure suitable for unweighted meta-analytic data (Crouzeilles et al. 2016, 2017; but see the complementary variance-weighted meta-analytical approach below). Across 10,000 iterations, one lnRR estimate was randomly sampled from each study case, and the mean lnRR was recalculated. This generated a bootstrapped distribution of mean effect sizes, from which 95% confidence intervals were obtained using the 2.5th and 97.5th percentiles. Effects were considered

statistically significant when their confidence intervals did not cross zero. By sampling one lnRR estimate per study case in each iteration, this procedure prevented study cases with more estimates—whether resulting from multiple reference–impact contrasts, multiple biodiversity facets, or both—from contributing disproportionately to the global estimate. At the same time, repeated resampling allowed all available β-diversity facets within a study case to contribute across iterations. The bootstrap applied to the complete dataset therefore provided an integrative estimate of β-diversity change across the taxonomic, functional, and phylogenetic evidence available in our dataset, while preserving the representation of each facet in the available evidence.

The bootstrap was subsequently applied separately to impact type, study design, biological group, and biodiversity facet. For impact type, study design, and biological group, the sampled lnRR could represent any available β-diversity facet within the focal category, thereby retaining the multifaceted nature of the global analysis. For the biodiversity facet moderator, separate bootstrap analyses were conducted for each facet, and only lnRR estimates belonging to the focal facet were eligible for resampling. This allowed us to assess whether the direction emerging from the integrative analysis was consistent across individual biodiversity facets. To avoid interpreting poorly represented moderator levels, we restricted substantive interpretation to levels represented by at least 10 articles. Because each article contributed at least one study case and each study case contained at least one reference–impact contrast, this threshold also ensured a minimum of 10 study cases and 10 lnRR estimates per interpreted level. Levels below this threshold were reported for completeness but not further interpreted.

We assessed the robustness of our results and potential publication bias using a leave-one-out bootstrap resampling approach. We sequentially excluded one study case at a time and re-ran the bootstrap analysis (10,000 iterations each time) on the remaining dataset. This allowed us to evaluate whether the global mean lnRR and its confidence interval were sensitive to the exclusion of any particular study case. Stable mean lnRR estimates across iterations indicate that the global pattern is not driven by a single influential study case and that publication bias is unlikely to substantially affect our results. For each removal, we also calculated the change in mean lnRR as the difference between the leave-one-out bootstrap mean and the full-data bootstrap mean. Negative values therefore indicate study cases whose removal reduced the global mean, whereas positive values indicate study cases whose removal increased the global mean.

2.4 | Meta-Analytical Approach

We also fitted variance-weighted meta-regressions to assess whether the direction of our main inference was retained in the subset of data with available standard deviations and sample sizes (A = 39, SC = 45, lnRR = 196). For this subset, we calculated the sampling variance of each log response ratio as:

$$v_i = \frac{SD^2 impacted}{n\,impacted\ \overline{\beta}^2{}_{impacted}} + \frac{SD^2 reference}{n\,reference\ \overline{\beta}^2{}_{reference}},$$

where *SD*, *n*, and $\bar{\beta}$ are standard deviation, sample size, and mean β-diversity values for impacted and reference groups, respectively. We first fitted an intercept-only model to estimate the global mean lnRR. We then fitted separate models for biodiversity facet, impact type, study design, and biological group, using models without an intercept to estimate the mean lnRR for each moderator level. All models included study cases as a random effect and were fitted using restricted maximum likelihood.

We also assessed the influence of error that publication bias might produce in our meta-analytical approach. We used a funnel plot, which provides a graphical assessment of potentially missing effect sizes. In the absence of publication bias, plotting effect sizes against their standard errors is expected to produce an approximately symmetrical distribution around the overall effect estimate. Funnel-plot asymmetry was formally assessed using Egger's regression method, in which lnRR estimates were regressed against their standard error while accounting for the non-independence of estimates within study cases (Egger et al. 1997; Nakagawa et al. 2022).

Variance-weighted meta-regressions, the funnel plot, and Egger's regression were conducted in R using the *metafor* package (Viechtbauer 2010). Cluster-robust inference for the Egger's regression, accounting for the non-independence of effect sizes within study cases, was obtained using *clubSandwich* (Pustejovsky and Tipton 2018).

2.5 | Sensitivity Analyses

2.5.1 | Data Extraction

We extracted geographic information to estimate the spatial extent and central coordinate of each sampled region. Coordinates were extracted from the main text or tables when available or using the *digitize* package in R (Poisot 2011) to extract specific coordinates from available maps. If sampling sites could not be distinguished in the map, the sampled region was outlined using the outermost visible peripheral sites. For sampled regions without reported coordinates but with clearly identifiable geographic boundaries, we outlined the region in Google Earth and extracted these coordinates.

Spatial extent and the central coordinate of each sampled region were estimated from the available geographic coordinates. When the spatial configuration of sampling sites allowed an area to be delineated, coordinates were projected to a global equal-area coordinate reference system (EPSG:6933), spatial extent was calculated as the convex-hull area in km^2, and the central coordinate was defined as the polygon centroid. This area-based approach was also applied to riverine systems whenever their spatial configuration allowed a polygon to be delineated. For riverine systems with sampling sites distributed predominantly along a longitudinal axis, spatial extent was instead calculated as the cumulative length of the sampled reach in km, and the central coordinate was defined as the midpoint along this length. Area- and length-based extents were treated as distinct spatial descriptors and were not combined into a common extent measure. Reported spatial-extent values were used only when the article

explicitly stated that they referred to the sampled region rather than to the basin, catchment, or another broader spatial boundary. We compiled 116 sampled regions and retrieved geographic coordinates for 113 of them (Figure 2). Spatially interspersed and spatiotemporal study designs each contributed a single sampled region per study case, whereas spatially segregated designs could contribute multiple sampled regions. Spatial extent was characterized for 84 sampled regions representing 71 study cases. Of these, 69 regions (59 study cases) had area-based extents ranging from 2.85 to 4,513.28 km², whereas 15 regions (12 study cases) had length-based extents ranging from 18 to 939 km.

Geographic distance (km) was calculated for each reference–impacted contrast from spatially segregated study designs for which the central coordinates of both sampled regions were available. Distance was calculated as the great-circle distance between the paired central coordinates using the Haversine method. Because the calculation relied only on central coordinates, sampled regions with either area-based or length-based extents could be included. Thus, regions with area-based extents were represented by their polygon centroids, whereas riverine regions with length-based extents were represented by the midpoint of the sampled reach. Geographic distances ranged from 6.62 to approximately 3,387 km.

We also extracted eight bioclimatic variables from the WorldClim 2 database at a spatial resolution of 30 arc-seconds (Fick and Hijmans 2017) to quantify spatial climatic heterogeneity within each study case, used as a proxy for broader environmental heterogeneity. To standardize the spatial unit used for environmental extraction across study cases, area-based spatial-extent estimates (km²), whether calculated as convex-hull areas or obtained directly from the original articles, were converted to the radii of equal-area circles as $r = \sqrt{A/\pi}$, where A is the area-based spatial extent. The resulting radii were then used to construct circular buffers around the corresponding central coordinates. This procedure avoided combining known polygon geometries with unknown spatial shapes represented only by reported area values. Because representing length-based extents would require selecting an arbitrary buffer width, environmental extraction was restricted to study cases with area-based extents.

We then retrieved annual mean temperature (Bio1), temperature seasonality (Bio4), maximum temperature of warmest month (Bio5), minimum temperature of coldest month (Bio6), annual precipitation (Bio12), precipitation seasonality (Bio15), precipitation of wettest quarter (Bio16), and precipitation of driest quarter (Bio17). To estimate climatic heterogeneity, we performed a single PCA on a pooled pixel-by-variable matrix containing all WorldClim pixels extracted across study-case buffers, with pixels as rows and the eight bioclimatic variables as columns (Figure S2). Variables were centered and scaled to unit variance, and we retained the principal components that collectively explained at least 90% of the total variance. For each study case, climatic heterogeneity was quantified as the mean Euclidean distance of its pixels to their centroid in the retained multivariate environmental space.

Finally, we calculated the temporal window (years) for spatiotemporal study designs as the time elapsed between the reference and impacted sampling time points. When reference and impacted times were reported as time intervals, we first calculated the midpoint of each interval and then estimated the temporal window as the difference between the reference and impacted midpoints.

All spatial buffers and coordinate transformations were performed using the *sf* package in R (Pebesma 2018), geographic distances were calculated using the Haversine method implemented in *geosphere* (Hijmans 2022), and WorldClim 2 climatic data at 30-arc second resolution (Fick and Hijmans 2017) were handled and extracted using *terra* (Hijmans 2023).

2.5.2 | Data Analysis

To evaluate whether freshwater β-diversity change was sensitive to dataset composition and potential sources of variation, we organized the sensitivity analyses into two blocks. The first block assessed dataset composition. Thus, we repeated the bootstrap procedure for separate subsets of presence-absence data (A = 55, SC = 83, lnRR = 431) and quantitative data (A = 13, SC = 22, lnRR = 76), as well as for a subset excluding the most-represented biological groups (A = 20, SC = 30, lnRR = 248), which were fish and invertebrates. These analyses tested whether the global mean effect was retained after changing the composition of the dataset. The second block assessed whether lnRR varied with five broad-scale factors: spatial extent, geographic distance, absolute latitude, climatic heterogeneity, and temporal window. These continuous variables were evaluated using separate linear mixed-effects models, with study cases included as random effects to account for non-independence among multiple lnRR estimates derived from the same study case. All models were fitted in R using the *lme4* package (Bates et al. 2015). As expected, spatial extent and climatic heterogeneity were strongly correlated ($r = 0.686$, $p < 0.001$; Figure S3), but both analyses were retained because they capture related yet non-equivalent aspects of spatial scale and were evaluated as complementary sensitivity tests.

Sensitivity analyses involving these broad-scale factors were restricted to study cases for which the corresponding information could be obtained; therefore, sample size and study composition varied among analyses. Spatial extent was analyzed using only spatially interspersed and spatiotemporal study cases with area-based extent estimates expressed in km² (A = 35, SC = 50, lnRR = 121). Geographic distance was analyzed only for spatially segregated study designs, using the distance calculated between paired reference and impacted sampled regions (A = 12, SC = 21, lnRR = 245). Absolute latitude was analyzed for all study cases with available coordinates (A = 65, SC = 100, lnRR = 492). For spatially segregated study designs, latitude was calculated as the absolute value of the mean latitude of the reference–impacted contrasts. When coordinates were available for only one of the paired reference or impacted sampled regions, we used the available coordinate to represent the pair. Climatic heterogeneity

was analyzed using the same subset as the spatial-extent analysis. However, two study cases were excluded because inaccurate coordinates shifted their convex hulls into the sea (A = 33, SC = 48, lnRR = 116), which would have substantially distorted the estimated environmental conditions extracted from the transformed buffer radius. The temporal window was analyzed only for spatiotemporal study designs with time-interval information available (A = 21, SC = 37, lnRR = 54).

3 | Results

Across 495 reference–impact contrasts worldwide, freshwater ecosystems revealed a global signal of biotic differentiation under human-impacted conditions. The bootstrap resampling procedure produced consistently positive estimates, indicating higher β-diversity in impacted sampled regions (mean lnRR = 0.089, 95% CI = 0.048 to 0.133; Figure 3). A complementary meta-analytic model based on the subset of data with available variance estimates (196 reference–impact contrasts) was directionally consistent with the bootstrap result, yielding a positive but marginally significant outcome (estimate = 0.130, 95% CI = -0.013 to 0.273, p = 0.074; Table S1).

This positive lnRR pattern was retained across several moderator categories. It was observed for both taxonomic (mean lnRR = 0.085, 95% CI = 0.070 to 0.098) and functional facets (mean lnRR = 0.116, 95% CI = 0.092 to 0.140), as well as for land-use impacts (mean lnRR = 0.171, 95% CI = 0.048 to 0.294) (Figure 3). The same pattern was also found in spatially interspersed (mean lnRR = 0.129, 95% CI = 0.074 to 0.182) and spatiotemporal study designs (mean lnRR = 0.068, 95% CI = 0.015 to 0.135), and among the best-represented biological groups, fish (mean lnRR = 0.115, 95% CI = 0.058 to 0.183) and invertebrates (mean lnRR = 0.224, 95% CI = 0.129 to 0.320) (Figure 3). Other moderator categories either showed no clear directional change (Figure 3) or did not meet the minimum sample sizes for robust interpretation in terms of articles, study cases, or lnRR estimates (Figure S4). Moderator-specific meta-analytic models yielded broadly positive estimates, consistent with the bootstrap results, but statistical support was limited to functional β-diversity among well-sampled categories (estimate = 0.197, 95% CI = 0.054 to 0.340, p = 0.007; Table S1).

3.1 | Sensitivity Analyses

We assessed whether the global differentiation signal was retained when restricting the dataset to presence-absence data (Figure 4). The positive mean lnRR remained in this subset (mean lnRR = 0.139, 95% CI = 0.080 to 0.192), supporting the robustness of the overall differentiation pattern. For comparison, the smaller quantitative-data subset showed a contrasting negative pattern (mean lnRR = -0.087, 95% CI = -0.127 to -0.045), indicating a tendency toward biotic homogenization. Excluding fish and invertebrates revealed a negative mean lnRR among the remaining biological groups (mean lnRR = -0.096, 95% CI = -0.137 to -0.057), indicating that the overall

differentiation signal was sensitive to the assemblage composition of the dataset. Finally, despite broad variation in spatial extent, climatic heterogeneity, latitude, geographic distance in spatially segregated comparisons, and temporal window in spatiotemporal comparisons, none of these factors showed a detectable association with β-diversity change (Table S2).

3.2 | Publication Bias

The leave-one-out bootstrap of the full dataset showed that the global differentiation signal was robust to the removal of individual study cases. Mean lnRR estimates remained positive and generally close to the full-data bootstrap mean across removals (Figure S5). Changes in estimates indicated that most study cases had little influence on the overall mean, although a few removals reduced mean lnRR more strongly, suggesting that these study cases contributed to the magnitude, but not to the direction, of the global differentiation pattern (Figure S6). The funnel plot of the meta-analytical approach showed slight visual asymmetry (Figure S7), but this pattern was not statistically supported (Egger's test; $p = 0.824$, $df = 6.24$).

4 | Discussion

Our meta-analyses revealed a global trend towards biotic differentiation of freshwater ecosystems associated with human activities. Across the available reference–impact comparisons worldwide, β-diversity was generally higher under impact than reference conditions, with differentiation observed across taxonomic and functional facets. Among impact categories, land-use change was the only one showing a clear directional response, also indicating differentiation. We also show that the inferred direction of β-diversity change depends on how reference and impacted regions are compared, as spatially segregated comparisons had no consistent directional change. Importantly, the overall pattern emerged despite variation in the broad-scale spatial, climatic, latitudinal, and temporal variables evaluated. However, the direction of change varied with data type: differentiation was retained for presence–absence data, whereas quantitative data revealed biotic homogenization. The overall response was also sensitive to the taxonomic representation of the available evidence, with homogenization emerging after the most represented groups were removed. This overall differentiation aligns with recent evidence of global-scale differentiation (Keck et al. 2025). By integrating multiple β-diversity facets while explicitly evaluating ecological and methodological sources of variation, our synthesis contributes to understanding the context-dependent nature of biodiversity change and the contrasting directions of β-diversity responses reported worldwide (Blowes et al. 2024; Petsch et al. 2022; Rolls et al. 2023a).

The differentiation pattern contrasts with the expectation that human pressures favour dominant and generalist species across localities, thereby promoting regional

528 homogenization (McKinney and Lockwood 1999; Rahel 2002). However, local
529 simplification and regional differentiation are not mutually exclusive. Differentiation
530 may arise when α-diversity declines more rapidly than γ-diversity, as can occur when
531 species losses are unevenly distributed among localities (Blowes et al. 2024). Such
532 uneven local responses may, in turn, emerge through different ecological processes.
533 Contrasting local environmental conditions may promote differentiation by imposing
534 distinct environmental filters in freshwaters (Bürgés et al. 2026), while reduced
535 connectivity may limit dispersal and recolonization (Jiang et al. 2021). Stochastic
536 processes such as ecological drift may also contribute to divergent community
537 reorganization, as their relative importance increases when disturbances reduce local
538 population sizes (McFadden et al. 2023). Together, these processes may allow
539 communities to follow distinct compositional trajectories, ultimately promoting regional
540 differentiation.

541 Taxonomic and functional facets both revealed differentiation, indicating that the
542 overall pattern was evident in both species identities and trait composition. In turn,
543 phylogenetic responses could not be interpreted with the same confidence because this
544 facet remained poorly represented in the available evidence. Previous studies
545 encompassing different freshwater groups and impacted conditions have reported either
546 no detectable change across facets (Lindholm et al. 2020a, 2020b; Wojciechowski et al.
547 2017) or divergent responses among them (Ferreira et al. 2026; Gianuca et al. 2018).
548 Our cross-group synthesis instead showed that taxonomic and functional facets both
549 tended towards differentiation, consistent with similar patterns reported for freshwater
550 groups (Jiang et al. 2024). Together, the matching directional responses of taxonomic
551 and functional β-diversity suggest that community reorganization under human impacts
552 extends beyond species identities to the ecological attributes they represent, with
553 potential implications for ecosystem functioning.

554 Based on the available evidence, land use was the only impact category showing a clear
555 differentiation signal. Because land-use change encompasses different pressures and
556 intensities within the same sampled region (Petsch et al. 2021), variation within this
557 category may contribute to the emergence of differentiation. Agricultural activities can
558 alter nutrient and contaminant inputs (Elliott et al. 2024), urbanization can modify
559 hydrological and water-quality conditions (Gál et al. 2019), and mining can introduce
560 metal contamination into river systems (Macklin et al. 2023). Indeed, broader evidence
561 has shown that freshwater biodiversity responds differently across land-use types (Shen
562 et al. 2026). More generally, even highly stressed freshwater systems may experience
563 spatially heterogeneous pressures and environmental filtering, allowing community
564 responses to diverge and β-diversity to increase (Cortés-Guzmán et al. 2026). By
565 contrast, multiple impacts and hydrological alteration showed no clear direction. For
566 multiple impacts, this may partly reflect variable interactions among stressors, including
567 antagonistic effects (Jackson et al. 2016). In turn, the lack of a clear direction for
568 hydrological alteration—which was represented mainly by damming in our dataset—
569 may reflect the spatially and temporally dependent effects of impoundment, which can
570 promote either differentiation or homogenization as assemblages reorganize (Ferreira et

al. 2026). Together, these results show that β-diversity responses depend on the nature and combination of human pressures. Distinguishing specific impact types may therefore help identify when human pressures promote compositional convergence or divergence among localities and inform context-specific conservation planning.

Spatially interspersed and spatiotemporal comparisons indicated differentiation, whereas spatially segregated comparisons showed no consistent directional change. This contrast suggests that the consistency of β-diversity responses may depend on how reference and impacted assemblages are compared. Moreover, the differentiation pattern observed in spatially interspersed and spatiotemporal comparisons emerged despite variation in spatial extent and climatic heterogeneity, while absolute latitude showed no detectable association with β-diversity change across all designs. In spatially interspersed comparisons, differentiation occurred among impacted localities within the same broad regional setting as reference localities, where species pools may overlap. However, local environmental differences may constrain establishment, while hydrological connectivity may still limit dispersal among these localities. Freshwater connectivity is particularly complex because dendritic drainage networks and natural or anthropogenic barriers constrain dispersal pathways in running waters, whereas lakes and ponds form spatially discrete habitats with varying degrees of connectivity (Heino et al. 2015b). Connectivity is further shaped by flow regime, which influences both the degree and timing of hydrological connections while also modifying environmental conditions (Rolls et al. 2016). Together, these features may influence the set of potential colonists and the sequence of species arrivals and losses, producing site-specific assembly histories. Consequently, differences in assembly history may contribute to distinct species compositions even among nearby assemblages (Chase 2003). Human pressures may interact with these pre-existing ecological and historical differences, allowing communities to follow distinct compositional trajectories and contributing to differentiation within interspersed designs. In segregated comparisons, reference and impacted assemblages occupy distinct spatial settings and may differ more substantially in local environmental conditions, assembly histories, and regional species pools. These differences may contribute to the absence of a consistent directional response, rather than indicating that β-diversity is unresponsive to human pressures.

Within spatiotemporal comparisons, elapsed time between reference and impacted conditions showed no detectable association with β-diversity change, suggesting that time alone was not a consistent predictor of the observed responses. Although temporal comparisons have long been considered particularly informative for detecting biotic homogenization (Rahel 2002; Olden 2006), previous syntheses have revealed heterogeneous trajectories of spatial β-diversity across temporal scales, including decadal to centennial records (Blowes et al. 2024). The ecological context and historical contingency discussed above may help explain this lack of a consistent temporal relationship. As environmental conditions, hydrological connectivity, and additional disturbances vary through time, local assemblages with distinct ecological histories may follow different compositional trajectories. Consequently, similar temporal windows may encompass substantially different ecological changes, while longer intervals may

not necessarily produce greater compositional divergence or convergence among localities. Thus, elapsed time alone may be a poor proxy for the ecological changes occurring between reference and impacted conditions.

The inferred direction of β-diversity change was sensitive to data type. Presence–absence data indicated differentiation, whereas quantitative data indicated homogenization, showing that the inferred direction of β-diversity change may depend on the type of measure being considered. Occurrence- and abundance-based approaches can lead to different, and even opposite, inferences about biotic homogenization or differentiation (Buonaiuto et al. 2025), including in freshwater assemblages under human pressures (Shen et al. 2026). One possible explanation for this contrast builds on the concept of ecological "winners" and "losers" (McKinney and Lockwood 1999). Widespread species may become more abundant across localities, promoting quantitative homogenization, while different species may be gained or lost across localities, promoting occurrence-based differentiation. However, this interpretation remains hypothetical, and the contrast between data types should still be treated cautiously because quantitative data were available from only 13 articles and encompassed different descriptors. These articles nevertheless spanned multiple biological groups, reducing the likelihood that the contrast was driven solely by biological-group representation. Nevertheless, quantitative measures capture partly distinct aspects of community structure, and their responses may depend on differences in species dominance and abundance distributions among assemblages. Thus, the contrasting responses may reflect differences between occurrence- and abundance-based patterns of community reorganization, although heterogeneity among quantitative measures and the more limited evidence base warrant caution in interpreting this contrast.

A further caveat concerns limitations in the representation and availability of the compiled evidence. Fish and invertebrates were the most represented biological groups and both showed differentiation; accordingly, their exclusion shifted the pooled response toward homogenization. This sensitivity indicates that the global tendency toward differentiation should not be interpreted as equally supported across freshwater taxa. However, because the remaining evidence was based on substantially fewer study cases, this shift should not be interpreted as a general contrast between fish and invertebrates and the other groups. Moreover, other potentially important sources of variation could not be explicitly evaluated. In particular, α-diversity was not consistently available, preventing us from assessing whether regional differentiation was associated with uneven changes in local diversity, while variation in sampling grain may have influenced the magnitude of compositional differences detected among localities. Also, analyses of macroecological variables were necessarily restricted to subsets of the complete dataset according to data availability. Together, these limitations indicate that some sources of variation remain unresolved, while the broader differentiation pattern remains supported by the available evidence. Broader taxonomic representation and more consistently reported local and spatial information will be

necessary to better resolve the contexts under which freshwater assemblages undergo biotic homogenization or differentiation.

5 | Conclusion

Human impacts in freshwaters should not be expected to inherently promote biotic homogenization. Our synthesis instead indicated overall differentiation, with responses varying across impact types, study designs, data types, and taxonomic representation. Also, no detectable relationships were found with the broad-scale spatial, environmental, and temporal gradients evaluated. Occurrence-based estimates indicated differentiation, whereas quantitative estimates indicated homogenization, and the pooled response shifted toward homogenization when fish and invertebrates were excluded. Together, these results show that the inferred β-diversity responses depend on both the component of community structure measured and the taxonomic composition of the available evidence. In particular, the contrast between occurrence-based and quantitative estimates is consistent with impacted regions retaining distinct species identities while becoming more similar in relative abundance, potentially through the increasing dominance of widespread taxa. More broadly, we argue that the observed direction likely reflects the balance between processes that drive assemblages toward a common impacted state and those that maintain or amplify local ecological and historical differences or increase the influence of stochastic assembly. The predominance of differentiation across the full dataset suggests that the latter processes prevailed on average, although not uniformly across assemblages or components of community structure. This balance may be especially sensitive to context in freshwaters, where connectivity constraints and flow regimes jointly shape dispersal opportunities and local environmental conditions. Identifying when similar human pressures override ecological context and when they instead interact with it to generate contrasting responses may therefore be key to predicting whether freshwater communities within a region converge toward a common impacted state or diverge toward distinct ones.

Author Contributions

Vitor M. B. Ferreira: conceptualization, methodology, data curation, investigation, validation, formal analysis, visualization, writing – original draft, writing – review and editing. **Natália C. L. dos Santos:** investigation, validation, writing – review and editing. **Míriam P. Albrecht:** investigation, validation, writing – review and editing, supervision. **Bruno E. Soares:** conceptualization, investigation, validation, writing – review and editing, supervision.

Acknowledgments

The authors would like to thank Juliana Leal, Danielle Petsch, Vinícius Farjalla, Rafaela Granzotti, Gabriel Brejão, and Márcio Zikán for their contributions during Vitor M. B. Ferreira's doctoral appraisal committees. Vitor M. B. Ferreira would also like to thank the Soares Quantitative Ecology Lab for hosting him as a visiting graduate researcher at the University of Regina, Canada, during the development of this study.

The authors used ChatGPT (OpenAI; GPT-5.5 and GPT-5.6, accessed in 2026) to assist with drafting, editing, and debugging of the code used to analyze data, as well as to support grammar correction. All code was reviewed and validated by the authors.

Funding

Vitor M. B. Ferreira received grants during the development of this study through the Emerging Leaders in the Americas Program (ELAP), by Immigration, Refugees, and Citizenship, Canada (IRCC), and through a Bolsa Nota 10 doctoral fellowship from the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) – process number: E-26/202.403/2024.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and code supporting the findings of this study will be made publicly available in a repository upon publication of the peer-reviewed article.

References

Barton, P. S., S. A. Cunningham, A. D. Manning, H. Gibb, D. B. Lindenmayer, and R. K. Didham. 2013. "The Spatial Scaling of Beta Diversity." *Global Ecology and Biogeography* 22, no. 6: 639—647. https://doi.org/10.1111/geb.12031

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using *lme4*." *Journal of Statistical Software* 67, no. 1: 1—48. https://doi.org/10.18637/jss.v067.i01

Blowes, S. A., B. McGill, V. Brambilla, et al. 2024. "Synthesis Reveals Approximately Balanced Biotic Differentiation and Homogenization." *Science Advances* 10, no. 8: eadj9395. https://doi.org/10.1126/sciadv.adj9395

Buonaiuto, D. M., D. Barnett, D. M. Blumenthal, et al. 2025. "Using Plant Invasions to Compare Occurrence- and Abundance-Based Calculations of Biotic Homogenisation: Are Results Complementary or Contradictory?" *Global Ecology and Biogeography* 34: e70022. https://doi.org/10.1111/geb.70022

Bürgés, J., B. Gál, I. Szivák, et al. 2026. "Urbanization and Stream Identity Shape Beta Diversity and its Components: Evidence from Stream-Dwelling Macroinvertebrate Assemblages." *Hydrobiologia* 853: 3197—3215. https://doi.org/10.1007/s10750-026-06134-x

Campbell, S. E., and N. E. Mandrak. 2019. "Temporal Dynamics of Taxonomic Homogenization in the Fish Communities of the Laurentian Great Lakes." *Diversity and Distributions* 25, no. 12: 1870—1878. https://doi.org/10.1111/ddi.12986

Campbell, S. E., and N. E. Mandrak. 2020. "Functional Differentiation Accompanies Taxonomic Homogenization in Freshwater Fish Communities." *Ecology* 101, no. 12: e03188. https://doi.org/10.1002/ecy.3188

Chase, J. M. 2003. "Community Assembly: When Should History Matter?" *Oecologia* 136: 489—498. https://doi.org/10.1007/s00442-003-1311-7

Cortés-Guzmán, D., D. E. Bowler, M. A. E. Forio, et al. 2026. "Recovering European River Invertebrate Communities Homogenize or Differentiate Depending on Anthropogenic Stress." *Global Change Biology* 32: e70716. https://doi.org/10.1111/gcb.70716

Crouzeilles, R., M. Curran, M. Ferreira, D. Lindenmayer, C. E. V. Grelle, and L. Rey Benayas. 2016. "A Global Meta-Analysis on the Ecological Drivers of Forest Restoration Success." *Nature Communications* 7: 11666. http://dx.doi.org/10.1038/ncomms11666

Crouzeilles, R., M. Ferreira, R. L. Chazdon, et al. 2017. "Ecological Restoration Success is Higher for Natural Regeneration than for Active Restoration in Tropical Forests." *Science Advances* 3: e1701345. https://doi.org/10.1126/sciadv.1701345

765 Dornelas, M., J. M. Chase, N. J. Gotelli, et al. 2023. “Looking Back on Biodiversity
766 Change: Lessons for the Road Ahead.” *Philosophical Transactions of the Royal Society*
767 *B* 378, no.1881: 20220199 https://doi.org/10.1098/rstb.2022.0199

768 Egger, M., G. Davey Smith, M. Schneider, and C. Minder. 1997. “Bias in Meta-
769 Analysis Detected by a Simple, Graphical Test.” *BMJ* 315: 629—634.
770 https://doi.org/10.1136/bmj.315.7109.629

771 Elliott A. H., S. E. Graham, P. A. Franklin, and S. T. Larned. 2024. “Predicting Impacts
772 of Agricultural Land Use on Stream and River Biota: Method Review, Evaluation, and
773 Guidance.” *Environmental Reviews* 32, no. 4: 572—591. https://doi.org/10.1139/er-
774 2023-0134

775 Ferreira, V. M. B., N. C. L. dos Santos, J. C. G. Ortega, R. G. Lima, E. P. Caramaschi,
776 and M. P. Albrecht. 2026. “Damming Affects the Taxonomic but Not the Functional
777 Beta Diversity of Fish in a Neotropical Reservoir Ecosystem.” *Hydrobiologia* 853:
778 3019—3033. https://doi.org/10.1007/s10750-026-06118-x

779 Fick, S. E., and R. J. Hijmans. 2017. “WorldClim 2: New 1-Km Spatial Resolution
780 Climate Surfaces for Global Land Areas.” *International Journal of Climatology* 37, no.
781 12: 4302—4315. https://doi.org/10.1002/joc.5086

782 Gál, B., I. Szivák, J. Heino, and D. Schmera. 2019. “The Effect of Urbanization on
783 Freshwater Macroinvertebrates—Knowledge Gaps and Future Research Directions.”
784 *Ecological Indicators* 104: 357—364. https://doi.org/10.1016/j.ecolind.2019.05.012

785 Gianuca, A. T., J. Engelen, K. I. Brans, et al. 2018. “Taxonomic, Functional and
786 Phylogenetic Metacommunity Ecology of Cladoceran Zooplankton Along Urbanization
787 Gradients.” *Ecography* 41: 183—194. https://doi.org/10.1111/ecog.02926

788 Gross, N., Y. Le Bagousse-Pinguet, P. Liancourt, M. Berdugo, N. J. Gotelli, and F. T.
789 Maestre. 2017. “Functional Trait Diversity Maximizes Ecosystem Multifunctionality.”
790 *Nature Ecology & Evolution* 1: 0132. https://doi.org/10.1038/s41559-017-0132

791 Harrison, S., S. J. Ross, and J. H. Lawton. 1992. “Beta Diversity on Geographic
792 Gradients in Britain.” *Journal of Animal Ecology* 61, no. 1: 151—158.
793 https://doi.org/10.2307/5518

794 Heino, J., A. S. Melo, and L. M. Bini. 2015a. “Reconceptualising the Beta Diversity-
795 Environmental Heterogeneity Relationship in Running Water Systems.” *Freshwater*
796 *Biology* 60: 223—235. https://doi.org/10.1111/fwb.12502

797 Heino, J., A. S. Melo, T. Siqueira, J. Soininen, S. Valanko, and L. M. Bini. 2015b.
798 “Metacommunity Organisation, Spatial Extent, and Dispersal in Aquatic Systems:
799 Patterns, Processes and Prospects.” *Freshwater Biology* 60: 845—869.
800 https://doi.org/10.1111/fwb.12533

801 Heino, J., L. M. Bini, J. García-Girón, F. M. Lansac-Tôha, M. Lindholm, and R. J.
802 Rolls. 2024. “Navigating the Spatial and Temporal Aspects of Beta Diversity to

803 Facilitate Understanding Biodiversity Change." *Global Ecology and Conservation* 56:
804 e03343. https://doi.org/10.1016/j.gecco.2024.e03343

805 Hijmans, R. J. 2022. "geosphere: Spherical Trigonometry. [R Package]."
806 https://CRAN.R-project.org/package=geosphere

807 Hijmans, R. J. 2023. "terra: Spatial Data Analysis. [R Package]." https://CRAN.R-
808 project.org/package=terra

809 Hurlbert, S. H. 1984. "Pseudoreplication and the Design of Ecological Field
810 Experiments." *Ecological Monographs* 54, no. 2: 187—211.
811 https://doi.org/10.2307/1942661

812 Jackson, M. C., C. J. G. Loewen, R. D. Vinebrooke, and C. T. Chimimba. 2016. "Net
813 Effects of Multiple Stressors in Freshwater Ecosystems: A Meta-Analysis." *Global*
814 *Change Biology* 22: 180—189. https://doi.org/10.1111/gcb.13028

815 Jiang, X., C. Ding, S. Brosse, B. Pan, Y. Lu, and Z. Xie. 2019. "Local Rise of
816 Phylogenetic Diversity Due to Invasions and Extirpations Leads to a Regional
817 Phylogenetic Homogenization of Fish Fauna from Chinese Isolated Plateau Lakes."
818 *Ecological Indicators* 101: 388—398. https://doi.org/10.1016/j.ecolind.2019.01.041

819 Jiang, X., P. Zheng, L. Cao, and B. Pan. 2021. "Effects of Long-Term Floodplain
820 Disconnection on Multiple Facets of Lake Fish Biodiversity: Decline of Alpha Diversity
821 Leads to a Regional Differentiation Through Time." *Science of The Total Environment*
822 763: 144177. https://doi.org/10.1016/j.scitotenv.2020.144177

823 Jiang, X., D. Peng, J. Alahuhta, J. Heino, and Z. Li. 2024. "Eutrophication Modifies the
824 Relationships Between Multiple Facets of Macroinvertebrate Beta Diversity and
825 Geographic Distance in Freshwater Lakes." *Diversity and Distributions* 30: e13830.
826 https://doi.org/10.1111/ddi.13830

827 Keck, F., T. Peller, R. Alther, et al. 2025. "The Global Human Impact on Biodiversity."
828 *Nature* 641: 395—400. https://doi.org/10.1038/s41586-025-08752-2

829 Lansac-Tôha, F. M., L. M. Bini, J. Heino, et al. 2021. "Scale-Dependent Patterns of
830 Metacommunity Structuring in Aquatic Organisms Across Floodplain Systems."
831 *Journal of Biogeography* 48: 872—885. https://doi.org/10.1111/jbi.14044

832 Lindholm, M., J. Alahuhta, J. Heino, J. Hjort, and H. Toivonen. 2020a. "Changes in the
833 Functional Features of Macrophyte Communities and Driving Factors Across a 70-Year
834 Period." *Hydrobiologia* 847: 3811—3827. https://doi.org/10.1007/s10750-019-04165-1

835 Lindholm, M., J. Alahuhta, J. Heino, and H. Toivonen. 2020b. "No Biotic
836 Homogenisation Across Decades but Consistent Effects of Landscape Position and Ph
837 ON Macrophyte Communities in Boreal Lakes." *Ecography* 43: 294—305.
838 https://doi.org/10.1111/ecog.04757

839 Nekola, J. C., and P. S. White. 1999. “The Distance Decay of Similarity in
840 Biogeography and Ecology.” *Journal of Biogeography* 26: 867—878.
841 https://doi.org/10.1046/j.1365-2699.1999.00305.x

842 Macklin, M. G., C. J. Thomas, A. Mudbhatkal, et al. 2023. “Impacts of Metal Mining
843 on River Systems: A Global Assessment.” *Science* 381: 1345—1350.
844 https://doi.org/10.1126/science.adg6704

845 McFadden, I. R., A. Sendek, M. Brosse, et al. 2023. “Linking Human Impacts to
846 Community Processes in Terrestrial and Freshwater Ecosystems.” *Ecology Letters* 36:
847 203—218. https://doi.org/10.1111/ele.14153

848 McKinney, M. L., and J. L. Lockwood. 1999. “Biotic Homogenization: A Few Winners
849 Replacing Many Losers in the Next Mass Extinction.” *Trends in Ecology & Evolution*
850 14, no. 11: 450—453. https://doi.org/10.1016/S0169-5347(99)01679-1

851 Mori, A. S., F. Isbell, and R. Seidl. 2018. “β-Diversity, Community Assembly, and
852 Ecosystem Functioning.” *Trends in Ecology & Evolution* 33: 549—564.
853 https://doi.org/10.1016/j.tree.2018.04.012

854 Naeem, S., C. Prager, B. Weeks, et al. 2016. “Biodiversity as a Multidimensional
855 Construct: A Review, Framework and Case Study of Herbivory’s Impact on Plant
856 Biodiversity.” *Proceedings of the Royal Society B: Biological Sciences* 283, 1844:
857 20153005. https://doi.org/10.1098/rspb.2015.3005

858 Nakagawa, S., M. Lagisz, M. D. Jennions, et al. 2022. “Methods for Testing Publication
859 Bias in Ecological and Evolutionary Meta-Analyses.” *Methods in Ecology and*
860 *Evolution* 13: 4—21. https://doi.org/10.1111/2041-210X.13724

861 Olden, J. D. 2006. “Biotic Homogenization: A New Research Agenda for Conservation
862 Biogeography.” *Journal of Biogeography* 33: 2027—2039.
863 https://doi.org/10.1111/j.1365-2699.2006.01572.x

864 Olden, J. D., and T. P. Rooney. 2006. “On Defining and Quantifying Biotic
865 Homogenization.” *Global Ecology and Biogeography* 15, no. 2: 113—120.
866 https://doi.org/10.1111/j.1466-822X.2006.00214.x

867 Pebesma, E. 2018. Simple Features for R: “Standardized Support for Spatial Vector
868 Data.” *The R Journal* 10, no. 1: 439—446. https://doi.org/10.32614/RJ-2018-009

869 Petsch, D. K. 2016. “Causes and Consequences of Biotic Homogenization in Freshwater
870 Ecosystems.” *International Review of Hydrobiology* 101: 113—122.
871 https://doi.org/10.1002/iroh.201601850

872 Petsch, D. K., S. A. Blowes, A. S. Melo, and J. M. Chase. 2021. “A Synthesis of Land
873 Use Impacts on Stream Biodiversity Across Metrics and Scales.” *Ecology* 102, no. 11:
874 e03498. https://doi.org/10.1002/ecy.3498

875 Petsch, D. K., A. P. S. Bertoncin, J. C. G. Ortega, and S. M. Thomaz. 2022. “Non-
876 Native Species Drive Biotic Homogenization, but it Depends on the Realm, Beta

877 Diversity Facet and Study Design: A Meta-Analytic Systematic Review." *Oikos* 6:
878 e08768. https://doi.org/10.1111/oik.08768

879 Poisot, T. 2011. "The Digitize Package: Extracting Numerical Data from Scatterplots."
880 *The R Journal* 3, no. 1: 25—26. https://doi.org/10.32614/RJ-2011-004

881 Pustejovsky, J. E., and E. Tipton. 2018. "Small-Sample Methods for Cluster-Robust
882 Variance Estimation and Hypothesis Testing in Fixed Effects Models." *Journal of*
883 *Business & Economic Statistics* 36, no. 4: 672—683.
884 https://doi.org/10.1080/07350015.2016.1247004

885 Rahel, F. J. 2002. "Homogenization of Freshwater Faunas." *Annual Review of Ecology,*
886 *Evolution, and Systematics* 33: 291—315.
887 https://doi.org/10.1146/annurev.ecolsys.33.010802.150429

888 Rolls, J. R., J. Heino, and C. Chessman. 2016. "Unravelling the Joint Effects of Flow
889 Regime, Climatic Variability and Dispersal Mode on Beta Diversity of Riverine
890 Communities." *Freshwater Biology* 61: 1350—1364. https://doi.org/10.1111/fwb.12793

891 Rolls, R. J., J. Heino, D. S. Ryder, et al. 2018. "Scaling Biodiversity Responses to
892 Hydrological Regimes." *Biological Reviews* 93: 971—995.
893 https://doi.org/10.1111/brv.12381

894 Rolls, R. J., B. C. Chessman, J. Heino, et al. 2021. "Consequences of Hydrological
895 Alteration for Beta Diversity of Fish Assemblages at Multiple Spatial Scales." *Science*
896 *of The Total Environment* 798: 149170. https://doi.org/10.1016/j.scitotenv.2021.149170

897 Rolls, R. J., D. C. Deane, S. E. Johnson, J. Heino, M. J. Anderson, and K. E. Ellingsen.
898 2023a. "Biotic Homogenisation and Differentiation as Directional Change in Beta
899 Diversity: Synthesising Driver-Response Relationships to Develop Conceptual Models
900 Across Ecosystems." *Biological Reviews* 98, no. 4: 1388—1423.
901 https://doi.org/10.1111/brv.12958

902 Rolls, R., B. Wolfenden, J. Heino, G. L. Butler, and J. Thiem. 2023b. "Scale
903 Dependency in Fish Beta Diversity-Hydrology Linkages in Lowland Rivers." *Journal of*
904 *Biogeography* 50, no. 10: 1692—1709. https://doi.org/10.1111/jbi.14672

905 Sayer, C. A., E. Fernando, R. R. Jimenez, et al. 2025. "One-Quarter of Freshwater
906 Fauna Threatened with Extinction." *Nature* 638: 138—145.
907 https://doi.org/10.1038/s41586-024-08375-z

908 Shen, M., R. Klink, W. Xu, A. Sangouis, and J. M. Chase. 2026. "Land Use Influences
909 on Freshwater Biodiversity." *Global Ecology and Biogeography* 35: e70230.
910 https://doi.org/10.1111/geb.70230

911 Simões, N. R., L. S. M. Braghin, G. A. V. Duré, J. S. Santos, S. L. Sonoda, and C. C.
912 Bonecker. 2020. "Changing Taxonomic and Functional β-Diversity of Cladoceran
913 Communities in Northeastern and South Brazil." *Hydrobiologia* 847: 3845—3856.
914 https://doi.org/10.1007/s10750-020-04234-w

Socolar, J. B., J. J. Gilroy, W. E. Kunin, and D. P. Edwards. 2016. "How Should Beta-Diversity Inform Biodiversity Conservation?" *Trends in Ecology & Evolution* 31, no. 1: 67—80. https://doi.org/10.1016/j.tree.2015.11.005

Soininen, J., J. Heino, and J. Wang. 2018. "A Meta-Analysis of Nestedness and Turnover Components of Beta Diversity Across Organisms and Ecosystems." *Global Ecology and Biogeography* 27: 96—109. https://doi.org/10.1111/geb.12660

Srivastava, D. S., M. W. Cadotte, A. A. M. MacDonald, R. G. Marushia, and N. Mirotchnick. 2012. "Phylogenetic Diversity and the Functioning of Ecosystems." *Ecology Letters* 15: 637—648. https://doi.org/10.1111/j.1461-0248.2012.01795.x

Taylor, E. B. 2004. "An Analysis of Homogenization and Differentiation of Canadian Freshwater Fish Faunas with an Emphasis on British Columbia." *Canadian Journal of Fisheries and Aquatic Sciences* 61, no. 1: 68—79. https://doi.org/10.1139/f03-141

Viechtbauer, W. 2010. "Conducting Meta-Analyses in R with Metafor Package." *Journal of Statistical Software* 36, no. 3: 1—48. https://doi.org/10.18637/jss.v036.i03

Wan, X., W. Wang, J. Liu, and T. Tong. 2014. "Estimating the Sample Mean and Standard Deviation from the Sample Size, Median, Range, and/or Interquartile Range." *BMC Medical Research Methodology* 14: 135. https://doi.org/10.1186/1471-2288-14-135

Whittaker, R. H. 1960. "Vegetation of the Siskiyou Mountains, Oregon and California." *Ecological Monographs* 30, no. 3: 279—338. https://doi.org/10.2307/1943563

Wojciechowski, J., J. Heino, L. M. Bini, and A. A. Padial. 2017. "Temporal Variation in Phytoplankton Beta Diversity Patterns and Metacommunity Structures Across Subtropical Reservoirs." *Freshwater Biology* 62: 751—766. https://doi.org/10.1111/fwb.12899

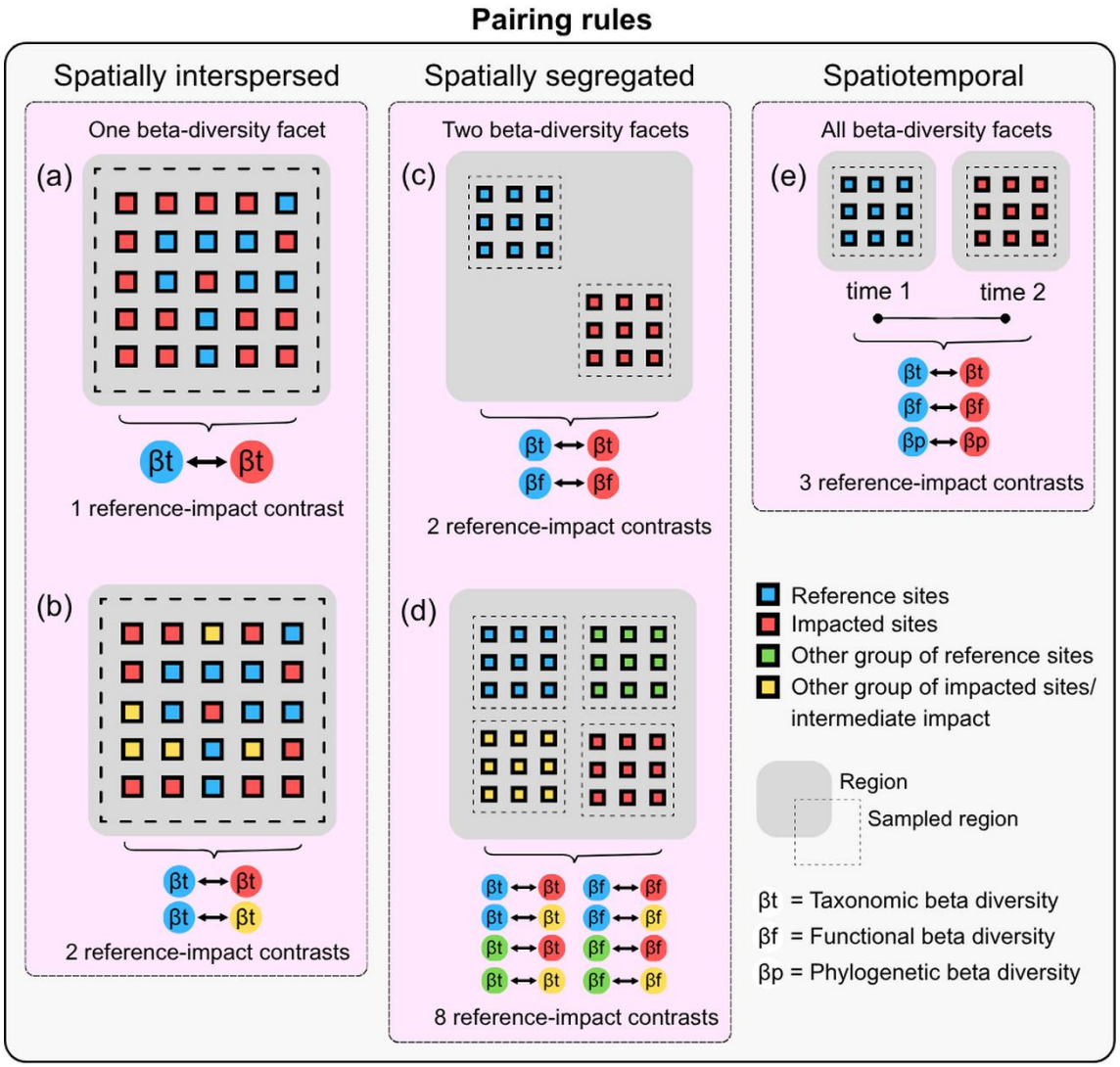
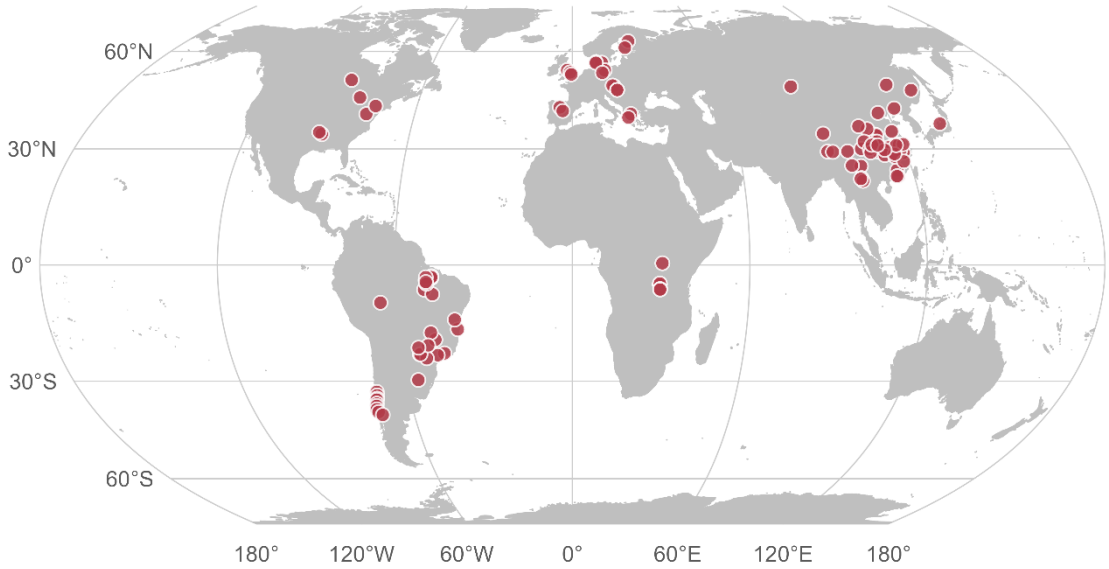


FIGURE 1 | Schematic examples of the pairing rules used to generate reference–impact contrasts. Each pairing between a reference and an impacted observation generated one contrast for each biodiversity facet available. In spatially interspersed designs, reference and impacted sites occurred within the same sampled region, generating one contrast when one reference group, one impacted group, and one facet were available (a), or two contrasts when the reference group was compared with two impacted groups, including an intermediate-impact group (b). In spatially segregated designs, reference and impacted groups occurred in separate sampled regions. One spatial pairing with two available facets generated two contrasts (c), whereas all four pairings between two reference and two impacted groups, each with two facets, generated eight contrasts (d). In spatiotemporal designs, pairings were restricted to the two temporal endpoints—the reference condition at the initial time point and the impacted condition at the final time point. When three facets were available, this single temporal pairing generated three contrasts (e).



964

965 **FIGURE 2 |** Global distribution of 113 sampling regions included in the meta-analysis.
966 Three sampled regions were not mapped because coordinates were unavailable.

967

968

969

970

971

972

973

974

975

976

977

978

979

980

981

982

983

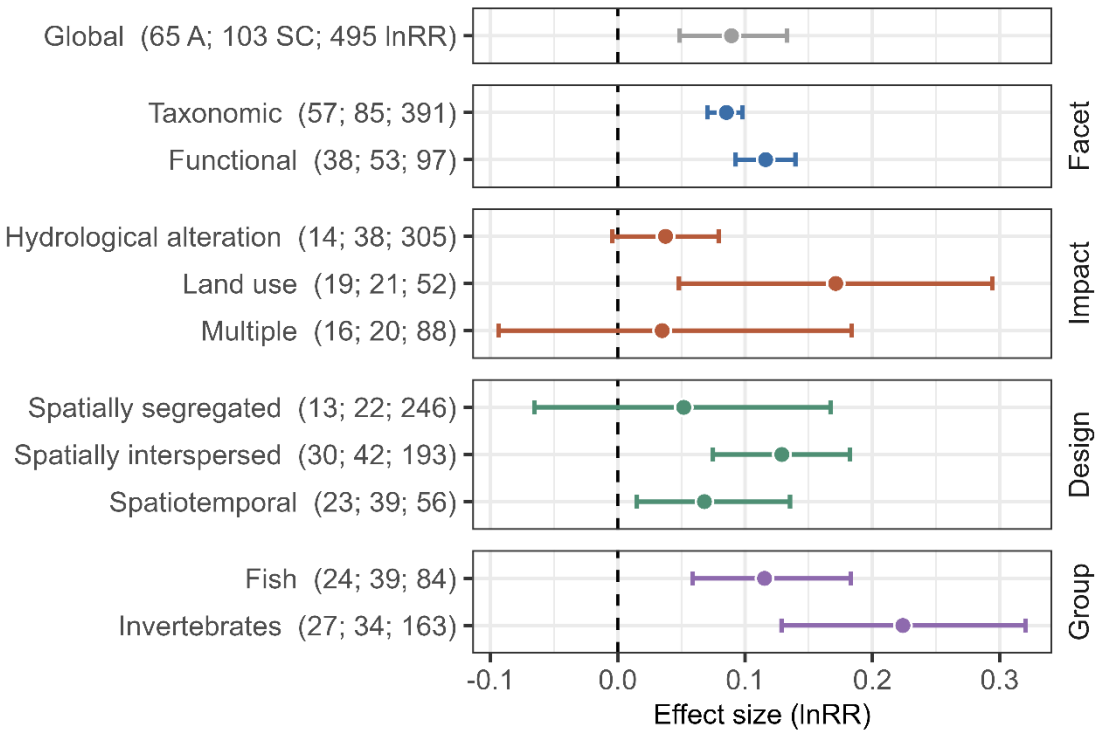


FIGURE 3 | Mean log response ratio (lnRR) and 95% confidence intervals obtained from the bootstrap resampling procedure for the global dataset, biodiversity facets, impact types, study designs, and biological groups. Numbers in parentheses indicate articles included in the meta-analysis (A), study cases (SC) and lnRR estimates. In each bootstrap iteration, one lnRR estimate was randomly sampled per SC. Positive mean lnRR values indicate biotic differentiation, whereas negative values indicate biotic homogenization. Confidence intervals overlapping zero, indicated by the dashed vertical line, were interpreted as showing no clear directional change. Only categories represented by at least 10 articles, 10 study cases and 10 lnRR estimates are shown.

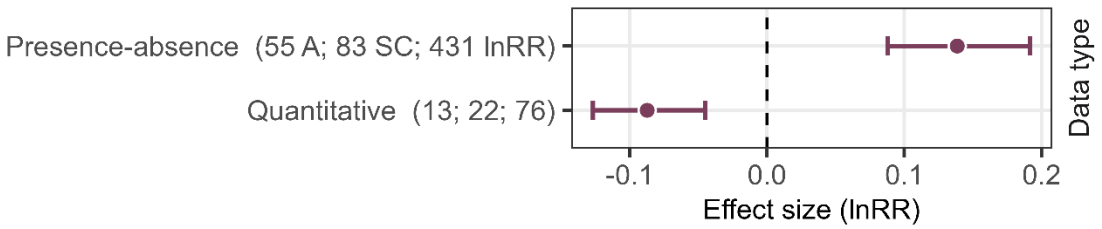


FIGURE 4 | Mean log response ratio (lnRR) and 95% confidence intervals obtained from the bootstrap resampling procedure for community data-type (presence-absence and quantitative). Numbers in parentheses indicate articles included in the meta-analysis (A), study cases (SC) and lnRR estimates. In each bootstrap iteration, one lnRR estimate was randomly sampled per SC. Positive mean lnRR values indicate biotic differentiation, whereas negative values indicate biotic homogenization. Confidence intervals overlapping zero, indicated by the dashed vertical line, were interpreted as showing no clear directional change.

Supplementary Material for

Global Evidence for Biotic Differentiation Amid Contrasting Freshwater β-Diversity Responses to Human Impacts

Running title: Freshwater β-Diversity Change

Vitor M. B. Ferreira et al.

Corresponding author: Vitor M. B. Ferreira

vitormbf2@gmail.com

SUPPLEMENTARY FIGURES

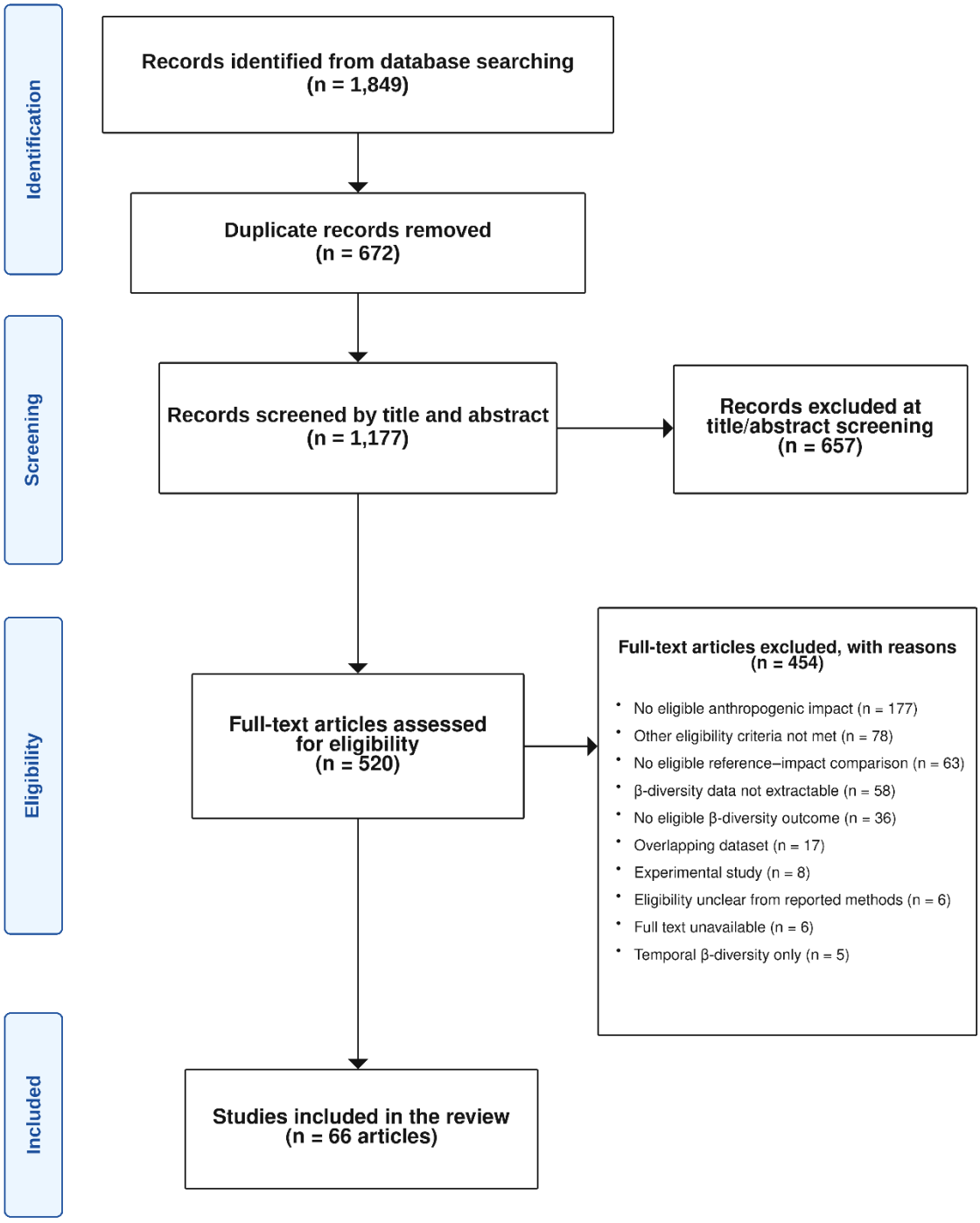


FIGURE S1 | PRISMA flow diagram of the literature search and study selection process.

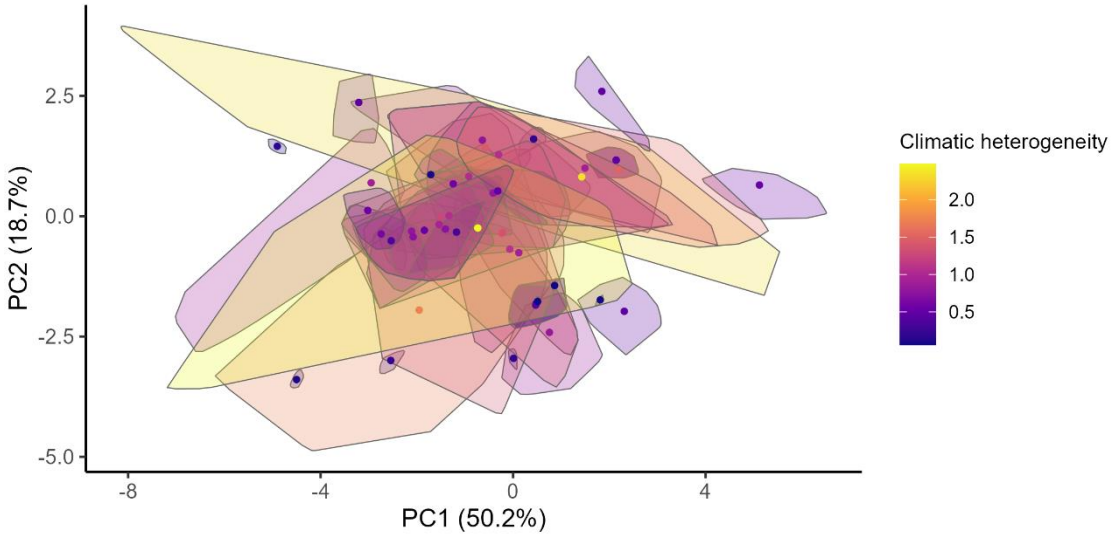


FIGURE S2 | Principal component analysis of climatic variables across study cases. Polygons represent the environmental space occupied by study cases, points indicate their centroids, and colors denote climatic heterogeneity.

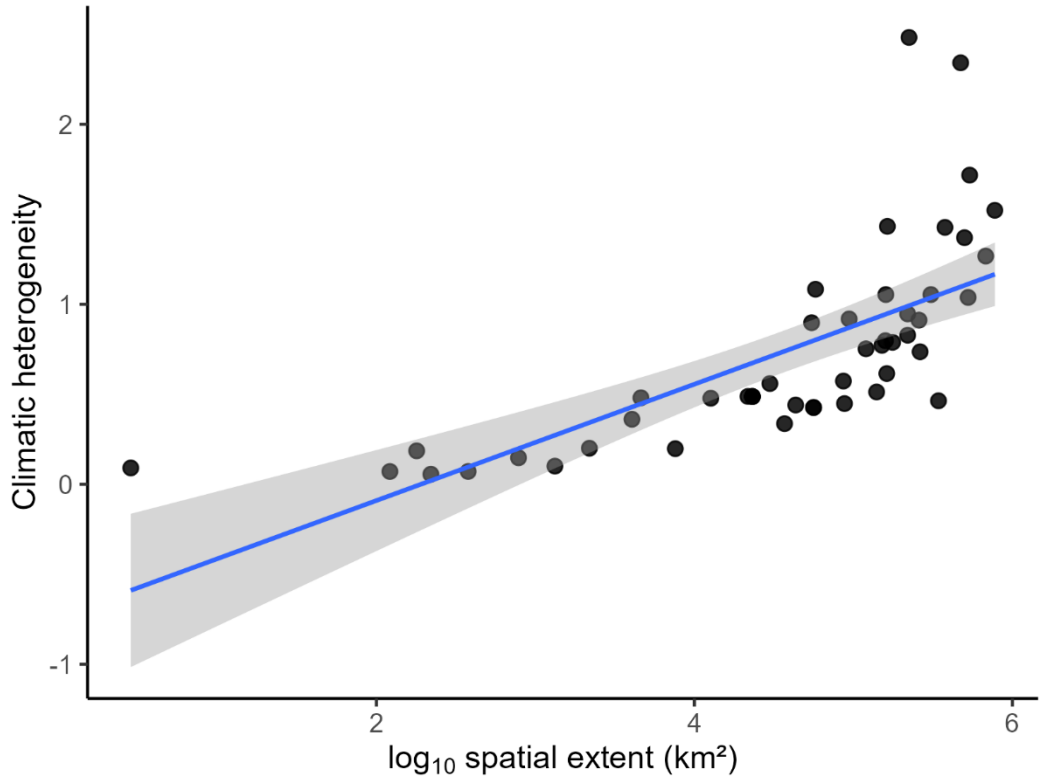


FIGURE S3 | Relationship between spatial extent and climatic heterogeneity across the study cases included in the sensitivity analyses. Spatial extent was log10-transformed, and climatic heterogeneity was derived from eight WorldClim bioclimatic variables.

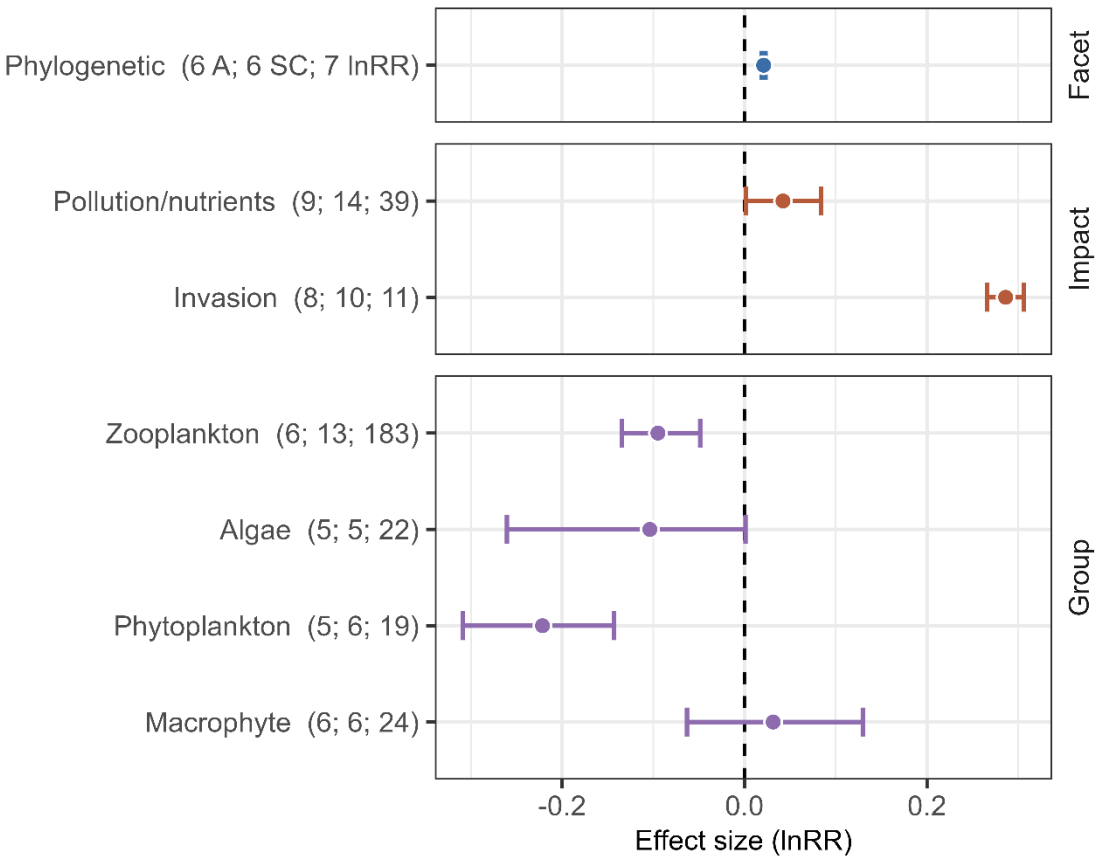


FIGURE S4 | Mean log response ratio (lnRR) and 95% confidence intervals obtained from the bootstrap resampling procedure for poorly represented categories (fewer than 10 articles). Positive mean lnRR values indicate biotic differentiation, whereas negative values indicate biotic homogenization. Confidence intervals overlapping zero, indicated by the dashed vertical line, were interpreted as showing no clear directional change.

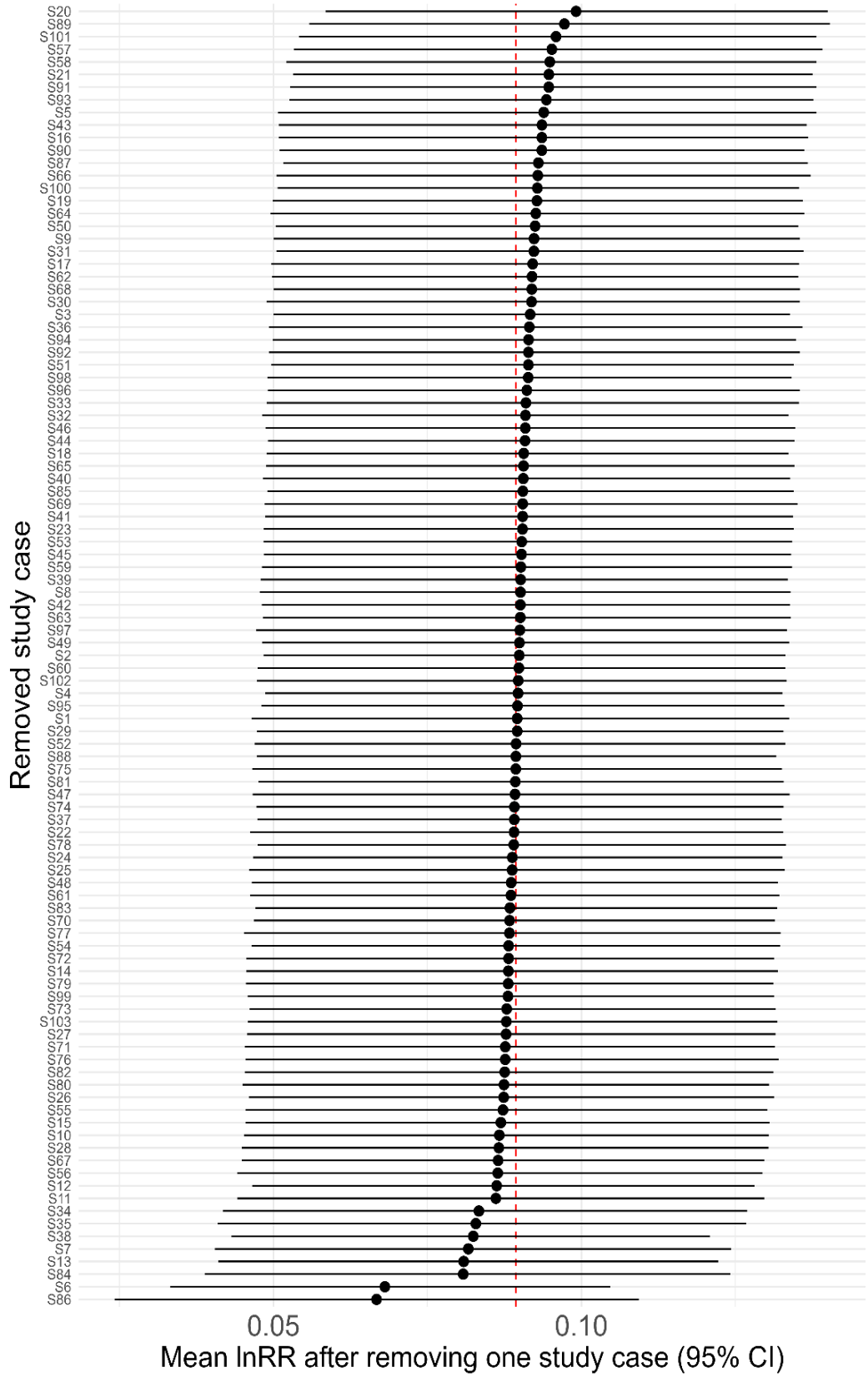


FIGURE S5 | Leave-one-study-case-out sensitivity analysis. Points show the mean lnRR after removing each study case, horizontal lines indicate 95% confidence intervals, and the red dashed line represents the overall mean lnRR from the complete dataset. Study-case identifiers can be found in our dataset.

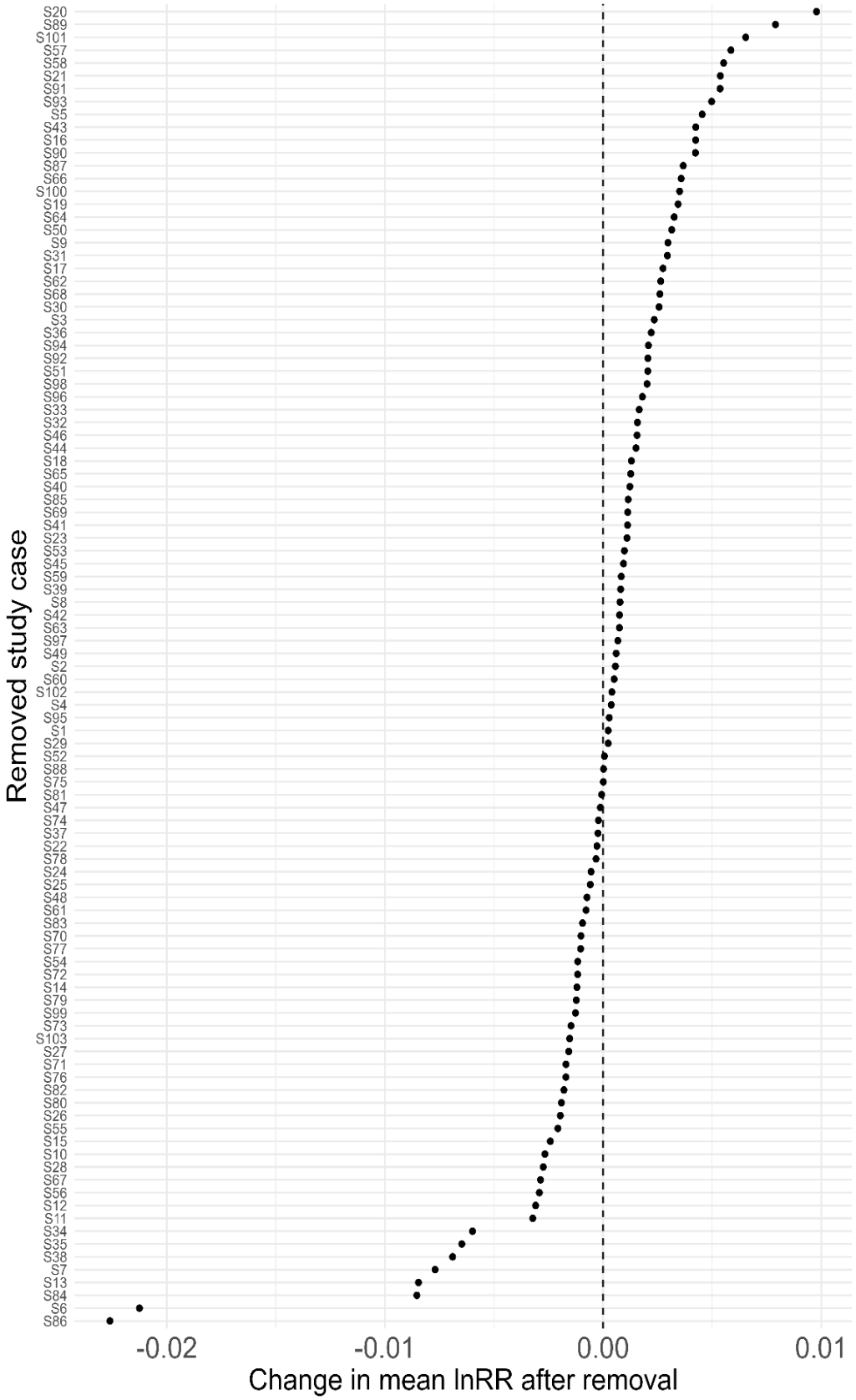


FIGURE S6 | Change in mean lnRR after removing each study case. Points represent the difference from the overall mean lnRR, and the dashed line indicates no change. Study-case identifiers can be found in our dataset.

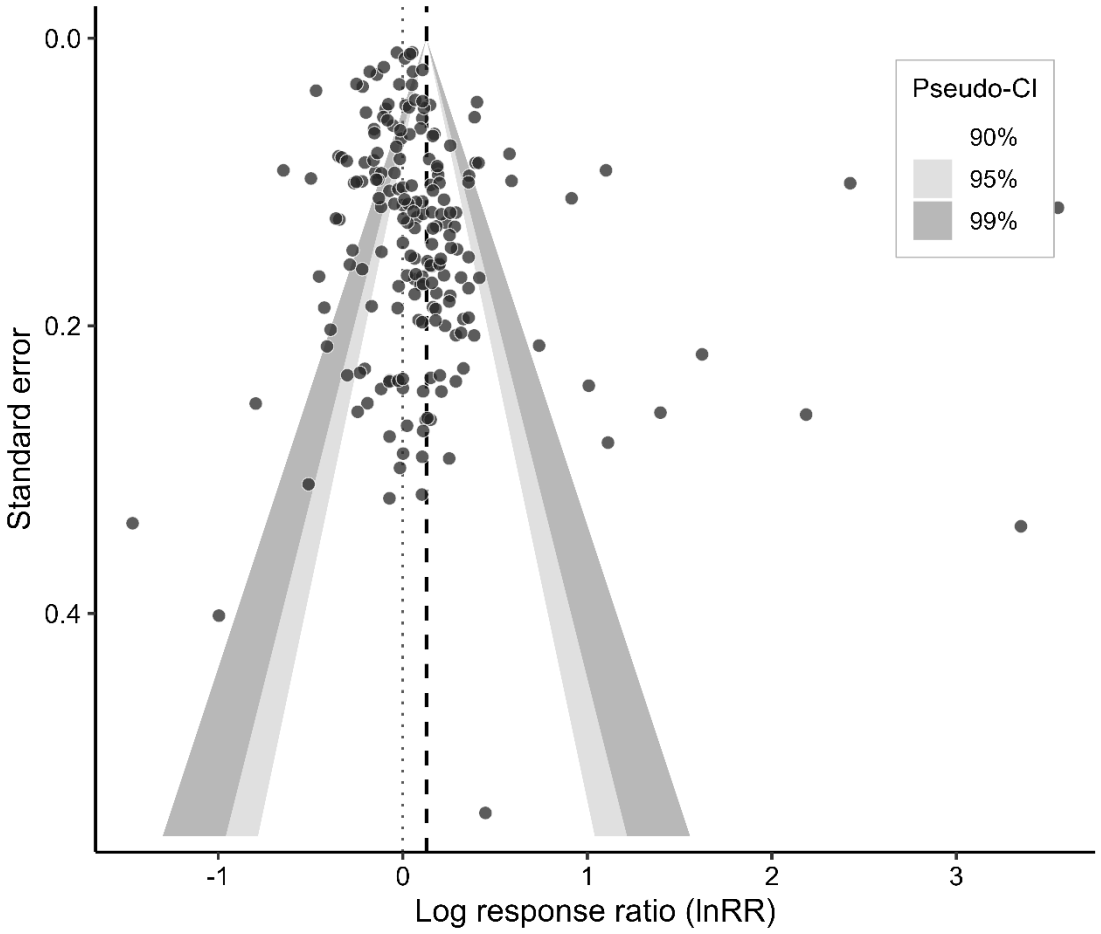


FIGURE S7 | Funnel plot of log response ratios (lnRR) against standard errors, with 90%, 95%, and 99% confidence-intervals. The dashed line indicates the overall mean effect, and the dotted line indicates no effect (lnRR = 0).

SUPPLEMENTARY TABLES

TABLE S1 | Results of random-effects meta-analytic models for the global effect and moderator levels. Estimates are log response ratios (lnRR), with positive and negative values indicating biotic differentiation and homogenization, respectively. SE = standard error; CI = 95% confidence interval; RH = residual heterogeneity (Q for the global model, and QE for moderator-specific models). Asterisks indicate $p < 0.05$ for categories within moderators.

Moderator	Estimate	SE	z value	p value	CI lower	CI upper	RH →	p value
Global	0.1305	0.0732	1.7828	0.0746	-0.0130	0.2739	3294.40	< 0.0001
Facet							3230.10	< 0.0001
Functional	0.1974	0.0732	2.6974	0.0070*	0.0540	0.3408		
Phylogenetic	-0.1343	0.0785	-1.7098	0.0873	-0.2882	0.0197		
Taxonomic	0.1178	0.0726	1.6216	0.1049	-0.0246	0.2602		
Impact							3032.01	< 0.0001
Hydrological alteration	0.1187	0.1656	0.7168	0.4735	-0.2058	0.4432		
Invasion	0.6286	0.2824	2.2257	0.0260*	0.0751	1.1821		
Land use	0.1714	0.1217	1.4086	0.1590	-0.0671	0.4098		
Multiple	0.0284	0.1261	0.2253	0.8217	-0.2188	0.2756		
Pollution/nutrients	-0.1235	0.3424	-0.3609	0.7182	-0.7945	0.5475		
Design							3209.90	< 0.0001
Spatiotemporal	0.1033	0.1308	0.7900	0.4295	-0.1530	0.3186		
Spatially segregated	0.2258	0.1491	1.5144	0.1299	-0.0664	0.5181		
Spatially interspersed	0.0955	0.1139	0.8385	0.4018	-0.1277	0.3186		
Group							3157.31	< 0.0001
Algae	-0.1103	0.3617	-0.3050	0.7604	-0.8193	0.5986		
Fish	0.1091	0.1342	0.8130	0.4162	-0.1540	0.3722		
Invertebrates	0.1787	0.1046	1.7081	0.0876	-0.0263	0.3837		
Macrophyte	0.1649	0.5106	0.3230	0.7467	-0.8359	1.1657		
Phytoplankton	-0.0172	0.3783	-0.0455	0.9637	-0.7587	0.7243		
Zooplankton	0.0076	0.5103	0.0149	0.9881	-0.9925	1.0077		

TABLE S2 | Results of linear mixed-effects models testing potential confounding variables associated with β-diversity change. Estimates represent the slope of each predictor on log response ratios (lnRR), with positive and negative values indicating increasing and decreasing lnRR, respectively. Study cases were included as a random intercept in all models. SE = standard error; df = degrees of freedom; N = number of unique predictor values represented in each model. Multiple lnRR observations may share the same predictor value.

Fixed-effect	Estimate	SE	df	t value	p value	N
Absolute latitude	-0.0026	0.0032	96.4194	-0.804	0.423	110
Spatial extent	-0.0225	0.0195	41.4934	-1.153	0.255	47
Distance	0.0017	0.0510	189	0.035	0.972	39
Clim. heterogeneity	-0.0104	0.051	39.9404	-0.204	0.8391	45
Temporal window	0.0014	0.0024	26.6064	0.602	0.552	20