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Defining River Regional Landscape Classifications for Implementing Spatially Extensive Brazilian Stream Assessments

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Keywords: benthic macroinvertebrates | ecoregion | lotic ecosystems | river typology

ABSTRACT

Aim: To determine whether regional classification—river typology or ecoregion—better captures the ecological variability of tropical stream ecosystems for biomonitoring purposes.

Location: Three hundred and forty-eight streams located in the São Francisco, Mata Atlântica and Paraná hydrologic units, Minas Gerais state, southeastern Brazil.

Time Period: Data were collected between 2003 and 2019 as part of regional freshwater monitoring programs conducted by different research projects.

Major Taxa Studied: Benthic macroinvertebrate assemblages.

Methods: We evaluated two spatial and hierarchical classification systems: (1) river typology, based on climate, topography and lithology and (2) ecoregions, which consider natural features such as landforms, vegetation, land use and cover and hydrography, to

†In memoriam. He was actively involved in the conceptual foundation and the first round of review, providing substantial contributions to this manuscript. Following his passing, we have chosen to dedicate this work to his memory in recognition of his contribution.

explain variation in benthic macroinvertebrate assemblages. We asked: which landscape classification (i.e., river typology or ecoregion) was more effective at representing patterns in benthic macroinvertebrate assemblages for stream bioassessment in Minas Gerais state, Brazil?
Results: Level III Ecoregion was the most adequate regional landscape classification for benthic macroinvertebrate data, as it has a better ability to differentiate ecological patterns between groups.
Main Conclusions: Ecoregions offer more ecologically meaningful spatial units for classifying rivers in tropical regions such as southeastern Brazil. Our approach fosters alignment between scientific assessment, legal frameworks and environmental governance, thereby advancing more informed and effective conservation strategies for freshwater ecosystems.

1 | Introduction

Rivers are dynamic systems influenced by natural and anthropogenic forces, leading to continuous changes along their longitudinal gradients (Callisto et al. 2019; Fausch et al. 2002; Macedo et al. 2016; Vannote et al. 1980). Alterations in their hydromorphology, water quality, or quantity can compromise the condition of these ecosystems, ultimately influencing the ecosystem services provided to humans (Mello et al. 2020; Santos et al. 2021). Modern bioassessment systems require the classification of rivers into categories that exhibit limited variability in both community composition and environmental factors (Kolada et al. 2017). Such classification frameworks play a crucial role in ecological assessments because they establish the baseline against which the degree of human alteration of ecosystems can be evaluated (Borgwardt et al. 2019).

Holistic approaches that link the multiple spatial extents necessary for maintaining lotic ecosystem biodiversity are essential for successfully managing these ecosystems (Ligeiro et al. 2013; Tukiainen et al. 2023). These holistic approaches support spatial classifications of water bodies by linking environmental characteristics to the structure and composition of lotic biodiversity (Ferronato et al. 2021; Johnson 2000; Lopes et al. 2010). In addition, freshwater biota are affected by several anthropogenic pressures at various spatial extents and temporal scales, and are often used for assessing river ecological quality (Allan 2004; Macedo et al. 2014). For these classifications to be effective, the selected variables must meaningfully influence biotic community composition. In other words, the similarity of biotic communities from the same group (within-type similarity) should be higher than that of communities from different groups (between-type similarity) (Jupke et al. 2022).

For biomonitoring purposes, river typology and ecoregion classification serve as standard tools for categorising and understanding patterns in freshwater ecosystems; however, they differ in their approach and focus. River typology classifies river segments using abiotic variables to group biological communities, aiming to identify similarities in ecosystem types (Agra et al. 2019; Jupke et al. 2022). River typology involves a fixed set of variables based on geomorphological, climatological and lithological variables to categorise river segments. However, the EU members can use additional parameters, such as geomorphology, land use and water quality, to refine river typology (Jupke et al. 2022). In contrast, ecoregion classification defines broader geographical regions based on environmental characteristics, such as geology and vegetation, aiming to establish patterns and reference conditions for freshwater ecosystems (Agra et al. 2019; Omernik and Griffith 2014). Whereas river typology targets segment-specific characteristics to assess local variations, ecoregion captures regional-scale patterns of land use and climate, as well as regional management based on ecological data (Moog et al. 2004). Level III Ecoregions are based on the proportional distribution of vegetation physiognomies and climate types within each mapped unit, while Level IV Ecoregions are based on landform pattern discontinuities such as climate patterns, dominant vegetation physiognomies and land use distribution (Omernik and Griffith 2014). The use of ecoregions in biomonitoring has emerged as a valuable strategy for improving the ecological relevance and spatial resolution of bioassessment programs (Stoddard 2004). By aligning assessment protocols with ecoregions, biomonitoring programs across large regions can achieve greater accuracy and comparability across spatial extents (Herlihy et al. 2020; Stoddard et al. 2008), enhancing their capacity to inform management and conservation.

Stream biological assessments are often based on multimetric indices (Callisto et al. 2019) and predictive models (Feio and Poquet 2011) that are sensitive to natural variability and anthropogenic pressures (Martins et al. 2018). Therefore, incorporating landscape classifications enhances accuracy by accounting for natural environmental heterogeneity (Agra et al. 2019). Thus, both ecoregion and typology approaches play useful roles in environmental monitoring and conservation efforts, providing valuable frameworks for understanding and managing river ecosystems (Omernik et al. 2017; Solheim et al. 2019). Furthermore, more local patterns and characteristics, such as river typology and ecoregion, should also be considered to monitor water body conservation status at a finer resolution, because abiotic and biotic conditions often vary markedly within river basins (Kaufmann et al. 2022; Omernik and Griffith 2014).

Among the many taxa that compose lotic ecosystem biodiversity, benthic macroinvertebrates are one of the most ubiquitous and diverse groups of organisms in these systems (Bonada et al. 2006; Cortes et al. 2013). The structure of benthic macroinvertebrate assemblages serves as an effective proxy for the overall structure and function of lotic ecosystems (Madureira et al. 2024). However, despite the large amount of available spatial data, these are provided at various spatial extents, from different sources, and through diverse acquisition methodologies, which may not be compatible with biomonitoring studies (Mello et al. 2020). Therefore, it is necessary to identify which type of a priori classification approach for sites can be useful for implementing biomonitoring nationally.

We addressed the question: which water body classification (i.e., river typology or ecoregion) more effectively represents benthic macroinvertebrate assemblage patterns for stream bioassessment? As a case study, we utilised an extensive biological database gathered over 16 years to test which regional landscape classification showed better within-type and between-type similarities and which was better for differentiating benthic macroinvertebrate assemblages in Minas Gerais state, Brazil. We hypothesize that Level III Ecoregions are more suitable to assess the differences between benthic macroinvertebrate assemblages, predicting that this classification has higher within-type similarities and lower between-type similarities when compared to the other classification types. Despite their widespread use in biomonitoring, few studies have compared the performance of ecoregion and river typology classifications in tropical regions. This gap limits the development of effective, regionally adapted frameworks for ecological monitoring. Our study addresses this need by testing which classification approach better captures benthic macroinvertebrate patterns in diverse tropical streams of southeastern Brazil.

2 | Methods

2.1 | Study Area

The study area includes the Pandeiros, Jequitaí, das Velhas, Pará, Araguari, Grande and Paranaíba basins—tributaries of the São Francisco and Paraná Rivers—as well as watersheds within the Mata Atlântica hydrologic unit. This region encompasses the main hydrologic units of the state of Minas Gerais (586,528 km^2) (Figure 1; Table 1). From 2003 to 2019, benthic macroinvertebrate samples were collected in 19 sites through various research projects led by the Laboratory of Ecology of Benthos, Universidade Federal de Minas Gerais (LEB/UFMG) (Agra et al. 2019; Callisto et al. 2021; Castro et al. 2019; Feio et al. 2015; Garuana et al. 2020; Linares et al. 2021; Macedo et al. 2022; Martins et al. 2018, 2020; Silva et al. 2017) and Technology Innovation Center, National Service of Industrial Learning (CIT-SENAI) (Ferreira et al. 2017) and were successfully analysed together for biomonitoring purposes (Cordeiro et al. 2025). Because the classifications are based on contemporaneous sampling, interannual variability is unlikely to affect the analyses. In fact, previous studies with the same dataset have shown that the temporal variability in the benthic macroinvertebrate assemblages is minimal when compared to the effect of local and regional scale variables, allowing them to be successfully compared (see Cordeiro et al. 2025; Feio et al. 2015; Firmiano et al. 2021; Linares et al. 2025).

The sites are distributed across the São Francisco, Mata Atlântica and Alto Paraná hydrologic units (Abell et al. 2008), which primarily encompass the Cerrado and Atlantic Forest biomes (IBGE 2024). The study area encompasses a geologically diverse framework composed of sedimentary, metamorphic and volcanic rock assemblages that span multiple geotectonic contexts—from Archean greenstone belts and granitoid complexes to Proterozoic sedimentary basins and Cenozoic volcanic-sedimentary covers (Brazil 2004). This geological complexity underpins a heterogeneous landscape marked by a succession of mountain ranges, plateaus, depressions and valleys (IBGE 2019). The region exhibits a north–south climate gradient, ranging from tropical humid conditions in the south of the state, characterised by three dry

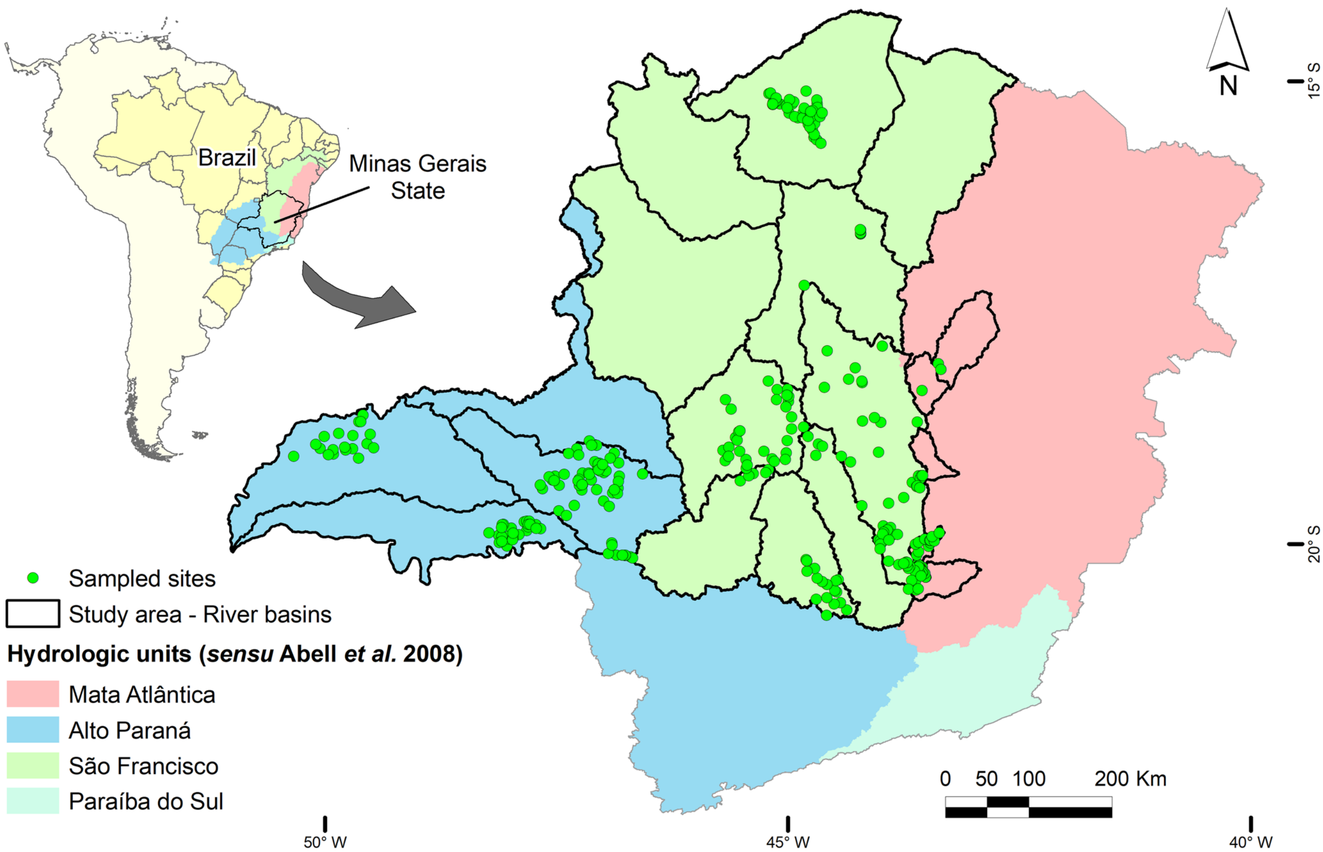

FIGURE 1 | Study area showing the hydrologic units and sampled sites.

TABLE 1 | Total number of sites in the study area.

Hydrologic unit	River basin	No. of sample sites
São Francisco	Pandeiros	46
	Jequitaí and Pacuí	5
	Três Marias Reservoir	31
	Pará	16
	São Francisco	5
	Paraopeba	10
	das Velhas	101
Mata Atlântica	Jequitinhonha	2
	Araçuaí	4
	Piracicaba	13
	Santo Antônio	1
	Piranga	4
Alto Paraná	Araguari	64
	Paranaíba	19
	Grande	27

months, hot semi-humid conditions in the central portion, and six dry months in the extreme north of the area (IBGE 2002). The varied climate zones and altitudinal gradients in the study area create distinct hydrological regimes including intermittent headwater streams in semi-arid uplands, perennial mid-elevation streams under semi-humid conditions, and lowland rivers in humid tropical zones (Almagro et al. 2024). This environmental mosaic influences not only streamflow permanence and seasonality but also the ecological dynamics of aquatic biota (Almagro et al. 2024). Industrial and mining activities are concentrated in the southern São Francisco hydrologic unit, particularly in the Quadrilátero Ferrífero region (Ferreira et al. 2017).

2.2 | Biological Samples and Water Quality

Benthic macroinvertebrate sampling employed Surber or kick nets (30 cm aperture, 500 μm mesh and 0.09 m^2). At each site, between 3 and 20 samples were collected from the most representative habitats and then combined into a single composite sample for each site, the number of samples per site varying with the objective of each of the projects that fed our database (see Agra et al. 2019; Callisto et al. 2021; Castro et al. 2019; Feio et al. 2015; Ferreira et al. 2017; Garuana et al. 2020; Linares et al. 2021; Macedo et al. 2022; Martins et al. 2018, 2020; Silva et al. 2017). The samples were fixed in the field with a 70% alcohol solution and individuals were deposited in the Reference Collection of Benthic Macroinvertebrates at the Institute of Biological Sciences, Federal University of Minas Gerais, or in the Center for Innovation and Technology, National Service of Industrial Learning (CIT-SENAI). The samples were washed in 1.00 mm, 0.50 mm and 0.25 mm sieves in the laboratory. All individuals were identified mainly at the family level with the aid of taxonomic keys (Hamada et al. 2014; Merritt et al. 2008; Mugnai et al. 2010; Pérez 1988). Insect individuals were identified to family, whereas Mollusca were identified to class, and Annelida to subclass. This identification standard was chosen because these are the taxonomic levels used in most Brazilian benthic macroinvertebrate studies and in biomonitoring programs by governmental agencies and decision makers (Costa et al. 2023). Macroinvertebrate abundances were fourth root transformed to minimise the influence of different sample sizes (Feio et al. 2014). Only biological data obtained during the dry season (between May and September) were used to facilitate the sampling and identification of the macroinvertebrates (see Linares et al. 2025). For sites sampled multiple times, the date with the highest taxa richness was selected (Cordeiro et al. 2025).

Water quality data (total phosphorus—mg/L, total nitrogen—mg/L, and turbidity—NTU) was also compiled for each site, using standardised methods for biomonitoring in the region (Lipps et al. 2023).

2.3 | Landscape Classification

2.3.1 | River Typology Classification

For each of the stream segments of our study area, we extracted variables characterising land use and cover, climate, topography and lithological groups from a GIS (Walz and Stein 2014; Wilson et al. 2007) (Table S1). Climatic data (50-year climatic reference) were obtained from Worldclim (Fick and Hijmans 2017) and encompass variables related to temperature (e.g., annual mean temperature, maximum temperature of warmest month, isothermality, and so forth) and precipitation (e.g., annual total precipitation, precipitation seasonality, precipitation of coldest quarter, and so forth). Topographic features (e.g., altitude, terrain roughness index, and so forth) were derived from the Shuttle Radar Topography Mission (30 arc-sec) (USGS 2015). The lithological groups (Table S2) were defined based on the Geological Map of Minas Gerais (Brazil 2004), a 1:1,000,000-scale map, as described by Ferreira et al. (2017). The grouping of lithologic units was based on the similar response to surface processes such as erosion, weathering and leaching (e.g., pelitic rocks, volcanic rocks, carbonate rocks, and so forth) (Ferreira et al. 2017).

We developed our river typology following System B of the European Union Water Framework Directive (EU-WFD) (European Union 2000), which provides a flexible, regionally adaptable classification based on statistically selected environmental variables. This approach is particularly well-suited for areas with high lithological, geomorphological and climatic diversity, as is the case in our study region. Rather than applying fixed thresholds, System B enables Member States—or, by analogy, regional researchers—to define typologies using locally relevant abiotic gradients (European Union 2000). In our case, the typology was developed from an initial set of 22 candidate variables, selected to reflect key drivers of stream structure and function across multiple spatial extensions (Table S3). To construct the typologies, we followed a three-step procedure. (1) We first reduced multicollinearity among continuous data (i.e., climatic and topographic variables) using Spearman rank correlations, excluding correlated variables with $|r| > 0.7$ (Dormann et al. 2013). (2) Next, we applied the Grouping Analysis tool in ArcGIS 10.4 (ESRI 2016) to perform a spatial clustering based on retained continuous variables. This *K*-means clustering algorithm ensures that features within groups are as similar as possible, while maximising differences between groups. Cluster effectiveness was evaluated using the Calinski–Harabasz pseudo-*F*-statistic, with the highest value indicating that two major environmental clusters best captured spatial differentiation (Table S4) (Warchalska-Troll and Warchalski 2022). (3) Finally, we refined the classification by subdividing these two environmental clusters according to underlying lithological categories (e.g., alkaline, sedimentary, and so forth), which represent nominal data. As a result, we defined two hierarchical typology levels: (1) Level 0 River Typology represents a spatial cluster derived from continuous climate and topographic variables. (2) Level 1 River Typology incorporated lithological categories into each of the two major environmental clusters, resulting in a more detailed classification.

2.3.2 | Ecoregion Classification

The delineation of ecoregions was based on a hierarchical, physiographic framework, integrating multiple spatial layers representing the natural landscape heterogeneity of the study area, comprising landforms, vegetation, land use and cover and hydrography (Table S5). This process involved overlaying geospatial data to integrate quantitative information (Borges and Macedo 2024), following the hierarchical model of Omernik and Griffith (2014). Levels III and IV Ecoregions differ primarily in their spatial resolution and environmental detail. Level III Ecoregions represent broader biogeographic zones characterised by overarching patterns of climate and dominant vegetation formations. In contrast, Level IV Ecoregions provide finer spatial resolution by incorporating more detailed physiographic elements such as landforms, slope variation, lithology and localised vegetation mosaics (Omernik and Griffith 2014).

At Level IV Ecoregions were established at a fine spatial resolution, guided primarily by discontinuities in landform patterns and further refined by overlaying additional environmental layers. Climate patterns, dominant vegetation physiognomies, and land use distribution exhibited strong spatial correlations with topography in most instances (Borges and Macedo 2024). The following datasets and criteria were used:

- *Landform units*: Defined based on geomorphological structures (e.g., plateaus, escarpments, depressions, mountainous massifs), using physiographic maps (Brazil 2006), and added elevation data (USGS 2015). These units served as the base layer for delimitation.
- *Climatic zones*: Based on the Köppen classification (Aw, Cwb, etc.) from Alvares et al. (2013), reflecting variations in rainfall seasonality and thermal regime.
- *Vegetation physiognomies*: Derived from Scolforo and Carvalho (2006), including vegetation formations, such as seasonal semideciduous forests, wooded savannas, rupestrian grasslands and urban use, whose distribution is tightly linked to geology and elevation.
- *Hydrography*: Main river networks and watershed boundaries were mapped using the ANA (2020) database, ensuring that ecoregion boundaries aligned with hydrological compartments.

Each ecoregion was manually delineated through visual interpretation of overlaid thematic maps, emphasising zones of ecological and physiographic coherence. These boundaries were further validated by identifying abrupt changes in combinations of elevation, landforms, climate and vegetation patterns. River networks and watershed boundaries were overlaid with topographic, climatic and vegetation data to ensure that ecoregion boundaries respected natural hydrological divisions and helped refine transitions between landform units (Borges and Macedo 2024).

At Level III, a broader aggregation was implemented by grouping Level IV units with similar proportions of vegetation types and climatic zones. This produced a regionalized classification more suited for spatial assessments over larger extents, while retaining the environmental logic of the finer resolution mapping. To further characterise Levels IV and III, the Brazilian biomes (IBGE 2024) were also used (Borges and Macedo 2024). This multi-criteria approach ensures that each ecoregion reflects integrated landscape units with ecological relevance, rather than administrative or arbitrary boundaries (Omernik and Griffith 2014).

2.3.3 | Reference Site Selection for Stream Classification Validation

To evaluate the effectiveness of river typology and ecoregion classifications, we selected minimally disturbed reference sites (Stoddard et al. 2006; Whittier et al. 2007). Reference sites were selected based on two primary criteria: (1) land use and land cover within the upstream catchment and (2) key water quality parameters.

Land use and cover data (e.g., forest, savanna, pasture, and so forth) were obtained from the MapBiomas online platform (Projeto Mapbiomas 2025), with a 30-m spatial resolution (Souza et al. 2020). We also calculated the Catchment Disturbance Index (CDI), a weighted metric based on the proportion of urban, agricultural and pasture land uses (Ligeiro et al. 2013). Water quality data were obtained following the procedures outlined in the 'Biological samples and water quality' section.

Sites were retained if they met the following conditions:

1. We retained sites without urban infrastructure and mining areas, and with Catchment Disturbance Index (Ligeiro et al. 2013) scores below 40.
2. We excluded sites not meeting Class II Brazilian legal limits for freshwater (Brazil 2005) for dissolved oxygen and turbidity (< 5.0 mg/L and > 100 NTU, respectively). Class II primarily corresponds to water intended for human consumption after simplified treatment and protection of aquatic life.

This selection reduced the confounding effect of alterations in macroinvertebrate assemblages caused by anthropogenic disturbance, rather than differences resulting from the different abiotic characteristics such as geology or climate (Stoddard et al. 2006; Whittier et al. 2007). These criteria were similar to those established in other Cerrado studies to ensure our reference site selection was comparable to theirs (e.g., Agra et al. 2019; Cordeiro et al. 2025; Macedo et al. 2016; Martins et al. 2018; Silva et al. 2017). These reference sites were subsequently used to evaluate how effectively each stream classification system captured minimally disturbed conditions.

2.4 | Data Analyses

To determine which classification system best supported biological assessment of rivers (e.g., reference values) using benthic macroinvertebrate assemblages, we applied two methods to compare within-type/ecoregion and between-type/ecoregion macroinvertebrate similarities for each classification: ANOSIM (which gives more weight to between-type dissimilarities) and Classification Strength (CS; which gives more weight to between-type similarities) (Jupke et al. 2022). Both tests express the difference between the mean rank of between-type/ecoregion similarities and the mean rank of within-type/ecoregion similarities (Van Sickle 1997). For each classification, we only tested those categories with at least five sites in our database, and tested them at all classification levels (Levels III and IV Ecoregion; Typology Levels 0 and 1).

To assess whether the different classification levels (Levels III and IV Ecoregion; Typology Levels 0 and 1) effectively differentiate benthic macroinvertebrate assemblages, we ran a pairwise permutational multivariate analysis of variance (Pairwise PERMANOVA). Level 0 river typology, which separated the sites into only two categories, was tested using a standard PERMANOVA. All analyses were run using the "vegan" package (Oksanen et al. 2019) in R software (R Development Core Team 2018).

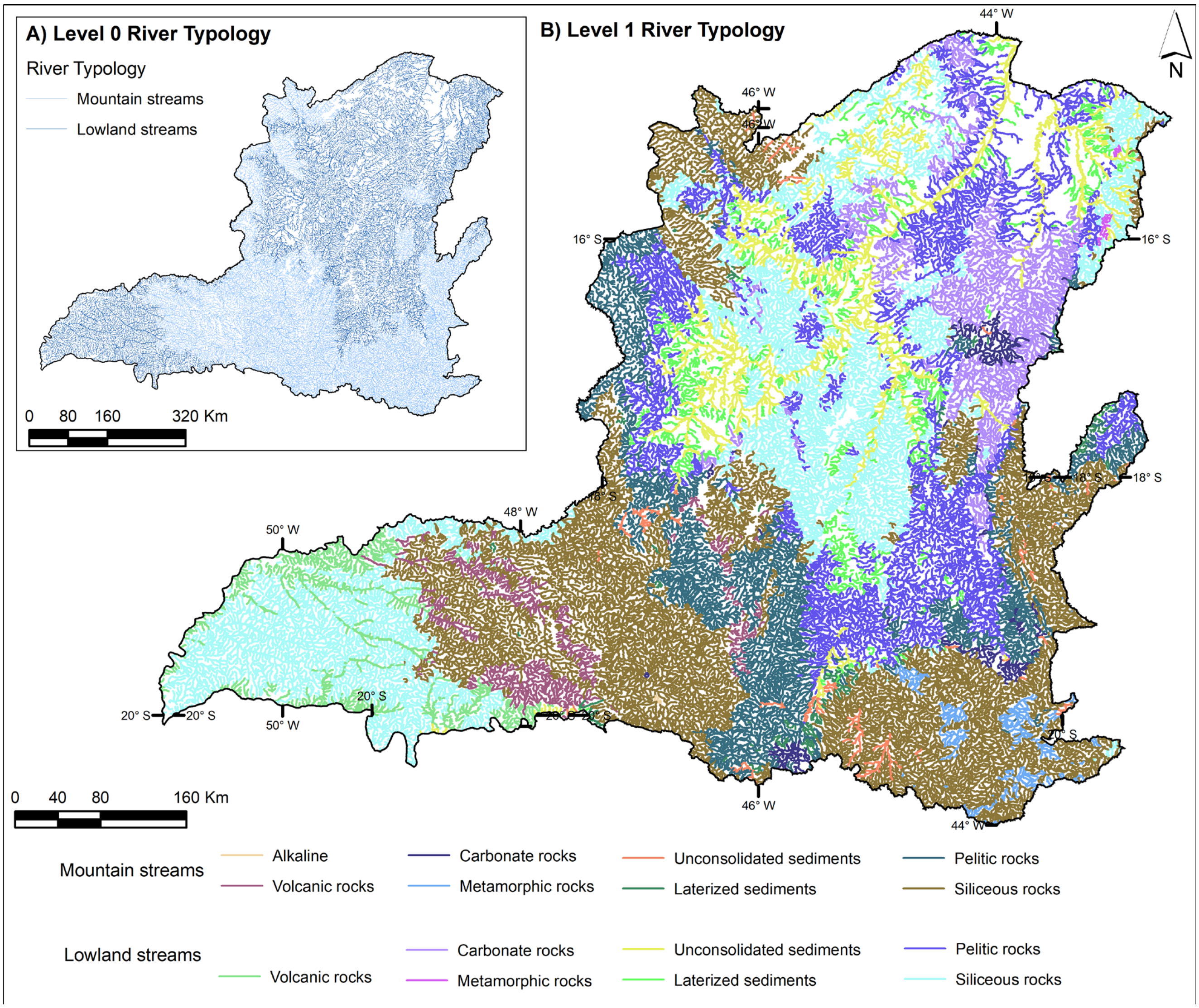

FIGURE 2 | Spatial distribution of the (A) mountain and lowland stream types across the study area (two stream types) and (B) mountain and lowland river types joined with eight lithological classes (15 stream types).

3 | Results

3.1 | Stream Typology

Spearman's rank correlation identified five variables for the river typology construction: altitude, average annual temperature, annual precipitation and terrain roughness (Table S3). The spatial cluster analysis, based on *K*-means clustering using four continuous environmental variables (temperature, precipitation, altitude and terrain roughness), identified two distinctly marked stream types: mountain and lowland. This classification corresponded to the highest Calinski–Harabasz index value, indicating that the two clusters best captured environmental separation (Table S4). Mountainous streams occurred at higher elevations (average of 858 m), with greater rainfall (average of 1,459 mm) and lower temperatures (average of 20.1°C). In contrast, lowland streams occurred at lower elevations (average of 542 m) with less rainfall (average of 1,123 mm) and warmer temperatures (average of 22.6°C) (Figure 2B). Finally, the mountain and lowland stream types were combined with the eight lithological classes in Minas Gerais, yielding 15 distinct river types (Figure 2A).

3.2 | Ecoregion Classification

Level IV Ecoregions were primarily delineated by landform and climate patterns, followed by land use and hydrographic features. This process identified 80 Level IV Ecoregions, each characterised by distinct landscape attributes (Table S6), distributed across the study area (Figure 3B). Level III Ecoregions were integrated mainly through the integration of

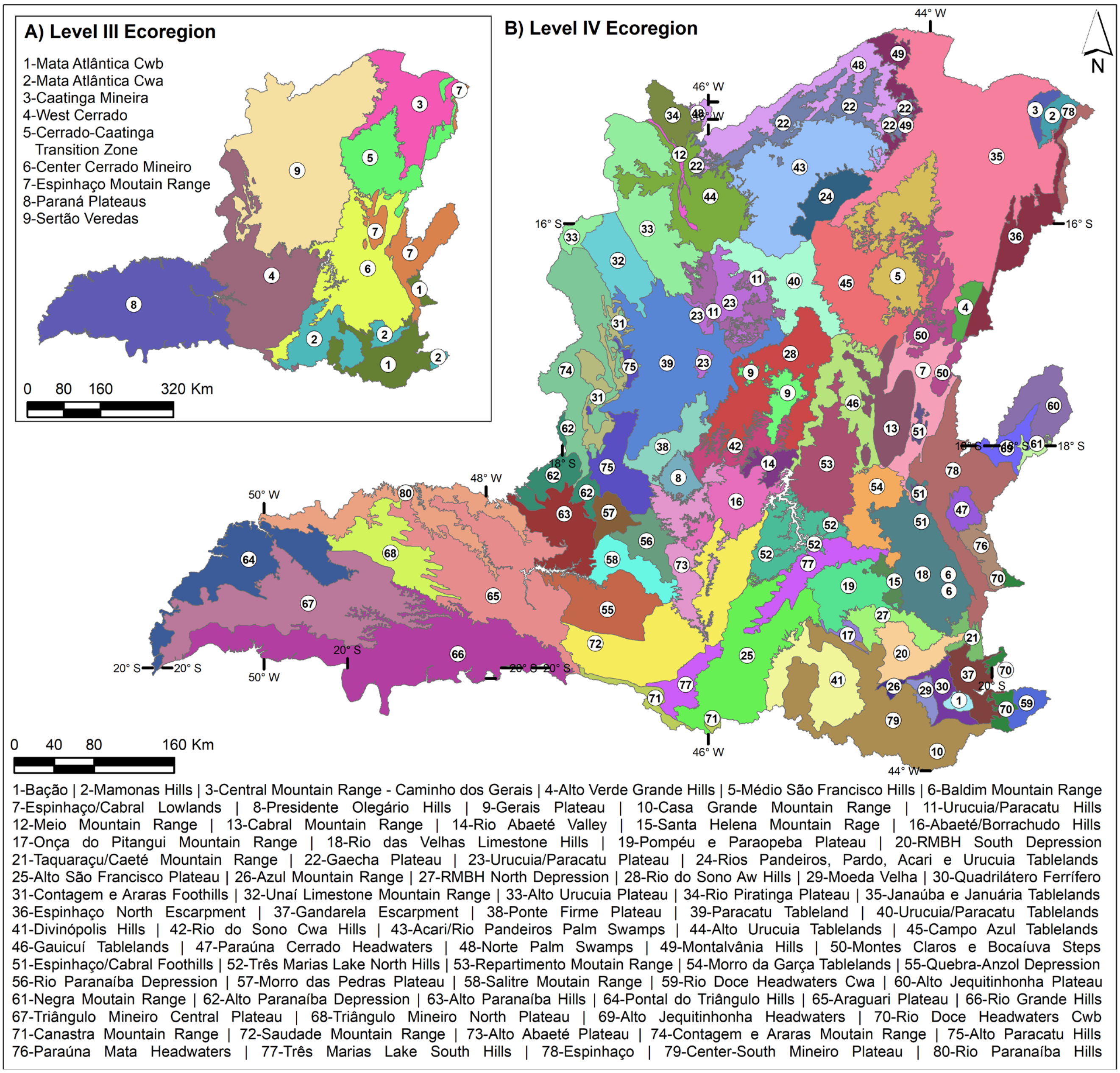

FIGURE 3 | Spatial distribution of the (A) Level III Ecoregions (nine regions) and (B) Level IV Ecoregions (80 regions).

the Cerrado, Atlantic Forest and Caatinga biomes and their associated climatic characteristics (Table S6), yielding a total of nine Level III Ecoregions (Figure 3A).

3.3 | Validation Coverage Across Classification Types

We used 118 validation sites, distributed throughout the study area (Figure 4, Tables S7 and S8), enabling the validation of varying proportions of watercourses assigned to river typology and ecoregions. Level 0 River Typology achieved full validation coverage (100% of the sites) (comprising two types), whereas at Level 1 River Typology, approximately 67% of the watercourses were validated. Regarding the ecoregions, 64% of Level III Ecoregions were validated, while only 17% of the Level IV Ecoregions had sufficient validation coverage (Figure 4E).

3.4 | Biological Validation of Stream Typology and Ecoregion

Level IV Ecoregion exhibited the highest overall dissimilarity among benthic macroinvertebrate assemblages, followed by Level III Ecoregion, Level 1 River Typology and Level 0 River Typology (Table 2). This result is evidenced by the ANOSIM and CS values: Level IV Ecoregion presented the highest values (ANOSIM $R = 0.422$; $CS = 0.072$), followed by Level III Ecoregion ($R = 0.371$; $CS = 0.063$), Level 1 River Typology ($R = 0.327$; $CS = 0.057$) and Level 0 River Typology ($R = 0.290$; $CS = 0.019$). ANOSIM values above 0.5 indicate strong separation between groups; values between 0.25 and 0.5 indicate weaker separation with some overlap; and values below 0.25 suggest no discernible difference. Similarly, CS values > 1.0 indicate high classification strength, values between 0.5 and 1.0 indicate moderate strength, and values < 0.5 reflect weak classification strength. Although all classifications revealed

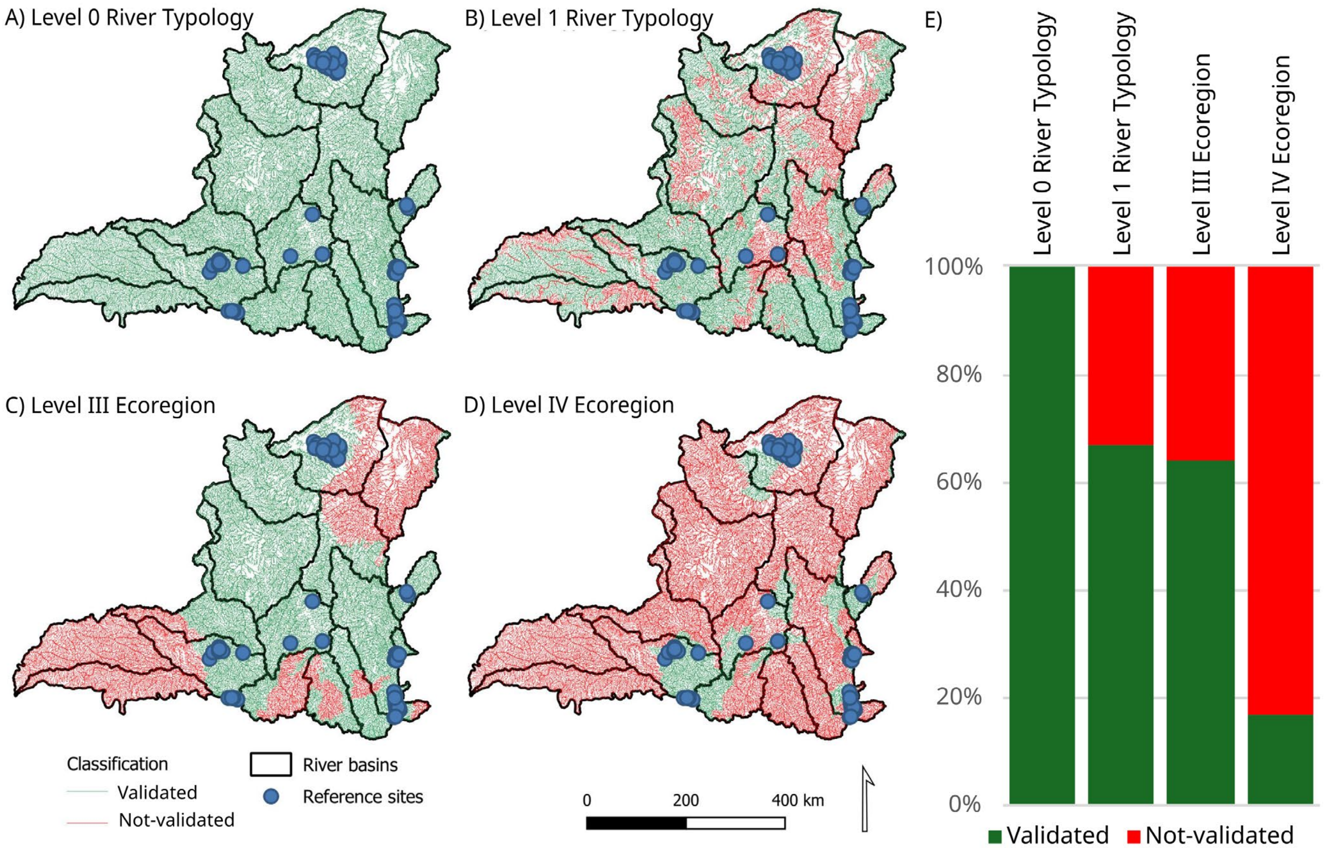

FIGURE 4 | Spatial distribution of validated stream segments for (A) Level 0 River Typology, (B) Level 1 River Typology, (C) Level III Ecoregion and (D) Level IV Ecoregion. (E) Proportion validated per landscape classification.

TABLE 2 | ANOSIM R statistics and classification strength (CS) values for each tested stream classification type.

Classification type	Number of categories	Proportion of validated sites	ANOSIM—R	CS
Level 0 River Typology	2	100%	0.290	0.049
Level 1 River Typology	15	67%	0.327	0.057
Level III Ecoregion	9	64%	0.372	0.066
Level IV Ecoregion	80	17%	0.422	0.073

Note: All values are significant.

some degree of group separation, Level IV Ecoregion performed best based on statistical criteria.

Regarding the capacity to differentiate the benthic macroinvertebrate assemblages, Level 0 River Typology showed a significant difference ($F = 9.6716$; $p = 0.001$) between the two tested categories (Mountainous vs. Lowland). Level 1 River Typology (Table 3) was unable to distinguish the benthic macroinvertebrate assemblages in five of the 15 category pairs tested. Level III Ecoregion (Table 4) successfully distinguished all the tested pairs of categories, indicating a better ability to distinguish ecological patterns between groups. Level IV Ecoregion (Table 5) failed to distinguish the benthic macroinvertebrate assemblages between seven of the 21 category pairs tested.

TABLE 3 | Pairwise permutational multivariate analysis of variance comparing the taxonomic composition of benthic macroinvertebrate assemblages in the tested categories of Level 1 River Typology.

Tested pairs (Level 1 River Typology)	*F*	*p*-adjusted
Mountainous Siliceous Rocks vs. Mountainous Pelitic Rocks	1.762846	0.330
Mountainous Siliceous Rocks vs. Lowland Pelitic Rocks	2.649379	**0.015**
Mountainous Siliceous Rocks vs. Lowland Siliceous Rocks	4.126819	**0.015**
Mountainous Siliceous Rocks vs. Mountainous Metamorphic Rocks	4.561596	**0.015**
Mountainous Siliceous Rocks vs. Lowland Unconsolidated Sediments	7.239724	**0.015**
Mountainous Pelitic Rocks vs. Lowland Pelitic Rocks	1.151711	1.000
Mountainous Pelitic Rocks vs. Lowland Siliceous Rocks	1.878778	0.375
Mountainous Pelitic Rocks vs. Mountainous Metamorphic Rocks	3.627651	**0.015**
Mountainous Pelitic Rocks vs. Lowland Unconsolidated Sediments	3.602391	**0.015**
Lowland Pelitic Rocks vs. Lowland Siliceous Rocks	1.846362	0.225
Lowland Pelitic Rocks vs. Mountainous Metamorphic Rocks	4.466345	**0.015**
Lowland Pelitic Rocks vs. Lowland Unconsolidated Sediments	4.511995	**0.015**
Lowland Siliceous Rocks vs. Mountainous Metamorphic Rocks	7.281938	**0.015**
Lowland Siliceous Rocks vs. Lowland Unconsolidated Sediments	1.990606	0.195
Mountainous Metamorphic Rocks vs. Lowland Unconsolidated Sediments	10.330614	**0.015**

Note: Values in bold show significant differences.

4 | Discussion

Level III Ecoregion emerged as the most suitable regional landscape classification for benthic macroinvertebrate data. Although it showed lower values than the Level IV Ecoregion in both ANOSIM and CS, it consistently distinguished the benthic macroinvertebrate assemblages in all its categories. While Level IV Ecoregion classification does show the highest internal dissimilarity, the lack of differentiation of the benthic macroinvertebrates limits its utility for biomonitoring purposes.

The better fit of ecoregions compared to typology may be related to using benthic macroinvertebrates as our model organisms. As most benthic macroinvertebrates have terrestrial life stages with aerial locomotion, they are affected directly by landscape attributes such as land use, vegetation cover and topography (Giehl et al. 2024; Perkin et al. 2020). Previous studies in the region showed that benthic macroinvertebrate taxa distributions were influenced by landscape resistance (Firmiano et al. 2021). This highlights the importance of dispersal corridors and other management actions that reduce landscape resistance, especially in regions dominated by anthropogenic activities (Cote et al. 2017; Linares et al. 2025).

TABLE 4 | Pairwise permutational multivariate analysis of variance comparing the taxonomic composition of benthic macroinvertebrate assemblages in tested categories of Level III Ecoregion.

Tested pairs (Level III Ecoregion)	*F*	*p*-adjusted
Espinhaço Mountain Range vs. West Cerrado	0.4368035	**0.010**
Espinhaço Mountain Range vs. Center Cerrado Mineiro	0.5542617	**0.010**
Espinhaço Mountain Range vs. Mata Atlântica Cwb	0.7281908	**0.010**
Espinhaço Mountain Range vs. Sertão Veredas	1.1279524	**0.010**
West Cerrado vs. Center Cerrado Mineiro	0.5061533	**0.010**
West Cerrado vs. Mata Atlântica Cwb	0.9703418	**0.010**
West Cerrado vs. Sertão Veredas	1.2611368	**0.010**
Center Cerrado Mineiro vs. Mata Atlântica Cwb	1.2603875	**0.010**
Center Cerrado Mineiro vs. Sertão Veredas	1.0650907	**0.010**
Mata Atlântica Cwb vs. Sertão Veredas	2.2632347	**0.010**

Note: Values in bold show significant differences.

TABLE 5 | Pairwise permutational multivariate analysis of variance comparing the taxonomic composition of benthic macroinvertebrate assemblages in tested categories of Level IV Ecoregion.

Tested pairs (Level IV Ecoregion)	*F*	*p*-adjusted
Canastra Mountain Range vs. Três Marias Lake South Hills	0.446079	**0.042**
Canastra Mountain Range vs. Gandarela Escarpment	0.75362	**0.021**
Canastra Mountain Range vs. Espinhaço	0.4403318	**0.021**
Canastra Mountain Range vs. Salitre Mountain Range	0.4054526	0.063
Canastra Mountain Range vs. Repartimento Mountain Range	0.3001453	0.882
Canastra Mountain Range vs. Acari/Rio Pandeiros Palm Swamps	0.7087848	**0.021**
Três Marias Lake South Hills vs. Gandarela Escarpment	0.6671834	**0.021**
Três Marias Lake South Hills vs. Espinhaço	0.4229921	0.042
Três Marias Lake South Hills vs. Salitre Mountain Range	0.4295399	**0.021**
Três Marias Lake South Hills vs. Repartimento Mountain Range	0.219291	1.000
Três Marias Lake South Hills vs. Acari/Rio Pandeiros Palm Swamps	0.7185899	**0.021**
Gandarela Escarpment vs. Espinhaço	0.49086	**0.021**
Gandarela Escarpment vs. Salitre Mountain Range	0.5240362	**0.021**
Gandarela Escarpment vs. Repartimento Mountain Range	0.6774457	**0.021**
Gandarela Escarpment vs. Acari/Rio Pandeiros Palm Swamps	2.2567265	**0.021**
Espinhaço vs. Salitre Mountain Range	0.281844	0.189
Espinhaço vs. Repartimento Mountain Range	0.3183145	0.441
Espinhaço vs. Acari/Rio Pandeiros Palm Swamps	0.7687854	**0.021**
Salitre Mountain Range vs. Repartimento Mountain Range	0.3419832	0.189
Salitre Mountain Range vs. Acari/Rio Pandeiros Palm Swamps	0.9320918	**0.021**
Repartimento Mountain Range vs. Acari/Rio Pandeiros Palm Swamps	0.4733567	**0.021**

Note: Values in bold show significant differences.

Because ecoregions consider these attributes, this may explain their better congruence with benthic macroinvertebrate composition.

Our findings underscore the limitations of applying bioassessment frameworks developed in temperate regions—such as those used in Europe and North America—to tropical river systems. For instance, in Minas Gerais, altitude emerged as a more influential factor in delineating river types than geological classes. Similar patterns have been observed across South America, with studies in Bolivia (Moya et al. 2011), Chile (Fuster et al. 2012) and Argentina (Pero et al. 2020), as well as in temperate contexts such as Germany (Lorenz et al. 2004), where a clear distinction between lowland and upland streams was evident. Lithology, due to its largely qualitative nature, appears to have limited explanatory power concerning biological variation (Ferréol et al. 2005). In contrast, classification schemes based on geomorphology and climate, as used in the ecoregion framework, are more effective in capturing ecological patterns in Brazilian streams, suggesting a more suitable path for regional adaptations.

This method addresses this gap by providing a scientifically grounded basis for identifying priority areas for restoration and conservation (Vynne et al. 2022). It can support decision-making in water resource planning, environmental licensing and territorial zoning, and it can also strengthen monitoring systems by incorporating landscape classifications and physical habitat indicators into existing assessment protocols. Despite relying on simple metrics, the typological framework was intentionally designed for replicability and national applicability. Like the multimetric indices proposed by Oliveira et al. (2011) and Pereira et al. (2016), it balances ecological relevance with operational feasibility. The selected variables—altitude, precipitation, temperature and terrain ruggedness—are well-established drivers of benthic macroinvertebrate assemblages, enabling the development of scalable stream classification. Ideally, this methodology should be expanded to broader geographic extents (e.g., South America). In this way, it would be possible to compare ecological status assessments between different regions and countries (Borgwardt et al. 2019). Such landscape classification would benefit from spatially extensive ecological research on the impacts of multiple pressures on streams by aggregating data comparable across large regions or countries (Borgwardt et al. 2019; Solheim et al. 2019). We acknowledge, however, a limitation in the distribution of reference sites used to validate the typology. Because the sites were drawn from existing monitoring networks not designed for this purpose, their representativeness across all typological and ecoregional units is uneven. Despite this, they span major environmental gradients and enable meaningful comparative analyses. Therefore, we hope our results can be used to develop biological assessment methods in Minas Gerais and to assess further aquatic ecosystem biodiversity for rivers and streams throughout South America.

5 | Conclusions

Level III Ecoregion proved to be the most effective classification for our data, as it balanced a relatively high classification strength with the capacity to distinguish the benthic macroinvertebrate assemblages between its tested categories consistently. This finding underscores the utility of regionalized classifications in enhancing the ecological relevance of biomonitoring programs. The practical implications of this work include supporting freshwater ecosystem assessment, guiding reference site selection, and informing watershed management and conservation planning. Furthermore, this framework may serve as a model for expanding stream classification schemes across other tropical regions, where ecological assessments remain underdeveloped. Future research could build on this foundation by incorporating climate projections and functional ecological indicators. By critically adapting international frameworks and proposing context-specific innovations, we offer a robust and scalable tool for watershed management and conservation in Brazil.

Author Contributions

D.R.M., M.J.F. and P.F.C. conceived the study. D.R.M., D.G.F.P., M.S.L. and P.F.C. performed the formal analyses. D.R.M. and M.C. secured funding. P.F.C., M.S.L. and D.R.M. developed the methodology and conducted data analysis. D.R.M. and M.J.F. supervised the work. M.C., M.J.F. and R.M.H. validated the results and provided critical guidance. P.F.C. wrote the first draft of the manuscript. D.R.M., D.G.F.P., M.C., M.J.F., M.S.L. and R.M.H. contributed to reviewing and editing. R.M.H. passed away during the editorial process and, therefore, was unable to approve the final version. All remaining authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The database used for this research is available in Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** List of variables for the river typology approach with their relative units and sources. **Table S2:** Lithological synthesis of Minas Gerais according to Ferreira et al. (2017). **Table S3:** Spearman rank correlations for river typology variables. **Table S4:** Pseudo *F*-statistic summary. **Table S5:** List of variables for the ecoregion approach with their relative units and sources. **Table S6:** Predominant landscape attributes for Level III and Level IV Ecoregion classification. **Table S7:** Distribution of reference sites per river typology or ecoregion. **Table S8:** Number of reference sites per typology or ecoregion level. **Data S1:** jbi70096-sup-0002-DataS1.gpkg. **Data S2:** jbi70096-sup-0003-DataS2.rar.