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eDNA Metabarcoding to Monitor Fish Communities in a Large River Floodplain

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ABSTRACT

Freshwater ecosystems are highly biodiverse and provide essential services that support both ecosystem health and economic sustainability. Despite their ecological significance, these ecosystems are disproportionately affected by the global biodiversity crisis. Large river floodplains constitute a fundamental component of freshwater ecosystems, sustaining fish biodiversity, growth, and reproduction. Yet, these floodplains face mounting threats from anthropogenic pressures, including physical modifications and land conversion for agriculture. In this context, there is an urgent need for scalable biomonitoring methods to more effectively assess floodplain ecosystems, which present methodological challenges due to their heterogeneous and dynamic nature. Traditional fish monitoring methods, however, are often invasive, costly, and resource-intensive. In contrast, environmental DNA (eDNA) metabarcoding presents a noninvasive, cost-effective, and scalable alternative. This study compares eDNA metabarcoding and electrofishing for fish community biomonitoring in the floodplain of Lake St. Pierre, the largest floodplain habitat along the St. Lawrence River. We assessed the effectiveness of these methods in monitoring fish community diversity and composition, as well as the influence of floodplain sectors and a gradient of land use from natural wetlands to annual (row) crops. eDNA metabarcoding detected a broader range of species than electrofishing, while both methods consistently identified abundant species. The two methods yielded uncorrelated diversity indices and distinct community compositions. Fish eDNA community composition was strongly associated with floodplain sectors, whereas land use within these sectors had a weaker influence on community diversity and composition. Our findings highlight eDNA metabarcoding as a valuable tool for characterizing broad patterns of fish communities in floodplain ecosystems. This method provides an additional tool to traditional methods for monitoring and conserving threatened floodplain habitats. However, careful consideration of study scale is essential to ensure effective conservation outcomes in these hydrologically dynamic environments.

1 | Introduction

Recent studies have highlighted the rapid loss of biodiversity in freshwater ecosystems (Albert et al. 2021; Sayer et al. 2025), with wetland habitats and fish species being among the most threatened within these environments. Floodplain wetlands are areas periodically inundated by river or lake overflow, direct rainfall, or rising groundwater levels (Junk et al. 1989). These habitats are essential for fish communities, supporting biodiversity (Welcomme 2000; Stoffers et al. 2022), growth (Tonkin

et al. 2011), and population recruitment (Górski et al. 2011). The dynamic ecosystems of floodplains offer diverse temporary habitats that serve as ideal spawning and nursery grounds, providing shallow, sheltered waters, abundant food, and protection from strong currents and predators (Holland and Huston 1985; Poizat and Corivelli 1997; Baber et al. 2002; Balcombe et al. 2007). Known for being nutrient-rich environments (Spink et al. 1998; Gordon et al. 2020), floodplains support productive plant (Deschamps et al. 2023), phytoplankton (Lehman et al. 2008), and zooplankton communities (Faggotter et al. 2013), which in turn promote juvenile fish growth and survival (Douglas et al. 2005; Pratt et al. 2023). Seasonal flooding connects rivers and floodplains, facilitating fish migration, dispersal, and maintaining genetic diversity (Fernandes et al. 2014; Stoffels et al. 2016). In addition to fisheries and biodiversity, floodplains also contribute to other ecosystem services such as climate regulation, water purification, flood regulation, and economic growth (Lynch et al. 2023).

Despite their critical importance to support fish populations and maintain overall ecosystem health, floodplains are increasingly threatened by human activities. Since the 1970s, over one-third of the world's wetlands and floodplains have been lost at a rate twice that of forests, primarily due to extensive physical alterations such as drainage, land conversion, and excessive irrigation (Tockner et al. 2002; Gardner and Finlayson 2018; Fluet-Chouinard et al. 2023). Such alteration of natural habitats has led to reduced river length and habitat diversity, disrupted lateral and longitudinal connectivity, and decreased retention of nutrients and sediments (Tockner et al. 2008). These changes have ultimately led to the degradation or complete loss of critical habitats essential for maintaining healthy riverine fish communities (van Puijenbroek et al. 2019; Su et al. 2021). Intensive land use, such as agriculture, is an important driver of floodplain degradation (Tockner et al. 2002; Knox et al. 2022; Morrison et al. 2023). Furthermore, agricultural activities negatively impact aquatic food webs by introducing stressors like nutrient pollution (Del Rossi et al. 2023), sediment runoff (Mateo-Sagasta et al. 2017), pesticides and other contaminants (Rad et al. 2022), and altered hydrology (Blann et al. 2009). Excess nitrogen and phosphorus from fertilizers can lead to eutrophication (Khan and Mohammad 2014), causing oxygen depletion and disrupting fish and invertebrate populations (Chislock et al. 2013). Sediment runoff increases water turbidity, harming benthic habitats, and fish reproduction (Kemp et al. 2011), while pesticides can reduce prey availability (Schäfer 2019) and bioaccumulate up the food chain (Katagi 2010). Lake St. Pierre (LSP), a biodiverse fluvial lake along the St. Lawrence River that includes a UNESCO Biosphere Reserve, is a clear example of such degradation. Since the 1950s, the LSP floodplain has undergone extensive agricultural intensification, resulting in the loss of wildlife habitats (Jobin and Brodeur 2023). Submerged aquatic vegetation in the lake itself has sharply declined over the past two decades (Hudon et al. 2018; Laporte et al. 2023), contributing to deteriorating spawning and nursery habitat for multiple fish species (Giacomazzo et al. 2023; Paquin et al. 2024). Intensive agricultural practices, coupled with river regulation and changes in flood dynamics, have disrupted seasonal connectivity, reduced water quality, and impeded reproductive success for several fish species (Foubert et al. 2020), all of which likely contributed to shifts in fish community composition (Laporte et al. 2025). Nearly 25% of the world's cropland is located in floodplains (Dryden et al. 2021), highlighting the urgent need for scalable biomonitoring methods to better assess and protect these ecosystems.

Fish communities in floodplains are usually surveyed using electrofishing and nets (Gunzburger 2007). Such "traditional methods" based on morphology are invasive, disrupt habitats, and are often lethal (Snyder 2003; Portt et al. 2006). Moreover, traditional sampling methods for quantitatively monitoring fish assemblages are both challenging and expensive, particularly in large river systems (Zajicek and Wolter 2018), and offer a low probability of detecting rare species (MacKenzie et al. 2005). Large river floodplains constitute particularly challenging environments for sampling, characterized by highly heterogeneous, complex, and temporally variable habitats (Erős et al. 2019). Growing evidence suggests that environmental DNA (eDNA) metabarcoding offers an efficient solution to the limitations of traditional sampling methods (Fediajevaite et al. 2021; Gehri et al. 2021). eDNA involves collecting genetic material from environmental samples without directly isolating or interacting with organisms (Taberlet et al. 2012). This method is increasingly recognized as a valuable tool for assessing biodiversity, community dynamics, and ecosystem-level processes (Deiner et al. 2021). eDNA sampling is noninvasive, cost-effective, and highly sensitive, capable of detecting species even at low densities (Lawson Handley 2015). However, eDNA metabarcoding is subject to its own limitations, including primer bias, PCR amplification, DNA degradation, contamination, dilution, and transport (Beng and Corlett 2020). In lotic ecosystems, eDNA can be quickly transported over distances ranging from a few meters to several kilometers from its source (Civade et al. 2016; Pont et al. 2018). Lateral and longitudinal transport of DNA particles could potentially limit the efficacy of eDNA metabarcoding in dynamic hydrological conditions found in floodplain ecosystems. The extent to which eDNA reflects the diversity and composition of local fish assemblages remains poorly documented in floodplains, where relatively few eDNA studies have been conducted compared to other freshwater systems.

In this study, we investigated the use of eDNA metabarcoding to monitor fish communities in the floodplain of the St. Lawrence River, Canada, where various forms of extensive and intensive agriculture are found, and where agricultural land use has recently intensified, shifting from perennial crops to annual crops. First, we compare eDNA metabarcoding estimates of community diversity and composition to those obtained with a traditional sampling method (boat electrofishing). Then, we tested the ability of eDNA metabarcoding to identify patterns of fish community diversity and composition associated with different geographical sectors of the floodplain and with distinct land use practices in the flooded areas. Finally, we compare the importance of land use in shaping fish community composition relative to spatial and other environmental factors.

2 | Materials and Methods

2.1 | Sample Collection

2.1.1 | Study Sites

Lake St. Pierre, a 50,000-hectare enlargement of the St. Lawrence River in Québec, Canada, is part of the Great Lakes-St. Lawrence drainage basin, the world's largest

freshwater system (Bartolai et al. 2015). The lake supports diverse wetland ecosystems, providing critical habitat for numerous aquatic species. Each spring, flooding driven by snowmelt and precipitation inundates adjacent agricultural and natural lands, creating a vast 28,000-hectare floodplain, the largest wetland in the St. Lawrence system (Hudon et al. 2018). LSP wetlands are considered a RAMSAR wetland of international importance and comprise a UNESCO Biosphere Reserve as previously mentioned. The hydrology of the Lake St. Pierre floodplain is complex and influenced by a combination of inputs from the main stem of the St. Lawrence River, its large tributaries, and numerous smaller streams that drain into the floodplain. The relative contribution of these water sources varies spatially between and within sectors of the LSP and fluctuates over the course of the spring flood period (Clermont et al. 2023; Campeau et al. 2024).

Agriculture is central to the region's economy. Agricultural areas cover approximately 23% of the floodplain and are occupied by mostly small, family-owned farms (Jobin and Brodeur 2023). Since 1950, land use in the floodplain has shifted from primarily perennial crops and pastures to predominantly annual crops—such as corn and soybeans—which accounted for 86% of agricultural land use in 2016 (Jobin and Brodeur 2023). In this context, an interdisciplinary ecosystem-scale sampling program involving experimental manipulations was designed and conducted by a strategic research cluster involving many researchers from three institutions (in original French: le Pôle d'expertise multidisciplinaire en gestion durable du littoral du lac Saint-Pierre), to investigate the environmental, agricultural, and socioeconomic impact of land use intensification in the LSP floodplain (Watson et al. 2024).

Our study examines 30 sites spanning a gradient of land use across four sectors of the LSP floodplain in 2019 and 2022, for a total of 47 sampling locations (Figure 1). All sampling sites were located within the 2-year flood recurrence zone of Lake Saint-Pierre, which represents the most frequently inundated portion of the floodplain. While the broader floodplain extends to the 100-year recurrence zone as defined by Jobin and Brodeur (2023), our study focused on areas subject to regular seasonal flooding. Although our sampling design does not cover the entire floodplain in a spatially uniform manner, it was structured by blocks (regions), each containing the same land use gradient. This allowed us to test local land use effects within hydrologically and spatially coherent units. With the collaboration of local farmers, conventionally managed corn-soy fields were manipulated using improved practices. The improved cropland practices consisted of combinations of cover cropping and perennial buffer strips along fields. Corn-soy fields were also sown with grasses/fodder to revert to a less intensive form of land use (perennial crops). Besides cropland, natural wetlands such as wet meadows and forested swamps were also sampled. The resulting land use gradient consists of the following land use categories, from annual crops with conventional management to natural habitat through improved systems and meadows established in large plots:

- Cropland—CM (Conventional Management). Annual crop of corn and soybean.
- Cropland—IM (Improved Management). Annual crops of corn and soybean are intercropped with ryegrass (*Lolium multiflorum*), winter wheat (*Triticum aestivum*), and legumes (*Lotus corniculatus*, *Trifolium repens*, *Vicia villosa*, and *Melilotus officinalis*). In cases where high flood years prevent planting an annual crop, bare soils are covered using a mixture of species. Perennial buffer strips of *Phalaris arundinacea* are also sown along ditches located at field margins.
- Temporary Meadows. Recently sown forage cropping.
- Permanent Meadows. Old forage cropping at least 5 years old
- Marsh. Wet meadows not used for agriculture. (*Phalaris arundinacea*, *Glyceria grandis*...)
- Forested Swamps. Silver maple (*Acer saccharinum*) and black ash (*Fraxinus nigra*) swamps

The study sites were sampled during spring flooding of 2019 (15 May–7 June) and in 2022 (27 April–5 May). Water samples for eDNA extraction and environmental parameters were taken at each site. In 2022, sites were sampled twice during flooding; we used the first sample (collected closest in time to electrofishing: 11 April–23 April) unless it failed to produce a sufficient number of usable sequences. In such cases, samples collected slightly later (May 10–17) were used instead. For a subsample of sites, fish were also sampled via electrofishing in both 2019 (30 April–7 May) and 2022 (11 April–23 April).

2.2 | Environmental Parameters

For the purpose of our study, we focus on turbidity and Normalized Difference Vegetation Index (NDVI) to characterize the different land use categories and confirm the existence of a gradient of habitat quality. Land use practices can strongly influence water turbidity and the amount of submerged vegetation in the lake, both of which have been shown to modulate fish species richness and abundance in LSP during the summer season (Giacomazzo et al. 2020; Giacomazzo et al. 2023). Turbidity was measured using a multiparameter probe (H198290, Hanna Instruments) carried aboard a double kayak. We then used NDVI during dry months as a proxy of submerged vegetation during flooding. NDVI is a commonly used metric to assess terrestrial vegetation health and density (Huang et al. 2021), measured from spectrometry data at two specific wavelengths: red and near-infrared (NIR), gathered by sensors on satellites. NDVI is calculated as follows:

$$\mathrm{NDVI} = (\mathrm{NIR} - \mathrm{Red}) \div (\mathrm{NIR} + \mathrm{Red})$$

NDVI correlates with vegetation conditions on the ground, with NDVI values ranging from −1 to 1. As vegetation density increases, so does the NDVI. We used NDVI values calculated by the European Union's Copernicus Land Monitoring Service information, which provides NDVI values every 10 days at 300-m resolution for our study area. For each site and sampling year, we calculated the mean NDVI from May to December of the year preceding sampling. Both turbidity and NDVI strongly

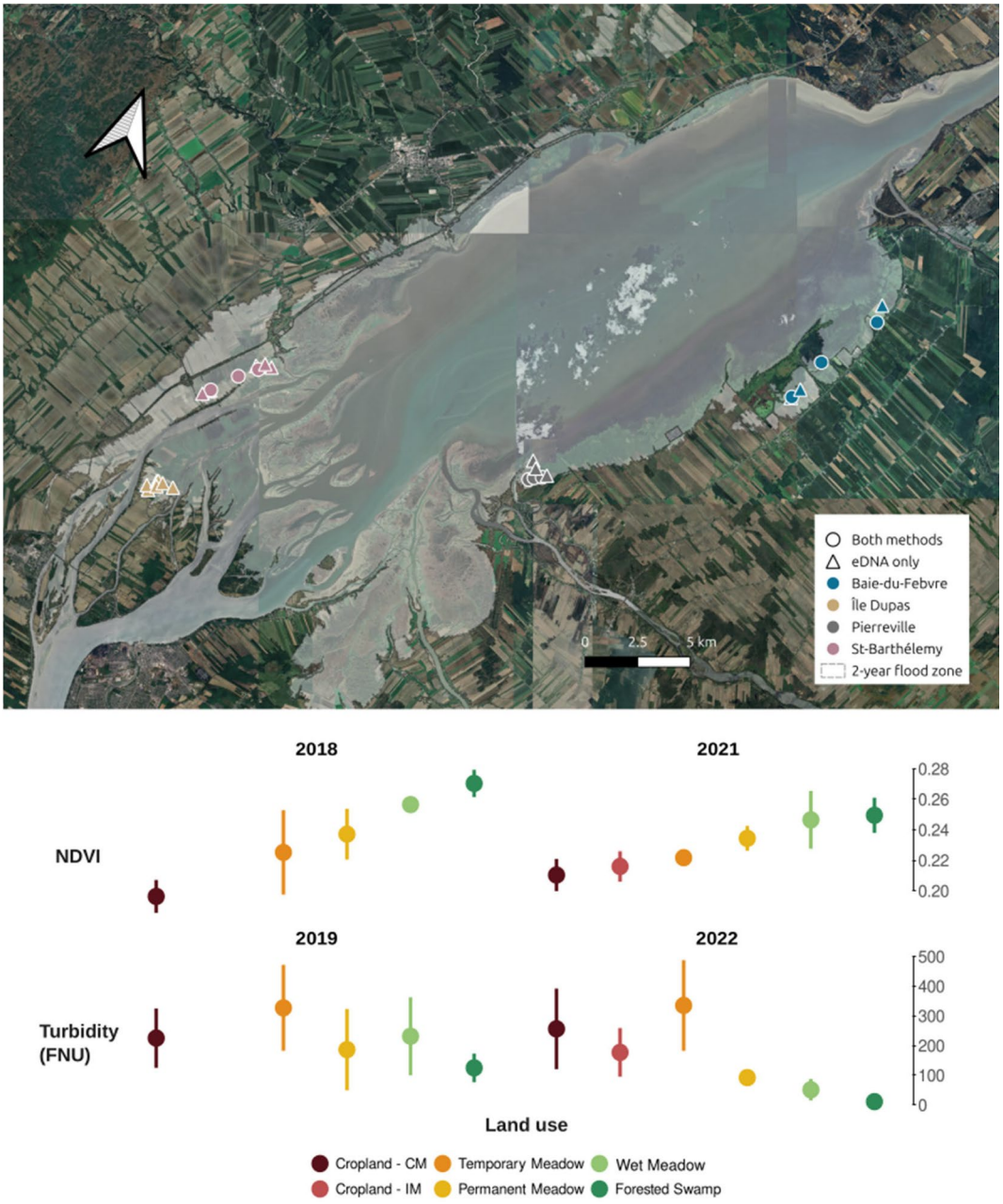

FIGURE 1 | Map of the LSP floodplain. Each symbol on the map represents a sampling location, with symbol color indicating the sector, and symbol shape specifying whether the location was sampled using eDNA only or both eDNA and electrofishing. The white overlay denotes the limits of the 2-year flood zone of LSP. Normalized Difference Vegetation Index (NDVI) and turbidity values for the various land use categories are displayed below the map, with symbols representing the means and their corresponding 95% confidence intervals.

varied between land use categories, with croplands having lower NDVI values and higher turbidity values than natural habitats (Figure 1).

We also estimated water level (a proxy for depth) at each site to account for flood timing and intensity. Water level was modeled using bathymetric and LIDAR data, which were combined to create a unified digital elevation model (DEM) for LSP and its floodplain. Daily water levels at the lake inlet were estimated using hydrometric data from a station of the National Hydrological Service of Canada located near Sorel, at the western end of the lake. Model accuracy was validated through field measurements and orthophotos taken by drones over flooded areas during the spring 2019 flood period (Campeau et al. 2024).

2.3 | Electrofishing

Fish communities were sampled by electrofishing during the flooded period of 2019 (30 April–7 May) and 2022 (11 April–23 April). At each site, fish were sampled by means of a Wisconsin-type electrofishing boat (Novotny and Priegel 1974) equipped with a Smith-Root 2.5 GPP shocker (Paquin and Brodeur 2021). The boat moved at a constant velocity for 20 min along a trajectory covering approximately 650 linear meters. Surfacing fish

were retrieved by two operators equipped with dip nets, one at the front of the electrofishing boat and the other in an auxiliary boat that trailed the electrofishing boat to retrieve fish missed by the first operator. Captured fish were identified to species and measured (maximum total length; ±1 mm). Sampling was carried out at water depths ranging from 0.5 to 2 m. Fishing focused on the three most easily accessible sectors of the floodplains (Pierreville, Saint-Barthélemy, and Baie-du-Febvre). Raw abundances were converted to relative abundances (site-specific proportions) to be consistent with eDNA analyses. Electrofishing was the only traditional method that could be consistently applied across our sites due to the dense flooded vegetation that precluded the use of nets. Our comparison thus reflects method performance under field-constrained conditions typical of many floodplain environments.

2.4 | eDNA Sample Collection, Extraction, and Sequencing

Water samples were collected in spring of 2019 (15 May–7 June) and 2022 (27 April–5 May). Samples of 1 L were taken at each site using sterile cubitainers, triple-washed with site water. Cubitainers were immersed directly from the side of the boat used to access sites. Water was filtered using two filters arranged in series (Whatman GF/D filters with a mesh size of 2.7 μm and Sterivex filters with a mesh size of 0.22 μm). Depending on the amount of organic matter in the water, filtered volumes varied between 100 mL and 500 mL. No significant correlations were found between 2022 samples filtration volume and either total read counts (Pearson's $r = 0.21$, $p = 0.31$) or species richness (Pearson's $r = 0.16$, $p = 0.44$). This methodology comprising two types of filters aimed to separate particles and organisms as filters were used for other genetic markers than the 12S analysis reported here. This approach differs from the more common use of 1.2 μm filters in fish-focused studies targeting the 12S marker, and may influence the composition of captured eDNA (Snyder et al. 2023). Although all filters were extracted, amplified, and sequenced separately, for the purpose of this analysis we combined the sequences collected by the two filters to obtain a single composite sample of all eDNA from a water sample. Prior to DNA extraction, Sterivex filters were recovered from their casing and cut into small pieces, following the protocol described by Cruaud et al. (2017). GFD filters were also cut into small pieces under sterile conditions. Filter pieces were then transferred to a PowerBead tube of the DNeasy PowerSoil Pro kit (QIAGEN, Netherlands) for DNA extraction following the manufacturer's instructions for QIACube instruments (QIAGEN). Extractions and amplifications were done at the Laurentides Forestry Center (CFL) of Natural Resources Canada. One laboratory blank negative control (i.e., PowerBead tube containing reagents only) was included in each extraction batch of 24 samples, but no field blank negative controls were included in the sampling design, again because we added a 12S analysis to a protocol that originally focused on microbial communities. Blanks were amplified and sequenced following the same procedure as samples. DNA concentration was assessed with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, MA, USA) using the dsDNA BR assay kit (Life Technologies, CA, USA). DNA extracts were diluted to a concentration of 4 ng/μL with 10 mM Tris pH 8.0 solution using the QIAgility automated system (QIAGEN) (no dilution applied if DNA concentration of the extract was lower than 4 ng/μL).

Library preparation for Illumina sequencing was conducted following the manufacturer's protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) with some modifications. The first PCR reaction was set up using primers targeting the fish 12S rRNA gene (MiFish-U primers: F: GTCGGTAAAACTCGTGCCAGC; R: CATAGTGGGGTATCTAATCCCAGTTTG) (Miya et al. 2015) attached to Illumina overhang adapters. PCR reactions were set up by mixing 25.0 μL of 2X HotStarTaq Plus Master Mix (QIAGEN, Germany), 0.5 μL of each 10 μM HPLC-purified primer (Invitrogen, USA), 16.5 μL of RNase-free water (QIAGEN), and 7.5 μL of DNA diluted at 4 ng/μL, for a total volume of 50 μL. PCR amplification was conducted using a touchdown thermocycling protocol (Pitz et al. 2020) with some modifications. The annealing temperature was set at 65°C and decreased by 1.5°C every cycle for 10 PCR cycles, and then 30 additional PCR cycles were performed at an annealing temperature of 50°C. The first 10 PCR cycles consisted of 30 s at 94°C, 30 s at the annealing temperature (65°C–1.5°C at each cycle), and 90 s at 72°C, whereas the 30 additional cycles consisted of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C. The touchdown thermocycling protocol was preceded by a 5 min denaturation step at 95°C and followed by a final elongation step of 10 min at 72°C. PCR products were visualized on an agarose gel. PCR products were purified using magnetic beads solution (Agencourt AMPure XP, Beckman Coulter Life Science, CA, USA); then, unique indexes were added to each sample using the Nextera XT Index Kit v2 following Illumina's protocols. After index PCR, the amplicons were purified again with magnetic beads, quantified using the Synergy Mx Microplate Reader (BioTek Instruments Inc., Winooski, VT, United States), and combined at equimolar concentration. Paired-end sequencing (MiSeq reagent kit V3, 2 × 300 bp) was performed on an Illumina MiSeq platform at the Next Generation Sequencing Platform of the CHU de Québec-Université Laval Research Centre.

2.5 | Bioinformatics

Sequences were processed with the Barque metabarcoding pipeline version 1.7.4 (https://github.com/enormandeau/barque), which includes steps for sequence filtration, trimming, dereplication, and annotation (Mathon et al. 2021). The raw sequences were trimmed using trimmomatic v0.36 for quality (options: LEADING =20, TRAILING =20, SLIDINGWINDOW = 20:20, MINLEN =100, and CROP =200). Trimmed reads were then merged using flash v. 1.2.11 (options: −t = 1, −z, −m = 30, −M = 280). Only merged reads possessing both forward and reverse amplicon primer sequences were kept using the 03_split_amplicons.sh script from Barque 1.7.4 (https://github.com/enormandeau/barque). Chimeric reads were removed with vsearch v. 2.15.2 (options: –uchime_denovo and –nonchimeras). Remaining reads were aligned using a 97% minimum similarity threshold on the database available in Barque compiled from the MitoFish database (Iwasaki et al. 2013), the GENBank database (Benson et al. 2013), and the Barcode of Life database (Ratnasingham et al. 2024). Sequences ambiguously identified

due to high similarity were further analyzed using a detailed list of species documented in Québec. Ambiguous classification involving taxa not previously documented in the region were assigned to species reported in Québec when possible. In the case of ambiguous classification involving multiple species reported in Québec, the sequences were not assigned to the species level (Cyprinidae with no genus or species assignments) and were subsequently excluded during the bioinformatics pipeline. All remaining species-level assignments corresponded to taxa previously documented in Québec. Species with sequence counts representing less than 0.001% of a sample's total reads were removed to adequately eliminate false positives (García-Machado et al. 2023). Sequences that aligned with non-fish species were discarded from the results, as were sequences that were assigned to fish but not to species level (less than 0.1% of total sequences). After bioinformatics processing, sampling sites containing less than 1000 reads assigning to fish species were replaced with samples collected later in the spring (5 samples collected from 10 May to 17 May) or discarded if no suitable samples were available. We found no significant difference in fish community composition collected by eDNA between the two sampling periods (perMANOVA comparing April 27–May 5 to May 10–17 samples; p value = 0.36). After completing all bioinformatics data processing steps, the total number of sequences assigned to fish species was 1,061,890 in field samples and 21 in blank samples. Given the low number of reads in the blank samples, ASVs were retained in the dataset. The site by species matrix was converted to relative abundances by dividing the number of reads of each species in a sample by the total read number for that sample.

2.6 | Data Analysis

2.6.1 | Differences Between Sampling Methods

Species list and the relative abundances across sites are presented (see Figure 2). To compare fish community composition sampled by electrofishing and eDNA metabarcoding, only sites sampled within a short time interval with the two sampling methods were retained, resulting in a total of 19 samples (total number of samples: eDNA $n = 47$, electrofishing $n = 34$, both = 19). The species list and relative abundances for the subset of 19 sites with matching data are provided in Figure S1. We first compared the correlations between community indices calculated from both datasets, using either all available species or only shared species detected by both methods, and considering different dimensions of diversity. Alpha diversity was calculated as the expected number of species, the exponent of the Shannon index (Hill 1973). Beta diversity at the site level (assemblage unicity) was estimated with the "Local contribution to beta diversity" (LCBD) index (Legendre and De Cáceres 2013). Phylogenetic diversity was calculated using the standardized effect size of Faith's phylogenetic diversity (Webb et al. 2002). All data analyses were performed in the R software version 4.3.3 (R Core Team 2021). Alpha diversity was calculated using the package "vegan" version 2.6 (Oksanen et al. 2024), beta diversity using the package "adespatial" version 0.3 (Dray et al. 2024), and phylogenetic diversity using the package "picante" version 1.8.2 (Kembel et al. 2010). Differences in fish community composition were then assessed using Principal Coordinate Analysis (PCoA). The PCoA was performed using the Gower distance on square root-transformed relative abundances. The Gower distance was chosen because the electrofishing dataset contained sampling locations with no fish occurrences, "true zeros", that the Gower distance can accommodate (Gower 1971). The R package "ape" version 5.8 (Paradis and Schliep 2019) was used for PCoA. For readability, species names are noted as codes on ordination plots (see Figure 1 and Table S1 for full species list). We also performed a permutational multivariate ANOVA (perMANOVA) with 9999 permutations on the Gower distance matrix to test for differences in community composition between sampling methods using the adonis function in the R package "vegan" version 2.6 (Oksanen et al. 2024).

2.6.2 | Fish Community Sampled by eDNA Metabarcoding

We used the full eDNA dataset ($n = 47$ samples from 29 sites) to assess how fish communities are structured by land use categories, LSP sectors, and linear environmental variables associated with land use (NDVI and turbity). For diversity analyses, we used the same calculations for alpha, beta, and phylogenetic diversity as above for comparing eDNA metabarcoding and electrofishing datasets. We used generalized linear mixed models (GLMMs) to investigate the relationships between diversity metrics and land use (a fixed effect and a factor), with "site" nested within "sector" as random effects to account for the non-independence of sites within the same sector (formula in R language: diversity metric ~ land use + (1|Sector/Site)). We used a gamma distribution (link = log) for both alpha and beta diversity, and a Gaussian distribution for phylogenetic diversity. Furthermore, we tested for an effect of land use by comparing the goodness of fit of each model to a null model (formula = Metric ~1 + (1|Sector/Site)) using a likelihood ratio test. The R package "glmmTMB" version 1.19 (Brooks et al. 2017) was used to fit GLMMs.

The relationships between fish community composition sampled by eDNA and environmental variables—including categorical factors (land use categories and LSP sectors) and linear variables (NDVI and turbidity)—were analyzed using PCoA, perMANOVA, discriminant analysis, and distance-based redundancy analysis (db-RDA). PCoA's computation used a square root-transformed relative abundance matrix (i.e., Hellinger transformation which is recommended for eDNA data (Laporte et al. 2021)) and a Euclidean distance matrix (i.e., 'Hellinger distance'). We tested the effects of LSP sectors and land use categories using perMANOVA with 9999 permutations. When assessing the effect of land use categories, permutations were stratified by sectors to account for a priori identified differences across sectors. Discriminant analysis was used to classify sites into land uses or sectors, performing 999 permutations with the function CAPdiscrim of the R package "BiodiversityR" version 2.16 (Kindt and Coe 2005). The parameter m = 0 was used, allowing the number of PCoA axes evaluated by the discriminant analysis to be optimized for maximum separation among factor levels. Finally, we used a db-RDA to explore the relationships between community composition, LSP sectors, and environmental variables associated with land use (turbidity and NDVI), while controlling for differences in water levels between sampling locations. To understand the portion of variance explained by the sector

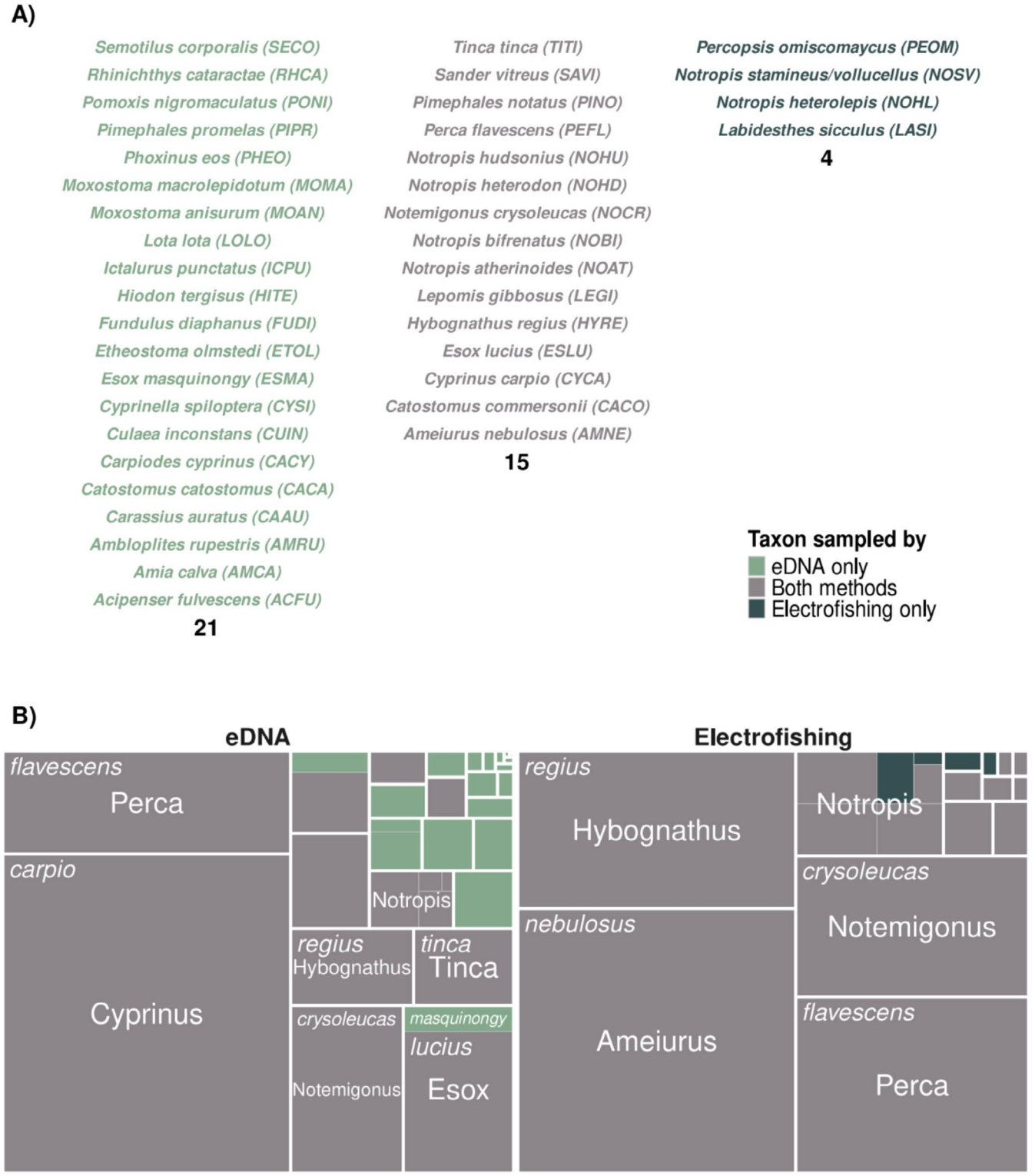

FIGURE 2 | Fish species sampled by eDNA metabarcoding and electrofishing. (A) Species list across all samples (eDNA $n = 47$, electrofishing $n = 34$) based on survey methods. Fish species sampled exclusively by eDNA metabarcoding (in green), sampled by both methods (in gray), and sampled exclusively by electrofishing (in blue). (B) Treemap displays of each species in either dataset (eDNA vs. electrofishing), with the size of the squares representing relative abundance.

variable compared to the environmental variables (e.g., water level, turbidity, and NDVI), we performed a variance partitioning using the "varpart" function and tested the statistical significance of the explained portions of variation using the "anova.cca" function in the "vegan" R package version 2.6 (Oksanen et al. 2024). We also performed the same db-RDA and variance partitioning with the electrofishing count data to verify that our inferences about the effects of land use and sectors were similar with both sampling methods. All community composition analyses were repeated using a

presence-absence matrix, which yielded similar conclusions to those derived from relative abundance data.

3 | Results

3.1 | Differences Between Sampling Methods

Electrofishing and eDNA metabarcoding found different species lists when considering all sites. Electrofishing found a total of 19 fish species, with four found uniquely with this method (Figure 2A). These four species include *Notropis heterolepis*, which was also found with metabarcoding, but since only 3 sequences were assigned to this species, it was removed during bioinformatic data cleaning. eDNA metabarcoding found a total of 36 fish species (after data filtration), with 21 found uniquely with this method (Figure 2A). Out of the 40 total fish species sampled by the two methods, 15 (37.5%) were found by both methods (Figure 2A). Dominant species in terms of relative abundance sampled by electrofishing were *Hybognathus regius*, *Ameiurus nebulosus*, *Perca flavescens*, and *Notemigonus crysoleucas* (Figure 2B). In contrast, dominant species sampled by eDNA metabarcoding were *Perca flavescens*, *Cyprinus carpio*, *Hybognatus regius*, and *Esox lucius* (Figure 2B). Although the dominant species differed in part between the two methods, the most abundant species (the largest squares in Figure 2B) were always detected with both methods (Figure 2B).

Alpha diversity, beta diversity, and phylogenetic diversity showed low correlations between the two methods with 0.27, 0.05, and −0.16, respectively, when considering all species (Figure 3A), and 0.23, 0.04, and 0.04, respectively, when considering only shared species between the two methods (Figure 3A). The first two PCoA axes represented 56.3% of the total variance in fish community composition (Figure 3B). Fish community composition sampled by the two methods overlapped on the first two PCoA axes (Figure 3B), but significant differences are highlighted by the perMANOVA using "method" as a predictor of composition (p value < 1e-04, $R^2 = 0.097$).

3.2 | Fish Community Sampled by eDNA Metabarcoding

We found no relationship between fish diversity metrics (alpha, beta, and phylogenetic diversity) and land use categories (Figure 4, Table 1). Beta diversity varied between the different LSP sectors, with Baie-du-Febvre having a higher and St-Barthélemy a lower beta diversity relative to the Île Dupas and Pierreville sectors (Figure 4D, Table S2). The PCoA, perMANOVA, and discriminant analyses all showed that fish community composition varied between the LSP sectors. The first two axes of PCoA (Figure 5A) represented 42.9% of the total variance in fish community composition. The perMANOVA testing for differences in fish community composition between sectors had a p value of 1e-04 and an R^2 of 20.5% (Table 2). The discriminant analysis classifying sites in the different LSP sectors based on their community composition had a p value of 0.002 and an overall classification performance of 63.8% (Figure 5A). The best classified sector was St-Barthélemy with 76.5% of sites successfully classified, and the worst classified sector was Baie-du-Febvre with 50% (Figure 5A). None of the multivariate analyses showed important variation in fish community composition among land use categories; the PCoA showed important overlap between land use categories (Figure 5B), the perMANOVA found no significant effect of land use (p value = 0.325; Table 2), and the discriminant analysis classifying sites into the different land use categories performed rather poorly (p value = 0.321 and overall classification performance = 34%; Figure 5B). All sites were classified into the Cropland–CM category, the most common land use class in the dataset (Figure 5B). Finally, an db-RDA relating fish community composition to environmental variables explained 20.9% of the total variance in fish community composition with its first two axes (p value for db-RDA1 axis = 0.036 and db-RDA2 = 0.816; Figure 5C). LSP sectors and water level were most strongly related to the first db-RDA axis explaining the most variance (Figure 5C). Finally, variance partitioning showed that LSP sectors, water level, turbidity, and NDVI explained 17.6% of the variance in fish community composition, with 2.6% attributable to environmental parameters (alone p value = 0.066, alone +shared p value = 0.006) and 11.3% to LSP sectors (alone p value = 0.001, alone + shared p value = 0.001, Figure 5D).

4 | Discussion

Electrofishing and eDNA metabarcoding sampling yielded different species lists and identified distinct dominant species (in terms of ranked abundance) in the fish community of the LSP floodplain. Nevertheless, abundant species were detected by both methods. Community datasets obtained with the two sampling methods showed no correlation in diversity indices and exhibited different community compositions. Overall, fish community composition and beta diversity differed among LSP sectors, whereas alpha and phylogenetic diversity did not. Land use within LSP sectors did not have a detectable effect on fish diversity or community composition.

4.1 | Differences Between Sampling Methods

Our findings indicate that electrofishing estimated a lower overall fish diversity than eDNA metabarcoding in the LSP floodplain (Figure 2). The inability of electrofishing to detect certain species may stem from low abundance or patchy distribution (Van Driessche et al. 2024). Abundant species were consistently detected by both sampling methods (Figure 2B), supporting the idea that species missed by electrofishing might be attributable to low species abundance or sampling bias. Our analysis suggests that metabarcoding reliably captures a broader range of species richness compared to electrofishing in a floodplain ecosystem, and species accumulation shows that this pattern isn't solely attributable to differences in sampling effort (Figure S2). Previous studies have demonstrated that eDNA often outperforms traditional methods in direct comparisons of species detection, especially for rare and hard-to-detect species (Fediajevaite et al. 2021; Gehri et al. 2021; Keck et al. 2022). Furthermore, numerous factors can influence estimates derived from conventional sampling methods such as electrofishing (Andres et al. 2023). Electrofishing efficiency is influenced by sampling area dimensions (Pottier et al. 2020), turbidity (Lyon

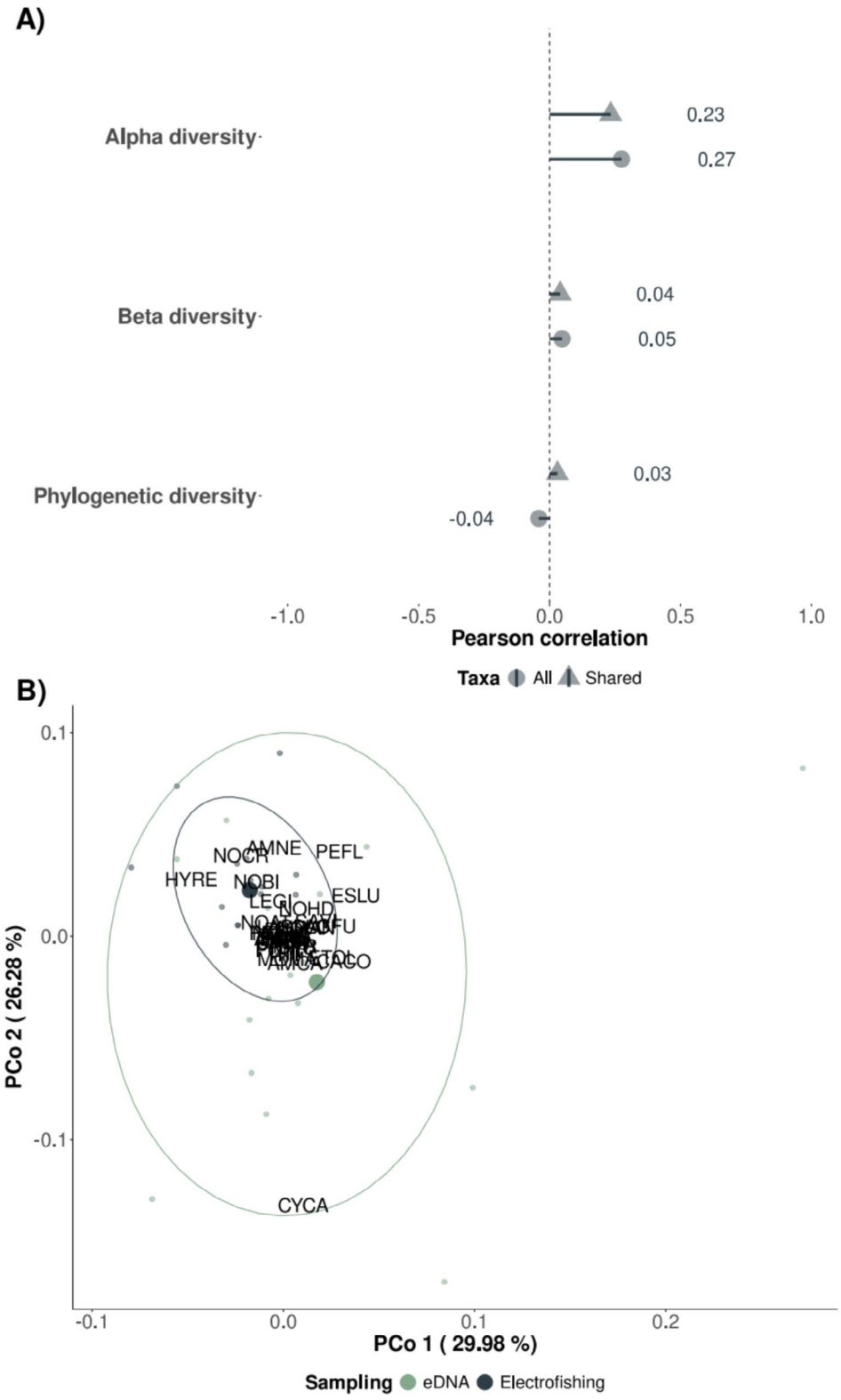

FIGURE 3 | Fish community diversity and composition estimated with eDNA metabarcoding and electrofishing. (A) Correlation coefficients between the two sampling methods for various diversity indices (Alpha diversity = exp(Shanon), Beta diversity = LCBD, and Phylogenetic diversity = standardized effect size of Faith's phylogenetic diversity). The triangles represent the correlations calculated using all available taxa, whereas correlations were calculated using only shared taxa sampled by the two methods are represented by circles. (B) Community composition of the sites represented with a PCoA. Small circles represent individual sampling sites, whereas larger circles denote group means. Lines indicate 95% confidence interval ellipses. Green symbols correspond to eDNA metabarcoding samples, and blue symbols correspond to electrofishing samples.

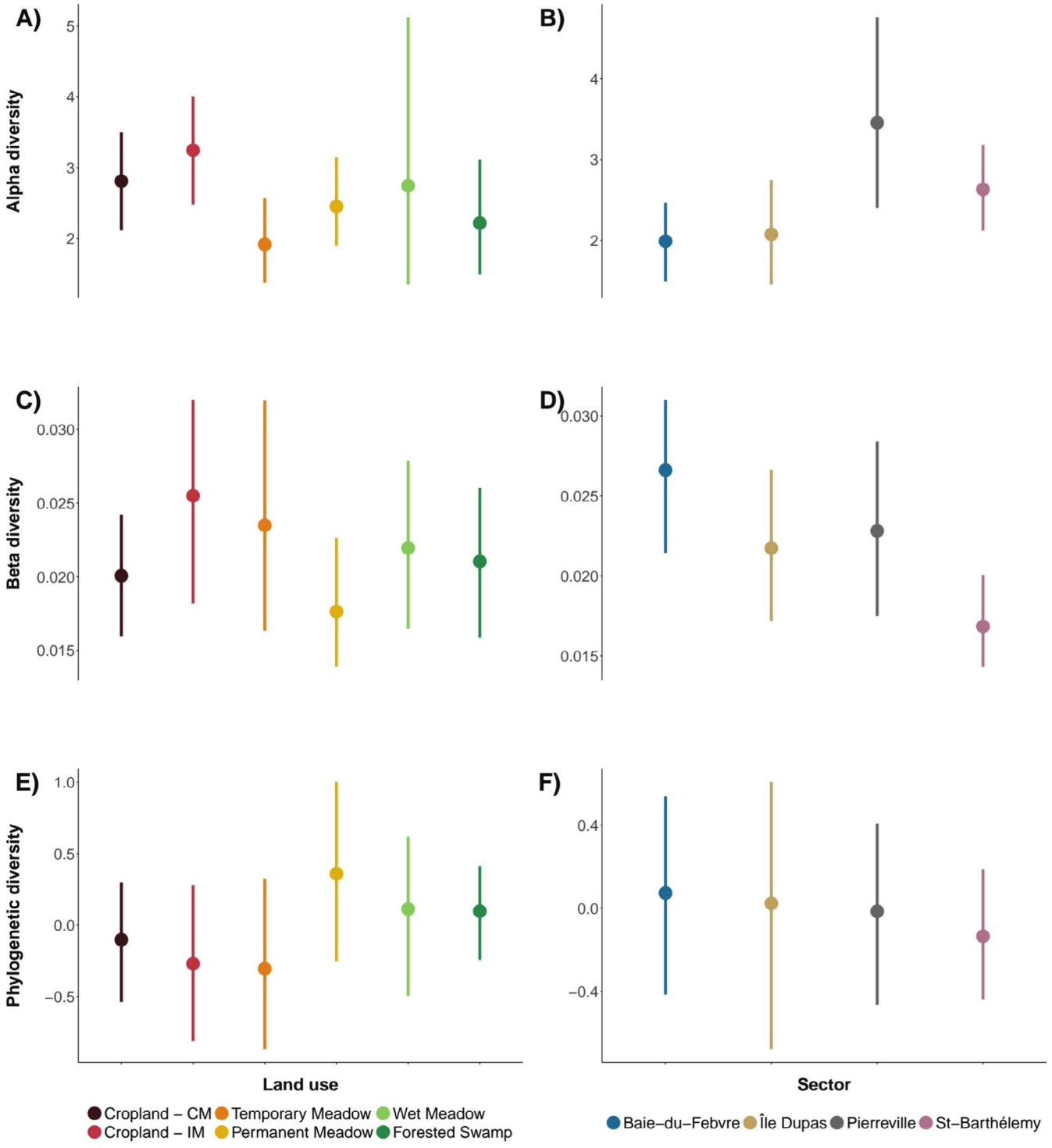

FIGURE 4 | Fish community diversity sampled by eDNA metabarcoding. Panels A, C, and E represent diversity indices (Alpha diversity = exp(Shanon), Beta diversity = LCBD, and Phylogenetic diversity = standardized effect size of Faith's phylogenetic diversity) for the different land use categories. Panels B, D, and F represent diversity indices for the sectors of LSP. Symbols represent means and 95% confidence intervals.

et al. 2014), as well as fish size, morphology, and behavior (Dolan and Miranda 2003).

In contrast, eDNA metabarcoding failed to detect certain species present in the reference database (*Percopsis omiscomaycus*, *Notropis heterolepis*, and *Labidesthes sicculus*), possibly due to primer biases or insufficient concentration for successful extraction. Primer bias could be mitigated by incorporating additional primers or sequencing mock communities (Boivin-Delisle et al. 2021; Van Driessche et al. 2024). Primer bias is not the only limitation of eDNA metabarcoding; the production and degradation of eDNA are influenced by several environmental factors (Barnes and Turner 2016). Environmental conditions, such as pH and temperature, can significantly affect the rate of eDNA production and degradation, thereby influencing the measured concentrations (Strickler et al. 2015; Jo et al. 2019;

TABLE 1 | Likelihood ratio tests for generalized mixed models testing the effect of land use on community metrics. Alpha diversity (exp(Shanon)) is referred to as alpha, beta diversity (LCBD) as beta, and phylogenetic diversity (standardized effect size of Faith's phylogenetic diversity) as phy.

Models	Formula	Df	Chisq	Chi Df	*p*
Null alpha	Alpha ~1 + (1|sector/site)	4			
Alpha	Alpha ~ land use + (1|sector/site)	9	3.447	5	0.632
Null beta	Beta ~1 + (1|sector/site)	4			
Beta	Beta ~ land use + (1|sector/site)	9	4.139	5	0.53
Null phy	Phy ~1 + (1|sector/site)	4			
Phy	Phy ~ land use + (1|sector/site)	9	4.433	5	0.489

Caza-Allard et al. 2022). These variations can occur within a single freshwater habitat and are particularly pronounced in large ecosystems like the St. Lawrence River (Bernos et al. 2023), leading to spatial variability in eDNA dynamics across the habitat that could partly account for differences across sectors. Moreover, lotic systems are known to transport and homogenize eDNA particles (Thalinger et al. 2021; Wood et al. 2021). These processes might play a significant role in hydrologically dynamic environments such as the LSP floodplain, which receives inputs from distinct water masses of the St. Lawrence River (Caza-Allard et al. 2022) and several other river systems (Richelieu, Saint-François, Yamaska, Nicolet, and l'Assomption rivers) (Clermont et al. 2023; Campeau et al. 2024). Yet, several studies have shown that eDNA metabarcoding can detect fine-scale variation in fish communities in large river systems (Berger et al. 2020; Cantera et al. 2022; Laporte et al. 2022). Inherent limitations of both sampling methods contribute to discrepancies in species detection and can also influence observed abundances, further differentiating the fish community compositions identified by eDNA and electrofishing.

Fish communities sampled using eDNA and electrofishing yielded different species lists, but both approaches consistently detected abundant species. Comparing communities using only species sampled by the two methods revealed significant differences (perMANOVA p value = 4e-04), indicating that differences in their relative abundance contributed to community composition disparities and further distinguished the two sampling methods. While efforts were made to align electrofishing and eDNA sampling within the same spring flood event, we acknowledge that there was some delay between sample collection. This may lower congruence between methods, as fish community composition in the floodplain is known to change rapidly during the flood pulse (Mingelbier et al. 2005). Among these abundant species, *Hybognathus regius* and *Ameiurus nebulosus* were both found in higher relative abundance through electrofishing. The small size of *Hybognathus regius* may limit the amount of eDNA in the sampled water compared to electrofishing estimates. We observed that eDNA metabarcoding found higher relative abundance for larger fish species compared to electrofishing (Figure S3). Given that fish size/body mass is positively correlated with eDNA production, this supports the notion that eDNA techniques may be more suitable for estimating fish biomass rather than density (Maruyama et al. 2014; Yates et al. 2021). Additionally, benthic species like *Ameiurus nebulosus* are particularly susceptible to electrical currents due to their highly sensitive electroreceptor organs (Peters et al. 2007), which could explain their greater prevalence in electrofishing samples. *Cyprinus carpio* and *Perca flavescens* were found in higher relative abundance through eDNA sampling. Visual observations confirm that *Cyprinus carpio* is abundant in the St-Barthélemy sector of the LSP floodplain, where large individuals could be seen near the surface when collecting water samples. However, electrofishing detected only a limited number of specimens in this sector and others. The discrepancy between eDNA and visual observations and electrofishing may be attributed to the avoidance behavior of *Cyprinus carpio*, a species with highly developed sensory capabilities (Kim and Mandrak 2017; Piczak et al. 2023). Our findings align with a previous study in the upper St. Lawrence River, which also suggests that eDNA provides better detection of *Cyprinus carpio* populations than traditional sampling methods (Maracle et al. 2024). However, the timing of *Cyprinus carpio* migration—typically in May—more closely aligned with the eDNA sampling period and may have contributed to the observed detection patterns for this species. The higher relative abundance of *Perca flavescens* detected through eDNA monitoring may be linked to their spawning activity. *Perca flavescens* typically spawn in early spring, shortly after the ice cover melts (Whiteside et al. 1985), and the LSP floodplain is a key spawning habitat for this species that used to be highly abundant in the system (Mailhot et al. 2015). The spawning event and subsequent hatching likely increased eDNA concentrations in the environment due to the release of gametes and the presence of larvae, which are not included in our electrofishing dataset. Recognizing the inherent biases within complementary methods allows for more accurate assessments of communities.

4.2 | Fish Community Sampled by eDNA Metabarcoding

Our results demonstrate that eDNA monitoring in the LSP floodplain effectively detected differences in fish community diversity and composition across sectors. Beta diversity patterns revealed that shallower sampling units support more distinct fish communities (Table S3). During flooding, these shallower habitats are located farther from the permanently aquatic areas of the lake before the spring flood, which may reduce the likelihood of fish accessing them directly. Additionally, we cannot rule out the potential influence of eDNA transported upstream from tributaries entering the floodplain, which may contribute to the observed signal. While we have not directly quantified eDNA transport, we found no obvious association

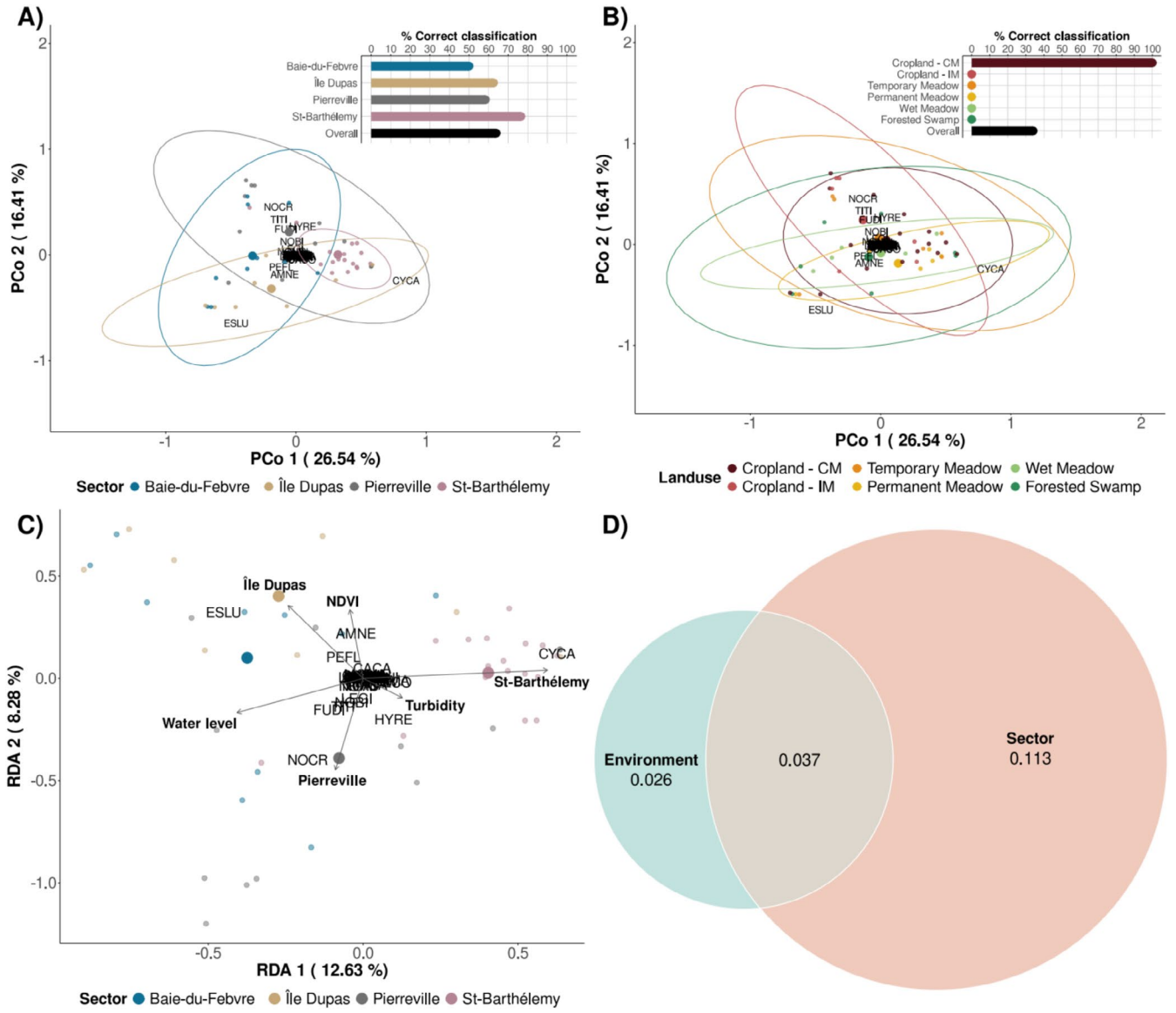

FIGURE 5 | Drivers of fish community composition sampled by eDNA metabarcoding. (A, B) Fish community composition visualized with PCoA. Small circles represent sampling site, whereas larger circles represent group means. Lines represent 95% confidence interval ellipses. Colors represent either LSP sectors (A) or land use categories (B). The two panels also depict percent of correct classification of discriminant analysis for each category. (C) The first two axes of the db-RDA relating LSP sectors, NDVI, turbidity, and water level (variables included in the model are represented with arrows pointing to bold text) with fish community composition. Small circles represent sampling sites, whereas larger circles indicate group means (sectors). Colors indicate LSP sectors. (D) Results of the variance partitioning analysis. The size of the circles is proportional to the amount of variance explained by the group of variables. Environmental variables (in blue) include NDVI, turbidity, and water level.

between the diversity of water sources flooding a sector (as reported in Campeau et al. 2024) and the resulting variance in community composition and diversity across sites within a sector. For example, DUPA is flooded by a single water source (the Saint-Lawrence River), yet shows as much variability as sectors flooded by diverse inputs (BAIE and PIER). BART sites are flooded by both the Saint-Lawrence River and a number of small streams, yet this sector is the least variable. Therefore, while it is impossible to conclude with certainty, it is unlikely that eDNA transport from upstream sources has a significant influence on our estimates of fish community composition in this dataset. Previous studies identified compositional shifts in the littoral fish community during late summer between the LSP and its archipelago using both traditional methods and eDNA metabarcoding (Foubert et al. 2018; García-Machado et al. 2022). The St-Barthélemy and Île Dupas sectors are found in the archipelagos of the LSP. Using pairwise perMANOVA (Table S4), we identified the St-Barthélemy sector as the primary driver of compositional differences among the LSP floodplain sectors. To our knowledge, this is the first study to document a shift in fish community diversity and composition across sectors of the LSP floodplain. Our findings emphasize the utility of eDNA metabarcoding as an effective tool for monitoring broad-scale patterns in fish communities within large river floodplain ecosystems.

Capturing more localized changes in diversity and composition due to, for example, local conditions of a specific flooded field,

TABLE 2 | Results of perMANOVA testing for the effects of LSP sectors and land use on fish community composition.

	Df	SS	R^2	F	p
Formula: Distance matrix ~ sector					
Sector	3	6.09	0.21	3.7	<0.0001
Residuals	43	23.57	0.8		
Total	46	29.66	1		
Formula: Distance matrix ~ land use (with permutation blocks based on sector)					
Land use	5	3.18	0.11	0.99	0.33
Residuals	41	26.48	0.89		
Total	46	29.66	1		

could prove challenging in highly dynamic environments such as floodplains. Indeed, we found no significant effect of land use or associated environmental variables on fish community diversity or composition. However, we observed a weak effect of arable land (as defined by the UN Food and Agricultural Organization (FAO), which in our study represent the land use categories: cropland-CM, cropland-IM, and temporary meadows) in shifting community composition (perMANOVA p value = 0.063, Table S5) and in reducing phylogenetic diversity (likelihood ratio test p value = 0.051, Table S6). Land use is a well-documented driver of fish diversity in freshwater ecosystems (Chen and Olden 2020; Camana et al. 2024). Land use has also been shown to influence fish diversity and composition in some floodplain ecosystems (Arantes et al. 2018; Arantes et al. 2019; Carlson Mazur et al. 2022). The absence of land use effects in our study could result from methodological limitations, the presence of weak effects relative to other drivers, or a combination of both. A greater number of samples along the land use gradient may be more appropriate for detecting potential effects. Currents and diffusion could have homogenized eDNA across the land use gradient within sectors of the LSP floodplain. In lotic environments, eDNA can be transported over long distances, with the source organism potentially located several kilometers upstream from the detection point (Laporte et al. 2020; Thalinger et al. 2021; Van Driessche et al. 2024). Such long-distance transport is unlikely to significantly impact community composition estimates across LSP sectors. However, as noted above, regarding within-sector differences, we cannot rule out the possibility that eDNA transport from upstream sources and the adjacent field could impact our ability to detect differences in community composition between neighboring sites with different land uses. In addition, recent work has shown that fish communities in LSP exhibit spatial dependence over distances of up to ~2.5 km (Campeau et al. 2024). Our sampling design aimed to capture a range of habitat and land use conditions within sectors of the LSP, but did not address spatial autocorrelation directly, as sampling sites were relatively few and clustered in space. Future studies could benefit from incorporating approaches to account for spatial dependence more explicitly. One limitation of our study is the absence of field blank controls during sample collection. While strict laboratory protocols and a conservative bioinformatic filtering strategy were used, the lack of field blanks prevents us from fully ruling out contamination introduced during sampling or transport. Such contamination, if present, could lead to the false detection of taxa, thereby inflating estimates of species richness or introducing artificial similarity between sites. Future studies should include field blanks to ensure robust community estimates and minimize the risk of false positives in biodiversity assessments. While both electrofishing (Figure S4) and eDNA sampling suggest relatively weak effects of land use in the system compared to LSP sectors, we recognize that recent findings suggest a more nuanced picture. Indeed, recent studies in the LSP showed that land use and vegetation structure influence fish communities and reproduction, with more natural and vegetated floodplain habitats supporting higher diversity and egg abundance (Campeau et al. 2024; Paquin et al. 2024). These results might stem from: (1) the broader electrofishing dataset reported in Campeau et al. 2024, allowing the authors to distinguish the effects of corn versus soy fields (combined in this study); (2) possible life-stage-specific associations with habitat types that may not be fully captured by eDNA metabarcoding (e.g., eggs and larvae missed by eDNA); or (3) eDNA transport or the movement of adult and juvenile fish during the flood season which may contribute to homogenization and reduce the spatial resolution of the eDNA signal. Other previous studies also found effects of catchment forest cover on fish community diversity and composition in floodplains (Arantes et al. 2018; Arantes et al. 2019; Huo et al. 2023), but these studies were conducted in tropical and subtropical regions, which support highly diverse fish communities in terms of taxonomy and functional traits. Moreover, these studies have examined land use effects primarily at the catchment scale, whereas our study specifically contrasts communities between adjacent agricultural and natural floodplain areas—a localized land-use comparison approach still rare in eDNA metabarcoding applications (but see Erős et al. (2024) for an analysis across multiple floodplain water bodies). Detecting land use effects on fish communities in hydrologically complex systems such as floodplains may require more refined spatial and temporal sampling, regardless of the method used.

Understanding the factors that influence fish community diversity and composition is crucial for the effective management and conservation of threatened floodplain ecosystems. In this study, we demonstrate that eDNA metabarcoding effectively captures broad-scale patterns in fish community composition within these highly dynamic environments. As such, this tool seems appropriate for monitoring temporal biodiversity trends in heterogeneous floodplains with spatially structured community composition. Several studies have reported high spatial resolution of eDNA metabarcoding for monitoring fish communities in large rivers, including the Saint-Lawrence (Berger et al. 2020; Laporte et al. 2022). During flood events, this fine-scale signal may be obscured due to increased water volume and mixing from multiple sources. We do not have direct evidence of such mixing; however, future work should assess the relative importance of DNA transported from upstream and lateral sources in the particularly complex hydrological dynamics characteristic of floodplain environments. As floodplains worldwide face increasing threats from deforestation, agriculture, urbanization, hydropower development, and mining, eDNA metabarcoding can complement traditional sampling methods to monitor these valuable habitats.

Author Contributions

V.F., F.G., M.R., V.M., and G.C. conceptualized the study. All authors contributed to data acquisition (field and/or lab work). L.A. performed data analyses with input from V.F. and M.R. L.A. drafted the manuscript, with all other authors providing feedback and revisions.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequences (fastq format), electrofishing and environmental data along with associated metadata will be deposited in the Dryad data repository DOI:10.5061/dryad.3bk3j9kzf. Scripts used for bioinformatics and data analysis are available upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Detected fish species taxonomic classifications, codes, common names, and detection method for each fish species in the dataset. **Table S2:** Likelihood ratio tests for generalized mixed models, testing the effect of LSP sectors on community beta diversity. Beta diversity (LCBD) is referred to as beta. **Table S3:** Likelihood ratio tests for generalized mixed models, testing the effect of water level on community beta diversity. Beta diversity (LCBD) is referred to as beta. **Table S4:** Comparison of sector fish community composition using pairwise perMANOVA using 9999 permutations on Euclidean distance matrix of squared relative abundances. **Table S5:**. Results of perMANOVA testing for the effects of arable land (grouping of land use categories, arable land: cropland—CM, cropland—IM, and temporary meadows; non-arable land: permanent meadows, marsh, and forested swamp) on fish community composition. **Table S6:** Likelihood ratio tests for generalized mixed models, testing the effect of arable land (grouping of land use categories, arable land: cropland—CM, cropland—IM, and temporary meadows; non-arable land: permanent meadows, marsh, and forested swamp) on community phylogenetic diversity. Phylogenetic diversity (standardized effect size of Faith's phylogenetic diversity) is referred to as phy. **Figure S1:** Fish species sampled by eDNA metabarcoding and electrofishing. (A) Species list across matching samples (eDNA $n = 19$, electrofishing $n = 19$) based on survey methods. Fish species sampled exclusively by eDNA metabarcoding (in green), sampled by both methods (in gray), and sampled exclusively by electrofishing (in blue). (B) Treemap displays of each species in either dataset (eDNA vs. electrofishing), with the size of the squares representing relative abundance. **Figure S2:** Species accumulation curves. Relationship between sampling effort (number of sites) and species richness detected by each method is shown. Curves are presented separately for eDNA and electrofishing. Shaded areas represent the standard deviation around the mean richness at each level of effort. **Figure S3:** Fish species maximum length. Each species sampled by both methods was classified as more abundant in either sampling method by comparing their mean relative abundance. Small dots represent the maximum length of species, whereas larger dots represent group mean maximum length and lines represent 95% confidence intervals. Fish species maximum sizes were extracted from Su et al. 2021. **Figure S4:** Drivers of fish community composition sampled by electrofishing. (A) The first two axes of the db-RDA relating LSP sectors, NDVI, turbidity, and water level (variables included in the model are represented with arrows pointing to bold text) with fish community composition. Small dots represent sampling sites, whereas larger dots indicate group centroids (sectors). Colors indicate LSP sectors. (B) Results of the variance partitioning analysis. The size of the circles are proportional to the amount of variance explained by the groups of variables. Environmental variables (in blue) include NDVI, turbidity, and water level.