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ORIGINAL ARTICLE

DNA metabarcoding captures different macroinvertebrate biodiversity than morphological identification approaches across a continental scale

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Abstract

DNA-based aquatic biomonitoring methods show promise to provide rapid, standardized, and efficient biodiversity assessment to supplement and in some cases replace current morphology-based approaches that are often less efficient and can produce inconsistent results. Despite this potential, broad-scale adoption of DNA-based approaches by end-users remains limited, and studies on how these two approaches differ in detecting aquatic biodiversity across large spatial scales are lacking. Here, we present a comparison of DNA metabarcoding and morphological identification, leveraging national-scale, open-source, ecological datasets from the National Ecological Observatory Network (NEON). Across 24 wadeable streams in North America with 179 paired sample comparisons, we found that DNA metabarcoding detected twice as many unique taxa than morphological identification overall. The two approaches showed poor congruence in detecting the same taxa, averaging 59%, 35%, and 23% of shared taxa detected at the order, family, and genus levels, respectively. Importantly, the two approaches detected different proportions of indicator taxa like %EPT and %Chironomidae. DNA metabarcoding detected far fewer Chironomid and Trichopteran taxa than morphological identification, but more Ephemeropteran and Plecopteran taxa, a result likely due to primer choice. Overall, our results showed that DNA metabarcoding and morphological identification detected different benthic macroinvertebrate communities. Despite these differences, we found that the same environmental variables were correlated with invertebrate community structure, suggesting that both approaches can accurately detect biodiversity patterns across environmental gradients. Further refinement of DNA metabarcoding protocols, primers, and reference libraries–as well as more standardized, large-scale comparative studies–may improve our understanding of the taxonomic agreement and data linkages between DNA metabarcoding and morphological approaches.

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KEYWORDS
benthic macroinvertebrates, bioassessment, DNA Metabarcoding, indicator taxa, morphological

1 | INTRODUCTION

The unprecedented decline of global freshwater biodiversity has prompted an urgent need to predict the extent of its declines, drivers of change, and modifications to ecosystem functioning (Jackson et al., 2016; Tickner et al., 2020). Benthic macroinvertebrates are routinely used in aquatic biomonitoring to track environmental health due to their sensitivity to changes in water quality, including those brought on by anthropogenic causes (Barbour et al., 1999; Kenney et al., 2009). Governments worldwide use standardized aquatic biomonitoring approaches to guide regulations on acceptable pollutant levels, freshwater management, and conservation at large spatial and temporal scales (e.g., U.S. Environmental Protection Agency National Aquatic Resource Survey [EPA NARS, 2022], Canadian Aquatic Biomonitoring Network [CABIN, 2022], Europe's Water Framework Directive [WFD, 2022]). Despite the global need for rapid bioassessment, these routine biomonitoring programs typically employ traditional morphology-based taxonomic identification, a costly and time-consuming approach that requires substantial taxonomic expertise and can produce subjective taxonomic identifications (Yu et al., 2012). Because biomonitoring is a foundational tool for environmental decision-making, it is critical that the current methods used for obtaining biodiversity data are both efficient and robust.

Recent advances in DNA-based technologies can overcome many of the challenges associated with traditional, morphological-based biomonitoring techniques (Baird & Hajibabaei, 2012; Blackman et al., 2019; Bush et al., 2019). DNA metabarcoding requires the isolation, amplification, and sequencing of organismal DNA from environmental samples, which include environmental DNA (eDNA) samples that include trace DNA of organisms in the water; or bulk benthic samples that include community DNA of whole organisms (Deiner et al., 2017). Advantages of DNA metabarcoding over traditional approaches for biomonitoring include standardized and scalable procedures via high-throughput sequencing, enabling more samples to be processed and automated, and reproducible identification of taxa from reference databases (Porter & Hajibabaei, 2018). These advantages create the potential for spatially and temporally expanded, time-efficient, and streamlined biomonitoring that is more consistent across research groups. Yet, the taxonomic assignment that metabarcoding relies upon is still subject to inaccuracies because of reference database incompleteness and primer bias (Keck, Couton, & Altermatt, 2022). However, DNA metabarcoding methods can be tuned to optimize taxon detection in ways that morphological methods cannot by (1) using multiple primer sets to overcome primer bias (Elbrecht et al., 2017; Hajibabaei et al., 2019), and (2) using taxonomy-free approaches to overcome reference database gaps (Apothéloz-Perret-Gentil et al., 2021).

Traditional morphology-based taxonomic assignment currently sets the baseline for biomonitoring programs, but is subject to individual taxonomic expertise, availability of identification reference material, and condition of the sample, which can lead to large discrepancies in taxonomic assignments (Haase et al., 2010). Additionally, morphology-based methods have several other drawbacks: (1) they focus only on morphologically identifiable biodiversity, ignoring meiofauna, and cryptic taxa; (2) they often identify individuals from a sub-sample, so not every individual is identified; and (3) they routinely discard juvenile or damaged individuals that cannot be identified because they lack characteristic morphological features (Cordier et al., 2021; Elbrecht et al., 2017). While there are clear advantages and disadvantages of both morphological and metabarcoding methods, it is both timely and critically important to clarify how DNA metabarcoding and morphological identification differ in detecting aquatic biodiversity and to identify their sources of bias and error (Bush et al., 2019).

There is growing interest from researchers and practitioners in using DNA metabarcoding as a supplement to, or a replacement for, traditional morphological identification approaches (Pawlowski et al., 2020). However, standardized comparisons of morphological and DNA metabarcoding approaches across large spatial scales are lacking for aquatic benthic macroinvertebrates (Duarte et al., 2021; but see Gibson et al., 2015; Keck et al., 2022). Further, most studies are not standardized to scalable sampling protocols with comparable measurements or are not open-sourced. These shortcomings could stem from the general challenge of completing comparative aquatic research across large spatiotemporal scales (Goodman et al., 2015). Despite the known advantages, biases, and limitations of both DNA metabarcoding and traditional morphological approaches (Duarte et al., 2021), widespread adoption of DNA metabarcoding for biomonitoring hinges on its ability to match existing identification methodologies. Resource managers are often bound by legislative mandates to use morphological identification approaches in benthic macroinvertebrate assessments and are hesitant to adopt DNA metabarcoding because of its lack of comparability to current methodologies and existing long-term biomonitoring data sets (Bush et al., 2019; Poikane et al., 2016). Thus, it is critically important to better understand how both approaches perform across large spatial scales using standardized, scalable methods.

Here, we compare the biodiversity and community structure of stream benthic macroinvertebrates detected by traditional morphological identification and DNA metabarcoding using data from the National Ecological Observatory Network (NEON). NEON is a continental-scale ecological monitoring program designed to collect and provide open ecological data from sites across the United States (Keller et al., 2008). The NEON platform enables a paired comparison of aquatic macroinvertebrate biodiversity using both DNA

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metabarcoding and traditional morphological identification across broad spatial and temporal scales. Our approach was fourfold: first, we compared macroinvertebrate taxonomic richness detected by both methods; second, we compared macroinvertebrate taxonomic composition and indicator taxa identified by both methods; third, we compared macroinvertebrate assemblages detected by both methods; fourth, we investigated if environmental variables are associated with differences in site-level variation in communities identified by both methods. By investigating how these two approaches differ in detecting aquatic macroinvertebrate taxonomic richness, diversity, and assemblages, we hope to illuminate how to interpret the information provided by these two approaches, their sources of bias and error, and whether they complement each other, such that they better capture total aquatic macroinvertebrate biodiversity when paired.

2 | METHODS

2.1 | Data sources and processing

We used NEON's data portal (https://www.neonscience.org/data) to download both the "Macroinvertebrate Collection" (DP1.20120.001), and "Macroinvertebrate Metabarcoding" (DP1.20126.001) datasets (2022 release version, timespan of 2018-06-01 – 2020-09-01, downloaded on 22 July 2022). Briefly, the "Macroinvertebrate Collection" (hereafter, morphological) dataset comprises benthic macroinvertebrate samples collected three times per year (spring, summer, and fall) at wadeable stream sites (1 km long stream reach) using habitat-appropriate sampling devices (e.g., Surber sampler for riffles and runs, D-frame sweep net for pools). Eight samples were collected for morphological identification, five in the dominant habitat type (e.g., riffle, run, pool, step pools), and three in the sub-dominant habitat type which are determined by prior habitat mapping efforts. Three samples were collected for DNA metabarcoding in the dominant habitat type. Morphological samples were preserved in ethanol in the field and then shipped to a taxonomy lab (Rithron Associates Inc. or EcoAnalysts Inc.) for identification using morphological characteristics. Samples were subject to taxonomic precision by comparing whole-sample identifications with a second taxonomist.

The "Macroinvertebrate Metabarcoding" (hereafter, DNA metabarcoding) dataset comprises benthic macroinvertebrate samples collected using the same methods and dominant habitat as the morphological dataset. Samples were shipped to a commercial lab (Jonah Ventures, 2020) for DNA extraction, PCR amplification, sequencing, and bioinformatics. Briefly, community samples were homogenized with a hand immersion blender and extracted using the Qiagen DNeasy Powersoil Kit. All samples were amplified using two primers from the CO1 gene (CO1 F230 fragment, Gibson et al., 2015; Folmer et al., 1994; CO1 BE fragment Hajibabaei et al., 2012). PCRs were conducted as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 40 s at 94°C, 1 min at 46°C, 30 s at 72°C, and a final elongation at 72°C for 10 min. Samples were purified using Exo1/SAP, and pooled, normalized, and indexed. The average fragment length for the library was determined on a TapeStation, and then sequenced on an Illumina MiSeq using the v2 500-cycle kit (Product Ref: MS-1022002). The sequencing run included 10 pM of library with a 15% PhiX spike-in as a control. Sequencing success and read quality was verified using FastQC v0.11.8, and reads were demultiplexed using Illumina-utils v2.6 (iu-demultiplex; https://github.com/merenlab/illuminautils) with default settings. A custom reference database was generated using NCBI GenBank, and taxonomy was assigned to Exact Sequence Variants (ESV) compiled from Usearch. For full methodological details, see Appendix S1 or NEON's documentation for each method (Morphological: https://data.neonscience.org/data-products/DP1.20120.001#documentation; Metabarcoding: https://data.neonscience.org/data-products/DP1.20126.001#documentation).

We considered samples taken by both sampling approaches at the same time, in the same location within the dominant habitat type, and at the same site as "paired," such that they should capture similar benthic macroinvertebrate biodiversity ($n = 179$ sample-level comparisons across 24 sites). However, because the two samples collected were in different locations with a sub-habitat type, and because macroinvertebrate taxa are heterogeneously distributed (even within the same habitat), we expected to observe some variation in macroinvertebrate taxa between the two samples (Barnes et al., 2013; Bush et al., 2019).

To fit environmental covariates to community data, we downloaded field site metadata that included average annual temperature (°C) and precipitation (mm/year), elevation (m), and watershed size (km^2). We downloaded water quality data from NEON's data portal (DP1.20288.001) that included conductivity (μS/cm) turbidity (FNU), dissolved oxygen (%), pH, chlorophyll a (μg/L), and fluorescent dissolved organic matter (fDOM, QSE).

We used NEON's 24 wadeable stream sites with at least 1 year of paired data between the morphological and metabarcoding data sets. These sites range from 18° latitude in the Tundra domain of Alaska to 65° latitude in the Atlantic Neotropical domain of Puerto Rico (Figure 1a, see Table S1 for site metadata). Data were filtered to taxonomy tables that indicate the specific taxa identified from each method and further filtered to include only taxa from the Phylum Arthropoda. Arthropoda are the most used in biomonitoring programs because of their sensitivity to environmental changes (Chang et al., 2014). Samples were paired by matching the "sampleID" column from the morphological dataset to the "dnasampleID" column in the metabarcoding data set. Comparisons between each method were made at three different taxonomic resolutions (i.e., order, family, genus), to represent different levels of biomonitoring programs and macroinvertebrate studies used to detect changes in communities and their responses to environmental conditions (Jones, 2008; Martin et al., 2016). We did not compare methods at the species level because 80% of taxa in the morphological dataset and 68% of taxa in the metabarcoding dataset were not identified to species.

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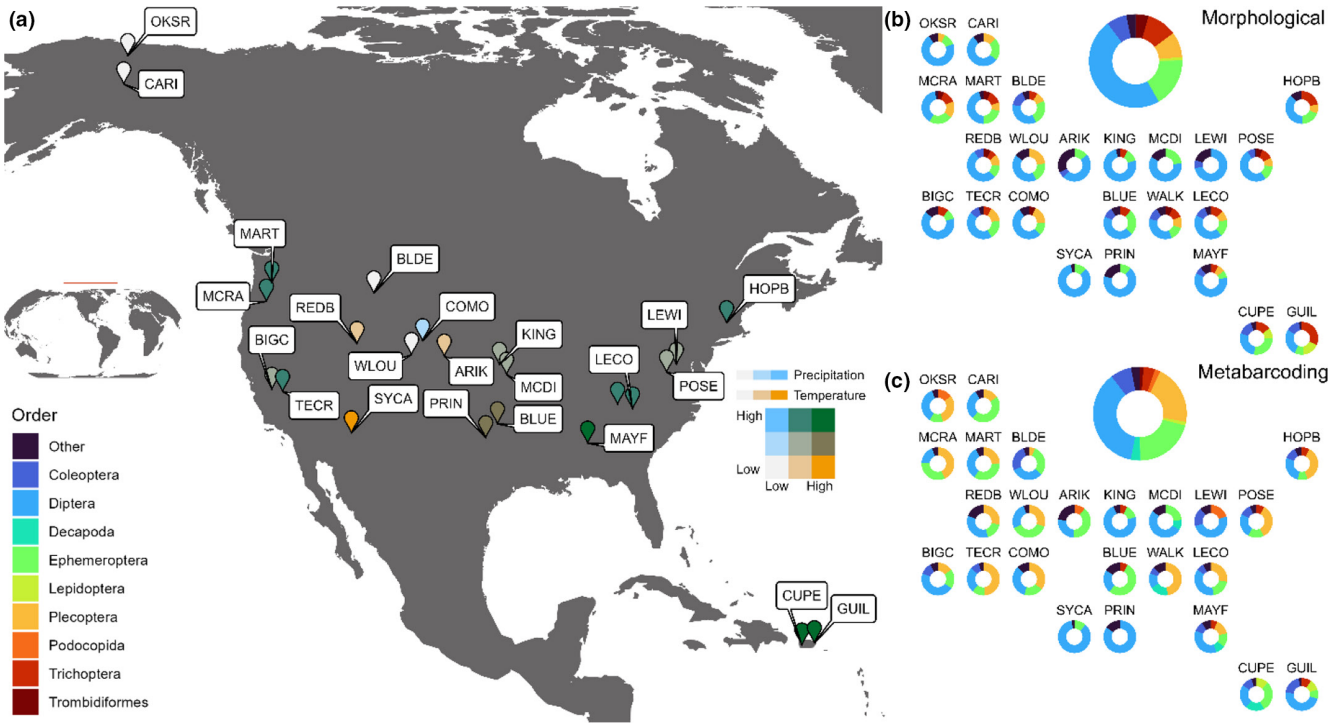

FIGURE 1 Map of NEON sites used in this study (a) color of the site indicates local climate. Relative proportion of taxa at the Order resolution across all sites (Gamma diversity, large donut), and at each site (Alpha diversity, small donuts) for the morphologically identified dataset (b), and DNA metabarcoding dataset (c). Sites are arranged in approximate geographic positions in B and C, with four letter abbreviations (Table S1). Orders with <1% relative proportion were grouped into the "other" category.

2.2 | Taxonomic richness

To compare the macroinvertebrate taxonomic richness detected by both approaches, we examined both alpha (i.e., total species detected at an individual site) and gamma (i.e., total number of taxa detected across sites) diversity of paired samples ($n = 179$ comparisons). First, to test whether one identification approach detected higher alpha diversity than the other, we used the log-ratio (Hedges et al., 1999). Briefly, we calculated the log-ratio $ln(A/B)$, where A is the total alpha diversity detected by DNA metabarcoding and B is the total alpha diversity detected by the morphological identification. The log-ratio is an effect size index, and the value here is positive when A is greater than B, negative when B is greater than A, and zero when A and B are equal. We used a linear mixed model with the site as a random effect in an intercept-only model to test whether the mean log-ratio was significantly different from zero across the three different focal taxonomic resolutions. Second, we used Pearson correlations to evaluate the similarity between alpha diversities detected by DNA metabarcoding and morphological identification across the three different focal taxonomic resolutions. Lastly, we calculated genera accumulation curves to evaluate the rate at which each identification method accumulates new genera. We used the R packages "lmerTest" (Kuznetsova et al., 2017) to conduct linear mixed models, "stats" (R Core Team 2022) to conduct Pearson's correlations, and "vegan" (Oksanen et al., 2022) to create genera accumulation curves.

2.3 | Taxonomic composition and indicator taxa

To compare macroinvertebrate taxonomic composition (i.e., the taxonomic identities of detected individuals) detected by each approach, we first calculated the relative proportion of each unique taxa detected across all sites at the three focal taxonomic resolutions as the frequency of occurrence of each unique taxa/total frequency of all unique taxa × 100. Second, we calculated three relative fractions: diversity detected by morphological identification only, diversity detected by DNA metabarcoding only, and the shared diversity detected by both approaches. Fractions were calculated following the approach outlined in Keck, Blackman, et al. (2022). Briefly, each fraction of diversity was divided by the total diversity detected by both approaches (see Figure 7 for a graphical description). Next, we used a beta regression to test for differences in the diversity detected between each fractional group (morphological only, metabarcoding only, and shared), including each fractional group as an independent variable. In cases where relative proportion values contained zeros and ones (genus and order taxonomic resolution), we transformed data following $(y \cdot (n-1)+0.5)/n$ where y is the fraction of diversity and n is the sample size (Smithson & Verkuilen, 2006). We performed post hoc pairwise comparisons across groups using least-squared means to test for significant differences among the fractional groups, using the "betareg" (Cribari-Neto & Zeileis, 2010) and "emmeans" (Lenth, 2022) packages in R.

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Second, to compare indicator taxa detected by each approach, we examined two biotic integrity metrics commonly used in biomonitoring: percent of total taxa in the community from the orders Ephemeroptera, Plecoptera, and Trichoptera (%EPT), and percent of total taxa in the community from the family Chironomidae (%Chironomidae). These taxa are often used for detecting changes in stream conditions associated with pollution and other disturbances because of their sensitivity to disturbances (Herman & Nejadhashemi, 2015). Generally, a high proportion of %EPT indicates good stream health, and a high proportion of Chironomids indicates poor stream health (Compin & Céréghino, 2003; Serra et al., 2017). We calculated the proportion of both %EPT and %Chironomidae for each unique sampleID ($n = 179$) across both identification approaches. We divided the number of unique genera detected within each group by the total number of distinct genera detected in the sample and used Pearson correlations to compare how DNA metabarcoding and morphological identification perform at detecting these indicator taxa (Emilson et al., 2017).

2.4 | Benthic macroinvertebrate assemblages

To compare macroinvertebrate assemblages detected by each approach, we used a multivariate ordination analysis. Benthic macroinvertebrate assemblages were grouped by the unique paired sample ID's ($n = 179$), and Jaccard dissimilarity distances were calculated between communities detected by DNA metabarcoding and morphological identification. We used nonmetric multidimensional scaling (NMDS) to ordinate communities using $k = 3$ dimensions and 1000 maximum iterations using the "vegan" package in R (Oksanen et al., 2022). To test whether there was a difference in the centroids and dispersion of assemblages detected by the two approaches, we used a Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson & Walsh, 2013; Oksanen et al., 2022). To test the correlations between environmental variables and NMDS configurations, we used two separate ordinations for each approach and discarded assemblage data from Oksrukuyik Creek, Alaska (OKSR) because no water quality data existed for it. We used the env.fit function in the "vegan" package in R to fit environmental covariates to each method-specific ordination and estimate the strengths and directions of their correlation with the NMDS configuration. We performed all statistical analyses in R version 4.1.3 (R Core Team 2022).

3 | RESULTS

3.1 | Taxonomic richness

Across all 24 NEON wadeable streams, gamma diversity was higher from the DNA metabarcoding identifications when compared with morphological identifications. DNA metabarcoding detected more than twice as many taxa compared with morphology across the focal order, family, and genus taxonomic levels (Figure 2). For the morphological approach, over half of the diversity detected was also detected by DNA metabarcoding across all taxonomic levels (blue bars, Figure 2). In other words, DNA metabarcoding detected more unique taxa that were not detected by morphological identification. The mean log-ratio values did not significantly differ from zero at the genus (linear mixed model, intercept = −0.041, Z-value = −0.613, $p = 0.546$) or family (linear mixed model, intercept = 0.111, Z-value = 1.827, $p = 0.081$) taxonomic resolutions (Figure 3). However, the mean log-ratio value of 0.114 was significantly different from zero at the order level (linear mixed model, intercept = 0.1464, Z-value = 3.793, $p < 0.001$), indicating that DNA metabarcoding detected more Arthropod orders at the alpha diversity scale. Pearson correlations between alpha diversity detected by each approach showed that DNA metabarcoding alpha diversity was significantly positively correlated with morphological alpha diversity at all three taxonomic resolutions (Figure 4). With increasing taxonomic resolution, the strength of the association between DNA metabarcoding alpha diversity and morphological alpha diversity increased (e.g., $R = 0.24$ at the order resolution and $R = 0.44$ at the genus resolution, Figure 4). Visual inspection of the number of points above the 1:1 line indicates that DNA metabarcoding detected more unique orders and families than morphological identification. Genera accumulation

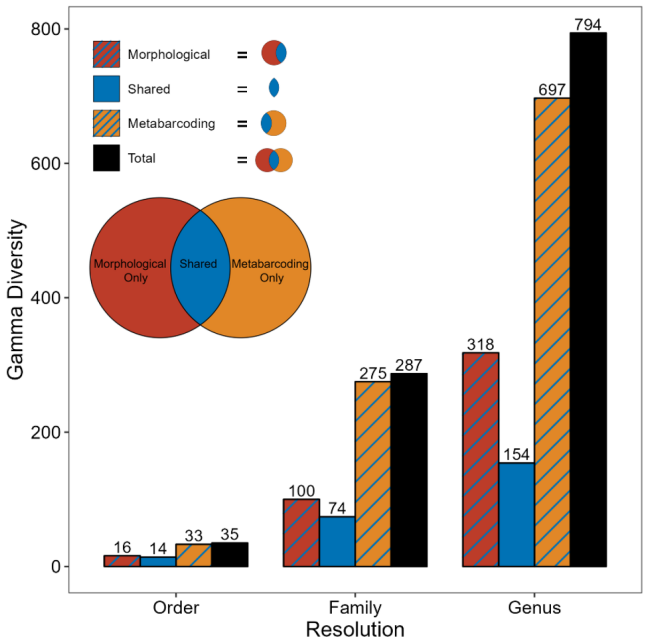

FIGURE 2 Total richness (Gamma diversity—the total number of taxa identified by each approach) across all sites at the Order, Family, and Genus taxonomic resolution. Venn diagram indicates how the data are conceptualized into different fractions of diversity detected by each approach. Legend further indicates how each bar represents different portions of the Venn diagram. Morphological and Metabarcoding bars are represented with a striped pattern to indicate the estimates of diversity including taxa detected by both approaches.

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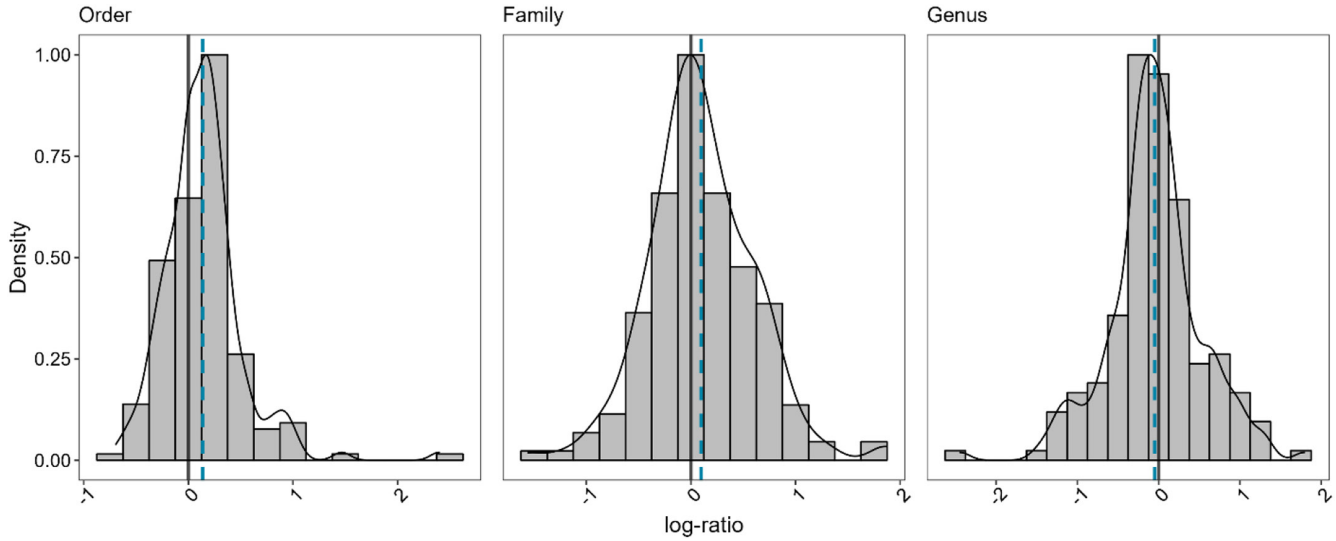

FIGURE 3 Histograms and density estimates of the log-ratio between the total diversity detected by DNA metabarcoding and the total diversity detected by morphological identification. Panels indicate which taxonomic resolution the log-ratio analysis was performed at. Blue dashed line indicates the mean log-ratio for that taxonomic resolution. Note differences in the x-axis scale between taxonomic resolutions.

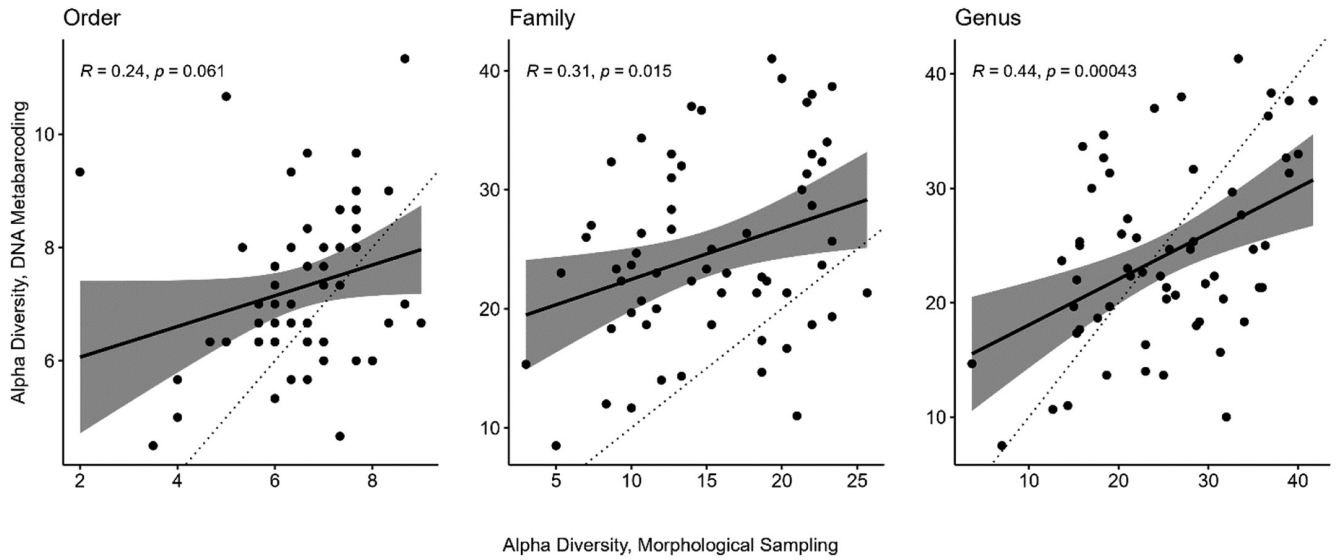

FIGURE 4 Pearson correlations between taxonomic richness detected by the two identification approaches (DNA metabarcoding, y-axis, and traditional morphological identification, x-axis). Solid line indicates the line of best fit, and the dotted line indicates 1:1 fit for visual comparison between approaches. Shaded areas surrounding solid line represent 95% confidence interval. Note differences in the x-and-y-axis scales between taxonomic resolutions.

curves suggest that DNA metabarcoding detects taxa at a faster accumulation rate than morphological data (Figure 5). The curve for morphological sampling indicates that this approach detected a high proportion of common genera, indicated by the steep initial slope and early plateau, suggesting that the overall genera diversity that can be captured by this method can be saturated with relatively few samples (Figure 5). On the other hand, the genera accumulation curve for DNA metabarcoding indicates that this approach detected a higher proportion of rare genera, indicated by the long slope and absent plateau, suggesting that the overall genera diversity that can be captured by this method has not yet reached saturation (Figure 5).

3.2 | Taxonomic composition and indicator taxa

Comparisons of proportions of major taxonomic groups across three focal taxonomic resolutions showed that DNA metabarcoding detected a lower proportion of Diptera, Trichoptera, and Trombidiformes taxa compared with morphological identification. However, DNA metabarcoding detected a greater proportion of Coleoptera, Decapoda, Ephemeroptera, and Plecoptera (Figures 1 and 6, and Figure S5).

The proportion of diversity detected varied across different fractional groups (morphological only, shared, and DNA metabarcoding only) and taxonomic resolutions (order, family, genus; Figure 7). For

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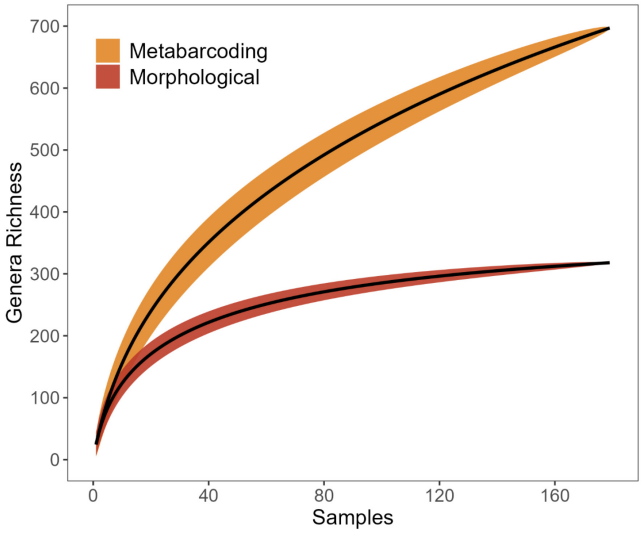

FIGURE 5 Genera accumulation curves for DNA metabarcoding and morphological identification methods for benthic macroinvertebrates across 179 unique sampling occasions at 24 sites across the U.S. Curves are estimated based on the richness of unique genera detected by each method. Shaded areas represent ±2 standard deviations.

example, the proportion of diversity detected by each individual approach increased with more specific taxonomic resolutions: a greater proportion of diversity was detected by only DNA metabarcoding and only morphological identification at the genus taxonomic level than order (Figure 7). The proportion of diversity detected by both approaches decreased with more taxonomic resolution: a greater proportion of diversity was detected by both approaches at the order level than genus (Figure 7). Specifically, across all 179 unique paired samples at the 24 NEON sites, the mean percent of taxa shared between both approaches was 59.7%, 35.2%, and 23.0% at the order, family, and genus taxonomic resolution, respectively (Figure 7, Figure S1). At the order level, the fraction of shared diversity detected by both approaches was significantly higher than both the fraction of diversity detected by morphological identification only (beta regression interaction $p<0.001$) and DNA metabarcoding only (beta regression interaction $p<0.001$; Figure 7). Additionally, the fraction of diversity detected by DNA metabarcoding only was significantly higher than the fraction of diversity detected by morphological identification only at the order taxonomic level (beta regression interaction $p<0.001$). At the family taxonomic resolution, the fraction of taxa detected by only morphological identification was significantly lower than both the shared taxa detected by both approaches (beta regression interaction $p<0.001$), and the taxa detected by DNA metabarcoding (beta regression interaction $p<0.01$). At the genus level, the fractions of diversity detected by only morphological identification and only DNA metabarcoding were both significantly higher than the shared diversity detected by both approaches (beta regression interactions $p<0.001$). Fractions of diversity did not greatly vary spatially across sites at either of the three taxonomic resolutions (Figures S2–S4).

Pearson correlations indicated that both the %EPT and %Chironomidae detected with DNA metabarcoding were significantly positively correlated with the %EPT and %Chironomids detected with morphological identification (Figure 8). %EPT was more highly correlated than %Chironomidae, and more points were above the 1:1 line, suggesting that DNA metabarcoding was better at detecting EPT taxa (Figure 8). However, more points were below the 1:1 line with %Chironomids, indicating that morphological identification was better at detecting Chironomidae (Figure 8). %EPT and %Chironomidae varied spatially across sites, with some sites detecting higher proportions of these indicator taxa with morphological sampling and vice-versa (Figures S6 and S7).

3.3 | Benthic macroinvertebrate assemblages

The NMDS ordination reached successful convergence after 36 iterations (stress = 0.172, $k=3$). At $k=2$, no convergence was reached after 1000 iterations and stress values were above 0.2; thus, we retained the results from the NMDS ordination at $k=3$ (Clarke, 1993). PERMANOVA analysis indicated that the assemblages detected by each approach were significantly different ($R^2=0.0306$, $F=11.225$, $p=0.001$; Figure 9). Assemblages detected by morphological identification tended to be more tightly clustered than assemblages detected by DNA metabarcoding (Figure 9).

3.4 | Environmental covariates

Separate method-specific ordinations fit with environmental covariates indicated that all variables were significant in explaining the variation in our NMDS ordination (Figure S8, Table S2). Temperature ($R^2=0.65$ Metabarcoding, $R^2=0.72$ Morphological), conductivity ($R^2=0.52$ Metabarcoding, $R^2=0.44$ Morphological), and elevation ($R^2=0.35$ Metabarcoding, $R^2=0.29$ Morphological) were the strongest predictors for both approaches (Table S2). Chlorophyll a ($R^2=0.16$ Metabarcoding, $R^2=0.13$ Morphological) and turbidity ($R^2=0.08$ Metabarcoding, $R^2=0.09$ Morphological) were the weakest predictors for both approaches. Vectors for each environmental covariate point toward the same sites between methods, indicating that the same environmental variables drive site-level variation in the communities as represented in the NMDS ordination (Figure S8).

4 | DISCUSSION

Freshwater biodiversity loss is accelerating rapidly across the globe (Lynch et al., 2023), making reliable and scalable biodiversity assessment frameworks a requirement for monitoring, decision-making, and conservation efforts (IPBES, 2019; Tickner et al., 2020). DNA metabarcoding shows potential for streamlined, cost-effective, and highly accurate biodiversity assessments that are scalable across ecosystems (Buchner et al., 2021; Stein et al., 2014). However,

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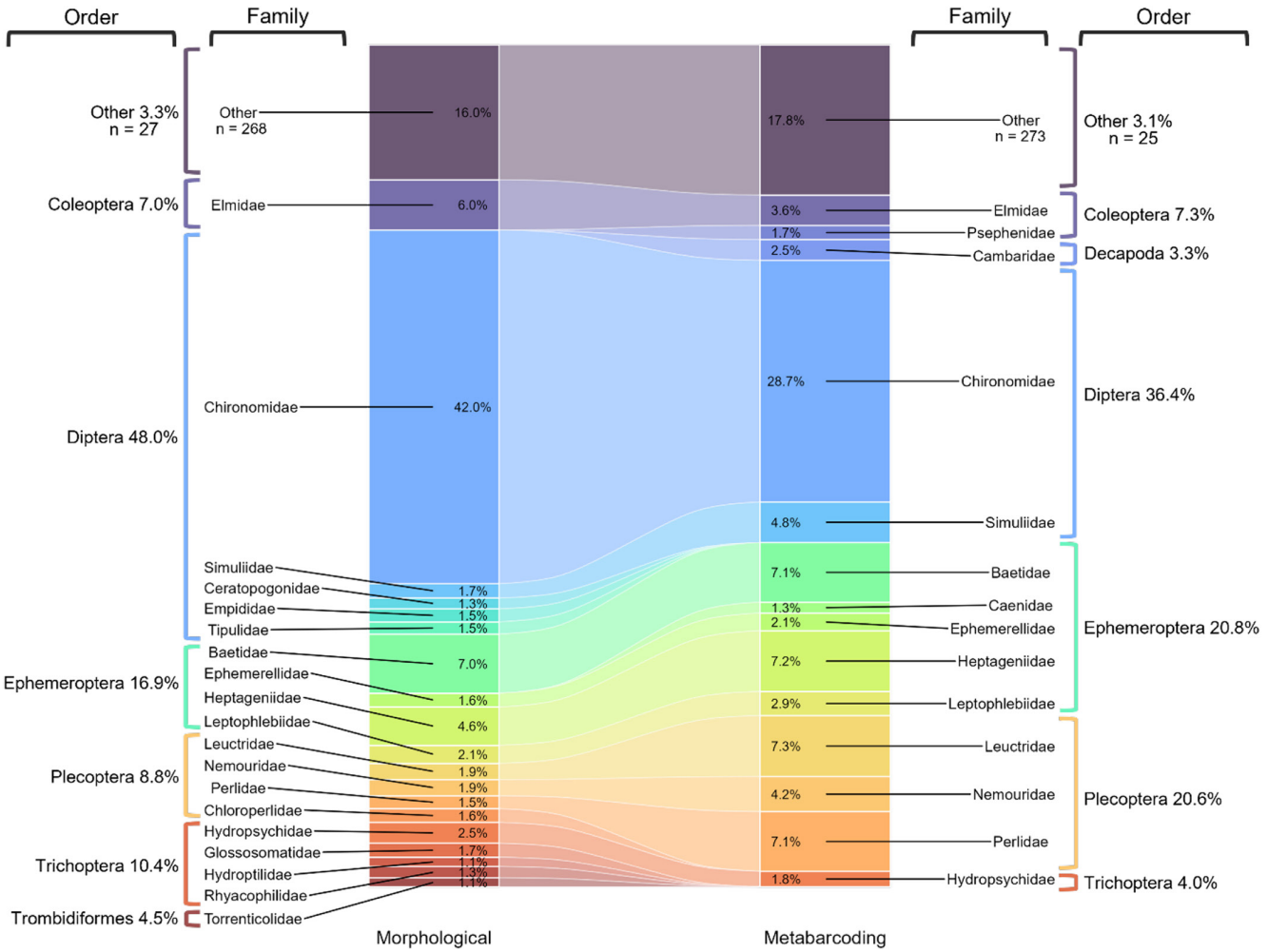

FIGURE 6 Relative proportions of taxa detected across all sites by morphological and DNA metabarcoding identification approaches. Stacked bars indicate proportions of taxonomic groups detected by each approach (frequency of occurrence of each unique taxa/total frequency of all unique taxa × 100). Flows between the stacked bars connect the same families. The outer brackets and percentages indicate proportions of taxa aggregated at the order taxonomic resolution. Families with proportions smaller than 1% were grouped into the "Other" category.

large-scale comparative studies between DNA metabarcoding and morphological approaches are lacking, but important in linking these two approaches to ensure the continuity of long-term biomonitoring datasets and further refine DNA metabarcoding approaches (but see Brantschen et al., 2021; Elbrecht et al., 2017; Emilson et al., 2017). Here, we compared benthic macroinvertebrate biodiversity detected by DNA metabarcoding and traditional morphological approaches to illuminate whether they provide similar taxonomic identification across a broad spatial scale using scalable, open-source data. We found that DNA metabarcoding detected twice as many unique taxa than morphological identification, but that the two approaches detected different macroinvertebrate taxonomic composition and assemblages. We also found detection biases for important indicator taxa like %EPT and %Chironomidae between the two methods. Our work highlights key tradeoffs between DNA metabarcoding and morphological identifications, suggesting a data fusion approach could leverage the strengths of each approach to better capture macroinvertebrate biodiversity (Pawlowski et al., 2018).

DNA metabarcoding and morphological identification detected similar numbers of taxa but from different taxonomic groups. While DNA metabarcoding detected twice as many taxa as traditional approaches across all sites (gamma diversity), both approaches detected similar taxonomic richness at the local or site scale (alpha diversity). Additionally, genera accumulation curves provided further support that DNA metabarcoding detected rare and unique taxa. The morphological genera accumulation curve plateaued at ~300 genera, while the curve for DNA metabarcoding peaked at ~700 genera and never plateaued. This could indicate some taxonomic familiarity bias by individuals identifying the taxa (i.e., they were more likely to detect shared taxa and miss rare taxa across the NEON network). On the other hand, richness could be inflated with DNA metabarcoding due to false or inaccurate taxonomic assignments because of the limited availability and quality of reference databases (Keck, Couton, & Altermatt, 2022). Regardless, detecting similar numbers of taxa between approaches at the local scale is not informative if the taxa identified are different, as indicated by our results.

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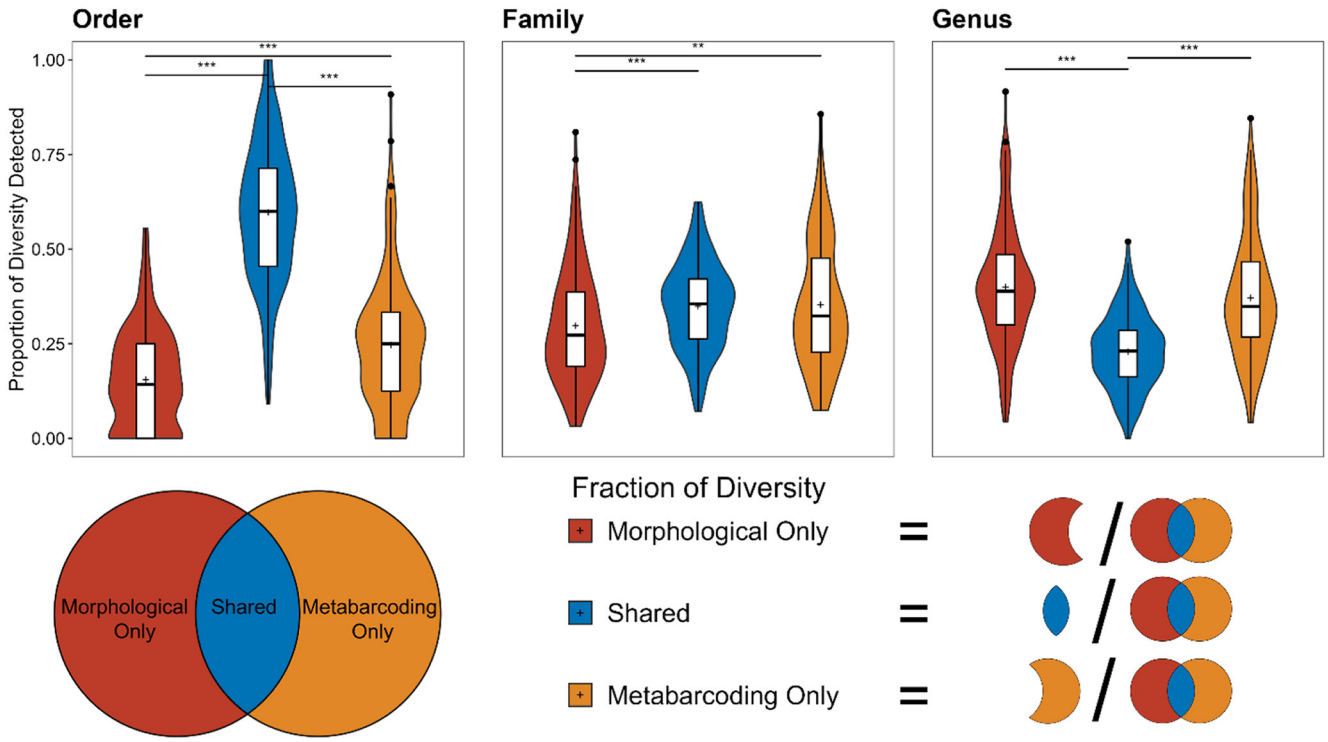

FIGURE 7 Relative fraction of diversity detected by morphological identification only, shared diversity detected by both approaches, and DNA metabarcoding only across different taxonomic resolutions. Boxplots and violin plots indicate full ranges and + symbols indicate means. Connecting lines with significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) indicate significant differences in means from beta regression. Venn diagram and the derivation of the different fractions of diversity are depicted below.

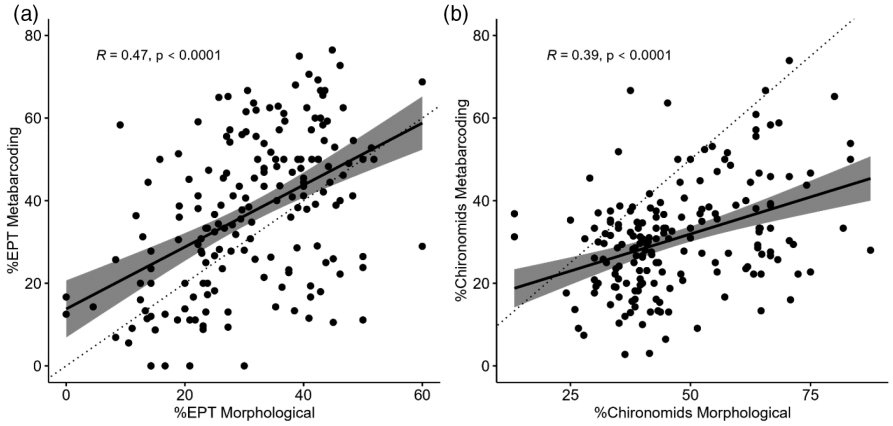

FIGURE 8 Pearson correlations between % Ephemeroptera, Plecoptera, and Trichoptera orders (%EPT, a) and %Chironomids (b) detected between both identification approaches (DNA metabarcoding, y-axis, and traditional morphological identification, x-axis). Solid line indicates the line of best fit, and the dotted line indicates 1:1 fit for visual comparison between approaches. Shaded areas surrounding the solid line represent 95% confidence interval. Note differences in the x-and-y-axis scales between panels a and b.

Macroinvertebrate taxonomic composition is essential to making inferences about stream health and ecosystem function because specific taxonomic identities indicate healthy or poor-quality stream ecosystems (Pander & Geist, 2013). We found that DNA metabarcoding and morphological identification detected different taxonomic composition and communities across different taxonomic resolutions at the local scale. Only an average of 35% and 23% of taxa matched across sites at the family and genus taxonomic resolution, respectively (Figure S1). The low agreement between these two approaches could be explained by a combination of incomplete reference libraries used to assign taxonomic identities (Bush et al., 2020; Weigand et al., 2019), primer and amplification biases where some taxonomic groups are unequally or poorly amplified (Duarte et al., 2021; Elbrecht & Leese, 2015; Hajibabaei et al., 2019; Leese et al., 2020), differences in the number of samples taken for each approach (NEON, 2022), or DNA metabarcoding's ability to detect

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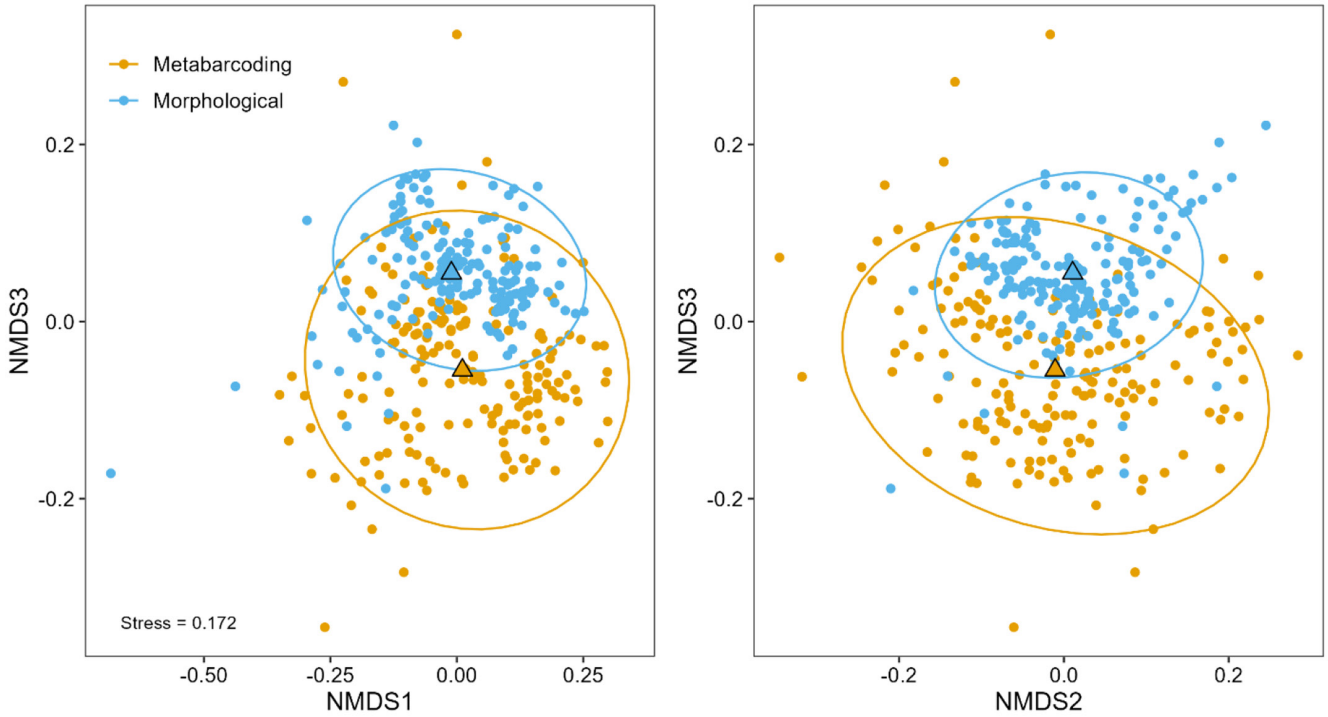

FIGURE 9 Nonmetric multidimensional scaling (NMDS) ordination of macroinvertebrate communities based on Jaccard dissimilarities for each of the taxonomic identification approaches (DNA metabarcoding and traditional morphological identification). The left plot shows NMDS axes 1 vs. 3, and right plot shows NMDS axes 2 vs. 3. The triangle symbols represent the centroid of each identification approach.

small arthropods (e.g., copepods and ostracods) that are not typically targeted by morphological approaches. This low agreement complicates resource managers' ability to compare aquatic biodiversity detected between approaches across broad spatial scales. Indeed, this underscores why many prospective users of DNA metabarcoding remain hesitant to adopt this approach: existing long-term datasets collected with traditional approaches capture a different subset of taxa than DNA metabarcoding. To boost the adoption of DNA metabarcoding in biomonitoring, co-developing monitoring tools with stakeholders that address the errors and biases in both genomic and traditional methods is critically important to linking existing datasets with DNA metabarcoding data and making them comparable (Aylagas et al., 2020). In some applications, it might be appropriate for DNA metabarcoding to act as a complementary approach with morphological identification, instead of a replacement, to detect rare taxa that are missed by morphological identification (Keck, Blackman, et al., 2022). Further, as reference databases become more comprehensive or taxonomy-free approaches gain traction (Apothéloz-Perret-Gentil et al., 2017), and as sequencing technologies advance to the point that metabarcoding can be replaced by shotgun sequencing for detection of organisms, DNA metabarcoding will be able to deliver on the promise of providing a near-census picture of all the biodiversity in a sample or system (Compson et al., 2020; Ficetola & Taberlet, 2023). This will usher in new possibilities for merging morphological and genomic approaches.

We also found that the two approaches exhibited detection bias for some taxonomic groups, including important indicator taxa. In this study, morphological identification detected a higher relative abundance of Diptera, Trichoptera, and Trombidiformes taxa compared with DNA metabarcoding, whereas DNA metabarcoding detected a higher relative abundance of Coleoptera, Decapoda, Ephemeroptera, and Plecoptera taxa. This finding is significant because these taxa are included in two widely used bioassessment indices, %EPT and %Chironomidae, where %EPT is typically used to assess stream health and %Chironomidae is used to assess how impaired a system is. These taxonomic groups are commonly used as indicator taxa in biomonitoring and assessment efforts to make inferences about water quality and stream health (Bonada et al., 2006; Buss et al., 2002). Because DNA metabarcoding detected far fewer pollution-tolerant Chironomidae and more EPT taxa than morphological identifications, conflicting inferences could be drawn about stream condition when using one approach over another. For example, %EPT and %Chironomid derived from DNA metabarcoding would indicate an overall healthier stream condition for most sites compared with morphological sampling because of the higher percentage of EPT taxa and lower percentage of Chironomids (Figures S6 and S7). These detection biases in benthic macroinvertebrate indicator taxa could be explained by primer choice, as some primers–including F230 and BE, primers that are specifically used in the NEON DNA metabarcoding pipeline–are known to underperform at detecting Dipteran taxa (Leese et al., 2020), while other primers are known to recover more EPT and Chironomidae taxa (Hajibabaei et al., 2019). Indeed, strong congruence between eDNA and morphological identification approaches was achieved using

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different primers than NEON's, but still struggled to detect important indicator taxa (Brantschen et al., 2021). Consequently, using multiple primer sets to minimize amplification bias is recommended to improve DNA metabarcoding's performance at detecting indicator taxa (Elbrecht et al., 2019).

Our study also provides important insights into environmental variables that drive variation in macroinvertebrate community composition observed from the two approaches. We found that the average annual temperature and precipitation, elevation, watershed size, conductivity, turbidity, dissolved oxygen, pH, chlorophyll a, and fDOM were all significant in explaining the variation in our NMDS ordinations, with temperature, elevation, and water conductivity explaining most of the variation for both approaches. Indeed, climatological factors such as temperature and precipitation drive benthic macroinvertebrate community production and composition (Patrick et al., 2019). Additionally, aquatic communities are structured by fluvial processes that shape local habitat characteristics, which vary with elevation (Rezende et al., 2014). Further, macroinvertebrates have varying tolerances to salinity in freshwater systems. Therefore, salinity plays a significant role in structuring communities (Shackleton et al., 2019) and is becoming a greater threat as freshwater ecosystems are becoming more saline due to road salt use and urbanization, particularly in higher latitudes (Kaushal et al., 2021). Our results suggest that both approaches can accurately detect environmental factors that structure macroinvertebrate community composition across the continental scale gradient of our study despite the major differences in the taxonomic identities detected by each approach. Future work should seek to better understand what drives macroinvertebrate community changes across broad spatial scales using DNA metabarcoding, given that macroinvertebrate communities are vulnerable to numerous stressors like pollution, habitat alteration, and climate change.

In conclusion, DNA metabarcoding and morphological identification captured different subsets of macroinvertebrate biodiversity in our study. Our results suggest that DNA metabarcoding for benthic macroinvertebrates should be considered as a different approach to traditional morphological identification rather than a replacement, corroborating the findings of a recent meta-analysis (Keck, Blackman, et al., 2022). While both approaches capture similar levels of alpha diversity, each method has different taxonomic biases, including important indicator taxa like %EPT and %Chironomidae. The two approaches could be used in tandem to better capture benthic macroinvertebrate biodiversity or to explore novel bioassessment tools that can utilize data from both approaches (e.g., food web modeling; Compson et al., 2018; Makiola et al., 2020). While DNA metabarcoding offers improved detection of macroinvertebrate taxonomic richness and diversity, widespread adoption among biomonitoring programs remains low because of its lack of comparability to existing morphological-based databases, and lack of method co-development and clear communication of project objectives among stakeholders and researchers (Aylagas et al., 2020; Bush et al., 2019; Hering et al., 2018; Sepulveda et al., 2020). Indeed, clearly defining program goals can help end-users identify "the right tool for the right job", or which approach or combination of approaches can be used to ensure the success of the program. Here, we presented a large comparison of standardized DNA metabarcoding and morphological identification approaches and found large differences in gamma diversity and the biodiversity detected between the two approaches. However, both approaches detected the same environmental drivers of community composition. Therefore, considering the fundamental errors and biases between the two approaches is critically important for future comparative studies. Ultimately, to improve freshwater biomonitoring, we suggest biomonitoring programs employ a data fusion approach that retains the strengths and corrects for the weaknesses of both approaches to obtain more accurate information on freshwater biodiversity.

AUTHOR CONTRIBUTIONS

Contributed to the conception and/or design of the study: SMW, ZGC, DCA; contributed to the acquisition, analysis, or interpretation of the data: SMW, ZGC, MCM, MHB VS; contributed to the writing of the manuscript: SMW, MCM, KTH.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Biological dataare open access and available at https://data.neonscience.org/data-products/DP1.20126.001 and https://data.neonscience.org/data-products/DP1.20120.001; Water Quality data are open access and available at https://data.neonscience.org/data-products/DP1.20288.001; Code for this article can be found at https://doi.org/10.5061/dryad.b2rbnzsmx.

ETHICS STATEMENT

The work presented here has not been published elsewhere, is not under consideration for publication at another journal.

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