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eDNA Is a Useful Environmental Monitoring Tool for Assessing Stream Ecological Health

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ABSTRACT

Environmental DNA (eDNA) is increasingly used in biodiversity assessments, but there remain uncertainties regarding its congruence with data based on traditional approaches involving habitat sampling and morphological-based taxonomy. Using eDNA for biomonitoring has several advantages, including improved processing efficiencies and precision of taxonomic identification. In contrast, traditional biomonitoring is time-consuming and expensive, often limiting the number of sites monitored. Establishing that eDNA-derived metrics are congruent with their traditional equivalents on a national scale would support its wider use in biomonitoring. Our study compared ecosystem health assessments made by traditional biomonitoring techniques to those using eDNA from 53 sites throughout Aotearoa New Zealand. Because eDNA sampling was not done concurrently with benthic sampling at most sites, we used the average community composition at each site based on previous sampling occasions. We also allocated species identified by eDNA to the traditional level of identification to allow comparisons with eDNA data identified to broader taxonomic groups. We assessed similarities between the three datasets and found a high degree of correlation and convergence between biotic indices calculated from the different methods. eDNA did, however, appear to under-represent some taxa, reflecting challenges in matching barcodes with an often-incomplete sequence library. eDNA data did not always perform better in terms of showing the effects of land use on invertebrate community composition, but all datasets produced similar patterns. Multivariate analyses (redundancy analysis and variation partitioning) identified congruent relationships between environmental and spatial variables with the invertebrate community structure described by the three methods. eDNA data replicated the environmental responses and showed the same overall patterns in community composition as the traditionally collected data. We suggest that eDNA biomonitoring can complement traditional methods, and will perform at least as well as traditional data at detecting patterns in invertebrate community composition and ecosystem health at a national scale.

1 | Introduction

Environmental DNA (eDNA) is rapidly transforming how ecologists and managers conduct biodiversity assessments (Takahashi et al. 2023). This biomonitoring tool has benefitted from recent technological advancements which have reduced the cost of analysis whilst increasing the sensitivity of detection (Pawlowski et al. 2021). eDNA is increasingly being used for biomonitoring aquatic organisms at the species and community levels in a wide range of different habitats including coastal areas

such as estuaries, along with inland lakes and rivers (Hering et al. 2018; Pawlowski et al. 2018; Takahashi et al. 2023). This new method has the potential to revolutionize assessments of ecological health for environmental monitoring by detecting multispecies assemblages, but there remain uncertainties about how congruent eDNA indicators are with data collected using traditional biomonitoring techniques.

The use of eDNA in aquatic science has greatly increased since first introduced for species detection in filtered water samples during the late 2000s (Ficetola et al. 2008; Takahashi et al. 2023). Unlike traditional biological sampling, where only a known area of streambed is sampled for aquatic invertebrates, eDNA is shed from organisms and washed downstream with regular mixing and resuspension, leading to a "conveyor belt" of biodiversity information (Deiner et al. 2017). In a less metaphorical sense, Pont et al. (2018) likened the movement of eDNA in river networks to that of fine suspended sediment. eDNA thus offers a potentially unparalleled way for ecologists and environmental managers to enhance biodiversity assessments. While traditional sampling of a known area of streambed may be appropriate for assessing small-scale responses to environmental stressors such as point source discharges or local changes in habitat conditions, characterizing biodiversity at a larger sub-catchment scale with eDNA may have more relevance to catchment managers interested in understanding the effects of larger scale stressors such as land-use change, or assessments of catchment wide restoration activities. An additional benefit to the use of eDNA is obtaining high resolution and accurate biodiversity data with taxonomic identification to species level (Ruppert, Kline, and Rahman 2019). eDNA is also less invasive and has become more cost effective than traditional sampling methods due to improved technology increasing processing efficiencies (Sigsgaard et al. 2015; Qu and Stewart 2019). Compared to traditional methods, more eDNA samples may be collected during a monitoring interval at a cost that is cheaper or equivalent to that currently spent on routine sampling (Yao et al. 2022). Because of these many advantages, the use of DNA-based identifications over more traditional techniques has received much attention for both fish (e.g., Civade et al. 2016; Pont et al. 2018; David et al. 2021) and freshwater invertebrates (e.g., Stein et al. 2014; Turunen et al. 2021). However, more research is required to validate eDNA-based stream biomonitoring as an effective surrogate for traditional methods based on benthic sampling and morphological-based taxonomic identification of macroinvertebrates.

Owing to globally prevalent pressures on ecosystems and biodiversity, environmental legislation has become more demanding in its information needs regarding the status of the environment. One example is the European Water Framework Directive (WFD) which has greatly expanded the range of biological communities and aquatic mesohabitats used to monitor surface water status in Europe (Reyjol et al. 2014). Coupled with a need to better quantify returns on investment of environmental actions (Upton 2022; Jaeger and Scheuerell 2023), these growing information needs have aligned with the development of eDNA as a potential biomonitoring tool (Baird and Hajibabaei 2012; Hering et al. 2018). One possible application of eDNA in Aotearoa New Zealand (ANZ) is for mandatory State of the Environment (SoE) monitoring of streams and rivers. eDNA biomonitoring has the potential to offer an accurate and cost-effective tool for regional authorities tasked with monitoring freshwater ecological status. These regional authorities (hereafter councils) have legal obligations under the Resource Management Act (New Zealand Government 1991) and the more recent National Policy Statement for Freshwater Management (NPS-FM; New Zealand Government 2020) to assess the ecosystem health of waterways and monitor temporal trends. The NPS-FM identifies 21 water quality and ecological attributes to monitor and introduced the concept of freshwater management units (FMU), which represent smaller spatial scales than those previously monitored. The NPS-FM requires councils to now assess the state of waterways representative of individual FMUs, as opposed to their region, which is likely to increase the size of council monitoring programs. Thus, the increased information demands of environmental legislation such as the NPS-FM makes eDNA monitoring attractive because traditionally, biomonitoring of streams and rivers in ANZ and internationally has relied strongly on the collection of freshwater macroinvertebrates (Stark et al. 2001; Carter, Resh, and Hannaford 2017).

In ANZ, national guidelines have been designed to minimize variability in collecting and processing macroinvertebrate samples (Stark et al. 2001; NEMS 2022). However, sample processing is generally the most time-consuming and expensive part of monitoring because it relies on well-trained taxonomists to accurately identify a wide range of different invertebrate taxa. Three macroinvertebrate attributes are required to assess ecosystem health in ANZ: the Macroinvertebrate Community Index (MCI), its quantitative variant (QMCI), and the Average Score Per Metric (ASPM). These indices have traditionally been based on morphological-based taxonomic identification of macroinvertebrates and established taxa-specific tolerance values which indicate sensitivity to stressors such as organic pollution and nutrient enrichment (Stark 1985). While specific tolerance scores are available for many genera of aquatic insects, other groups (Oligochaeta, Nematoda, and Platyhelminthes) have a paucity of autecological information available, compounded by a lack of taxonomic knowledge. Due to the lack of identification keys to species-level for many groups and the costs involved in gaining accurate species information based on morphological-based taxonomy, most invertebrates are identified to coarser levels: Genus (most aquatic insects), Order or Class (Crustacea), and even higher levels such as Phyla (Nematoda and Platyhelminthes) using the NEMS (2022) protocols. Despite these limitations, the current macroinvertebrate indices used in ANZ have been shown to be effective at detecting stream degradation (Stark 1985; Collier, Ilcock, and Meredith 1998; Collier 2008), although there has been no attempt to assess if their eDNA-derived equivalents are congruent and appropriate for biomonitoring.

The three macroinvertebrate indices (MCI, QMCI, and ASPM) codified in the NPS-FM are compulsory attributes for councils to monitor in ANZ. This creates little incentive to attempt more detailed macroinvertebrate identifications with potential implications for addressing biodiversity loss (Kim and Byrne 2006). Many studies show that relatively high levels of identification (e.g., to Family) are sufficient for environmental monitoring (Hewlett 2000; Marshall, Stewart, and Harch 2006; Lovell et al. 2007). However, a coarse level of identification may result in a loss of information, reducing our ability to detect patterns in macroinvertebrate assemblages and identify stressors driving anthropogenic change. This problem may affect how biodiversity

conservation priorities are set (Tsyrlin et al. 2023) and greatly reduce our understanding of impacts if some groups are highly responsive to environmental gradients when considered at finer taxonomic levels (Andújar, Arribas, and Gray 2018; Carew et al. 2022). Thus, establishing that eDNA is effective for existing biomonitoring purposes would support the further development of this tool for collecting highly resolved biodiversity data.

Our study aimed to compare ecosystem health assessments made by traditional methods (habitat sampling, morphological-based taxonomy) to those made using eDNA. Similar comparative work has shown congruency between bioassessments conducted using traditional morphological identifications and those using either metabarcoded DNA filtered from water samples (eDNA) or extracted from invertebrate samples (Stein et al. 2014; Elbrecht et al. 2017; Turunen et al. 2021).

We subsequently tested the following four hypotheses:

1. Both biomonitoring techniques (NEMS vs. eDNA) would produce very similar, and complementary values for the three common biotic indices (MCI, QMCI, and ASPM).
2. Macroinvertebrate community data collected using the NEMS methods would discriminate between landcover classes the least, while data collected using eDNA would perform better, reflecting a greater proportion of the overall invertebrate community being sampled.
3. eDNA-derived biotic indices would discriminate the most between landcover data, reflecting the enhanced ability to sample the upstream invertebrate community, and improved taxonomic resolution from the metabarcoded species data.
4. Using species-level information obtained from eDNA data to summarize the macroinvertebrate community would result in stronger relationships between environmental and spatial variables than that for community data collected by traditional techniques grouping taxonomic data due to the improved taxonomic resolution.

Showing that eDNA-collected data displays similar differences in invertebrate community composition across environmental gradients (including landcover) when compared with data collected using traditional NEMS methods would further justify the increased use of eDNA-based ecological indicators. Such results would have major implications for environmental managers seeking to implement more extensive monitoring programs with greater accuracy and efficiency. These outcomes would further highlight that eDNA has the potential to meet our biomonitoring needs under more demanding legislation (such as the NPS-FM in ANZ or the WFD in Europe) that seeks to help achieve desired environmental goals.

2 | Methods

2.1 | Field and Laboratory Methods

eDNA samples were collected from 53 sites located within 16 Regional and District Council boundaries throughout ANZ (Figure 1). A wide range of waterways, with very large gradients in stream size (flow), land cover, physical characteristics like slope or distance inland, and reach characteristics like shade and native riparian vegetation, were sampled between December 2020 and August 2022. Most sites (31) were sampled in the austral autumn (March to May), while 22 sites were sampled in summer (December to February). Only four sites were sampled in winter (August). To minimize possible dilution of eDNA, all samples were collected during base flows, where stream flows had not increased following rain for at least a 2-week period. At each site, six replicate eDNA samples were collected using Wilderlab V2 eDNA mini kits and filtering up to 1 L of water through filters (pore size = 1.2 μm designed to trap DNA material) attached to sterile 60 mL syringes. Six replicates were used as this number of filters has been shown to optimally detect the majority of fish and invertebrate species present at an individual site (Melchior and Baker 2023). Following filtration, excess water was forced from the filter by pushing air through the same syringe, and material collected on the filter was preserved with 350 μL DNA/RNA shield solution (Zymo Research, Irvine, CA, USA). Sample details (total volume filtered, GPS coordinates of the location) were recorded before being sent to a laboratory (Wilderlab Ltd., Wellington, NZ) for analysis. For DNA extraction and purification, 200 μL of each sample were loaded into a Genolution GD141 cartridge and run on the Genolution Nextractor NX-48S system using the standard extraction settings. DNA quality/quantity analysis, indexing, and amplification were carried out in single-step quantitative PCR reactions on an Applied Biosystems QuantStudio 1 qPCR instrument. DNA extracts were PCR-amplified using eight fusion-tag mitochondrial and nuclear rRNA assays for the detection of vertebrate, invertebrate, plant, microeukaryote and microbial DNA (see Table S1 for primer sequences). Fusion tag primers included Illumina P5 and P7 adapter sequences, Illumina TruSeq sequencing primer binding region (forward primer only), unique 8 bp index sequences, and locus specific primers, respectively. PCR reactions were carried out in duplicate, with each reaction containing 5 μL SensiFAST 1 × LoRox SYBR Mix (Bioline), 0.25 μL forward primer (10 μM), 0.25 μL reverse primer (10 μM), 0.5 μL BSA (10 mg ml^{-1}, Sigma Aldrich), 2 μL deionized water and 2 μL template DNA. qPCR cycling conditions included an initial denaturation of 3 min at 95°C, followed by 40 cycles of 5 s at 95°C, 10 s at the annealing temperature specified in Table S1, and 15 s at 72°C (see Wilkinson et al. 2024 for further information on molecular methods).

The resultant DNA sequences were matched against a global reference sequence database primarily compiled of trimmed reference sequences downloaded from GenBank (Benson et al. 2010) and the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007). Matching sequences were assigned at the lowest common ancestor level (see Smith et al. 2024 for further details). This gave information on the taxonomic composition of the sample, based on the DNA sequences that could be identified, as well as the read counts of the genetic material (Table S1). These read counts were used as a potential "proxy" for the relative abundance of different taxa, which is used in calculations of the QMCI.

Benthic invertebrates had also been sampled for invertebrate communities from the 53 sites as part of local council SoE

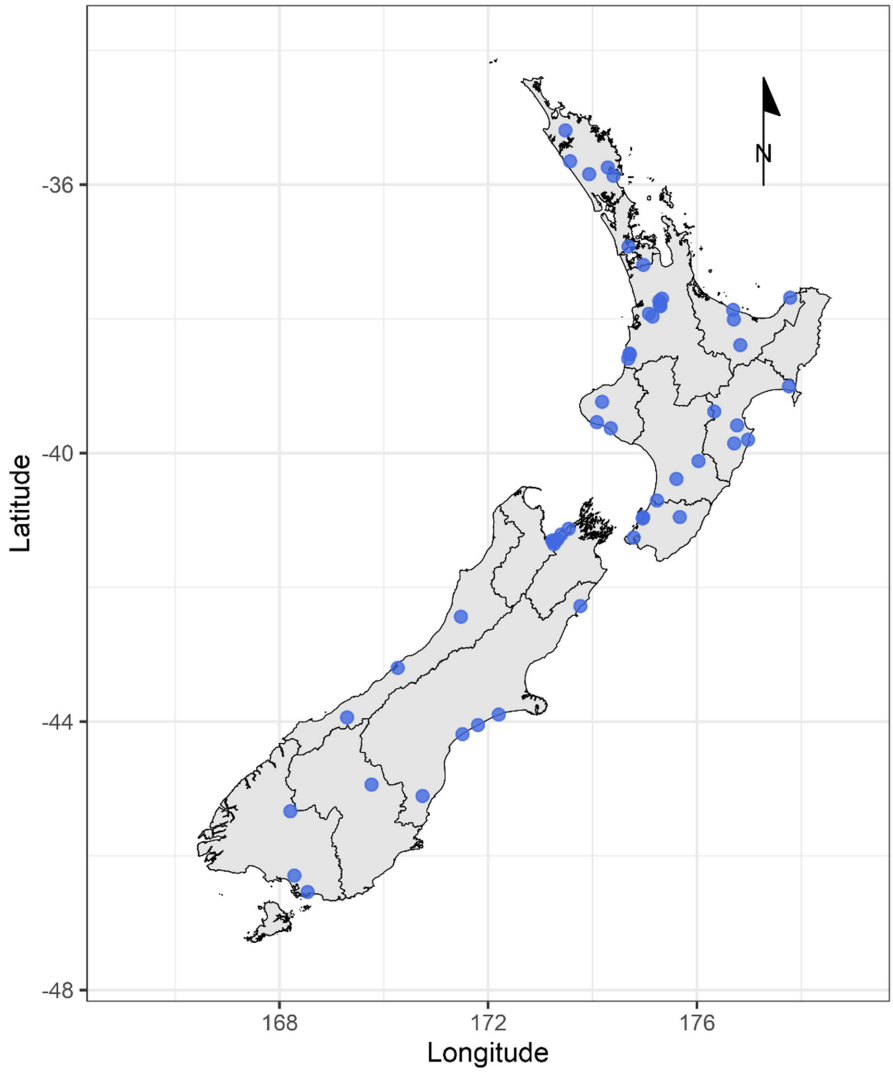

FIGURE 1 | Map showing the location of eDNA sampling sites throughout Aotearoa New Zealand (ANZ) in each of the 14 Regional Council or Territorial Local Authority boundaries.

invertebrate monitoring programs, or one-off surveys. However, eDNA sampling was not done concurrently with benthic sampling at most sites. Examination of the temporal differences between traditional and eDNA sampling showed that kick net samples were collected at a median time of 99 days prior to the eDNA samples, with a 20%–80% percentile range of 8–395 days. Although invertebrate communities display an inherent degree of "stability" over time (Winterbourn 1997; Scarsbrook 2002), other studies (e.g., Larned, Snelder, and McBride 2016) have shown that invertebrate communities can change over time, especially when subject to various factors such as land use change, sedimentation, and water quality degradation. Although such changes were assumed to be minor when considering the relatively short time span between traditional sampling and eDNA sampling (within a few months of each other), the average community composition at each site was calculated using the past 5 years at 33 sites, or the average community composition over a three-year period at three sites. A further 17 sites only had one invertebrate and eDNA sample collected, but these sites were still used in the analysis to maximize the number of sites in this comparison.

Invertebrate samples were collected using a kick net (500 μm mesh size) following the semi-quantitative methods outlined in Stark et al. (2001). All samples were processed using either semi-quantitative fixed count method (39 sites) or coded abundance method (14 sites) as outlined by Stark et al. (2001). All fixed count data of individual taxa were converted to percentages of total counts at each site, while coded abundance data was first allocated to nominal abundance values based on the mid-range of the abundance categories (rare = 2; common = 12; abundant = 60; very abundant = 300; very very abundant = 500), before being converted to percentages of total counts. The use of these nominal abundance values enabled greater spatial coverage in the data, which was deemed more important than not including these sites in analyses using relative abundance data.

2.2 | Invertebrate Identification

A total of 3952 invertebrate taxa were identified from the eDNA database. International databases such as the World Register of Marine Species (https://www.marinespecies.org/about.php),

and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/) were used to reduce this list to 625 freshwater invertebrate taxa from one of 10 dominant invertebrate groups: bryozoa, cnidaria, crustacea, flatworms, insects, mites, mollusks, springtails, worms, and nematodes. This list was further reduced to 576 taxa, based on invertebrates being present in the New Zealand Organisms Register (NZOR: https://www.nzor.org.nz). This list was further refined to 561 taxa following the removal of some eDNA taxonomic identifications that were to orders (e.g., Trichoptera, Plecoptera, and Ephemeroptera) or families of aquatic insects (e.g., Gripopterygidae, Hydropsychidae, Helicopsychidae, and Conoesucidae) that were not present in the NEMS (2022) level of resolution. Care was also taken to ensure that all the invertebrates collected during routine SoE programs using a kick net were also taken to a similar level of identification. This ensured that comparisons being made between these two methods were based on a similar level of taxonomic resolution.

2.3 | Data Analysis

The two datasets summarizing invertebrate community composition from traditional sampling (NEMS 2022) or from eDNA gave us the ability to compare the effects of sample collection (traditional techniques or eDNA) and level of identification (identification to NEMS level, or to species using eDNA) to address three analytical questions (Table 1). Firstly, we wanted to examine similarities in the NPS-FM attributes (MCI, QMCI, and ASPM), their constituent parts (EPT richness, %EPT richness, and abundance), and taxonomic richness when calculated based on invertebrate communities collected and analyzed using traditional methods (NEMS) or eDNA. The MCI is the average tolerance scores of all taxa at a site, multiplied by a scaling factor (20) such that

$$\mathrm{MCI} = \frac{\sum_{i=1}^{i=S} a_i}{S} \times 20$$

where S = the total number of taxa in the sample, and a_i is the tolerance value for the ith taxon. Scores for the MCI can theoretically vary from 20 to 200.

The QMCI is calculated from count, (or in this case relative abundance) data such that:

$$\mathrm{QMCI} = \sum_{i=1}^{i=S} \frac{(n_i \times a_i)}{N}$$

where S = the total number of taxa in the sample, n_i is the abundance of the ith scoring taxa, a_i is the tolerance value for the ith taxon, and N is the total of the abundances.

The ASPM (Collier 2008) is calculated as the average scores for %EPT-abundance (normalized to 100), EPT-richness (normalized to 29), and the MCI (normalized to 200). A fundamental aspect of these metrics is their reliance on published tolerance values to organic enrichment. Taxa highly tolerant to organic enrichment score 1, while highly sensitive taxa score 10. Under the NEMS, all Oligochaetes are grouped, and thus score 1. However, if eDNA

TABLE 1 | Summary of the analyses conducted in this study comparing biotic metrics and community composition between samples when collected using traditional techniques according to the national monitoring standards for New Zealand (NEMS 2022), and samples using eDNA, but identified to the level of the NEMS (N_eDNA), and samples collected with eDNA and identified to the lowest possible level (eDNA).

			1. Differences in metrics		2. Effects of landuse		3. Community analysis	
Sample collection	ID	Abbreviation	Collection	Collection + ID	Collection	Collection + ID	Collection	Collection + ID
NEMS	NEMS	NEMS	Y	Y	Y	Y	Y	Y
eDNA	NEMS	N_eDNA	Y		Y		Y	
eDNA	eDNA	eDNA		Y		Y		Y

identified 10 species of Oligochaetes at a site, these would each score 1, and this would thus lower the MCI, QMCI and ASPM scores, giving different assessments of overall stream health.

We explored the effect of this more by comparing metrics calculated using NEMS methods (e.g., MCI, etc., calculated using grouped taxa such as Oligochaetes) to (a) metrics derived from eDNA sampling, but identified to the NEMS resolution (N_eDNA, i.e., the 10 species of Oligochaeta identified being all grouped), or (b) to metrics derived by eDNA sampling and by the lowest level of identification (usually species) obtained by eDNA (eDNA, i.e., each Oligochaeta scoring 1).

Similarities between the biotic indices calculated from the three datasets were first assessed by linear regression analysis. Where relationships appeared to be non-linear from visual inspection, a Generalized Additive Models (GAM) with a cubic-spline smoothing function was fitted. GAM were fitted using the *mgcv* R package (Wood 2011). We compared the Akaike information criterion (AIC) value of the resulting GAM with the linear regression. Models that had a $\Delta AIC > 2$ were determined to be sufficiently different and the model with the lowest AIC score plotted; where $\Delta AIC < 2$ we used the expected relationship (i.e., the linear model). The relationships for the MCI, QMCI and ASPM are shown in Figure 2; all remaining indices are plotted in Figure S1. We also used Bland–Altman analysis to assess the congruency of the metrics generated with NEMS and eDNA data (Giavarina 2015). This analysis evaluates the bias between the mean differences, and estimates an agreement interval, within which 95% of the differences of the second method, compared to the first one, fall. Data were analyzed as unit differences and plotted using the *blandr* R package (Datta 2017).

Secondly, we were interested in assessing the effects of landcover on both invertebrate community composition and on calculated biotic indices. This assessment reflects the fact that a central part of regional council monitoring is describing stream health in different land use classes to highlight the impact of anthropogenic stresses on invertebrate communities. We consequently compared differences in invertebrate community composition between the different River Environment Classification (REC; Snelder and Biggs 2002) landcover classes of Indigenous Forest, Exotic Forest, Pasture, and Urban when collected using either eDNA or traditional methods. As with the previous analysis, these analyses were done with data collected by NEMS methods, data collected by eDNA but analyzed to the NEMS level of resolution (N_eDNA), and data collected and identified using the eDNA level of resolution (eDNA). All

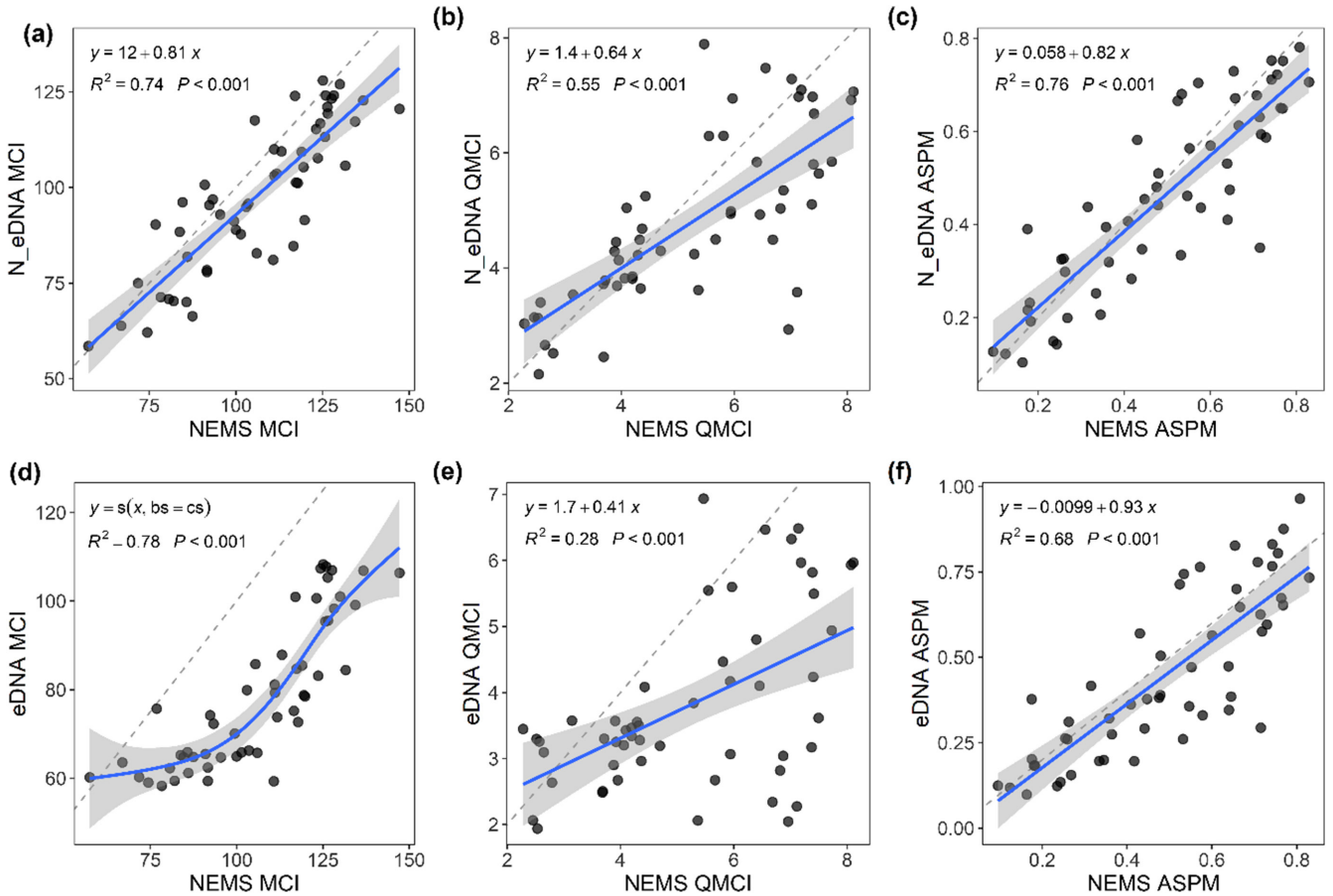

FIGURE 2 | Results from regression analyses showing the congruence of macroinvertebrate biotic metrics measured by kick-net sampling (NEMS) and eDNA. Using eDNA data at an equivalent level of taxonomic resolution (N_eDNA), (a–c) shows the linear regressions for (a) the Macroinvertebrate Community Index (MCI); (b) the Quantitative version of the MCI (QMCI); and (c) the Average Score Per Metric (ASPM) index. Using the lowest possible level of identification from the eDNA data (eDNA), (d) shows the non-linear relationship for the MCI with the NEMS data; (e, f) shows the linear relationships for the QMCI and ASPM. The dotted lines indicate the 1:1 relationship and gray shading indicate the 95% confidence interval for each regression.

community composition data in each data matrix was converted to percentages of each taxa based on total count data (NEMS) or total read count (eDNA) and square-root transformed (Hellinger transformation). A Bray–Curtis similarity matrix was then created and permutational multivariate analysis of variance (PERMANOVA) was used to explore differences in community composition according to the different REC land cover categories. The PERMANOVA models were analyzed using *adonis2* in the *vegan* R package (Oksanen et al. 2014) and *pairwise.adonis2* in the *pairwiseAdonis* R package (Martinez Arbizu 2020).

We also used generalized linear models (GLM) to examine differences in the four landcover classes and the calculated biotic metrics when calculated using each of the three methods (NEMS, N-eDNA, and eDNA). For proportion data (e.g., %EPT-richness) and the ASPM we used a quasibinomial distribution with a logit-link function. For the EPT richness data, we used a Poisson distribution with a log-link function. All other data assumed a Gaussian distribution—data were log-transformed to meet assumptions of normality and homoscedasticity. GLM models were fitted with the *glm* function in *stats* R package (R Core Team 2024).

Part of this second analysis was also to determine what effect the different sample collection and processing techniques had on the ability for councils to assess the ecological condition of sites using the three biotic indices (MCI, QMCI, and ASPM). This analysis was done as the NPS-FM requires councils to assess ecological state according to clearly defined bands from A (Excellent) to D (below the National Bottom Line). For example, the NPS-FM bands for the MCI are such that scores ≥130 are in the A band, while scores <90 are in the D band. The relevant NPS-FM bands for each of these three indices were subsequently calculated, and a chi-squared test was used to see whether the null hypothesis of there being no difference between the three methods and the numbers of sites allocated to each of the four NPS-FM bands was rejected.

Thirdly, we wanted to compare responses of the invertebrate community data collected by either eDNA or traditional techniques to a wide range of environmental factors. We used the REC (Snelder and Biggs 2002) as a digital representation of the river and stream network throughout ANZ. The REC comprises both waterway reaches (or segments) and their catchments. The digital river network is also combined with spatial data layers describing catchment and segment-scale characteristics describing climate, topography, hydrology, geology, and land cover (Booker and Snelder 2012; Leathwick et al. 2008). In particular, quantitative explanatory variables were obtained from the Freshwaters of New Zealand database (FWENZ; Leathwick et al. 2008), linked to the digital river network.

Meso-scale variables included distance to sea, average elevation, and average air temperature (Table 2). The physical location (geographic coordinates) of each site was also included in this analysis, as it can describe spatial patterns to biological data. Spatial structuring of macroinvertebrate community data using geographic coordinates was assessed using Principal Coordinates of Neighbors Matrices (PCNM) analysis (Borcard and Legendre 2002). PCNM geographic functions are a type of "Distance-Based Eigenvector Maps" (DBEMs) that are part of a wider family of methods also known as Moran's Eigenvector Maps (Borcard and Legendre 2002; Borcard et al. 2004; Dray, Legendre, and Peres-Neto 2006). PCNM descriptors (or axes) represent a spectral decomposition of the spatial relationships amongst the study sites (Borcard and Legendre 2002). This can be important where processes can structure communities at different spatial scales (Borcard et al. 2004). PCNM axes were generated from spatial coordinates using the *pcnm* function in the *vegan* R package (Oksanen et al. 2014).

We examined the strengths of relationships between the environmental and spatial variables with invertebrate community structure when collected by traditional techniques or by eDNA, but identified to the NEMS level of resolution (N_eDNA), or when collected and identified using eDNA.

We then performed constrained ordination on macroinvertebrate datasets using the spatial and environmental data described above. Distance-based Redundancy Analysis (db-RDA) was used for analysis of the community composition matrices as represented by either the eDNA species data, or by the generic and higher level of identification traditionally used in the NEMS (2022) protocol. db-RDA simultaneously analyses the community and explanatory data by combining an ordination method and multiple linear regression (Ter Braak 1986; Legendre and Legendre 1998). Taxa that were present at <3 sites were excluded prior to analysis. Macroinvertebrate data was Hellinger transformed prior to using the Bray–Curtis dissimilarity. We first compared AIC values for alternative approaches (not removing rare taxa and other transformations of relative abundance data: cubic root, log, and arcsine square root). In all instances, removing rare taxa improved model fit, and the Hellinger transformation was mostly optimal, except for a few instances where the ΔAIC <2. In these instances, we kept using the Hellinger transformation for consistency. These comparisons of AIC values confirmed the adequacy of the data processing pipeline used. We selected predictor variables for the db-RDA models from data representing spatial and environmental influence factors (Table 2) using a forward-selection method with the *ordiR2step* function in the vegan R package (Oksanen et al. 2014). The significance of selected predictors was tested using permutation tests that randomly permuted the invertebrate assemblage data, while holding the explanatory variables constant. The significance was determined from the number of random permutations in which the total inertia of the constrained axes (i.e., the explained variation) exceeded that of the original db-RDA. These steps resulted in eight PCNM axes and seven environmental predictors selected for the NEMS data, seven PCNM axes and seven environmental predictors selected for the N_eDNA data, and eight PCNM axes and nine environmental predictors selected for the eDNA data.

To visualize the results of the db-RDA models, we used a partial db-RDA approach where the influence of the spatial PCNM predictors was conditioned out. This helped to highlight the influence of selected environmental predictors on community composition. In these plots, we include the 10 most abundant taxa for the respective invertebrate community dataset to better understand the ecological implications of these analyses.

We also used variation partitioning analysis to compare the independent and shared influence of environmental and

TABLE 2 | List of explanatory variables used to investigate relationships between invertebrate communities identified to levels used in eDNA or traditional approaches and environmental variables. The variable types are either quantitative (Q) or categorical (C).

Scale	Factor	Abbreviation	Description and type
Macroscale	Climate	SegJanAirT	Segment Average Jan Air temp (Q)
	Climate	SegMinTNor	Segment Minimum Air temp (Q)
	Climate	USAvgTNorm	Average air temperature (°C) in the catchment, normalized with regards to Segment Summer Temperatures (Q)
	Climate	USDaysRain	Days/year with rainfall in the catchment greater than 25 mm (Q)
	Hydrology	SegFlow	Segment total annual runoff volume (Q)
	Hydrology	SegFlow4th	Segment total annual runoff volume, fourth root transformed (Q)
	Hydrology	SegFlowVar	Segment flow variability (Q)
	Hydrology	SegLowFlow	Mean annual 7-day low flow (m^3/s), fourth-root transformed (Q)
	Landuse	USGlacier	% of runoff from upstream glacial areas (Q)
	Landuse	USIndigFor	% of catchment in LCDB category (indigenous forest) (Q)
	Landuse	USLake	Lake Index (Q)
	Landuse	USNative	% of catchment in LCDB category (native vegetation) (Q)
	Landuse	USPasture	% of catchment in LCDB category (pasture) (Q)
	Landuse	USWetland	% of catchment in LCDB category (wetland) (Q)
Mesoscale	WQ	SegCluesLo	Modeled Clues N loading log-transformed (Q)
	WQ	SegCluesN	Modeled Clues N loading (Q)
	WQ	USCalcium	Average calcium concentration of rocks in the catchment, 1 = very low to 4 = very high (C)
	WQ	USHardness	Average hardness of rocks in the catchment, 1 = very low to 5 = very high (C)
	WQ	USPeat	Area of peat in upstream catchment (proportional) (Q)
	WQ	USPhosporu	Average phosphorus concentration of rocks in the catchment, 1 = very low to 5 = very high (C)
	Physical	DSAvgSlope	The average slope of the catchment below the segment (Q)
	Physical	DSDist2Coa	Downstream distance to coast (km) (Q)
	Physical	DSMaxLocal	The maximum local downstream slope (Q)
	Physical	Order	Strahler stream order (C)
	Physical	SegSlope	Average segment slope (m/m) (Q)
	Physical	SegSlopeSq	Square of average segment slope (m/m) (Q)
	Physical	USAvgSlope	The average upstream catchment slope (Q)
	Reach	SegRipNati	Segment riparian shade from native species (Q)
	Reach	SegRipShad	Segment riparian shade (proportional) (Q)
Microscale	Reach	ReachHab	Habitat Weighted average of proportional cover of local habitat (Q)
	Reach	ReachSed	Weighted average of proportional cover of bed sediment (Q)
	Spatial	X	GPS_Easting (Q)
	Spatial	Y	GPS_Northing (Q)

spatial predictors on community composition. For these analyses, we used the *varpart* function in the *vegan* R package (Oksanen et al. 2014). The significance of the unique fractions were tested by permutation of partial db-RDAs on the sets of variables under examination with the other set of variables included as co-variables (i.e., their effect was removed; Legendre and Legendre 1998). The significance of each independent variation component was permutation-tested using 1000 randomizations (Peres-Neto et al. 2006). The results of the variation partitioning were visualized using the *venneuler* R package (Wilkinson 2022).

We applied appropriate transformations to predictor variables to approximate a normal distribution prior to building the db-RDA and variation partitioning models. All environmental predictor variables were centred (subtracted from the mean) and standardized (scaled to unit variance). The explanatory variables were initially screened for collinearity ($r < 0.75$), in addition to using variance inflation factors (VIF) for the final db-RDA models (Kutner et al. 2004). Variables with a VIF >5 were deemed to have high collinearity and were removed from further analyses. Unless otherwise specified, all analyses were conducted in R (R Core Team 2024).

3 | Results

3.1 | Comparisons of Biotic Metrics

Regression analysis examined relationships between biotic metrics based on benthic macroinvertebrate samples collected and processed in the 53 sites according to the NEMS protocols and (1) samples collected by eDNA but identified to the level of NEMS (N_eDNA), and (2) samples collected by eDNA and identified to (usually) species level from the eDNA genomic library (eDNA). With the exception of taxonomic richness, there was generally a high degree of correlation between the biotic indices when calculated from standard methods (NEMS) and the N_eDNA or the eDNA data (Table 3).

The Macroinvertebrate Community Index (MCI) showed close congruency between the NEMS and N_eDNA data with a regression slope $b = 0.81$ and the model explaining 74% of the variance (Table 3; Figure 2a). In contrast, we identified a nonlinear relationship between the NEMS MCI and the eDNA MCI (Figure 2d). Using the generalized additive model (GAM) improved the AIC score by −12.9 when compared with the linear regression model. The GAM showed a good congruency between the eDNA MCI and the NEMS MCI when the latter exceeded scores of 100. However, when the NEMS MCI was <100, there was a much lower congruency between the scores.

There was less congruence for the quantitative equivalent (QMCI) of the MCI between the NEMS and N_eDNA data, with the linear regression model only explaining 55% of the variance with a slope of $b = 0.64$ (Figure 2b). A much lower level of congruence was also observed for the QMCI between the NEMS and eDNA data, explaining only 28% of the variance (Table 3; Figure 2e). The highest variance explained (77%) was between NEMS and N_eDNA data for the Average Score Per Metric (ASPM) index, with relatively close congruency of the regression slope ($b = 0.82$; Figure 2c). The ASPM also performed well with eDNA data (Table 3; Figure 2f), with an even closer regression slope to the 1:1 line ($b = 0.93$) and a *y*-intercept of nearly

TABLE 3 | Results of linear regression analyses showing the congruence of macroinvertebrate biotic metrics measured by traditional sampling (NEMS) and eDNA at an equivalent level of taxonomic resolution (N_eDNA), and eDNA data at the lowest possible level of identification (eDNA).

Comparison	Model	*b*-estimate	SE	*F*-value	*p*-value	R^2
NEMS vs. N_eDNA	Richness	0.254	0.116	4.79	0.033	0.086
	EPT-richness	0.719	0.095	56.7	<0.001	0.527
	%EPT-richness	0.789	0.077	105.0	<0.001	0.674
	%EPT-abundance	0.616	0.072	74.0	<0.001	0.592
	MCI	0.813	0.067	145.0	<0.001	0.740
	QMCI	0.638	0.080	62.9	<0.001	0.552
	ASPM	0.816	0.065	158.0	<0.001	0.756
NEMS vs eDNA[a]	Richness	0.088	0.271	0.107	0.745	0.002
	EPT-richness	1.145	0.157	53.4	<0.001	0.511
	%EPT-richness	0.744	0.066	126.0	<0.001	0.711
	%EPT-abundance	0.457	0.072	40.0	<0.001	0.440
	MCI[b]	0.676	0.062	118.0	<0.001	0.699
	QMCI	0.408	0.092	19.8	<0.001	0.279
	ASPM	0.933	0.090	107.0	<0.001	0.677

Note: The *b*-estimate refers to the slope parameter in the linear regression model $y = a + bx$. Biotic metrics in bold are compulsory for councils to measure under New Zealand environmental legislation (i.e., NPS-FM; New Zealand Government 2020).
[a]Results from regression analyses showing the congruence of macroinvertebrate biotic metrics measured by eDNA and NEMS are shown in Figure S1.
[b]The GAM (Figure 2d) produced a better fit than the linear regression model for the eDNA MCI.

TABLE 4 | Results of Bland–Altman analyses showing the bias (±95% confidence interval) introduced by eDNA at an equivalent level of taxonomic resolution (N_eDNA), and eDNA data at the lowest possible level of identification (eDNA) when compared to macroinvertebrate biotic metrics measured by traditional sampling (NEMS). For full results including Limits of Agreement (LOA) see Table S2 and Figures S2 and S3.

Comparison	Metric	Bias	Lower CI	Upper CI
N_eDNA vs. NEMS	Richness	0.21	−3.45	3.87
	EPT-richness	−0.19	−1.90	1.52
	%EPT-richness	−1.96	−4.76	0.84
	%EPT-abundance	−4.73	−10.07	0.61
	MCI	−8.22	−11.14	−5.29
	QMCI	−0.45	−0.77	−0.13
	ASPM	−0.03	−0.06	0.00
eDNA vs. NEMS	Richness	45.45	38.44	52.47
	EPT-richness	5.70	3.08	8.31
	EPT%-richness	−14.94	−17.50	−12.37
	EPT%-abundance	−18.95	−25.21	−12.69
	MCI	−26.99	−30.10	−23.87
	QMCI	−1.43	−1.85	−1.01
	ASPM	−0.04	−0.08	0.00

Note: Biotic metrics in bold are compulsory for councils to measure under New Zealand environmental legislation (i.e., NPS-FM; New Zealand Government 2020).

zero, despite the lower amount of variance explained by the NEMS equivalent (68%).

The Bland–Altman analyses supported the linear regression results, showing that in general, the degree of congruence between traditional sampling (NEMS) and eDNA at an equivalent level of taxonomic resolution (N_eDNA) was relatively high (Table 4). Taxa richness showed little bias due to the equivalent level of taxonomic resolution used for the N_eDNA data. However, the comparison of the traditional sampling data (NEMS) with the eDNA data showed stronger biases, with taxa richness greatly underestimated by the conventional methods (Table 4). Contrasting this result, the MCI scores were greatly reduced in the eDNA data (negative bias), possibly due to high diversity of non-insect taxa (e.g., Oligochaeta) with nominally low MCI tolerances scores. The multi-metric ASPM index performed relatively well with both comparisons of eDNA data, showing lower levels of negative bias (Table 4) and greater variance than explained in the linear regression models of ASPM (Table 3).

In general, after excluding taxa richness the presence-absence based metrics had similar % variance explained when compared with the semi-quantitative metrics for both the N_eDNA comparison (average $R^2 = 0.647$ vs. 0.633 respectively) and the eDNA comparison (average $R^2 = 0.640$ vs. 0.465 respectively).

The relatively poor relationship between taxonomic richness derived from the NEMS processing and N_eDNA (Table 3) was somewhat surprising, given that all taxa were identified to the same taxonomic level. However, examination of the number of taxonomic groups in each of the NEMS major invertebrate categories (e.g., Phyla, Order) showed that fewer taxa were identified from eDNA when grouped according to the NEMS level of identification for all groups containing more than one taxa (Table 5). In particular, Coleoptera, Crustacea, insects (Diptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, and Trichoptera) and Mollusca appeared under-represented in the N_eDNA data. For example, three mayfly genera (*Acanthophlebia*, *Arachnocolus*, and *Neozephlebia*) and the caddisfly *Pycnocentrella* were not recorded in the eDNA data, yet were recorded from the NEMS data. While *Arachnocolus* and *Pycnocentrella* were infrequently found at sites (and therefore potentially missed with a single eDNA sample), other taxa such as *Acanthophlebia* and *Neozephlebia* were reasonably widespread, and found at 10 and 14 sites respectively. Their absence from the eDNA data may simply reflect differences in sampling effort between the NEMS data (based on an average of three or (more usually) 5 years of data) versus a single eDNA sample. Notwithstanding this under-representation of some taxa in the N_eDNA data, it was clear that the use of eDNA revealed a much higher taxonomic richness than the NEMS data (Table 5).

3.2 | Effects of Landuse

Of the 53 sites, most (30) were in catchments classified as Pasture, and 18 were in catchments classified as Indigenous Forest. Three sites were in catchments classified as Urban, while the remaining two sites were in Exotic Forest. Results of PERMANOVA showed differences in community composition between the four landcover classes and the invertebrate communities analyzed using the NEMS protocols ($F_{3,49} = 3.85$, $p < 0.001$, $R^2 = 0.191$). Significant pairwise differences were observed between Indigenous Forest and Pasture ($F_{1,46} = 7.21$, $p < 0.001$, $R^2 = 0.135$), Indigenous Forest and Urban ($F_{1,19} = 5.78$, $p < 0.001$,

TABLE 5 | Summary of the number of taxa identified from the 53 study sites in according to the different methods of the traditional NEMS protocols for collection and identification, collection using eDNA but identification to the NEMS level (N_eDNA), or collection and identification of taxa using eDNA.

Taxonomic group	NEMS	N_eDNA	eDNA
Acarina	1	1	4
Bryozoa	1	1	13
Coelenterata	1	1	22
Coleoptera	16	**5**	**11**
Collembola	1	1	12
Crustacea	14	**9**	65
Diptera	52	**22**	66
Ephemeroptera	21	**15**	43
Hemiptera	6	**2**	5
Hirudinea	1	1	2
Lepidoptera	1	1	1
Megaloptera	1	1	3
Mollusca	23	**14**	36
Nematomorpha	1	1	4
Nematoda	1	1	22
Nemertea	1	1	7
Neuroptera	1	1	2
Odonata	10	**6**	7
Oligochaeta	1	1	63
Platyhelminthes	1	1	18
Plecoptera	14	**11**	30
Rhabdocoela	1	1	1
Trichoptera	45	**35**	114
Total taxa	214	132	551

Note: Bold values indicate when either the N_eDNA or eDNA taxa richness were less than that of samples collected and analyzed using the NEMS (2022) standards.

$R^2 = 0.233$), and Indigenous Forest and Exotic Forest ($F_{1,18} = 2.13$, $p < 0.05$, $R^2 = 0.106$). Slightly stronger differences were observed with the N_eDNA data ($F_{3,49} = 4.26$, $p < 0.001$, $R^2 = 0.207$), where pairwise differences existed between Indigenous Forest and Pasture ($F_{1,46} = 9.69$, $p < 0.001$, $R^2 = 0.174$) and Indigenous Forest and Urban ($F_{1,19} = 4.14$, $p < 0.001$, $R^2 = 0.179$). Invertebrate community composition also differed between the four landcover classes when assessed using the eDNA data identified to its lowest possible level of identification (eDNA), although these differences were not as pronounced ($F_{3,49} = 3.47$, $p < 0.001$, $R^2 = 0.175$). However, similar patterns existed in the data, with significant pairwise differences between Indigenous Forest and Pasture ($F_{1,46} = 7.40$, $p < 0.001$, $R^2 = 0.139$), Indigenous Forest and Urban ($F_{1,19} = 2.81$, $p < 0.01$, $R^2 = 0.129$), and Pasture and Urban ($F_{1,30} = 1.81$, $p < 0.01$, $R^2 = 0.057$).

Similar patterns were found in the assessment of landcover on biotic metrics calculated from data collected by the traditional NEMS methods and eDNA (Table 6). In all cases, the highest metric scores were in Indigenous or Exotic Forest, with the lowest scores in Urban catchments. Sites draining Agriculture had intermediate metric scores. The greatest differences in biotic indices between the four REC landcover classes were found in the indices derived from the eDNA data (average model $R^2 = 0.511$), followed by the N_eDNA data (average model $R^2 = 0.458$). Biotic indices derived from the NEMS methodology differed the least between the four landcover classes (average model $R^2 = 0.378$).

Following the assessment of landuse effects on the indices calculated from the three datasets, differences in the number of sites allocated to each of the NPS-FM bands was examined. The Chi-Squared tests used for this analysis showed that the number of sites in each of the four NPS-FM bands differed between observed and expected when calculated using either N_eDNA and eDNA (Table 7) for two of the three indices. For the MCI score calculated using the N_eDNA data, fewer sites than expected were in the A band, and more sites were in the B and C band, while for the eDNA data, the majority of sites were in the D band for the MCI (Figure 3). In contrast, the number of sites allocated to each of the four NPS-FM bands for the MCI score calculated by the NEMS methods did not differ from that expected by chance (Table 7). Significant differences occurred between observed and expected number of sites in the four NPS-FM bands for the QMCI when calculated using data from all three methods, but these differences were largest in the eDNA data, and smallest in NEMS data (Table 7). As with the MCI, the number of sites in the A band were highest for the NEMS data, but were lower when using N_eDNA or eDNA, while the number of sites in the D band were highest for these latter metrics (Figure 3). No significant differences were found between the observed and expected number of sites in the four bands for the ASPM when assessed using NEMS, N_eDNA, or eDNA data (Table 7).

3.3 | Quantifying Biological and Environmental Relationships

The observed large environmental gradients observed between the 53 study sites (Table S3) were expected to influence invertebrate community composition as a result of individual habitat preferences of all taxa identified, either to the level of the NEMS, or to lower taxonomic levels from the eDNA.

The results of the distance-based redundancy analysis (db-RDA) models highlighted some common environmental drivers between the invertebrate datasets, albeit with subtle differences. The forward-selection process in the db-RDA models showed that invertebrate communities recorded from kicknet samples using the NEMS methodology had seven environmental variables that explained variation in community structure: the proportion (%) of indigenous forest and native vegetation landcover upstream, the % riparian shading in the upstream reach, the upstream channel slope, the reach sediment index (a higher number indicating coarser benthic substrate), the mean January air temperature, and the distance to the coast (Table 8; Figure 4a). In the partial db-RDA model for NEMS macroinvertebrates

TABLE 6 | Results of the Generalized Linear Model (GLM) analysis show how the six biotic indices differed between the four REC landcover classes.R^2

Biotic index	NEMS	N_eDNA	eDNA
EPT richness	$F_{3,49}=9.731$, $p<0.001$ $R^2=0.351$	$F_{3,49}=10.089$, $p<0.001$ $R^2=0.367$	$F_{3,49}=9.980$, $p<0.001$ $R^2=0.378$
%EPT-richness	$F_{3,49}=12.117$, $p<0.001$ $R^2=0.395$	$F_{3,49}=14.428$, $p<0.001$ $R^2=0.438$	$F_{3,49}=26.605$, $p<0.001$ $R^2=0.592$
%EPT-abundance	$F_{3,49}=8.157$, $p<0.001$ $R^2=0.305$	$F_{3,49}=12.478$, $p<0.001$ $R^2=0.407$	$F_{3,49}=21.430$, $p<0.001$ $R^2=0.532$
MCI	$F_{3,49}=12.430$, $p<0.001$ $R^2=0.432$	$F_{3,49}=15.165$, $p<0.001$ $R^2=0.493$	$F_{3,49}=30.622$, $p<0.001$ $R^2=0.652$
QMCI	$F_{3,49}=8.766$, $p<0.001$ $R^2=0.349$	$F_{3,49}=15.855$, $p<0.001$ $R^2=0.558$	$F_{3,49}=11.412$, $p<0.001$ $R^2=0.411$
ASPM	$F_{3,49}=12.951$, $p<0.001$ $R^2=0.436$	$F_{3,49}=15.642$, $p<0.001$ $R^2=0.482$	$F_{3,49}=16.693$, $p<0.001$ $R^2=0.501$

Note: Table shows the *F*-ratio, *p*-value, and the pseudo R^2 value.

TABLE 7 | Results of chi-squared tests showing differences between observed and expected numbers of sites in each of the four National Policy Statement for Freshwater Management (NPS-FM) bands when calculated for three macroinvertebrate metrics using data derived from the NEMS, N_eDNA, and eDNA approaches.

		Variable		
Metric	**Statistic**	**NEMS**	**N_eDNA**	**eDNA**
MCI	$\chi 2$ test	10.86	17.46	81.88
	p-value	0.093	0.008	0.000
QMCI	$\chi 2$ test	23.91	30.79	78.51
	p-value	0.001	0.000	0.000
ASPM	$\chi 2$ test	3.70	4.54	4.12
	p-value	0.717	0.603	0.660

(Figure 4a), the first axis db-RDA1 significantly influenced community structure ($F_{1,37}=6.81$, $p<0.001$) and accounted for much of the variance explained by the model (43.6%). The second axis db-RDA2 also significantly influenced community structure ($F_{1,37}=2.85$, $p<0.05$) but explained less variance (18.3%). The environmental factors most positively associated with db-RDA1 were the reach sediment and native vegetation cover, whereas db-RDA2 was more associated with the mean January air temperature. The leptophlebiid mayfly *Deleatidium* and conoesucid caddisfly *Olinga* were abundant taxa positively associated with db-RDA1, whereas invertebrate taxa negatively associated with the same axis included the hydrobiid snail *Potamopyrgus*, the amphipod *Paracalliope*, and oligochaete worms.

The invertebrate communities characterized by the eDNA samples but identified to the NEMS level of taxonomic resolution (N_eDNA) had seven environmental variables that explained variation in community structure: the proportion (%) of native vegetation and glacial landcover upstream, the % riparian shading in the upstream reach, the substrate index, the mean January and upstream air temperatures, and the mean upstream annual rain days (Table 8, Figure 4b). In the partial db-RDA model for the eDNA NEMS macroinvertebrates (Figure 4b), both the db-RDA1 ($F_{1,38}=8.72$, $p<0.001$) and db-RDA2 ($F_{1,38}=3.77$, $p<0.001$) axes significantly influenced community structure, although db-RDA1 accounted for more of the variance explained by the model (45.6%). Environmental factors negatively associated with db-RDA1 included the substrate index and native vegetation cover. Three mayflies (*Deleatidium*, *Coloburiscus*, and *Ameletopsis*) were abundant taxa negatively associated with this axis, whereas the invertebrate taxa positively associated with the same axis included oligochaete worms, cladoceran microcrustaceans, and the cnidarian *Hydra*.

The invertebrate communities recorded from eDNA samples using the lowest possible level of identification had 10 environmental variables that explained variation in community structure: the proportion (%) of native vegetation, indigenous forest, and glacial cover upstream, the upstream mean channel slope, flow and substrate index, the maximum channel slope downstream, the mean upstream rain days in a year, and the mean January air temperature (Table 8; Figure 4c). In the partial db-RDA model for the eDNA macroinvertebrates with the lowest level of identification (Figure 4b), both axes presented in Figure 4c significantly influenced community structure with db-RDA1 ($F_{1,35}=5.80$, $p<0.001$) and db-RDA2 ($F_{1,35}=2.81$, $p<0.01$), in addition to db-RDA3 ($F_{1,35}=2.39$, $p<0.05$). However, the first axis accounted for much of the variance explained by the partial db-RDA model (31.2%). Environmental factors positively associated with the first axis included indigenous forest land cover and channel slope. An abundant macroinvertebrate taxon also positively associated with the first axis was the mayfly *Coloburiscus humeralis*, whereas taxa negatively associated with the same axis including the limbriculid

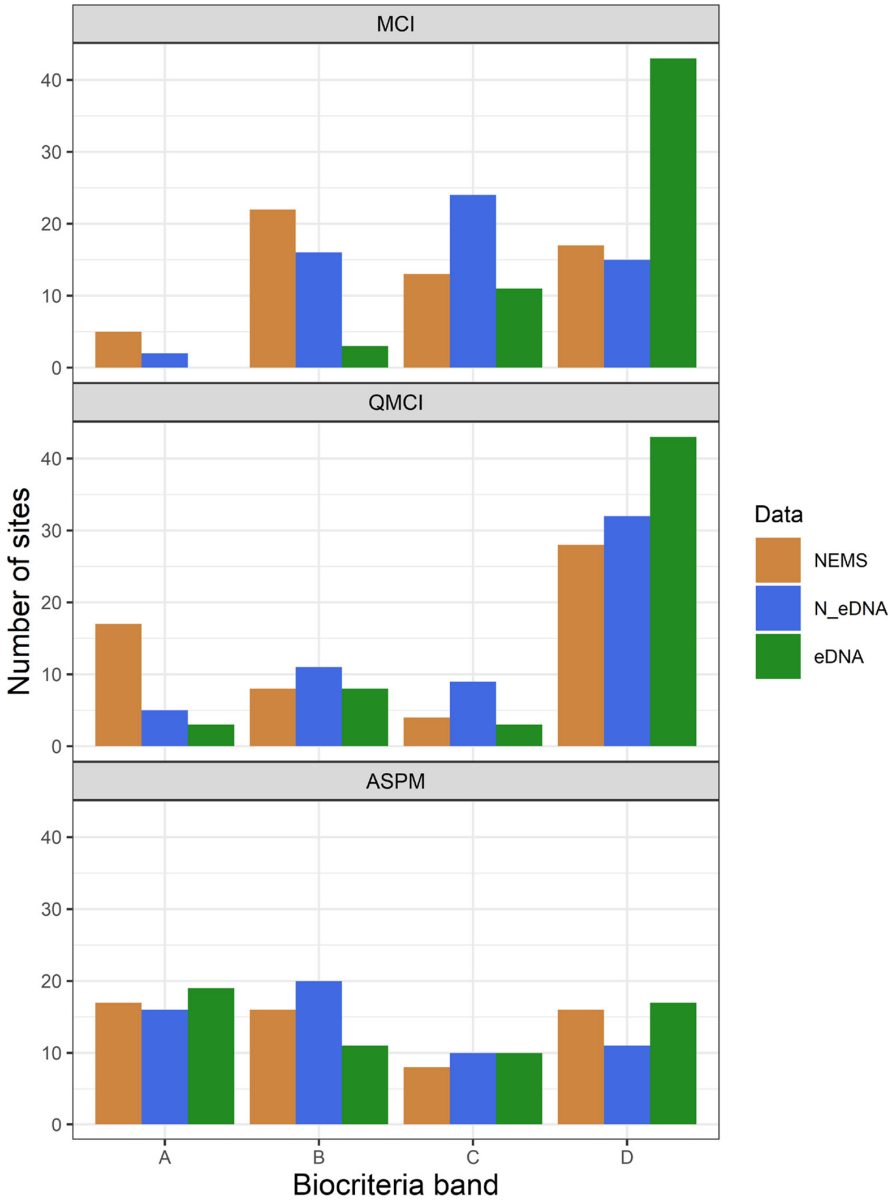

FIGURE 3 | Number of sites allocated to each of the four biocriteria bands (NPS-FM) used in ANZ for three macroinvertebrate indices (MCI, QMCI, and ASPM) when calculated using data from NEMS methods, N_eDNA, or eDNA.

worm *Lumbriculus variegatus* and several species of naidid worm such as *Chaeogaster diaphanus*.

There were three environmental predictors that were common to all three db-RDAs (Table 8). These were the mean predicted January air temperatures at the segment scale (indicating temperatures during the austral summer), the proportion of the upstream catchment in native vegetation, and the weighted score for sediment particles at the reach scale. Most of the environmental variables identified as important predictors for the NEMS data were also included in models for the N_eDNA and eDNA data, which included other predictors not identified in the NEMS data.

Variance partitioning analyses complemented the db-RDA models by separating the variation in community structure explained by the environmental and spatial predictors described above into their individual and shared components. For the macroinvertebrate communities collected and identified using the NEMS methodology, the environmental predictors independently explained 13.4% of the variation in community structure (Figure 5a; $F_{7,37}=2.23$, $p<0.001$), contrasted by 7.6% independently explained by the spatial predictors ($F_{8,37}=1.61$, $p<0.001$). The joint influence of environmental and spatial predictors explained 16.1% of the variation in community structure, with the remaining 62.8% unexplained as residual variation.

The macroinvertebrate communities recorded by eDNA to the NEMS level of taxonomic resolution (N_eDNA) showed that the environmental predictors independently explained 18.4% of the variation in community structure (Figure 5b; $F_{7,38}=2.74$, $p<0.001$), contrasted by 6.9% independently explained by the spatial predictors ($F_{7,38}=1.64$, $p<0.001$). The joint influence of

TABLE 8 | Summary of environmental predictors selected for db-RDA models testing the influence of the environment and spatial factor on macroinvertebrate communities measured using kick-net sampling (NEMS), eDNA at an equivalent level of taxonomy to the NEMS, and eDNA at the lowest possible level of identification.

Scale	Factor	Abbreviation	NEMS	N_eDNA	eDNA
Macroscale	Climate	**SegJanAirT**	Y	Y	Y
		USDaysRain		Y	Y
		USAvgTNorm		Y	
	Hydrology	SegFlow4th			Y
	Landuse	USGlacier		Y	Y
		USIndigFor	Y		Y
		USNative	Y	Y	Y
Mesoscale	Physical	DSDist2Coa	Y		
		SegSlopeSq	Y		
		USAvgSlope			Y
		DSMaxLocal			Y
	Riparian	SegRipShad	Y	Y	
Microscale	Reach	**ReachSed**	Y	Y	Y

Note: Y indicates inclusion in models; Bold text indicates environmental predictor that were consistently selected in the db-RDA models. See Table 2 for full explanations of the environmental predictors.

environmental and spatial predictors explained 12.6% of the variation in community structure, with the remaining 62.1% unexplained as residual variation. The environmental predictors were able to independently explain 5.0% more variation in community structure using the eDNA data when compared to the NEMS data.

The macroinvertebrate communities recorded by eDNA to the lowest possible level of identification (eDNA) showed that the environmental predictors independently explained 14.6% of the variation in community structure (Figure 5c; $F_{9,35}=2.05$, $p<0.001$), contrasted by largest amount (9.5%) independently explained by the spatial predictors ($F_{8,35}=1.74$, $p<0.001$). The joint influence of environmental and spatial predictors explained 11.1% of the variation in community structure, with the remaining 64.7% unexplained as residual variation. The overall gains in independently explaining community structure using environmental predictors with the eDNA data at the lowest possible level of identification were more modest (1.2%) when compared to the NEMS data.

4 | Discussion

Our results show that biotic metrics derived from eDNA are highly congruent with the same indicators generated from traditional biomonitoring methods relying on benthic sampling and morphological-based taxonomic identification. We found the greatest congruency when eDNA data was collated to the same level of taxonomic identification as the NEMS kicknet samples (N_eDNA). Metrics that were calculated from eDNA data at the lowest possible level of taxonomic identification generally showed less concordance with the NEMS metrics. This difference was more pronounced with the semi-quantitative QMCI that accounts for community composition by abundance or read count. This lower congruency between the QMCI when calculated from the NEMS and eDNA data may reflect the variability in DNA shedding rates from different invertebrates, and environmental degradation of genetic material as if flows downstream. As such, the read counts may not accurately reflect the actual abundance of organisms, as some taxa might be overrepresented while others may be underrepresented. Nevertheless, we found that the greatest differences in QMCI scores between landuse classes was observed from the N_eDNA or eDNA data, while the NEMS data showed the least difference. If we assume that landuse changes act as a large-scale stressor on invertebrate communities that can change the relative abundance of different taxa (e.g., Allan 2004), then the read count data may actually be providing more useful information about the relative abundances of taxa within a sub-catchment, as opposed to the reach-scale assessments of relative abundance that are obtained from NEMS sampling.

Some metrics performed better than others when compared with the NEMS equivalents. The multi-metric ASPM index was highly congruent with the NEMS data, and this result was consistent across the eDNA datasets. These differences in the performance of key metrics have management implications if biocriteria are applied to indices generated from eDNA data. Nonetheless, eDNA data was able to detect the same key environmental drivers of community composition, such as stream types by land use, and continuous environmental predictors in constrained ordinations. Overall, our results highlight that biomonitoring using eDNA has the potential to transform ecological studies and environmental management in Aotearoa New Zealand (ANZ) and internationally.

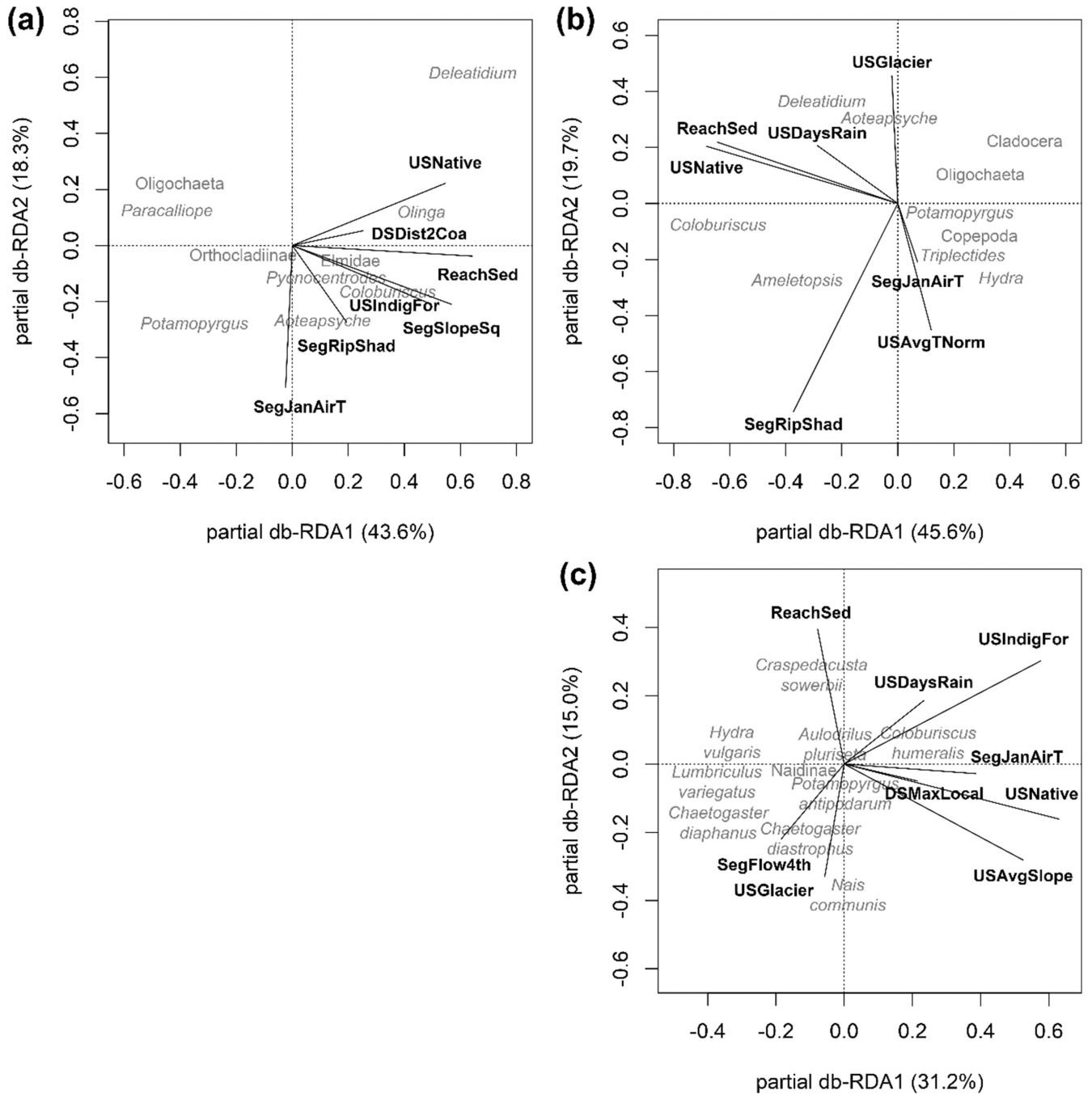

FIGURE 4 | Results from partial distance-based redundancy analyses (db-RDA) showing the influence of environmental predictors (black) on macroinvertebrate community data measured by (a) kick-net sampling (NEMS), (b) eDNA at an equivalent level of taxonomic resolution (N_eDNA), and (c) eDNA at the lowest possible level of identification (eDNA). The spatial Principal Coordinates of Neighbors Matrices (PCNM) predictors are conditioned out in these plots. The 10 most abundant macroinvertebrate taxa in each dataset are shown in gray.

4.1 | Comparisons of Biotic Indices and Taxon Richness

We found that most eDNA-derived metrics showed good congruency when compared to the traditional approach using kick-net sampling. Deficiencies in these comparisons can best be partially explained by limitations in current sequence libraries and ecological knowledge. First, we found that both average and total taxonomic richness was significantly higher from the eDNA data matrix than from either the NEMS data, or the eDNA taken to the same level of identification as NEMS (N_eDNA). This finding is similar to results of Elbrecht et al. (2017), Turunen et al. (2021), and Vourka, Karaouzas, and Parmakelis (2023) and is not surprising, as many invertebrate groups are only identified to coarse taxonomic levels when using the NEMS protocols. In contrast, Brantschen, Blackman, and Altermatt (2021) reported that eDNA sampling identified significantly fewer taxa per site than kick-net sampling from 92 rivers sites in Switzerland. They found that eDNA significantly under-represented groups such as Hemiptera, Arachnida and Coleoptera, and attributed this to a

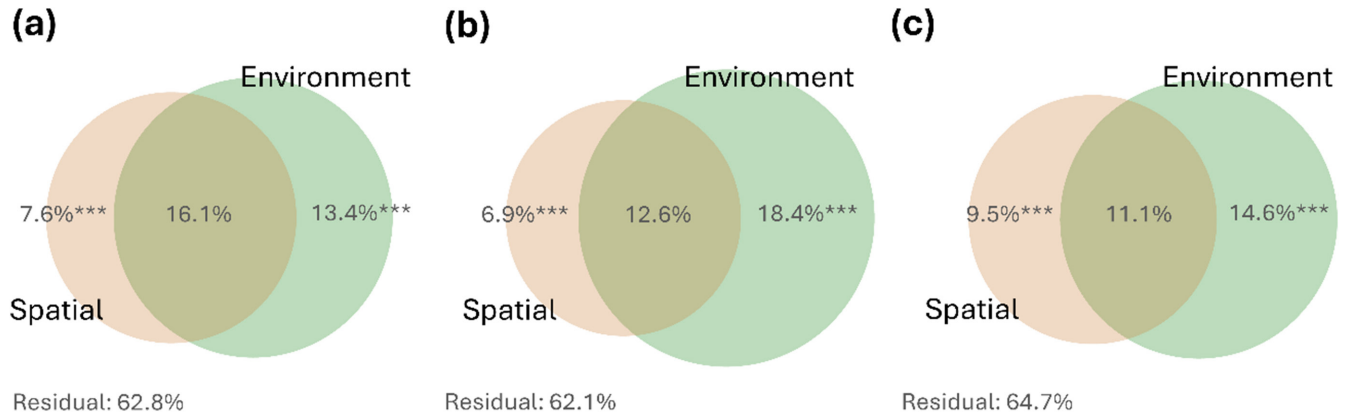

FIGURE 5 | Results from variation partitioning analyses showing the proportion of variation explained by environmental and spatial predictors for macroinvertebrate community data measured by (a) kick-net sampling (NEMS), (b) eDNA at an equivalent level of taxonomic resolution (N_eDNA), and (c) eDNA at the lowest possible level of identification (eDNA). ***$p < 0.001$.

mixture of their hydrophobic exoskeletons and their lower DNA shedding rates. We also found that the N_eDNA data identified fewer taxa than the NEMS data for all groups containing more than one taxa. Potential reasons for this reduced detectability include shortcomings of eDNA assays caused by incomplete barcode libraries or primer bias (Pawlowski et al. 2018), low quality DNA due to insufficient sample preservation, or unknown effects during sampling due to the low concentration and heterogeneous distribution of DNA molecules in the water column (Ficetola et al. 2015; Hering et al. 2018). Work is currently underway within ANZ to target specific invertebrate groups (e.g., crustaceans, caddisflies and mayflies); consistent with international efforts through the International Barcode of Life (iBOL) initiative to improve the ability for eDNA to adequately characterize biodiversity globally.

Secondly, we found that the MCI calculated with eDNA data showed a non-linear relationship with the MCI generated from NEMS data. Certain invertebrates, such as naidid worms are common in streams and are relatively well described in sequence libraries. However, the autecology of these species is often poorly understood, meaning that the currently used default tolerance score for Oligochaeta (1) may not be appropriate for all species. Assigning a single default score for a Class as diverse as the Oligochaeta is likely to greatly obscure their relationship with the environment. This may help explain why the MCI scores generated from the eDNA data were much lower than scores from the NEMS data, especially when the latter score was less than 100, indicating sites in "Fair" condition. A similar situation would also occur for many other species rich groups such as Hydra, Ostracods, Nematoda and Platyhelminthes, all of which have relatively low MCI tolerance scores. These high-level taxonomic groupings would be scored with their respective tolerance score only once at each site for both the NEMS and N_eDNA assessments, but would have been assigned the same low score for each species within a site using the eDNA. By doing so, these low-scoring taxa may have down weighted the overall MCI score when calculated using eDNA data. In contrast, the autecology of other invertebrate groups, such as the EPT taxa is much better understood, and thus their tolerance scores (usually assigned to genera) are likely to be more ecologically meaningful when assigned to species in the eDNA dataset.

A third reason for the potentially large difference in taxonomic richness between the three methods is that traditional NEMS sampling collects invertebrates from pooled replicate kick samples from a clearly defined area of stream bed. The resultant sample thus consists only of invertebrate taxa living in the sampled area, along with often large quantities of fine sediment and organic matter such as algae, macrophytes or leaf litter. Two technical challenges exist with processing these samples: finding individual taxa amongst this material, and then accurately identifying them. Furthermore, the NEMS methodology counts and identifies only the first 200 individuals in a sample. While the rest of the sample is scanned for rare taxa, it is likely that smaller invertebrates such as nematodes, micro-crustaceans, and water mites are simply missed.

These weaknesses sharply contrast with characterizing invertebrate communities at a site using eDNA. First, collecting replicate eDNA samples ($n = 6$ in the present study) is effectively sampling a much larger area of stream bed, as invertebrate eDNA is shed from all upstream areas. This increases sampling intensity, and collecting six replicates has been shown to capture up to 87% of total species richness at a site, when compared to the number of species collected from 16 replicates (Melchior and Baker 2023). Second, the ability to detect taxa present in each 1-L sample using PCR to detect and amplify DNA is likely superior to the equivalent visual 200+ fixed count (with a scan for rare taxa). eDNA techniques are not only sampling a greater area of stream for invertebrates, but can detect and identify taxa with greater efficiency and accuracy than traditional techniques. Depending on the reason for biomonitoring, this could offer greater benefits than traditional kick sampling, especially for large-scale biodiversity assessments, or identifying presence of pest organisms within catchments. However, for smaller reach-scale studies such as investing effects of point-source discharges, eDNA may not be as useful, as eDNA drifting from upstream areas could still be present in affected areas, and thus may mask any local loss of taxa.

Apart from taxonomic richness, other metrics showed a high degree of congruency across the environmental gradients that the sampling sites encompassed. The Average Score Per Metric (ASPM) performed the best of all metrics using data generated

from both the NEMS and the eDNA. The ASPM is a multi-metric index combining the MCI, EPT richness, and %EPT abundance. Its performance possibly reflects the sensitivity of EPT taxa to environmental stressors, as well as the weighted bias towards taxa occupancy based on the MCI and EPT richness. Another aspect of this metric is that it also includes the relative proportion of sensitive EPT taxa, which when combined with the other metrics, might help provide something of a "portfolio effect" (a statistical averaging effect) for estimating environmental conditions. The statistical favorable properties of the ASPM regressions (e.g., a slope close to 1 and an y-intercept of nearly 0) were reflected in the ability of this metric to show very good congruence in the number of sites assigned to different NPS-FM bands when comparing the three datasets. This result is helpful, as it implies that the multi-metric ASPM index is robust to the deficiencies in current sequence libraries and our knowledge of invertebrate autecology, so could be used in routine biomonitoring to effectively complement the traditional sampling approaches.

Another obvious advantage of eDNA was the much higher number of taxa identified from the 53 streams than was possible using the NEMS level of identification. This data can provide insightful taxonomic data on occurrence of endangered or data deficient species, and can identify species whose early larval stages cannot be separated morphologically. This advantage was highlighted by Turunen et al. (2021) who compared biotic indices derived from morphological and eDNA at 36 sites in northern Finland, and who also found that DNA metabarcoding produced far more extensive species level inventories for groups for which morphological identification was not feasible.

4.2 | Effects of Landuse

Catchment land use is a strong driver of instream responses (e.g., Allan 2004), underscoring the often-quoted adage "the valley rules the stream" (Hynes 1975). As such, we expected that the eDNA data would display the biggest differences in both overall community composition and calculated biotic indices, as the response of individual species to environmental variables were assumed to be more nuanced than the responses of taxa grouped to a higher level of taxonomic resolution. For example, Stein et al. (2014) compared biotic metrics calculated with data derived from morphological identifications or DNA barcoding to detect differences in stream condition of six paired stream sites in southern California with subtle impacts to their instream habitat conditions. The 16 biotic metrics calculated with morphological data showed subtle but not significant differences in community composition between reaches, but the statistical power of 10 of these metrics was higher when calculated using DNA.

Contrary to this expectation, however, was the finding that eDNA data did not necessarily perform better in terms of showing the effects of land-use on invertebrate community composition. Although differences in overall species composition amongst the land use classes were most apparent using the N_eDNA data, the eDNA data displayed the least difference in community composition. In contrast, the biggest differences between the calculated biotic indices and landcover were from the eDNA data, while the indices derived from the NEMS data differed the least. The reasons for these somewhat contradictory results are unclear, however all three datasets reported similar patterns in the data, with differences in both community composition and biotic indices between sites in Indigenous Forest and Agriculture, and Indigenous Forest and Urban. Such patterns agree with many other studies investigating the effects of land use on invertebrate communities, both within ANZ (Harding and Winterbourn 1995; Hall, Closs, and Riley 2001) and elsewhere (Allan 2004).

4.3 | Biological and Environmental Relationships

The macroinvertebrate data generated from eDNA identified similar environmental responses demonstrated by the NEMS samples collected with kicknets. Community analyses using constrained ordination showed that three environmental predictors consistently explained variation in community composition using the NEMS, N_eDNA, and eDNA approaches. One of these predictors, the modeled mean January air temperature, reflects a strong latitudinal gradient in climate. Another consistent environmental predictor was the proportion of upstream catchment land cover in native vegetation, aligning with our earlier assessment of land use influences, and with work by Death and Collier (2010), who found that the percentage of catchment vegetation in native forest had stronger relationships with invertebrate communities than segment or reach scale measures of riparian vegetation. Other vegetation predictors that contributed to at least two of the models included the proportion of upstream catchment land cover in indigenous vegetation, further supporting the earlier work by Death and Collier (2010). Modeled riparian shading at the segment scale was also another important driver of macroinvertebrate community composition, confirming observations of Burdon et al. (2020) that woody riparian vegetation can be a strong determinant of ecosystem health. The last environmental predictor that consistently explained variation in community composition was benthic sediment composition, represented as a weighted score of substrate categories. This substrate index is strongly influenced by deposited fine sediment, which has been identified as a key determinant of benthic macroinvertebrate community composition in ANZ (Burdon, McIntosh, and Harding 2013). Benthic substrate heterogeneity also helps determine the number of niches present (Beisel, Usseglio-Polatera, and Moreteau 2000), and national studies have highlighted that coarser substrates generally support greater richness and abundances of stream macroinvertebrates (Quinn and Hickey 1990).

Variation partitioning analyses showed the most variation in macroinvertebrate community composition was explained by the N_eDNA data, and least by the NEMS data. This possibly reflected the more accurate identification of taxa using the N_eDNA approach, coupled with phylogenetic conservation of macroinvertebrate-environment relationships at this coarser level of taxonomy. The variation partitioning analyses showed that the influence of spatial location was stronger with the eDNA data, most likely reflecting species distributions being more strongly driven by biogeography than taxa grouped at a coarser level of taxonomy. However, in spite of differences in the strengths of the variance partitioning models and the subtle

differences in the results of the constrained ordination, the use of eDNA data appeared to show the same overall patterns in invertebrate community composition.

Brantschen, Blackman, and Altermatt (2021) compared macroinvertebrate communities assessed with eDNA and kick-net sampling in 92 river sites throughout Switzerland, and also found strong congruence between both methods for assessments of total indicator community composition. Moreover, they found that the ecological classification of rivers based on indicator taxa resulted in similar biotic index scores for both the kick net and the eDNA data. Furthermore, a study by Turunen et al. (2021) in northern Finland found that impacts of mining on invertebrate community structure could only be shown using DNA metabarcoding, suggesting that the finer taxonomic resolution can improve detection of subtle impacts. These results and ours suggest that using eDNA can indeed provide reliable and potentially improved assessments of the ecological state of waterways as the use of traditional methods. Brantschen, Blackman, and Altermatt (2021) also eruditely highlighted that any mismatch between the two methods (eDNA versus morphological) does not mean that the one method is inherently more (or less) less accurate, as both approaches are proxies (each with their inherent error) of the true ecological state that is being estimated by sampling.

4.4 | Implications for Biomonitoring Using eDNA

Although we calculated biotic indices such as the MCI and QMCI from both NEMS and eDNA data, these indices were based on using the original tolerance values for each taxon based on the original work of Stark (1985) for hard bottomed streams, and Stark and Maxted (2007) for soft bottomed streams. Using these tolerance values would likely have resulted in a large loss of information inherent in biotic indices where individual tolerance values represent the sensitivity of specific taxa to anthropogenic stresses. For example, both the NEMS and N_eDNA data grouped all 63 oligochaete species into a single taxonomic group, with a low tolerance score. This misses the fact that many oligochaete species are likely to be characteristic of less impacted environments, and would consequently have a higher tolerance score. Allocating all oligochaete species to a low tolerance score would explain why the eDNA data classified more sites in the D band than either the NEMS or the N_eDNA data, as there could be multiple oligochaete species at a site, each scoring 1.

More work is needed to develop more nuanced tolerance values at the species level to improve the use of indices such as the MCI and QMCI with highly resolved taxonomic data from metabarcoding. Consistent with global trends, there has been a very large uptake in eDNA monitoring throughout ANZ, resulting in an extensive eDNA database from over 2500 sites nationwide. This database offers an unparalleled opportunity to generate new tolerance values for many of the invertebrate species identified by eDNA using the same iterative technique as used by Stark and Maxted (2007) and Greenwood (2015). Although eDNA data has recently been used to develop a taxonomic independent community index (TICI) for New Zealand streams that is highly correlated to the MCI, and by inference to overall stream "health" (Wilkinson et al. 2024), the TICI score is based on all organisms encountered in streams. Given the historic precedents and the current requirements of environmental legislation (NPS-FM), the reliance on indices such as the MCI creates a strong argument for the development of eDNA equivalents (i.e., MCI scores for metabarcoded data), based on macroinvertebrate species occurrences. This would enable environmental managers to monitor their sampling sites more efficiently using eDNA and/or metabarcoded invertebrate samples (Elbrecht et al. 2017), and still create assessments of stream health based on the antecedent metrics.

5 | Conclusions

Environmental DNA has the potential to revolutionize biomonitoring (Pawlowski et al. 2021; Takahashi et al. 2023). These improvements are needed to help address the growing global biodiversity crisis and increased information needs of environmental managers. eDNA provides significant benefits in terms of improved taxa identification, enhanced sampling (more sites in a day, more frequently), and ability to generate species lists for not only macroinvertebrates, but other organisms (e.g., fish) relevant to management, such as invasive species (Ruppert, Kline, and Rahman 2019; Yao et al. 2022). Our results suggest that eDNA biomonitoring gives complimentary results when compared with traditional methods and appears to perform at least as well as traditional data at detecting patterns in invertebrate community composition at a national scale. However, further work is required to develop more nuanced and accurate tolerance values at the species level for taxa that are presently identified to high taxonomic levels such as Class (e.g., Copepoda and Oligochaeta) or Phyla (e.g., Nematoda and Platyhelminthes). Development of new tolerance values at the species level is likely to greatly to improve the sensitivity of common biotic indices such as the MCI and QMCI which are used to describe stream health. These developments will enable better environmental monitoring and reporting outcomes, in a similar way, that eDNA is being used by European water managers to meet the increased information demands of the WFD (Fernández et al. 2019; Pont et al. 2021). These improvements will ultimately provide more accurate and timely information on the status of ecosystem health and biodiversity at spatial scales ranging from catchments and regions to nationally and globally.

Author Contributions

Alastair M. Suren and Shaun P. Wilkinson conceived the study; all authors helped acquire data; Shaun P. Wilkinson led the eDNA analysis and bioinformatics; Francis J. Burdon and Alastair M. Suren contributed to the data analysis; Alastair M. Suren and Francis J. Burdon wrote the first draft; all authors discussed the results and made edits.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.