Relationships between Riparian Forest Fragmentation and Biological Indicators of Streams

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Abstract: Anthropogenic activities, such as land use and land cover modifications in riparian areas, can alter the degree of fragmentation of riparian vegetation, lead to the degradation of stream habitats, and affect biological communities in the streams. The characteristics of the riparian forests can modify the condition of stream environments and the transporting mechanisms of materials, sediments, nutrients, and pollutants loaded from the watersheds. This study aimed to examine the relationships between forest fragmentation and three biological indicators of trophic diatom, benthic macroinvertebrate, and the fish assessment in the Nakdong River, Korea. Eighty-nine biological assessment sampling sites in the National Aquatic Ecological Monitoring Program of South Korea were identified. For each sampling site, riparian forest data within a 500 m radius were extracted from national LULC using GIS to compute fragmentation metrics using FRAGSTATS software. Four fragmentation metrics—number of forest patches, percentage of riparian forest cover (PLAND), largest riparian forest patch index (LPI), and riparian forest division index (DIVISION)—were correlated with the biological indicators. Also, due to severe spatial autocorrelation among observations, the fragmentation metrics and stream environmental variables were regressed to biological indicators using regression tree analysis. Our results indicate that the biological indicators were significantly associated with most forest fragmentation metrics. We found positive correlations of PLAND and LPI with biological indicators, whereas DIVISION was negatively correlated with biological indicators. Both correlation and regression tree analyses revealed that the biological conditions of streams were likely to be better if riparian forests are less fragmented. Particularly, stronger relationships were revealed between macroinvertebrates and fish with the fragmentation metrics of riparian forests than with benthic diatoms. However, these relationships varied with elevation, stream size, and slope conditions. The results of this study reinforced the importance of including riparian forests in the planning, restoration, and management of stream environments. These results also suggested that planners and managers may need to consider different strategies for different stream environments and topographic characteristics in managing riparian forests.

Keywords: forest fragmentation; biological indicators; landscape metrics; Nakdong River; riparian areas

1. Introduction

The relationship between land use and the biological condition of streams has been a primary research area in stream ecosystem studies. The negative impacts of land use on stream ecosystems have been well-documented and are considered a key concern for stream restoration planning and

management [1–6]. In previous studies, anthropogenic activities in watersheds were found to generate various types of pollutants, which were then transported into streams through runoff, eventually resulting in degradation of water quality and poor ecological conditions in streams. Other studies have shown that the characteristics of surrounding stream environments are important in determining the state of water quality and ecological conditions [7–9]. The landscape characteristics in riparian areas have significant impacts on the transport mechanisms of materials, sediments, nutrients, and pollutants loaded from the watersheds, and on the condition of stream environments [10–13]. Various types of riparian vegetation (e.g., riparian buffer, buffer strip, and vegetation strip) have been shown to have positive effects on both biological and nonbiological characteristics of streams, such as stabilizing stream banks [14–16], reducing nutrient and sediment loading from anthropogenic land uses from watersheds into streams [1,17,18], lowering stream water temperature by providing shade [19,20], providing habitats for aquatic and terrestrial organisms [21–23], enhancing ecological integrity and biodiversity [24,25], and mediating the negative impacts of land uses on streams in watersheds [10,26,27]. Given these various benefits, riparian vegetation has been widely used in many countries in accordance with guidelines or regulations in the U.S. (e.g., [28]), Canada (e.g., [29]), and Australia (e.g., [30]). Likewise, the Korean Ministry of Environment (MOE) has been enforcing preservation of riparian vegetation and the planting of various types of vegetation within 500 m buffers for wadable streams and 1000 m buffers for non-wadable streams since the introduction of regulations in 2007. In general, the effectiveness of riparian buffers improves when the width of the buffer is increased [31–33]. Some proposed maximum buffer sizes in the literature include 3 m [34], 5 m [35], 6 m [36], 9 m [35,36], 15 m [31], 30 m [37], and 300 m [38]. Jontos [39] proposed different maximum buffer sizes for particular purposes, such as 10 m for detrital input, 20 m for stream stabilization, 30 m for water quality protection, 150 m for flood attenuation, and more than 500 m riparian habitat development for riverside species, such as insects, macroinvertebrate, and riverside birds. From a river–watershed continuum perspective, Fischer and Fischenich [40] suggested a ~500 m buffer width for riparian habitats of riverside species (e.g., birds and relatively larger mammals).

Most streams and rivers in human-dominant landscapes have experienced serious riparian forests degradation worldwide over the preceding decades, resulting in loss and fragmentation of riparian forests [41–43]. The main driving factors of this degradation were human activities and land uses such as livestock grazing, agricultural land uses, and land developments [1,21,44–46]. In general, fragmentation is one of the critical spatial patterns in disturbed landscapes. Fragmentation is "the breaking of a whole into smaller pieces" [47] and is responsible for the loss of native species, invasion of exotic species, increased soil erosion, loss of biodiversity, and decreased water quality [9,47,48]. Particularly, fragmentation of riparian forest has been believed to affect the types and amounts of pollutants entering streams and rivers [49–51]. The degree of riparian forest fragmentation can alter the characteristics of hydrological and biochemical runoff processes by increasing flow velocity and decreasing residence time within riparian forests, which are critical for the efficiency of infiltration, interception, deposition, absorption, uptake, and evaporation in riparian areas, resulting in benthic substrates, dissolved oxygen level, and the concentrations of pollutant and nutrients in streams [52–55]. For example, the spatial pattern of riparian forest can affect the variability of coarse organic matter [56], leaf breakdown rates [57], concentration of TN [58], total suspended solids [59] and total inorganic N [60], as well as some other characteristics of stream water [58,61]. In turn, such changes to hydrological and biochemical runoff processes caused by altering spatial pattern (e.g., fragmentation, aggregation, etc.) of riparian forest may have a significant impact on water quality and aquatic biota (e.g., biological diversity and species dynamics) in direct and indirect ways [62–66]. Despite the fact that some studies investigated the effects of riparian forest fragmentation on biological communities in streams [66–68], a large portion of the relationship between fragmentation of riparian forests and biological indicators (e.g., diatom, macroinvertebrate, and fish) in streams at riparian scale remains unexplored.

Biological indicators have been widely used to detect the ecological impacts derived from changes of stream environments that originated from mostly anthropogenic activities, such as farming,

cultivating, and land uses in watershed or riparian areas [69,70]. Numerous studies reported that indicators of diatom, macroinvertebrate, and fish could effectively capture the impacts of land use changes and deforestation on streams within both watersheds and riparian areas [68,71,72]. Because different types of organisms have different relative tolerance to environmental changes, using multiple indicators of different organism groups can be more effective than using a single indicator of a group [42,73–75]. Benthic diatom has shown to be reliable indicators of organic pollution, eutrophication, and pollution [76,77]. As important components of stream ecosystems, macroinvertebrates are sensitive to degradation of water quality and habitats including temperature, substrate, vegetation, pH, droughts, food, and stream geomorphology [78,79]. Valle et al. [80] found that macroinvertebrate fauna is significantly influenced by land use types in riparian areas. Macroinvertebrate communities are also used to evaluate the biological integrity of streams because they are sensitive to both organic pollution and habitat changes [81]. Fish communities represent a variety of trophic levels and include availability of food resources originated from both aquatic and terrestrial ecosystems. Their position at the top of the aquatic food web helps to provide an integrated view of the watershed environment [69]. Fish communities respond to anthropogenic disturbances, providing a valuable biotic index for stream quality assessments [82]. Since the introduction of the index of biotic integrity (IBI) by Karr [69], an IBI-type model using fish assemblages has been adopted in many countries.

It is very common to observe that variables of stream and natural environments are not independent, rather they are dependent on each other. Using a dataset containing autocorrelation among observations may result in biased model estimation, poor statistical inference, and violation of the independency assumption in statistical analysis [83–88]. Spatial autocorrelation, so called spatial dependency, is an inherent property of most spatial landscapes and their characteristics [89], suggesting that the value measured at a certain location is not independent from values of surroundings. Numerous studies in ecology, geography, forest science, and hydrology have emphasized the importance of accounting for spatial autocorrelation [83–85,90]. It is necessary to take spatial autocorrelation into account when investigating the relationship between riparian forest fragmentation and biological indicators in the stream, unless there is evidence indicating that spatial autocorrelation among variables does not exist or is not significant. The issue of spatial autocorrelation can be resolved in multiple ways. One way is to use a subset of data collected from sampling points separated by a distance greater than the range of spatial dependency in observed values [83,91,92]. However, this method is not preferred for most cases because it is difficult to get a sufficient number of observations for analysis. Another possible way is to use spatial statistical analysis methods to take spatial autocorrelation into account without losing the number of observations. These methods include regression kriging [93,94], autoregressive modeling [95,96], spatial autocorrelation [84,97], spatial lag regression [84], spatial eigenvector mapping [98–100], and geographically-weighted regression [2,85,101,102]. In previous studies, these approaches have been shown to perform better than conventional least-square-based models [2,84,103–106]. Particularly, regression tree analysis has shown to be promising [83,92,107] because it is effective for handling the spatial autocorrelation problem [107] and easy to interpret [91].

This study aimed to investigate (a) the relationship of riparian forest fragmentation with biological indicators of streams, and (b) the role of stream environmental factors (e.g., elevation, stream size, and slope) on the relationship. Since every restoration and management plan must address the spatial patterns of riparian forests, it is critical to understand how these patterns and stream environmental factors are associated with the ecological communities of streams. It was hypothesized that riparian forest fragmentation and stream environmental factors are associated directly and indirectly with the filtering efficiency of riparian vegetation by altering flow velocity, deposition process, and residence time [108–110]. Despite its importance, fragmentation of riparian forests has not been fully investigated, particularly in association with biological indicators of streams [66,111]. Considering the growing degree of fragmentation of riparian forests in human-modified streams, we must understand how to minimize the adverse impacts of riparian forest fragmentation on stream ecosystems in different stream environments. Particularly, the results of this study can provide insights into preparing strategic

management and restoration plans for fragmented riparian buffer zones to maximize ecological functions and reduce the adverse effects of land uses in watersheds.

2. Materials and Methods

2.1. Study Area and National Aquatic Ecological Monitoring Program (NAEMP)

South Korea accounts for half of the Korean peninsula, with an area of approximately 100,210 km^2, located between 35°74′ N and 127°46′ E. The area has distinct seasons with individual seasonal characteristics. Summer is humid and hot and spans from June to August, during which rain is common, with a continuous rainy season in late June. Winter is dry and cold due to the northwest winds from Siberia. Average monthly temperatures range between −2.4 and 25.7 °C in the central region and between about 3.2 and 20 °C in the southern region. The uneven distribution of temperature and differences between regions are caused by the mountainous nature of the northern area and the presence of the ocean in the southern area. The average annual precipitation is between 600 and 1800 mm. There are also 2 to 3 typhoons in the North Pacific from June to October every year. These produce 50%–60% of the total annual rainfall in the summer season [112].

There are five major river systems in Korea: the Han, Nakdong, Geum, Youngsan, and Seomjin Rivers. For this study, the Nakdong River watershed was selected due to the greater variation in land use and remnant riparian forests, while also being less disturbed than other watersheds since it contains fewer dams. The Nakdong River is the longest river in South Korea, at approximately 525 km, and passes through the southeastern region with a drainage area of 23,384 km^2. Large agricultural areas and major cities including the City of Daegu (population size = 2.5 million) and the City of Pusan (population size = 3.6 million) are located along the main stream (Figure 1).
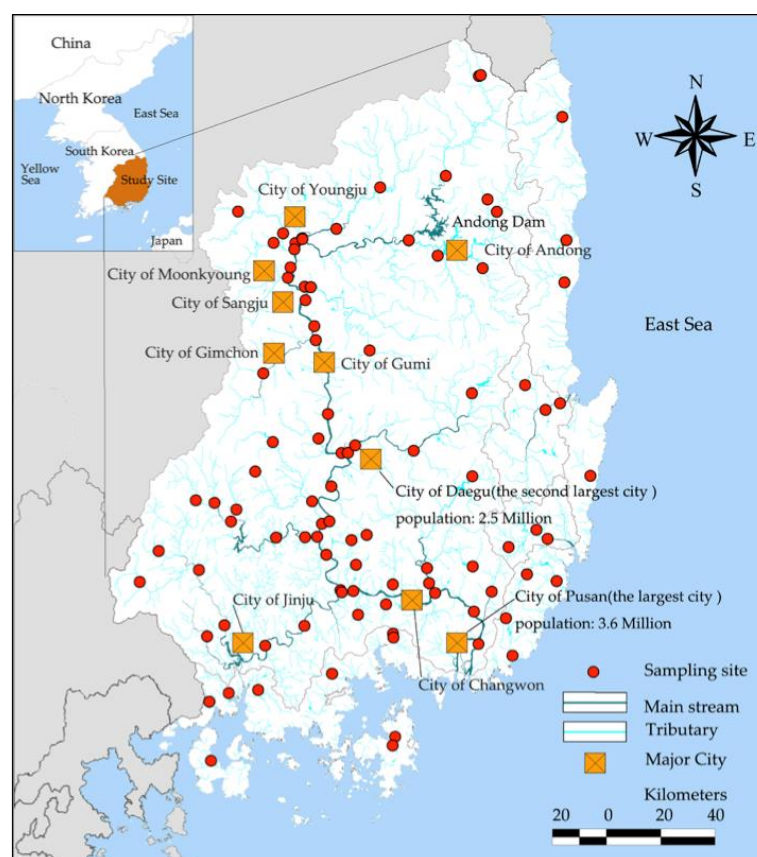
Figure 1. Distribution of sampling sites in the Nakdong River system under the National Aquatic Ecological Monitoring Program (NAEMP). Most major cities in the regions are located along the main streams.

The Ministry of Environment (MOE) in Korea has been monitoring the water quality and ecological status of streams and rivers across the country under the National Aquatic Ecological Monitoring Program (NAEMP) since 2007. The NAEMP is a comprehensive program addressing the wellbeing of various water body properties in surrounding areas including biochemical (e.g., BOD, COD, TN, TP, Chl-a, pH, DO, SS, etc.), geomorphological (e.g., velocity, width, and depth) characteristics, plants, and biological indicators (e.g., benthic diatoms, macroinvertebrates, and fish). All parameters and indicators are sampled twice per year (spring and fall) from 960 sites across the country, and all monitoring results are analyzed and archived in the monitoring database (for more detailed information on NAEMP, see Lee et al. [113]). The database has been widely used to analyze the status and spatiotemporal variations in stream environments and parameters, and the conservation and restoration of lotic systems in Korea [113]. Out of 149 sampling sites in the Nakdong River system, we selected 89 sampling sites monitored in 2012 under NAEMP by MOE for analysis, omitting first- and second-order streams. Large buffer size was not applicable to small streams because a 500 m buffer from sampling point exceeds catchment boundary for low-order streams.

2.2. Monitoring Program and Biological Indicators

The NAEMP classified scores ranging from 0 to 100 for each biological indicator including TDI (Trophic Diatom Index), BMI (Benthic Macroinvertebrate Index), and FAI (Fish Assessment Index), and scores are categorized as either class A "good", class B "fair", Class C "poor", and Class D "very poor", on the basis of monitoring results of the last five years, between 2006 and 2010. These classes should not be taken as an absolute biological status, rather it is a relative biological status in streams because the classification was based on the national wide distributions of each biological indicator with a similar number of observations in each class [33] (Table 1).

Table 1. Classifications of biological indicators including TDI (Trophic Diatom Index), BMI (Benthic Macroinvertebrate Index), and FAI (Fish Assessment Index) in NAEMP.

Class	Biological Status	TDI	BMI	FAI
Class A	Good	$60 \leq TDI \leq 100$	$80 \leq BMI \leq 100$	$87.5 \leq FAI \leq 100$
Class B	Fair	$45 \leq TDI < 60$	$60 \leq BMI < 80$	$56.2 \leq FAI < 87.5$
Class C	Poor	$30 \leq TDI < 45$	$45 \leq BMI < 60$	$25 \leq FAI < 56.2$
Class D	Very Poor	$0 \leq TDI < 30$	$0 \leq BMI < 45$	$0 \leq FAI < 25$

The TDI, as adopted by NAEMP, is a biotic index that evaluates the trophic status of stream ecosystems by calculating the proportion of benthic diatom taxa and compositions using a weighted mean sensitivity (WMS) measure by abundance (proportion) of species in samples and pollution sensitivity of species, as shown in Equations (1) and (2) [113–115]:

$$TDI = 100 - \{(WMS \times 25) - 25\} \tag{1}$$

$$WMS = \sum A_j \cdot S_j \cdot V_j / \sum A_j \cdot V_j \tag{2}$$

where WMS is the weighted mean sensitivity, j is the species of concern, A_j is the abundance (proportion) of species j in the sample (%), S_j is the pollution sensitivity ($1 \leq S \leq 5$) of species j, and V_j is the indicator value ($1 \leq V \leq 3$) [116].

The BMI (Equation (3)), as developed by the MOE for NAEMP, represents the health of macroinvertebrate communities in stream ecosystems, and incorporates the assigned number of species, unit saprobic value of the species, species frequency, and species indicator weight [113,117]:

$$BMI = \left\{ 4 - \sum_{j=1}^{n} S_j H_j G_j / \sum_{j=1}^{n} H_j G_j \right\} \times 25 \tag{3}$$

where j is the number assigned to species, n is the number of species, S_j is the unit saprobic value of species j, H_j is the frequency of species j, and G_j is the indicator weight value of species j [116].

The NAEMP developed the fish assessment index (FAI) using eight metrics originally proposed by Karr [69] based on the ecological characteristics of Korean fish assemblages. The metrics adopted by the NAEMP can be classified into four categories of fish assemblages: species composition, trophic composition, fish abundance, and individual health [113]. In the monitoring guidebook published by the MOE for NAEMP [118], the metrics used in the FAI include number of native species, number of riffle benthic species, number of sensitive species, percentage of tolerant species, percentage of omnivores, percentage of insectivores, number of native individuals, and percentage of fish abnormalities (e.g., tumors, lesions, wounds, unusual scale patterns, body color changes, and physical deformities such as curved spine or blindness). It is noteworthy that the FAI of Korean MOE places more weight on the introduction of invasive species in Korean aquatic ecosystems [119]. Fish types for metrics were identified on the basis of the monitoring guidebook of MOE for NAEMP [118]. The guidebook of MOE provides a specific scoring interval ranging from 1 (very poor) to 10 (excellent) for each metric, and the FAI can be computed by summing all eight metrics [116].

2.3. Scale of Riparian Forest Buffer

Specifying buffer widths is challenging, with proposed widths varying considerably in previous studies, depending on rainfall intensity, topography, hydrology, geology, vegetation types, and the purpose of buffer placement. On a broad level, a buffer width greater than 500 m is needed when considering multiple purposes, including nutrient control, bank stabilization, pesticide retention, stream water temperature, the amenity of river landscapes, and the provision of aquatic and terrestrial wildlife habitat. Based on riparian buffer studies in the literature, the Drinking Water Protection Act (1999) in Korea, and the implementation of riparian buffer zones in Korea, we selected 500 m as the buffer width for the study.

Boundary polygons of forest cover and water bodies in shape file format were extracted from LULC data released by MOE in 2009 and transformed into GRID files (10 m resolution) in a geographic information system (GIS). In the LULC file of MOE, vegetation is classified into three categories including grass, shrub, and tree covers, and only tree cover was used in the study. Buffers with a 500 m radius from 89 sampling sites in the study areas were created and overlaid on the extracted forest cover GRID file. Then, all tree cover GRIDs within the 500 m buffer from the 89 sampling sites were clipped and stored as separate GRIDs to compute fragmentation metrics.

2.4. Selecting and Computing Fragmentation Metrics

Fernandes et al. [21] suggested that the most common configurational characteristics of forest fragmentation are the number of large forest patches, overall forest patch size, and forest patch shape complexity, along with a misappropriate patch distribution within the riparian areas. As Jaeger [120] and Wang et al. [121] stated, there is no perfect spatial metric for landscape fragmentation measurement, and we have to instead use multiple metrics to describe them along various broad spatial aspects of forest fragmentation. Also, it is noteworthy that some metrics are highly correlated to each other [121–124]. To avoid this issue, we selected four base aspects to capture the characteristics of fragmentation: number of forest patches, proportion of forest, largest forest patch ratio, and spatial proximity of forest patches in riparian areas. The selected metrics are: (1) number of riparian forest patches (NP) [121,125–127], (2) percentage of riparian forest cover (PLAND) [121,125,126], (3) largest riparian forest patch index (LPI) [121,126,127], and (4) riparian forest division index (DIVISION) [120,128]. Landscape metrics selected to delineate the fragmentation of riparian forests were computed using FRAGSTATS (version 4.2; The University of Massachusetts, Amherst, US) at the class level [129], and the input to the program was the clipped riparian forest GRIDs (Table 2).

Table 2. Selected fragmentation metrics, their acronyms, and calculations [129] (see McGarigal et al., 2012 for more details on the metrics calculations).

Characteristics	Metrics	Calculation	Remarks
Number of riparian patches	NP	n	• Higher NP values indicate a greater degree of fragmentation.
Proportion riparian forest	PLAND	$(\sum_{i=1}^{n} a_i / A) \times 100$	• A value of 0 represents a landscape with no riparian forests. • A value of 100 means the entire buffer is covered by a large forest patch.
Largest riparian forest patch ratio	LPI	$\max_{i=1}(a_i) / A \times (100)$	• LPI approaches 0 when the patch is very small. • A value of 100 means the entire buffer area consists of a single large forest patch.
Spatial proximity of riparian forest patches	DIVISION	$\left\{ 1 - \sum_{i=1}^{n} (a_i / A)^2 \right\}$	• A value of 0 means the riparian buffer consists of a single large forest patch. • A value of 1 indicates that the total forest patch type consists of a single small one-cell patch

n = number of riparian forest patch, a_i = area of riparian forest patch i, and A = total buffer size.

2.5. Preliminary Test and Data Analysis

Prior to the main analysis, we conducted a preliminary test for normality of observed variables and linearity of biological indicators with stream environmental variables (i.e., elevation, slope, and stream size) and fragmentation metrics (i.e., NP, PLAND, LPI, and DIVISION). The QQ plots indicated that most variables were close to the normal distribution. To test linear relationships, we used 'GAM' and 'MGCV' packages in RStudio (available at https://www.rstudio.com). In the estimated GAMs (Generalized Additive Model) for biological indicators, EDF (estimated degree of freedom) values of almost all variables were close to 1, suggesting linear relationships of biological indicators with stream environmental variables and fragmentation metrics (EDF of 1 = perfect linear relationship; Wood, 2006). However, we observed significant non-linear relationships of elevation with biological indicators. Overall, there was no serious violation of the linearity assumption for using correlation analysis.

The preliminary analysis examined whether variables used in the study were spatially autocorrelated in ArcMap. The estimated Moran's *I* values [130] of the variables were greater than 0.5 ($p < 0.01$), indicating the presence of significant spatial dependency of the variables. Spatial autocorrelation of biotic/abiotic parameters in the stream was commonly observed in previous studies [131–134]. Therefore, we adopted a regression tree analysis to avoid the spatial autocorrelation inherent in the dataset while retaining the maximum number of observations. According to Cablk et al. [107], regression tree analysis is an effective method to model correlative relationships among variables even if autocorrelation exists in the dataset. At the same time, the results of regression tree analysis are relatively easy to interpret [91]. Regression trees provide better predictions than conventional regression models, and they are well suited for the analysis of complex ecological data [92,135,136]. We used the 'rpart' package in RStudio (available at https://www.rstudio.com) to estimate regression trees for TDI, BMI, and FAI with the 'ANOVA' method in the study.

3. Results

### 3.1. Descriptive Statistics and Spatial Distributions

The mean values of biological indicators TDI, BMI, and FAI, were 45.8, 65.63, and 48.86, respectively. According to NAEMP classification [137], the mean TDI was in "poor" condition, while BMI and FAI were in "good" and "fair" condition, respectively. BMI showed a relatively high mean value, whereas TDI had the lowest mean value (Table 3). Similarly, the minimum values of TDI, BMI, and FAI were 7.8, 13.1, and 12.5, whereas the maximum values were 76.3, 91.9, and 90.70, respectively. Standard deviations of BMI (18.35) and FAI (18.47) indicated relatively larger variances than TDI (16.06), indicating larger variances in BMI and FAI over sampling sites. Relatively lower minimum and maximum values of TDI as compared with BMI and FAI suggested that diatom status was relatively poorer than macroinvertebrate and fish in the study areas.

Table 3. Descriptive statistics of measured biological indicators and fragmentation metrics on riparian buffer scales (*n* = 89).

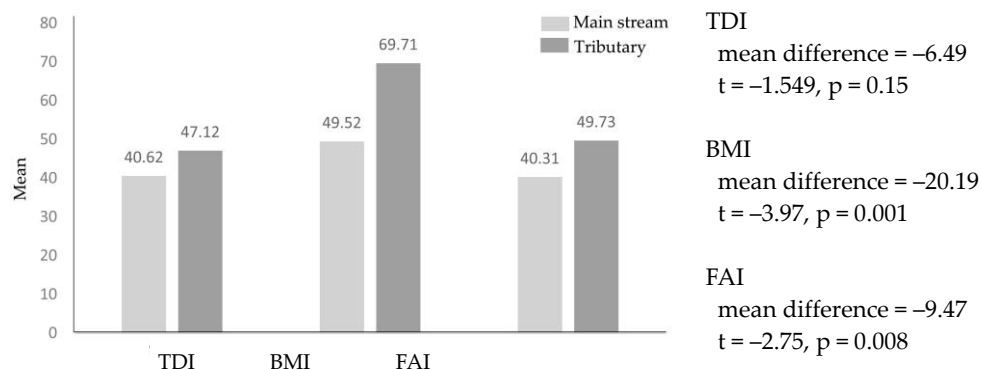| Variable		Min.	Max.	Mean	SD
Biological indicators	TDI	7.80	76.30	45.80	16.06
	BMI	13.10	91.90	65.63	18.35
	FAI	12.50	90.70	47.86	18.47
Fragmentation metrics	NP	1	23	7.58	4.41
	PLAND	0.24	78.42	33.41	20.82
	LPI	0.24	56.29	19.33	12.83
	DIVISION	0.67	1.00	0.92	0.07

The relatively low computed fragmentation statistics of forest cover suggests that some riparian forests were considerably fragmented. This is because higher values of PLAND and LPI indicate lower degrees of fragmentation, and higher values of NP and DIVISION indicate higher degrees of fragmentation. The NP and PLAND mean values were 7.58 and 33.41 respectively, whereas those of LPI and DIVISION were 19.33 and 0.92, respectively. The maximum values of NP, PLAND, LPI, and DIVISION were 23, 78.42, 56.29, and 1.0, respectively. This also confirms that riparian forests at some sites were significantly fragmented.

The spatial distribution in TDI values in the study sites showed that most of the very poor (class D) and poor (class C) classes were distributed in the main streams, and the tributary streams were predominately classified as fair (class B) and good (class A). Similarly, the distribution in BMI values showed that the very poor (class D) and poor (class C) classes are more common in the main streams, whereas fair (class B) and good (class A) classes are more common in other tributary streams. The FAI values of the main streams are often classified as very poor (class D) and poor (class C), whereas the tributary streams are often classified as fair (class B) and good (class A) (Figure 2). Despite their slight differences, classes C (poor) and D (very poor) of all biological indicators were observed along the main streams where major cities are located (Figures 1 and 2). Therefore, the water quality and biological conditions in main streams are often worse than in tributary streams (circled areas in Figure 2).
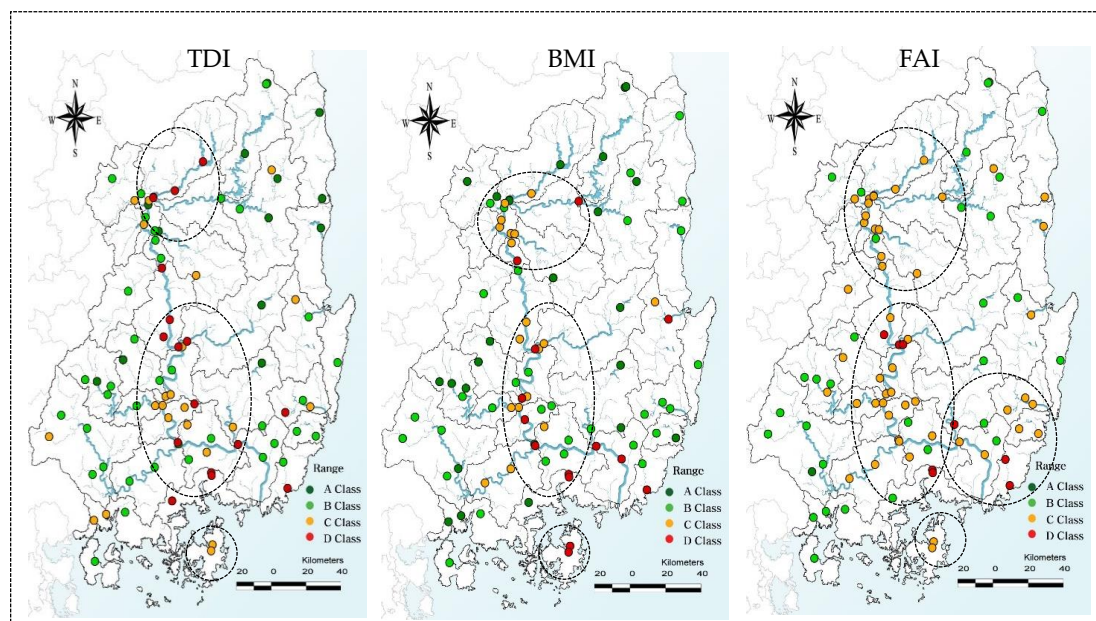
Figure 2. Spatial distribution of biological indicators of TDI (trophic diatom index), BMI (benthic microinvertebrate index), and FAI (fish assessment index) classes in the Nakdong River system. The circled areas in the figure represent the locations with the worst water quality and biological conditions in main streams.

We observed that the mean BMI and FAI values were significantly different between main streams and tributary, but not TDI (Figure 3). The mean BMI values of main streams and tributary were 49.52 and 69.71 respectively, and this difference was significant. Similarly, the mean difference of FAI between main and tributary streams was also significant. However, the mean TDI values between main and tributary streams were not significantly different.

TDI
  mean difference = −6.49
  t = −1.549, p = 0.15

BMI
  mean difference = −20.19
  t = −3.97, p = 0.001

FAI
  mean difference = −9.47
  t = −2.75, p = 0.008

Figure 3. Comparison of the mean TDI (trophic diatom index), BMI (benthic microinvertebrate index), and FAI (fish assessment index) between main stream and tributary by t-test.

3.2. Correlations of Biological Indicators with Forest Fragmentation and Stream Environments

The results of Pearson correlation analysis indicated that the TDI indicator (benthic diatoms) was significantly correlated with most fragmentation metrics of riparian forests. TDI was positively correlated with PLAND and LPI and negatively correlated with DIVISION (Table 4). The TDI was better when riparian forests were less fragmented. However, no significant relationship was observed between TDI and NP. The BMI indicator (macroinvertebrates) showed significant fragmentation indicators of riparian forests. BMI was positively correlated with NP, PLAND, and LPI, and negatively correlated with DIVISION. These correlations strongly indicate that the biological conditions of macroinvertebrates in streams are not likely to be good if riparian forests are severely fragmented.

Table 4. Pearson correlations between forest fragmentation metrics, stream environmental factors, and biological indicators.

| Category	Variables	TDI (Benthic diatoms)	BMI (Macroinvertebrates)	FAI (Fish)
Fragmentation metrics	NP	0.18	0.23 *	0.21
	PLAND	0.28 **	0.48 **	0.43 **
	LPI	0.30 **	0.37 **	0.35 **
	DIVISION	−0.22 *	−0.37 **	−0.34 **
Stream environments	Stream Order	−0.16	−0.46 **	−0.36 **
	Slope (%)	0.08	0.34 **	0.32 **
	Elevation (m)	0.35 **	0.38 **	0.45 **

NP = number of riparian patches, PLAND = proportion of forests in the riparian areas, LPI = largest riparian forest patch ratio, and DIVISION = spatial proximity of riparian forest patches. * $p < 0.05$, ** $p < 0.01$; $n = 89$.

Similarly, FAI was significantly related with NP, PLAND, LPI, and DIVISION. Thus, the poor biological conditions of fish in streams are significantly tied to a higher degree of fragmentation of riparian forests. These results suggest that the fragmentation of riparian forests is associated with poor biological community conditions including benthic diatoms, macroinvertebrates, and fish in streams. All biological indicators were more sensitive to PLAND, LPI, and DIVISION, but less sensitive to NP. Only BMI showed a significant relationship with NP, whereas TDI and FAI demonstrated significant relationships with all landscape metrics.

Stream order and slope did not show significant correlations with TDI. However, they were significantly correlated with BMI and FAI. Specifically, stream order was negatively correlated with BMI and FAI, while slope appeared to have positive correlations with BMI and FAI, respectively. The negative correlations of stream order with BMI and FAI suggested that biological conditions of macroinvertebrate and fish are likely poorer in larger streams (i.e., high-order streams) than in smaller streams (i.e., low-order streams). Positive relationships between slope and BMI and FAI indicated that biological conditions of macroinvertebrate and fish in streams are likely poorer in flat or mildly sloped areas. Similarly, elevation showed positive correlations with all biological indicators, suggesting all biological conditions in streams were likely better in high elevated areas than low elevated areas (Table 4).

3.3. Regression Trees for TDI, BMI, and FAI

The observations in the TDI regression tree were split by elevation into either a low elevation group or a high elevation group, suggesting high elevation could increase the TDI value by 16.92% (Figure 4). Membership to Node 2 implied that a high PLAND value (i.e., less fragmented riparian forests) increased the biological status of trophic diatom in the stream (i.e., TDI). The right side of the TDI regression tree suggested that less fragmented riparian forest increases the TDI value by about 6.71% in the high elevation areas. Observations at Node 13 revealed that lower stream orders are associated with higher TDI values. The R^2 of the estimated regression tree for TDI was 0.44, and the relative importance of the variables were PLAND (25%), elevation (23%), DIVISION (19%), LPI (18%), NP (6), stream order (5%), and slope (4%). DIVISION, LPI, and NP were not used in the estimated regression tree for TDI, but the estimated relative importance of the variables indicated that PLAND, DIVISION, and LPI were more important variables than NP, stream order, and slope. Overall, less fragmented riparian forests (i.e., high PLAND and LPI) were associated with better TDI status of streams, and the effects of riparian forest fragmentation depended on stream environment (i.e., elevation in this case; Figure 4).
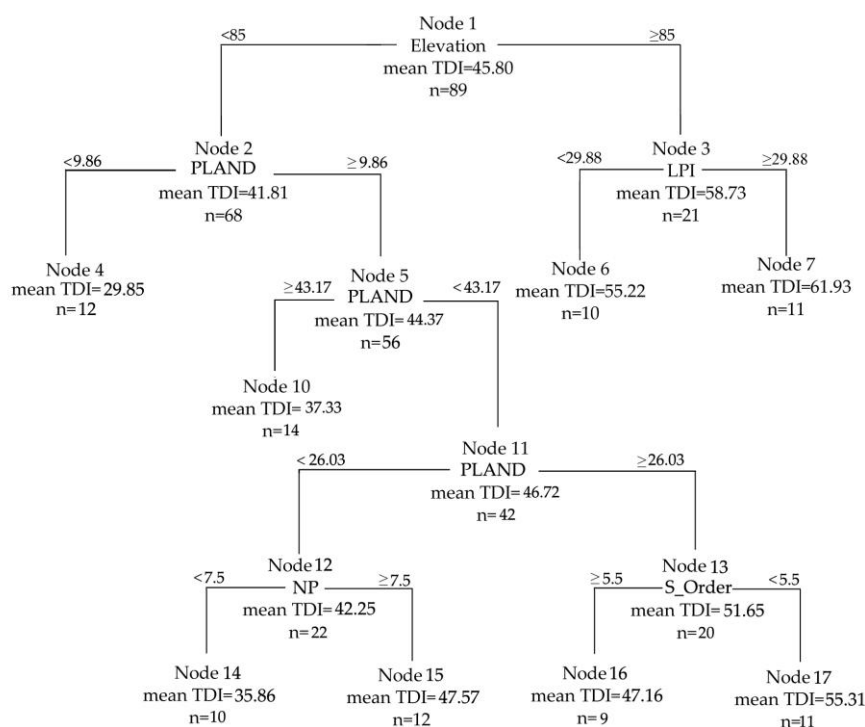
Figure 4. Regression tree for the TDI with stream environmental variables and fragmentation metrics of riparian forests ($R^2 = 0.44$).

The first split in the BMI tree indicated the significant effects of less fragmented riparian forest (i.e., high PLAND) on benthic macroinvertebrate stream conditions (i.e., 17.82% increase of expected BMI value; Figure 5). The observations in the low PLAND group were classified by stream order and suggested that lower stream order could considerably increase the BMI value (17.99%). Similarly, the split in Node 3 implied that higher stream elevation significantly increased the expected BMI value (10.95%). We observed an inconsistent slope effect on BMI at Node 2 and Node 6, indicating that the effect of slope on BMI could vary over other stream environmental variables (i.e., stream order and elevation) and fragmentation (i.e., PLAND). The R^2 of the estimated regression tree for BMI was 0.44, and the order of the variables by relative importance was PLAND (23%), DIVISION (19%), LPI (19%), slope (13%), elevation (13%), stream order (13%), and NP (1%).
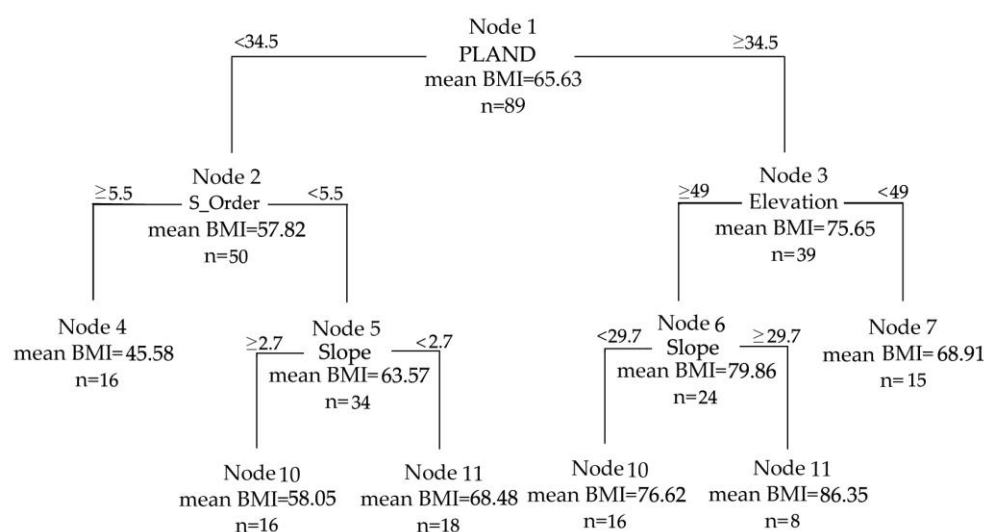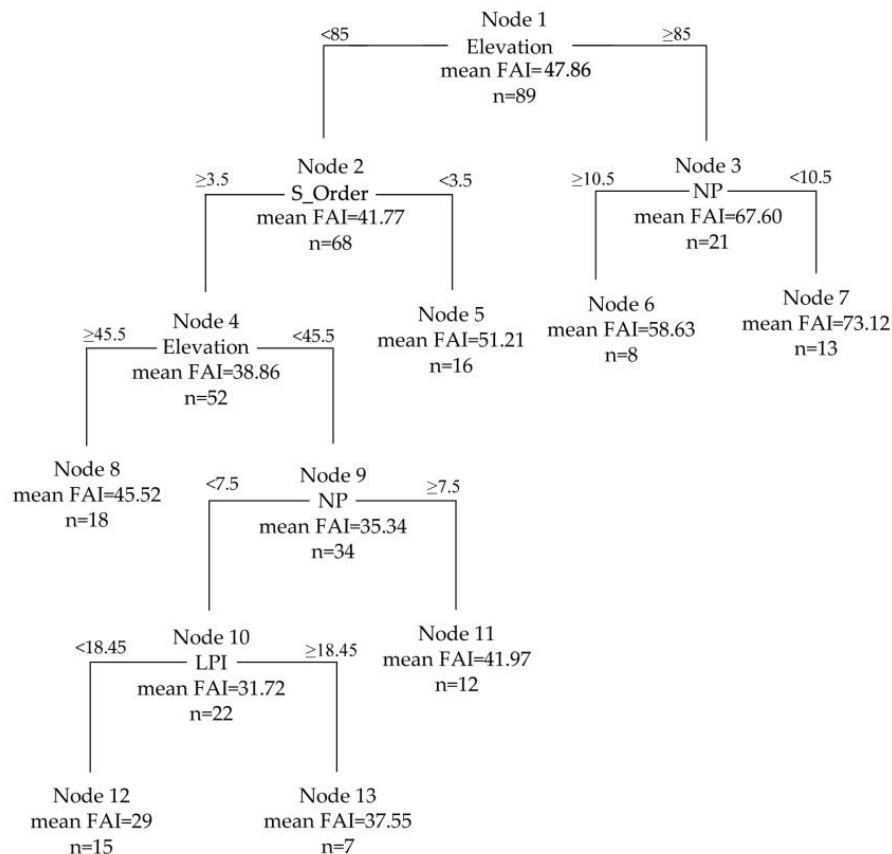

Figure 5. Regression tree for the BMI with stream environmental variables and fragmentation metrics of riparian forests ($R^2 = 0.44$).

We observed 25.83% greater FAI values in high elevation group than low elevation group in the FAI regression trees (Figure 6). FAI statuses were likely poor in the high-order streams. At the same time, we found contrasting effects of NP, suggesting that the effect of NP varied depending on the given conditions of stream environmental variables (i.e., elevation and stream order). The split at Node 10 revealed a positive relationship between a high largest-riparian forest patch ratio (i.e., LPI) with a better FAI status in a stream. The R^2 of the estimated regression tree for FAI was 0.54, and the order of the variables by relative importance was elevation (46%), PLAND (15%), DIVISION (11%), LPI (10%), stream order (8%), NP (8%), and slope (3%). In addition, we observed that the effects of fragmentation metrics on FAI were dependent on certain stream environmental conditions, such as elevation and stream order.

In conclusion, the regression trees analysis of the biological indicators in question illustrated the structured effects of fragmentation metrics and environmental stream variables on biological indicators. The regression trees for TDI, BMI, and FAI revealed the importance of elevation in explaining the spatial variance of biological indicators in the study area. Particularly, elevation was the first variable split in the estimated regression trees for TDI and FAI, and it was the second variable split in the regression tree for BMI. In addition, PLAND appeared to be the most important variable among the fragmentation metrics of riparian forests and showed positive effects on biological indicators in most cases. Particularly, LPI consistently showed positive effects on TDI and FAI. It seemed that the role of NP was not consistent for all biological indicators and within all stream environments. Rather, LPI varied between stream environments (e.g., elevation and stream order). Overall, the effects of riparian forest fragmentation metrics are dependent on the stream environments, except for FAI regression. Stream environmental variables were the primary splits in the regression trees, and the

effects of the fragmentation metrics might play a significant role within the particular circumstances of stream environments. Among stream environmental variables, slope did not appear as a main split factor while elevation and stream order were significant splitting variables at the first and second levels in the regression trees of BMI and FAI.

Figure 6. Regression tree for the FAI with stream environmental variables and fragmentation metrics of riparian forests ($R^2 = 0.54$).

3.4. Difference in Fragmentation between Main and Tributary Streams

Finally, we observed that there were more developed areas (e.g., farms, paddies, and urban areas) in the surrounding areas of main streams than around tributary streams. This makes sense since we know that human disturbance and land use change are concentrated around main streams rather than tributary streams. Such developments and land use changes in the surroundings of main streams inevitably cause disturbances on riparian forests, resulting in serious fragmentations of riparian forests. There were significant differences in mean values of NP, PLAND, LPI, and DIVISION between main streams and tributary streams, suggesting that the riparian forests of large main streams are more severely fragmented than those of tributary streams. Riparian forests of main streams showed significantly higher mean values of DIVISION and lower means values of NP, PLAND, and LPI, indicating more severely fragmented riparian forests of main streams than those of tributary streams (Table 5). The anthropogenic disturbances are responsible for more severely fragmented riparian forests in the surrounding areas of main streams. This might be because riparian forests are extremely vulnerable to anthropogenic impacts [138,139], resulting in a decreased positive function [140], which might be particularly evident at large main streams in this case.

Table 5. Comparison of the mean fragmentation metrics between main streams and tributary streams.

Fragmentation Metrics	Stream Classification	Mean	Mean Difference	t
NP	Main stream	5.67	2.40	2.93 **
	Tributary stream	8.07		
PLAND	Main stream	24.13	11.62	2.44 *
	Tributary stream	35.76		
LPI	Main stream	12.97	7.97	2.93 **
	Tributary stream	20.94		
DIVISION	Main stream	0.96	−0.04	−3.15 **
	Tributary stream	0.92		

NP = number of riparian patches, PLAND = proportion of forests in the riparian areas, LPI = largest riparian forest patch ratio, and DIVISION = spatial proximity of riparian forest patches. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

4.1. Fragmentation Metrics and Biological Indicators

Evidence in the literature indicates the positive effects of vegetative riparian buffer zones on streams, such as stabilizing stream banks, reducing nutrient and sediment loading from watersheds, regulating water temperature, providing habitats for aquatic and terrestrial organisms, enhancing ecological integrity and biodiversity, and mediating negative impacts of land use in watersheds [10,14,19,21,25,27,141,142]. However, most previous studies focused on the effectiveness of varying widths and types of riparian vegetation. The results of the correlation analysis in this study suggested that biological communities in streams are not only associated with riparian forest width and type, but also with the spatial configuration of riparian forests. Specifically, indicators of benthic diatoms, macroinvertebrates, and fish in streams are significantly correlated with various aspects of fragmentation of riparian forests.

The results of the correlation analysis indicated that the biological status of benthic diatoms (TDI) in streams was likely to be better in areas with more tree cover (i.e., high PLAND), largest forest patch (i.e., high LPI), and less isolation among forest patches in riparian areas (i.e., low DIVISION). In addition, the regression tree for TDI suggested that LPI can significantly enhance the biological condition of benthic diatoms in highly elevated areas (elevation ≥ 85 m), while in low elevation areas (elevation < 85 m) biological conditions of the diatom are largely determined by PLAND. As discussed in previous studies, increasing riparian forest area can increase rainfall interception, slow surface runoff speed, enhance infiltration time of surface water into soils, trap more sediments, and uptake more nutrients in riparian areas [143–145]. Our study indicated that these positive effects of riparian forests on water quality and biological communities in streams [146,147] might not be the same over space. In low elevation areas, water quality and the biological condition of benthic diatoms might be positively affected by the overall proportion of riparian forests, while in high elevation areas largest patch size is more critical for the diatom community than the proportion of forest.

Correlation analysis indicated that the status of BMI (benthic macroinvertebrate index) is positively related with fragmentation metrics of riparian forests, including the proportion of riparian forests, largest forest patch ratio, and the spatial proximity of riparian forest patches. Correlations of BMI with fragmentation metrics (except for NP) indicated that the condition of macroinvertebrates in streams was likely to be better with less forest fragmentation (i.e., high PLAND, high LPI, and low DIVISION). Furthermore, the regression tree of BMI reinforced the positive effect of the proportion of forests in riparian areas. The proportion of riparian forest appeared to be a more critical factor in explaining the variance of the biological condition of macroinvertebrate than stream environments (i.e., elevation, stream order, and slope) in the regression tree. Thus, it is evident that the biological condition of macroinvertebrates is positively influenced by the proportion of riparian forests in riparian areas. Macroinvertebrates are particularly sensitive to pollution, and their dependence on

terrestrial environmental conditions around the stream [147] plays a key role in the functioning of aquatic ecosystems, accounting for the deficiency between stream producers and stream consumers in streams [80,148]. In our study, BMI showed positive relationships with all fragmentation metrics, the highest positive correlation being with PLAND. And the BMI regression tree confirmed the importance of PLAND in the biological condition of macroinvertebrates in the stream. This is supported by Potter et al. (2004) [149], who reported that the health of invertebrate communities was positively correlated with high forest cover. A high proportion of riparian forest also can provide unique habitats, nutrients, refuges, and resources for both terrestrial and aquatic invertebrates [150]. The BMI regression tree also indicated that stream order (i.e., stream size) is a critical factor to the biological communities of macroinvertebrates when the proportion of forest is less than 34.5%, while elevation is a more significant factor when the proportion of forest is more than 34.5% in riparian areas.

Similarly, FAI was also negatively correlated with fragmentation metrics of riparian forest patches. The PLAND produced the highest correlation coefficient with the fish assessment index (FAI). Correlations of FAI with fragmentation metrics indicated that the status of fish in streams was likely to be better if riparian forests were less fragmented. However, the FAI regression tree suggested that the effects of forest fragmentation depend on stream environments, including elevation and stream order. In high elevation areas (≥85 m), forest patch number (NP) may enhance the biological status of fish in the stream. However, number of forest patches (NP) may decrease FAI values in low elevation stream environments. This suggests that the effects of forest fragmentation may vary due to different stream environments.

Severe riparian forest fragmentation is characterized by a relatively greater number of forest patches, a smaller percentage of forest coverage, a smaller largest-forest patch, and less proximity of forest patches. Compared to highly fragmented riparian forests, less fragmented forests may intercept more rainfall, slow surface runoff speed, enhance infiltration time of surface water into soils, trap more sediments, and uptake more nutrients in riparian areas [143–145]. Our study supports previous findings, as we observed higher scores for biological indicators as the degree of forest fragmentation decreased (i.e., high NP, PLAND, and LPI values, and low DIVISION values). This can be explained by the fact that the degree of land–water interface (i.e., function) partially depends on the spatial composition and pattern (i.e., structure) of riparian forests. From a landscape ecology perspective, riparian forests are essential landscape elements linking terrestrial ecosystems with stream ecosystems and regulating the flow of energy, materials, and organisms between the two [110,151–153]. The degree of fragmentation determines riparian forests' ability to capture pollutants and sediments heading from terrestrial environments into streams and can therefore enhance the protection of biological communities in streams [146]. As an integral component of riparian landscapes, forests can directly affect stream channel microclimates, local air temperature, humidity, and wind speed, which influence stream water temperatures [154–157]. For example, the difference in maximum summer water temperatures between forested and open terrain is 4–9 °C [158–161]. For example, the summer maximum difference could even reach 13 °C, as was followed after clearcutting of commercial conifer plantations in the southern interior of British Columbia, Canada [161]. Extremely fragmented riparian forests increase exposure to sunlight and wind, which can negatively affect biological integrity through temperature fluctuations and desiccation caused by forest removal in riparian areas [158,160,162,163]. However, the number of forest patches (NP) in riparian areas showed weak relationships with biological indicators. Thus, the critical fragmentation characteristics that determine the efficacy of riparian forests on biological conditions are the proportion of forests in the riparian areas, largest forest patch ratio, and isolation/proximity of forest patches (except NP). Some possible explanations for the weak relationships of NP with biological indicators might include non-linear effects [164], non-stationary effects [2,165], and constrained effects by more significant variables [166].

The study results indicating the negative impacts of fragmented riparian forests on biological indicators should be applied to restoration and management practices cautiously because other vegetation types (e.g., grasses and shrubs) have been shown to have similar effects. The negative

effects of highly fragmented riparian forests could be significantly moderated by developments in understory vegetation, such as grasses or shrubs [167–169]. The results of this study and previous investigations suggest that fragmentation of riparian forests might negatively affect the biological conditions of streams, but these effects could be mitigated by other types of riparian vegetation. However, we were unable to find guidelines recommending specific types of vegetation for particular purposes (i.e., trapping, infiltration, deposition, shading, etc.,) in the literature. In general, forests have been shown to be more effective at intercepting rainfall compared to other types of vegetation [170]. It is important to note that environmental planning must consider the whole watershed for water resource conservation [171,172], but nevertheless, fragmented and disturbed riparian forest restoration can be prioritized as a short-term action for immediate water quality improvement [173].

4.2. Effects of Stream Environments on the Relationships

Both correlation analysis and regression tree analysis indicated that elevation is a very critical variable for understanding the status of biological indicators and the effects of riparian forest fragmentation on biological conditions. The estimated regression trees for TDI, BMI, and FAI suggested that the effects of some fragmentation metrics were dependent on elevation and stream order (i.e., stream size). In the literature, elevation has been shown to be closely associated with transportation of pollutants and nutrients [174,175], sediments discharge [176–178], and a steep bed gradient [179]. Furthermore, each of these phenomena significantly impacts biological communities in streams. In general, the main trait of low elevation streams are their relatively large widths, high depths, high conductivity, poorly oxygenated water, high levels of nutrients, and high water temperature [133]. In addition, streams in low elevation areas are significantly affected by intense land use and human activities, resulting in a high concentration of nutrients, pollutants, and poor biological conditions [180]. In our study, the effects of riparian forest fragmentation on biological status in streams could greatly vary among elevation ranges (e.g., PLAND in TDI regression and NP in FAI regression). However, we are not sure this is also true for other river systems in other geographical locations where the intensity of anthropogenic activities and stream environments are significantly different. In addition, the cause of fragmentation might play significant roles in the relationships between fragmentation and biological status in streams. Fragmented riparian forests by natural processes might not have negative impacts on stream ecosystems, while human-originated fragmentation might affect stream ecosystems negatively, as we reported in the study. Nonetheless, further studies considering different vegetation types, buffer sizes, and various stream environments are needed to depict the complex nature of the relationships among fragmentation, stream environments, and the biological status of streams. Finally, we observed that there were more developed areas (e.g., farms, paddies, and urban areas) in the surrounding areas of main streams than around tributary streams. This makes sense since we know that human disturbance and land use change are concentrated around main streams rather than tributary streams. Such developments and land use changes in the surroundings of main streams inevitably cause disturbances on riparian forests, resulting in serious fragmentations of riparian forests.

5. Conclusions

We examined the relationships between riparian forest fragmentation and biological indicators in different stream environments on a large riparian buffer scale. The results demonstrate that stream biology characteristics are closely linked with forest fragmentation. The results of this study indicate that a higher degree of riparian forest fragmentation negatively affects biological status of benthic diatoms, macroinvertebrates, and fish in streams. Numerous previous studies reported that the amount of riparian vegetation is an important factor for water quality and biological status of streams. Our study suggests that the spatial structure of riparian vegetation is also critical for preserving biological communities in streams. However, the degree of negative effects of riparian forest fragmentation on the biological status of streams might vary across the given stream environmental variables. In order to plan effective stream-management policies, it is essential to understand the

relationships between biological indicators and forest fragmentation patterns at the riparian scale. As demonstrated in this study, streams with unfragmented forest have relatively rich ecological communities. However, the mechanisms by which the forest fragmentation process contributes to ecological communities needs to be elucidated in more detail. In this regard, integrating our findings into the Riparian Ecosystem Management Model (REMM) (developed by USDA [181,182]) can be an effective way to delineate the specific roles of riparian forest fragmentation on water quality and biological status of streams. Despite the fact that REMM has been shown to be a useful computer tool for simulating the key hydrologic and biogeochemical processes occurring in real world buffers [183,184], the model only takes into account vegetation types in riparian areas. Integrating spatial patterns (e.g., fragmentation) of riparian vegetation into the model would increase its accuracy and usage for different stream environments and geographical locations.

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