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ORIGINAL ARTICLE

Insect Conservation and Diversity

Comparing species accumulation within taxonomically well- and poorly resolved insect groups over 37 years

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Abstract

1. While long-term datasets are invaluable for tracking changes in abundance and species richness, they may still fail to fully capture the diversity of taxonomically unresolved groups, such as many insect taxa.
2. Here, we used a unique 37-year (1969–2006) freshwater macroinvertebrate time series, collected over seven sites in central Germany, to study biodiversity patterns and species accumulation over time. Focusing on taxonomically well-resolved insect groups (Ephemeroptera, Plecoptera, Trichoptera [EPT]) versus poorly resolved "dark taxa" (Diptera), we hypothesized that accumulation curves would saturate only in well-resolved groups.
3. Over the study period, Diptera showed a continuous rise in both abundance and species richness, with accumulation curves not yet reaching a plateau.
4. In contrast, EPT richness increased with no overall abundance changes, and their accumulation curves reached a plateau despite considerably more sampled individuals compared to Diptera.

5. Our findings reveal a critical blind spot in biodiversity monitoring: highly diverse yet poorly resolved groups like Diptera remain underrepresented, even in very long, high-resolution time-series.

KEYWORDS
aquatic, conservation area, insect decline, insect monitoring, long-term, pristine

INTRODUCTION

Insect populations have become the focal point of biodiversity monitoring efforts due to widespread concerns and mounting evidence corroborating their decline globally (Dirzo et al., 2014; Hallmann et al., 2017; Van Klink et al., 2020, van Klink et al., 2022, van Klink et al., 2024; Desquilbet et al., 2021; Kalinkat et al., 2021; Jähnig et al., 2020; Cabrerizo & Marañón, 2022, Boyle et al., 2025, Hallmann et al., 2025). As key contributors to ecosystem functions, such as pollination, nutrient cycling and food web stability, insect losses can have cascading effects across trophic levels (Wagner et al., 2021). Detecting and interpreting insect population trends is complicated, especially since the rapid loss of insect biomass can distort the outcomes of monitoring activities because richness and evenness often do not change (Baranov et al., 2020; Enns et al., 2023). Our understanding of the long-term interactions between abundance, evenness, and species richness remains limited and should be improved by generating and examining long-term monitoring time-series.

Species–time relationships based on long-term sampling offer crucial insights into gradual shifts in abundance, evenness, and richness, and can reveal whether trends are directional or cyclical. These insights are critical for designing effective monitoring strategies that balance taxonomic resolution, sampling effort, and spatial coverage (White et al., 2006). Observations of long-term changes across both megadiverse and less species-rich taxonomic groups can provide a better understanding of the impact of long-term community turnover dynamics, thereby allowing us to refine expectations for biodiversity baselines and its inherent variability.

To unravel such complexity, individual-based rarefaction curves (i.e., accumulation curves) offer a valuable framework to understand relationships among biodiversity metrics in assemblages sampled through time, showing how changes in abundance and evenness combine to determine the magnitude of richness changes (Blowes et al., 2022). For example, when evenness and abundance change in the same direction, their effects can amplify changes in species richness and steepen species accumulation curves. In contrast, opposing trends in these components can counterbalance the effects that constrain the magnitude of changes in species richness, making biodiversity trends difficult to detect (Blowes et al., 2022). This masking effect occurs because a decline in species evenness may be offset by an increase in overall abundance, resulting in little or no apparent change in species richness, even though the community structure has shifted significantly. Thus, to address the full scope of biodiversity trends in the Anthropocene, there is a pressing need for approaches that integrate multiple components of diversity to more accurately reveal the underlying patterns of ecological change.

To this end, comparing the impact of turnover dynamics on species accumulation in taxonomically rich and taxonomically poor groups is a promising avenue for exploration. One major constraint lies in the difficulty of achieving species-level identification for many insect specimens, especially in monitoring time-series. While well-resolved, indicator groups of insects like Ephemeroptera, Plectoptera, and Trichoptera are favoured in monitoring programmes, a substantial proportion of flying insects in fact belong to highly diverse, understudied and poorly resolved groups, often referred to as "dark taxa" (Awad et al., 2025). These include, among terrestrial insects, the parasitoid micro-Hymenoptera and "lower" Diptera (Nematocera), while only the latter are abundant in aquatic ecosystems (Srivathsan et al., 2023). In both terrestrial and aquatic environments, the "dark taxa" problem impedes species-level biodiversity assessments and the subsequent development of conservation priorities. "Dark taxa" may also distort observed species abundance distributions by underrepresenting rare or cryptic species, biasing estimates of evenness and richness, and masking true ecological dynamics (Bickford et al., 2007; Buchner et al., 2025).

In this study, we used a unique 37-year-long quantitative dataset on aquatic insects to better understand the influence of "dark taxa" on abundance, richness, turnover, and accumulation patterns, by analysing differences in those patterns between two types of taxonomic groups: a taxonomically resolved group (including Ephemeroptera, Plecoptera, Trichoptera—hereafter "EPT") and a taxonomically poorly resolved group (Diptera). We showcase the unique value of long-term monitoring sites by mobilizing a dataset on insects from the Breitenbach stream, in Hesse, Germany, which was continuously studied by the Schlitz Limnological station (Max Planck Society) from 1969 to 2010 (Wagner et al., 2011). This is one of the best quantitative datasets on insects globally, as samples were taken at high resolution with standardized methods (emergence traps) and identified to species when possible. A subset of this dataset already revealed an 82% decline in abundance in the Breitenbach in just 42 years (Baranov et al., 2020), with other data yet to be analysed. Here, we analyse data from seven traps along the Breitenbach, including both previously published and unpublished data from these sites. The main objective of the present study is to elucidate species–time relationships using long-term datasets.

Specifically, we hypothesize that species accumulation curves will only approach saturation in taxonomically well-resolved groups, like the EPT. In contrast, for taxonomically unresolved "dark taxa" such as nematoceran Diptera, we expect accumulation curves to continue

TABLE 1 Taxonomic group-specific trap observation periods and summary of organisms caught.

Group	Trap	No. years	Total abundance	Total species richness	Δ Abund. ± SE	Δ species richness ± SE	Δ turnover ± SE
Diptera	A	15	53,365	274	0.67 ± 0.37	−0.00 ± 0.25	−0.92 ± 0.27
	B	36	139,662	283	0.62 ± 0.10	0.46 ± 0.08	−0.10 ± 0.06
	C	13	13,641	180	1.80 ± 0.66	1.44 ± 0.48	**1.22 ± 0.49***
	E	17	35,569	238	1.59 ± 0.47	0.86 ± 0.31	−0.30 ± 0.30
	G	15	105,795	294	1.00 ± 0.22	0.22 ± 0.16	−1.10 ± 0.23
	I	9	2270	39	**−1.19 ± 0.48***	0.15 ± 0.34	−0.24 ± 0.39
	III	10	9180	108	1.06 ± 0.78	**1.33 ± 0.59***	1.15 ± 0.67
	Overall	36	359,482	402	**7.36 ± 0.15***** (Intercept)	**3.80 ± 0.08***** (Intercept)	**0.23 ± 0.08**** (Intercept)
					9.99 ± 1.38*** (1st degree)	**6.70 ± 0.73***** (1st degree)	−2.08 ± 0.73 (1st degree)
						−2.20 ± 0.73** (2nd degree)	−3.26 ± 0.63 (2nd degree)
EPT	A	14	208,785	76	0.43 ± 0.17	0.00 ± 0.04	0.08 ± 0.18
	B	37	643,183	90	−0.28 ± 0.05	0.02 ± 0.02	0.14 ± 0.06
	C	13	98,689	62	−0.76 ± 0.25	**0.09 ± 0.03***	0.69 ± 0.23
	E	17	235,327	82	−0.05 ± 0.11	0.06 ± 0.04	0.06 ± 0.12
	G	15	164,752	98	−0.07 ± 0.13	0.05 ± 0.03	0.12 ± 0.10
	I	9	117,690	51	0.86 ± 0.22	0.14 ± 0.05	0.51 ± 0.43
	III	9	116,367	53	0.62 ± 0.23	−0.02 ± 0.04	−0.48 ± 0.31
	Overall	37	1,584,793	115	**9.43 ± 0.11***** (Intercept)	**3.73 ± 0.04***** (Intercept)	**−1.18 ± 0.04***** (Intercept)
					0.09 ± 0.82 (1st degree)	0.45 ± 0.13 (1st degree)	**1.52 ± 0.35***** (1st degree)
						−0.27 ± 0.10 (2nd degree)	−0.26 ± 0.34 (2nd degree)

Note: Apart from a single year of missing EPT data from trap C, all other observation periods were complete. Traps specific models indicate the change (Δ) in metrics over time, while overall models were modelled with a second-degree polynomial. Significant values are shown in bold.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

rising over time, reflecting unresolved diversity and the persistent difficulty of capturing their full species pool. By comparing abundance, richness, turnover, and accumulation patterns between these groups, we aim to better understand how taxonomic resolution shapes biodiversity assessments.

METHODS

The data forming the basis of this paper were collected at the Breitenbach stream, located in a German nature reserve (50°39′42 N 9°37′26E) from January 1969 to December 2010. This study only uses data collected between 1969 and 2006, to make trends of Diptera and EPT communities more comparable, as collection of Diptera data stopped 5 years before EPT did, resulting in a total observation period of 37 years (Baranov et al., 2020).

Aquatic insects belonging to EPT and Diptera were collected on a weekly basis throughout the 37-year observation period (1969–2006) using stationary "greenhouse-like" emergence traps, which covered the whole stream and part of the riparian area over a length of 12 m. Sampling protocols transitioned in 1986: prior to this shift, all specimens were manually collected from the traps on a daily basis. From 1986 onwards, collection tents were installed within the greenhouse structures to automate the process, with insects captured in ethanol-filled jars and retrieved at weekly intervals (Wagner et al., 2011). Samples were morphologically identified to the species level and counted (Wagner et al., 2011). The initial dataset contained species counts from 10 traps. The observation period for each of the traps differed in length (Table 1). We chose to only retain traps with observation periods of at least 9 years, ensuring robust temporal patterns could be calculated. Consequently, all analyses were based on seven traps (Table 1). It is important to note that when we are referring to the "long-term dataset" we are talking about the entirety of the Breitenbach data (all years combined), as some of the individual trap time-series that covered less than 10 years do not strictly satisfy the definition of "long-term data" (Lindemayer & Likens, 2010).

The Breitenbach stream and its surroundings are largely free from direct human disturbance, except for a one-off spill of liquid

insecticides into the Breitenbach during the summer of 1986. Following the spill, traps A, E, and G all demonstrated a pronounced drop in EPT abundance in 1986 but a subsequent recovery to pre-spill abundance levels was observed within the next 4–5 years (Wagner et al., 2011). Physico-chemical variables never exceeded natural background levels, consistently staying within a "good" water quality class according to the EU Water Framework Directive 2000. The physical-chemical properties over time were: pH, 7.2 ± 0.6; O2, 11 ± 3 mg/L; ammonium, 0.007 ± 0.01 mg/L; nitrate, 0.8 ± 0.4 mg/L; and organic orthophosphate, 0.04 ± 0.05 mg/L (Wagner et al., 2011). Climate change had a direct impact, with stream temperature increasing by 1.88°C between 1969 and 2010 (Baranov et al., 2020). Mean and maximum annual discharge did not change significantly, but directional shifts in discharge patterns were recorded, particularly towards more "dry" years (Dietrich et al., 2023).

Taxonomic harmonization

To ensure consistent and accurate taxonomy across all insect groups, species names were mostly validated against the Global Biodiversity Information Facility database (GBIF, 2025); the taxonomy of the Limoniidae, Pediciidae, and Tipulidae followed (Oosterbroek, 2025). When necessary, species names were corrected to contemporary taxonomy. The validated taxa list was thereafter presented to group-specific taxonomic experts for a second and final round of species name validation; the taxonomy of the Limoniidae, Pediciidae, and Tipulidae was verified by Sigitas Podėnas; the Dolichopodidae was verified by Marc Pollet; and the remaining taxa were verified by Rüdiger Wagner and Marija Ivković. Experts provided the final species name used in all subsequent analyses. A comprehensive taxa list is presented in the accompanying dataset in the File S1.

Data cleaning and retention

All subsequent statistical analyses were conducted on EPT and Diptera groups separately. It is important to note that only Diptera taxa identified to the species or morphotype levels for more than 10 years were included in our analysis, not the total Diptera emergence. Hence the Chironomidae, Simuliidae, and Ceratopogonidae, while diverse and abundant in the Breitenbach, were not considered here since samples were discarded prior to identification in most of the cases (Wagner et al., 2011). Out of the 402 Diptera taxa, 88% (354) were identified to species, while the rest were identified to subspecies (2), subgenus (3), genus (42) or family (1). For the 115 EPT taxa, 93% (107) were identified to species, while the remaining 8 were identified to the genus level.

Biodiversity indices

For each validated species and operational taxonomic unit—including taxa identified at higher taxonomic levels—we used the annual sum of emerged individuals to calculate overall abundance and community metrics for both EPT and Diptera groups separately per year. Annual sums are commonly used in studies investigating inter-annual changes in communities (Hallmann et al. 2020). We also computed species richness (*N*), Shannon diversity, and Shannon's evenness using the vegan package (Oksanen et al., 2007) within the R statistical environment (R version 4.4.2, R Core Team). Inter-annual abundance-based species turnover, as a measure of the rate and direction of change in communities, was calculated using the *codyn* R package (Hallett et al., 2016).

Data analysis

First, we separately computed diversity profiles for EPT and Diptera using the *diversity* function in the SpadeR R package (Chao et al., 2016). We used both empirical estimates as well as bias-corrected Chao-Jost estimates to assess observed and potentially undetected species, respectively. For each trap, we calculated Hill numbers for orders (q) 0, 1, 2, 3, where $q = 0$ is species richness (i.e., each species weighted equally), $q = 1$ is Shannon entropy (i.e., each species weighted by their abundances), $q = 2$ is the inverse of Simpson entropy (i.e., more weight given to abundant species) and $q = 3$ is an inverse of the dominance index (Chao et al., 2014). Chao-Jost estimates describe the sensitivity of the different parameters of the biological diversity (Species richness, Shannon diversity, Simpson diversity) to the abundance of the organisms in the community. Changes in the abundance of the organisms, and their magnitude, allow for estimation of the theoretical values of the biodiversity parameters. Results were then visualized along with 95% confidence intervals.

Second, to assess sampling completeness and species richness ($q = 0$) across each trap, we constructed sample-based rarefaction curves for EPT and Diptera separately using the *iNEXT* function in the iNEXT R package (Hsieh et al., 2025). For each trap, we computed both interpolated and extrapolated rarefaction curves along with their associated 95% confidence intervals. To allow fair comparisons across traps with different sampling effort and frequency, we standardized all rarefaction curves by the number of individuals sampled over the entire observation period. Extrapolation was extended to approximately twice the observed sample size to estimate asymptotic species richness while maintaining prediction robustness. Chao-Jost numbers methodology followed Chao and Jost (2015).

To assess temporal trends in community dynamics, we analysed temporal trends of abundance, species richness ($q = 0$), and temporal turnover using hierarchical regression models implemented with the glmmTMB R package (Bolker, 2019). For each response variable, we implemented two modelling approaches. First, we constructed individual models for each trap, using year (standardized to improve model convergence) as the sole predictor variable. Next, we constructed an overall model which incorporated data from all traps and included standardized year as a fixed effect (modelled as a second-order polynomial to account for non-linearity in the response through time), trap

as a random effect, and a first-order autoregressive term (AR1) to account for temporal autocorrelation within each trap. To ensure robustness of the overall model, pulling from as many data points as possible, we calculated overall models only using the years for which data from multiple traps was available. Thus, while individual trap models span their own, trap-specific sampling period, overall models were constructed using data collected between 1974 and 1996. The model formulas were:

$$\text{Trap} - \text{specific model}: Response_i \sim year, where\ i\ represents\ trap$$

$$\text{Overall model}: Response \sim poly(year, df) + (1|trap) + ar1(0 + factor(year)|trap)$$

A polynomial term was included to capture potential non-linearity in the temporal trends of the response variables, and trap as a random effect accounts for potential trap-specific variations and the nested sampling design structure. We included trap-specific AR1 temporal dependence structures as we assumed that observations closer in time within each trap would be more correlated than those further apart. Thus, the AR1 autocorrelation term accounts for this inherent dependence.

Model distributions were selected based on the nature of the response variables: a negative binomial distribution was used for abundance data to account for overdispersion (initial Poisson models were assessed to be overdispersed), while Gamma distributions were used for species richness and Gaussian distributions for turnover, with "log" and "logit" links, respectively. Model validation was performed using DHARMa residual diagnostics; minor violations of model assumptions were identified, especially in residual versus predicted plots, and we offer the following explanations. First, we believe the patterns observed are largely structural in nature, arising from pooling traps with different observation period lengths and trend directions, rather than indicating fundamental model misspecification. To address this, we explored several alternative model specifications, including natural splines with varying degrees of freedom, random slopes and AR(1) autocorrelation structures and, while some alternatives partly reduced the deviations in the residuals versus predicted plots, the ecological conclusions remained consistent across all model specifications. We thus chose to retain the simpler polynomial models for interpretability, given that results were robust to model choice. Second, the quantile-quantile plots indicated adequate overall distributional fit and dispersion and outlier tests were non-significant, supporting the reliability of our inference. Third, we note that the DHARMa quantile regression tests used for the residuals versus predicted plots can be sensitive with moderate sample sizes. Predicted models were visualized using ggplot2 (Wickham et al., 2016) by combining predictions of both the overall and individual trap models. Since year was included as a standardized variable in all models, model predictions were back-transformed prior to plotting to ensure values were presented in their original units of measure.

To assess which Diptera families contributed most to their increases in abundance through time, we assessed each trap–family combination across the seven focal traps (A, B, C, E, G, I, III). For each trap–family combination with more than one year of data, the slope of abundance (annual total abundance per family within each trap and year) against year was estimated using Theil–Sen median-based linear regression with the mblm R package (Komsta, 2019). Trends were then classified based on the sign of the estimated slope as "Increase" (slope >0), "Decrease" (slope <0) or "Stable" (slope = 0).

To assess the relationships between changes in diversity components (abundance, richness), we followed Blowes et al. (2022) and computed year-on-year changes in the abundance (ΔN), species richness (ΔS), and rarefied species richness (ΔS_n), and used Pearson's correlations to explore the relationships between ΔS and ΔS_n and ΔS and ΔN for the entire EPT and Diptera dataset (overall), as well as on an individual trap basis (Figures S1–S4).

All data and code are available online (Baker et al., 2026).

RESULTS

Abundance, richness and turnover trends

Despite being far more abundant within the traps throughout the entire observation period (1,584,793 ind. vs. 359,482 ind.), EPT were much less taxonomically diverse than Diptera (115 species vs. 402 species; Table 1). The overall abundance of EPT did not change through time (EPT abundance estimate across all traps between 1974 and 1996 = 0.09, SE = 0.82, $p > 0.05$; Figure 1), while overall dipteran abundance strongly increased (overall Diptera abundance estimate across all traps between 1974 and 1996 = 9.99, SE = 1.38, $p < 0.001$; Figure 1). Overall species richness of both EPT and Diptera tended to increase through time, though Diptera richness trends plateaued towards the mid-1990s (second-degree polynomial = −2.20, SE = 0.73, $p < 0.01$) compared to EPT (second-degree polynomial = −0.27, SE = 0.10, $p < 0.01$). Increases in Diptera abundance was mostly driven by representatives of Dolichopodidae and Psychodidae increasing in abundance over time (Table 2). Turnover of EPT across all traps generally increased over the observation period (first-degree polynomial = 1.52, SE = 0.35, $p < 0.001$), whereas the turnover of Diptera was more complex, characterized by an initial increase followed by a strong decline (second-degree polynomial = −3.26, SE = 0.63, $p < 0.001$).

While trends summarized across all traps are important for providing overarching patterns (black lines in Figure 1), traps with different observational periods are likely to influence overarching trends despite our attempts to account for this in the model design. With this in mind, trap B can be used as a proxy for changes occurring across all traps, since it had the longest observation period which encompassed all years in which the other traps were sampled (Table 1). Correspondingly, and only focusing on the trends of trap B (yellow lines in Figure 1), there were much clearer contrasting temporal trends of abundance, species richness and turnover for EPT and Diptera. Specifically, EPT abundance at trap B significantly declined through time (EST = −0.28, SE = 0.05, $p < 0.001$), while Diptera abundance

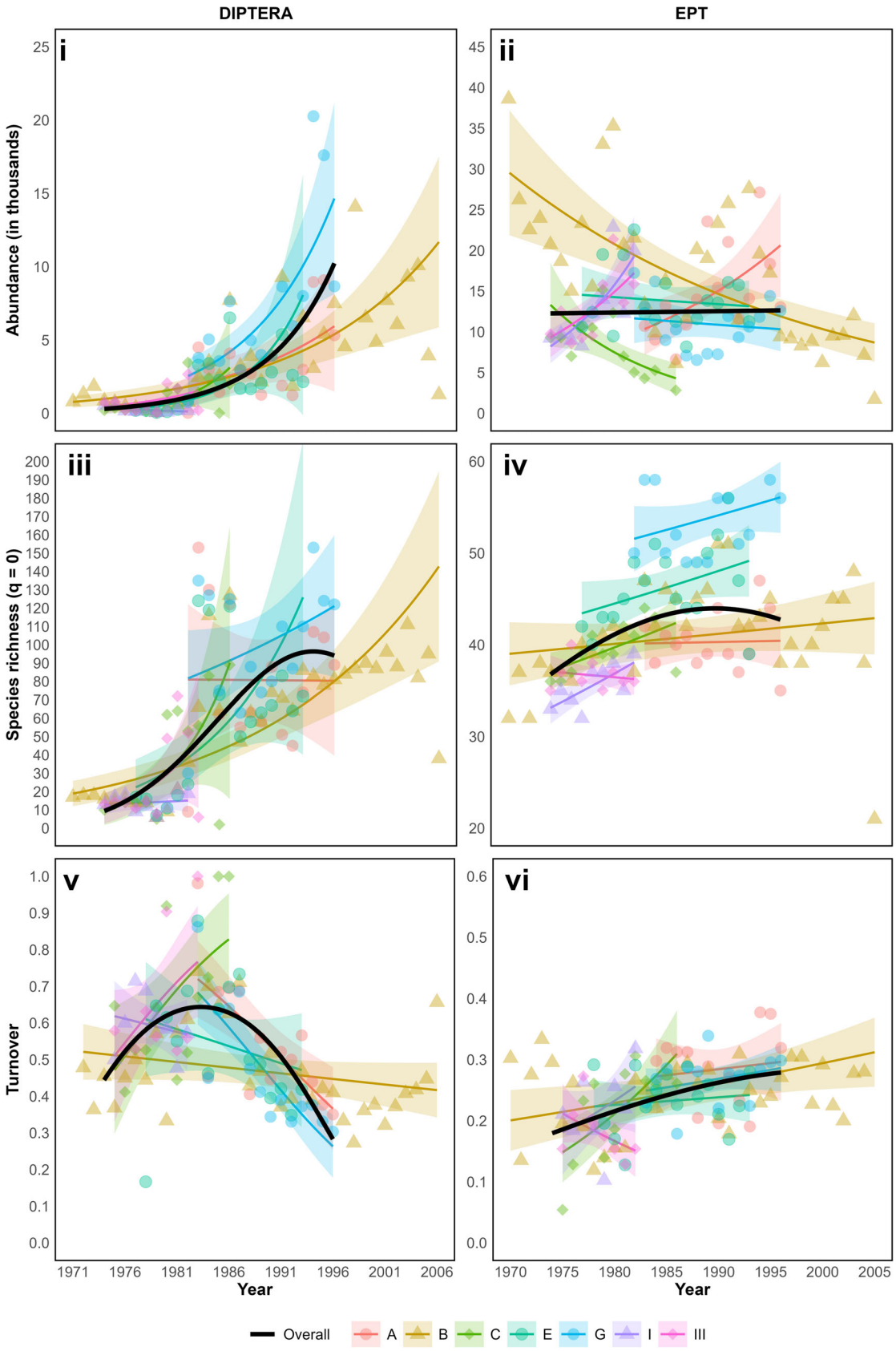

FIGURE 1 Abundance, species richness, and turnover trends of Diptera (i, iii, v) and EPT (ii, iv, vi) in seven of the longest running traps (9–37 years) and across all traps from 1974 to 1996.

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TABLE 2 Temporal trends in abundance for Diptera families across traps (A, B, C, E, G, I, III).

Trap	Family	Slope (change specimens/year)	Trend
A	Dixidae	−9.92	Decrease
A	Dolichopodidae	75.5	Increase
A	Empididae	5.1	Increase
A	Limoniidae	−509	Decrease
A	Pediciidae	11.2	Increase
A	Psychodidae	59.4	Increase
A	Ptychopteridae	0	Stable
A	Rhagionidae	−4.8	Decrease
A	Stratiomyidae	−2.65	Decrease
A	Tabanidae	−3.58	Decrease
B	Dixidae	22.6	Increase
B	Dolichopodidae	34.5	Increase
B	Empididae	12.2	Increase
B	Limoniidae	620	Increase
B	Pediciidae	27	Increase
B	Psychodidae	89.3	Increase
B	Ptychopteridae	12.2	Increase
B	Rhagionidae	−0.717	Decrease
B	Stratiomyidae	0.261	Increase
B	Tabanidae	0	Stable
B	Thaumaleidae	0.5	Increase
C	Culicidae	−2	Decrease
C	Dixidae	−16	Decrease
C	Dolichopodidae	−20.5	Decrease
C	Empididae	−20.1	Decrease
C	Ephydridae	−14.3	Decrease
C	Limoniidae	−230	Decrease
C	Pediciidae	49	Increase
C	Psychodidae	−1.07	Decrease
C	Ptychopteridae	76	Increase
C	Rhagionidae	−2	Decrease
C	Stratiomyidae	0.0833	Increase
C	Syrphidae	0	Stable
C	Tabanidae	−0.417	Decrease
C	Thaumaleidae	−0.5	Decrease
E	Dixidae	39.8	Increase
E	Dolichopodidae	−28.8	Decrease
E	Empididae	−4.54	Decrease
E	Limoniidae	150	Increase
E	Pediciidae	39	Increase
E	Psychodidae	64.4	Increase
E	Ptychopteridae	61.8	Increase
E	Rhagionidae	3	Increase
E	Stratiomyidae	−1.12	Decrease
E	Tabanidae	5	Increase
G	Dixidae	112	Increase
G	Dolichopodidae	87.1	Increase
G	Empididae	46.4	Increase
G	Limoniidae	2	Increase
G	Pediciidae	45.8	Increase
G	Psychodidae	162	Increase
G	Ptychopteridae	22	Increase
G	Rhagionidae	−6.33	Decrease
G	Stratiomyidae	1.5	Increase
G	Tabanidae	−2.1	Decrease
I	Psychodidae	−25.9	Decrease
I	Stratiomyidae	0.75	Increase
III	Culicidae	−2	Decrease
III	Dixidae	−155	Decrease
III	Dolichopodidae	754	Increase
III	Empididae	90.5	Increase
III	Psychodidae	−42.5	Decrease
III	Ptychopteridae	28	Increase
III	Rhagionidae	−10	Decrease
III	Stratiomyidae	34	Increase
III	Tabanidae	−1	Decrease
III	Thaumaleidae	−4	Decrease

Note: For each trap–family combination, the slope (change in abundance per year) was estimated using Theil–Sen median-based linear regression (mblm) of total annual abundance against year. Trends are classified as "Increase" (slope > 0), "Decrease" (slope < 0), or "Stable" (slope = 0).

increased (EST = 0.62, SE = 0.10, $p < 0.001$). Diptera species richness also significantly increased through time (EST = 0.46, SE = 0.08, $p < 0.001$), while EPT species richness showed no strong trend (EST = 0.02, SE = 0.02, $p > 0.05$). Contrastingly, the turnover of EPT significantly increased through time (EST = 0.14, SE = 0.06, $p < 0.05$), with Diptera turnover showing no significant trend (EST = −0.10, SE = 0.06, $p > 0.05$).

Species accumulation curves

Across the 37-year sampling period, Diptera species richness was 249.6% higher than that of EPT, despite Diptera having 77.3% less sampled individuals. Regardless of these stark differences in diversity, accumulation curves of Diptera at 359,482 individuals—the total number of Diptera sampled from all traps throughout all years (vertical red lines on Figure 2)—had not yet reached the asymptote (i.e., a plateau). Notwithstanding the impressive and unparallelled sampling effort needed to produce the dataset used in this study, Diptera diversity is

underrepresented and undersampled. Conversely, at the same number of individuals, the accumulation curves of EPT, for all traps, were approaching the asymptote. The diversity of the comparatively well-resolved EPT group was therefore effectively captured given the sampling effort employed. When focussing solely on the longest running trap, trap B, we found a similar pattern, with even much starker differences between the accumulation curves, where EPT species richness was almost asymptotic compared to the more vertical curve of Diptera (Figure 2).

Diversity profiles

There was no significant difference between the proposed (predicted) and empirical values of the Chao-Hill numbers at quartiles 0–3 (Species richness, Shannon diversity, Simpson diversity and inverse dominance) for EPT. Contrastingly, Diptera showed a significant difference in species richness ($q = 0$) and Shannon diversity ($q = 1$), with empirical values being significantly lower than predicted (Figure 3). Overall species richness of Diptera during the 36 years totalled 402 taxa, while the predicted Chao-Hill number of species was 506 (Figure 3). For the Diptera, we found significant differences between species richness ($q = 0$) and Shannon entropy ($q = 1$) in all traps except for "trap I", the trap with the shortest operating time of 9 years (Table 1). For all other traps with longer time series (10–36 years), differences between empirical and predicted Diptera diversity for $q = 0$ were statistically relevant (Figure 3, Traps A, B, C, E, G, III). Conversely, and apart from traps C (13-year operation) and G (15-year operation), differences between empirical and predicted EPT diversity for $q = 0$ were not statistically relevant (as indicated by overlapping confidence intervals), despite predicted EPT diversity always being higher than empirical EPT diversity (Figure 3). Overall species richness of EPT during the 37-year sampling period was 115 taxa, while the predicted Chao-Hill number of species was 148. For higher orders of q, empirical and predicted EPT diversity measures were mostly congruent, except in traps I and III (Figure 3).

Relationships between the changes in diversity components

Considering year-on-year changes in the abundance, species richness, and rarefied species richness of EPT and Diptera, all pairwise correlations (r) were highly significant. However, correlation strength varied between diversity components and taxonomic groups (Figure 4). Relationships between species richness (ΔS) and abundance (ΔN) were considerably flatter and more dispersed compared to the relationships between species richness (ΔS) and rarefied species richness (ΔS_n),

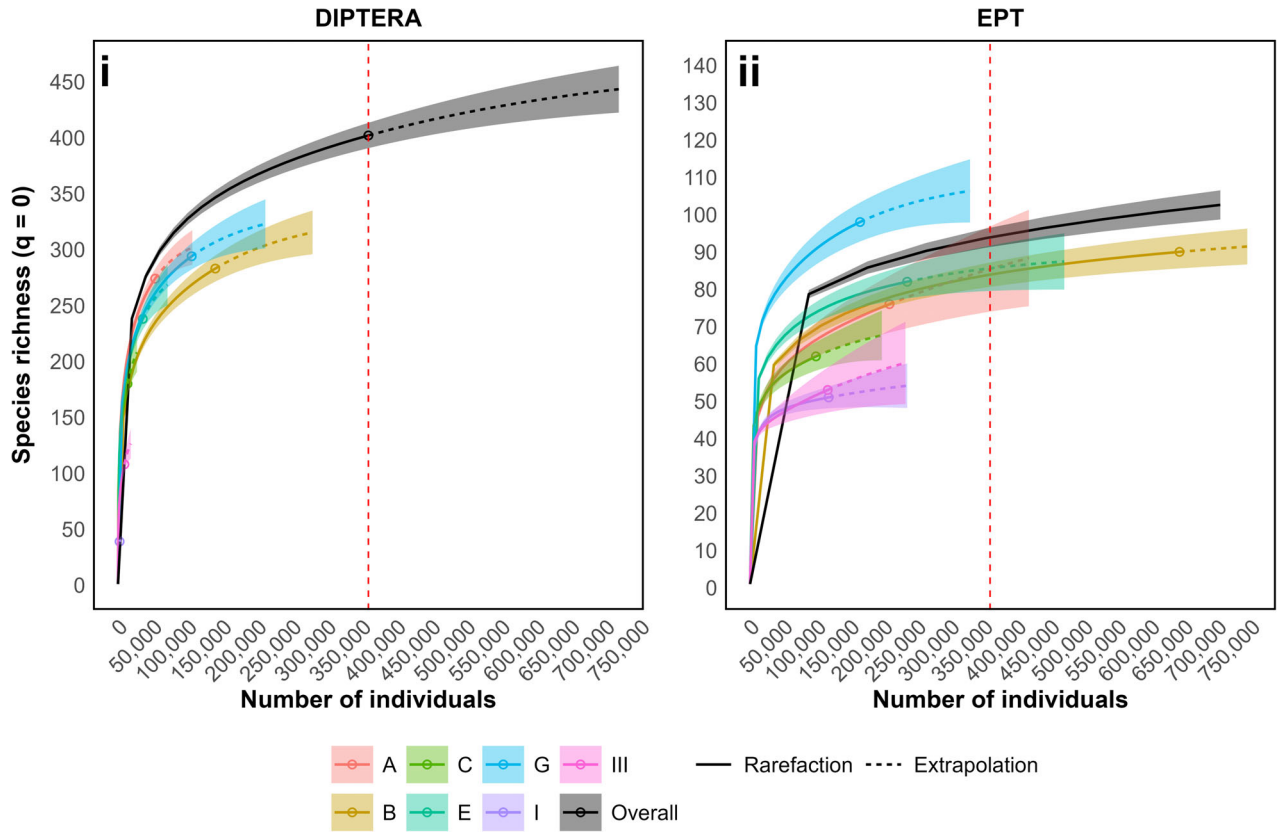

FIGURE 2 Overall and individual rarefaction of Diptera (i) and EPT (ii) across seven of the longest running traps. Solid lines represent empirical species richness, while dotted lines represent an estimate. The vertical red line indicates the total number of dipteran individuals captured throughout all years of sampling.

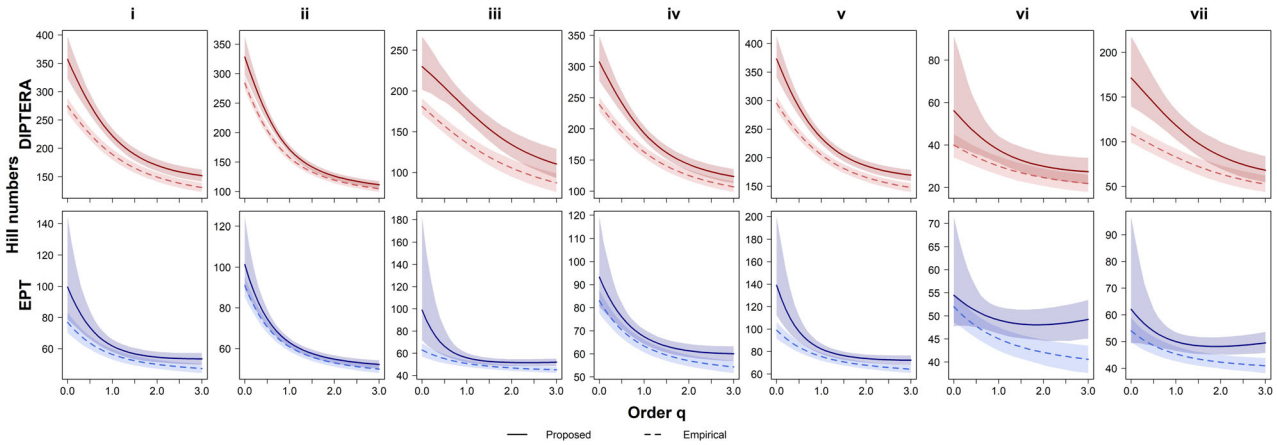

FIGURE 3 Diversity profiles for Diptera and EPT from Breitenbach (traps that were in operation ≥9 years). The empirical (dotted line) and estimated (Chao1, solid line) diversity profiles for communities as quantified by Hill numbers for values of the diversity order (q) from 0 to 3 with 95% confidence intervals (shaded areas based on bootstrap analysis of 100 permutations). Species richness is depicted by $q = 0$; Shannon diversity by $q = 1$; Simpson diversity by $q = 2$; inverse dominance index by $q = 3$. I - trap A; ii – trap B; iii – trap C; iv – trap E; v – trap G; vi – trap "I"; vii – trap "III".

which were predictably more tightly grouped (Figure 4). In Diptera, the correlation between the temporal change in species richness (ΔS) and abundance (ΔN) was 0.55, while the correlation between the temporal change in species richness (ΔS) and rarefied species richness (ΔS_n) was 0.82. In EPT, the pairwise correlation between species richness (ΔS) and abundance (ΔN) was 0.25, while the correlation between species richness (ΔS) and rarefied species richness (ΔS_n) was 0.85.

DISCUSSION

We found that the accumulation curves of EPT reached a plateau despite a rapid decline in abundance (as indicated by trap B model results), suggesting that their true diversity was likely captured within the 37-year observation period. Conversely, no such plateau was observed in Diptera. This mismatch likely results from the much higher taxonomic diversity of Diptera in the Breitenbach area, supporting the underrepresentation of these taxa in biodiversity research and suggesting a much larger proportion of rare species compared to EPT. Our results are in line with the previous research, pointing out that time-series length is an extremely important consideration when attempting to disentangle past, present, and future diversity patterns (Lindenmayer et al., 2012; White, 2019; Dolný et al., 2021, Wagner et al., 2021).

Principally, indicator species belonging to EPT, while being more abundant in the traps, are far less taxonomically diverse than Diptera. Thus, while the exceptional effort employed at the Breitenbach stream over the 37-year sampling period provided a very clear picture of local EPT diversity, our knowledge of Diptera diversity is far from exhaustive. Further, dipteran diversity increased throughout the observation period, contrasting that of the sensitive indicator species within EPT. This is a surprising result, although it parallels Baker et al. (2021), specifically since the primary stressor to the fauna in the Breitenbach is global climate change, with no other stressors directly influencing stream conditions and the inhabiting fauna (Baranov et al., 2020). Therefore, either the Diptera are (1) better able to cope with the prevailing conditions caused by global climate change, "outlasting" the EPT, or (2) the expansion of Diptera from warmer lowland regions has created a constant supply of new species that were able to become established in previously unsuitable conditions. Since the increases in Diptera we observed were driven by Psychodidae and Dolichopodidae, mostly warm-adapted and generalist taxa with predominantly riparian or semi-aquatic detritophagous larvae (Wagner et al., 2011), our results align more with the second explanation and support those from Baranov et al. (2020) and Baker et al. (2021), who both reported the expansion of the warm-adapted detritophagous (and filtrators') taxa.

The second possibility is also more in line with our results of the relationships between the changes in different diversity components, following Blowes et al. (2022). We found that both EPT and Diptera showed strong correlations between the year-on-year species richness change and rarefied species richness, but correlations between changes in abundance and richness were much flatter and less pronounced (Figure 4). We believe this difference to be primarily driven by the contrasting community-level trends observed between the EPT and Diptera over time. Specifically, EPT abundance declined as turnover increased and new generalist species arrived, at least at trap B (Baranov et al., 2020). Contrastingly, Diptera abundance and richness consistently increased through time, leading to a much higher correlation between the change in abundance and richness in Diptera (Blowes et al., 2022). Our results therefore corroborate previous results (Blowes et al., 2022; Santini et al., 2017) showing tight connections between the rarefaction of species richness and other biodiversity components and suggest that the faster accumulation of EPT

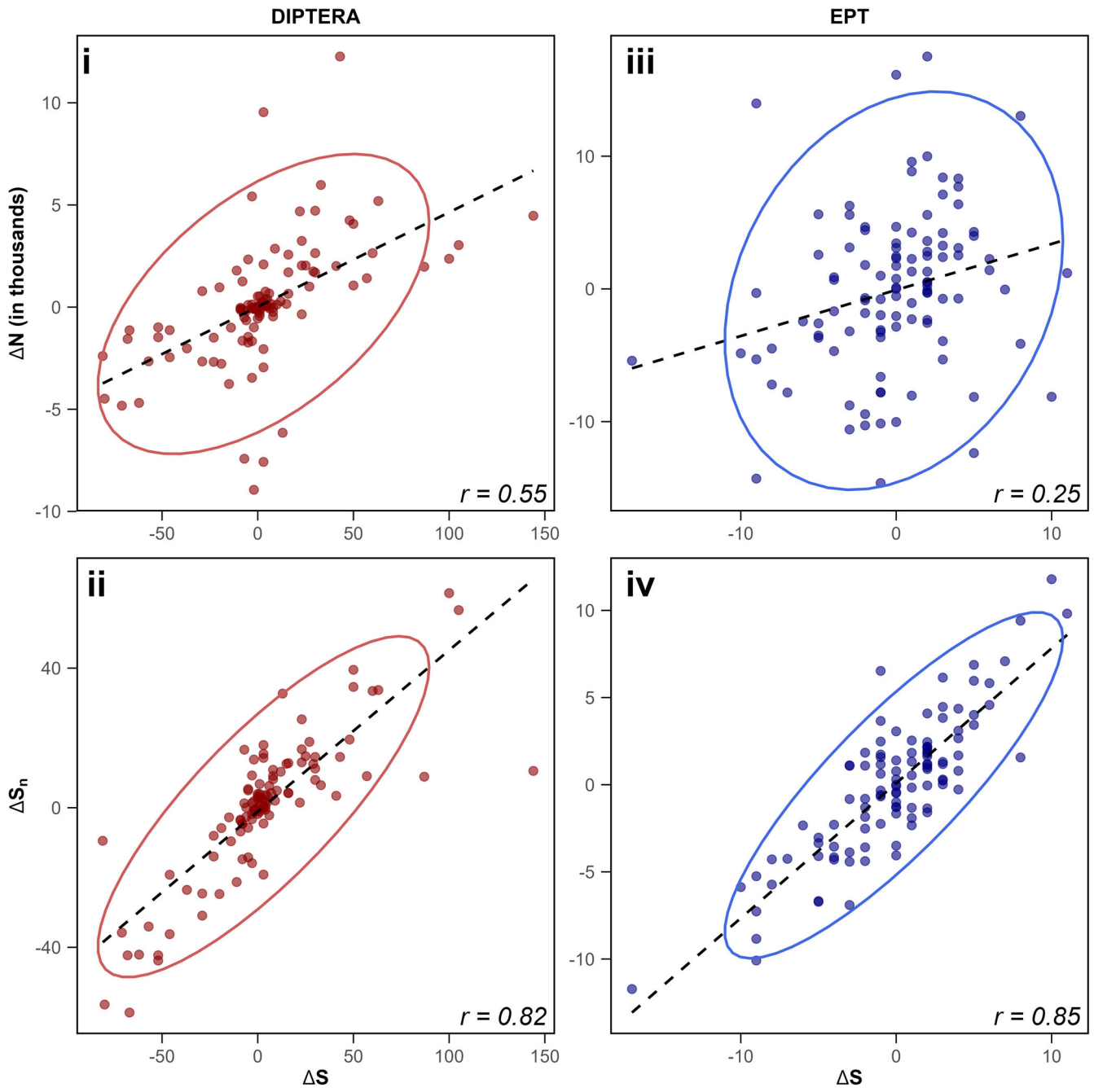

FIGURE 4 Pairwise Pearson correlations between different biodiversity components. Correlation coefficients (r) show change in species richness (ΔS) versus change in abundance (ΔN) (i & iii) and change in species richness (ΔS) and change in rarefied species richness (ΔS_n) (ii and iv) for Diptera (i and ii) and EPT (iii and iv), respectively. The ellipse in each panel represents the 95% confidence ellipse, summarizing the joint distribution of the two metrics across all trap-years under an assumed bivariate normal distribution. Points falling outside the ellipse correspond to trap-years in the tails of the distribution (≤5% of observations expected under bivariate normality).

diversity, represented by asymptotic curves, is not merely driven by a decline in their abundances through time.

Further, life-history differences between Diptera and EPT may also provide support for this finding. For instance, many EPT taxa are larger, are longer lived (>1 year) and are known to actively disperse (but at relatively short distances). Conversely, Diptera are generally smaller, shorter lived, and disperse passively to new environments via wind and/or stream drift (Marshall, 2012). Thus, the likelihood of different dipteran taxa passively dispersing to new habitats, depending on different climatic processes, is higher compared to EPT. Additionally, the fact that many Diptera taxa are polyvoltine, and that their microhabitats are more resilient to fragmentation, might have kept Diptera turnover rates low (Wagner et al., 2011). This would also explain the high turnover rates of EPT compared with Diptera, whereby dipteran diversity is likely cumulative (newer colonizers coexist with established species) leading to stable turnover, while EPT

are replaced by more tolerant species of the same groups and/or those of other groups like Diptera (Wagner et al., 2011).

Aquatic community turnover and succession

With this analysis, we obtained a comprehensive and complex picture of the non-linear responses of biodiversity to ongoing climate change, the primary anthropogenic driver impacting the Breitenbach stream catchment. Overall species richness of EPT did not change significantly, while that of Diptera increased. Species richness of Diptera increased significantly over time, while the turnover of Diptera increased and then decreased, similarly to the results of Radošević et al. (2025) obtained during a 15-year long Diptera monitoring campaign in a karst barrage lake system in Croatia.

Overall, these contrasting results are well aligned with the existing body of work, pointing to the displacement of specialist EPT taxa (Dudgeon et al., 2020; Baker et al., 2021; Green et al., 2022; Dorić et al., 2023; Haase et al., 2023). In our previous analysis of this dataset, we found that increasing temperatures and prevalences of dry years drove elevated turnover of EPT taxa, mostly caused by the displacement of the cold-adapted specialists with warmer-adapted generalists (Baranov et al., 2020). Previous studies have also shown that rhithral communities, initially dominated by grazers and/or scrapers, move towards being dominated by passive filter feeders and predators under increasing temperature and decreasing flow regimes (Baranov et al., 2020; Dudgeon et al., 2020).

Unfortunately, our knowledge of Diptera larval traits is still insufficient, except for a few well-studied families (Caterino & Recuero, 2024; Schmidt-Kloiber & Hering, 2025). Nevertheless, the majority of Diptera recorded in the Breitenbach over the years belonged to the families Empididae, Dolichopodidae, Psychodidae, Limoniidae, and Tipulidae. As a very broad generalization, all larvae of the Empididae and most of the Dolichopodidae are predators, while the larvae of the Limoniidae, Tipulidae, and Psychodidae are mostly detritophages and/or saprophages (De Jong, 2017; Grichanov & Brooks, 2017; Jourdan et al., 2019; Kvifte & Wagner, 2017; Wagner et al., 2011, 2024). Thus, increases in the abundance of Diptera, mostly driven by Psychodidae and Dolichopodidae, align well with our earlier results, demonstrating that reductions in discharge and increases in temperatures are likely leading to the higher availability of particulate dead organic matter, stimulating increases in passive filter feeders and predators (Baranov et al., 2020). It stands to reason, however, that an increase in dead organic matter will not only benefit filter feeders and predators but also detritophages and saprophages, such as many Diptera larvae (Dorić et al., 2023; Marshall, 2012).

Rarefaction and taxonomic resolution

Species accumulation curves confirmed our hypothesis that Diptera, as a poorly resolved "dark taxa" group, did not reach saturation, even in one of the world's most comprehensive long-term freshwater invertebrate datasets. However, taxonomically well-resolved EPT taxa showed clear saturation, suggesting their diversity was effectively captured during the 37-year observation period. This may be due to EPT being easier to identify, their use as indicator species, and their comparatively low overall diversity; Forbes et al. (2018) report that there are less than 50,000 EPT species worldwide, with 558 species known to occur in Germany (Morinière et al., 2017) versus 250,000 described Diptera species of which 9554 occur in Germany (Schumann, 2010). Furthermore, based on rarefaction of the Diptera sampled by the network of Malaise traps in Bavaria, Diptera species richness in Germany was estimated to be at least 15,000 species (Chimeno et al., 2022).

Consequently, rare Diptera species are less likely to be sampled and/or identified, leading to slower curve saturation. This result suggests that the observed EPT diversity closely approximates their true richness in the Brietenbach, while that of Diptera is still significantly underrepresented. We also were able to show that the diversity of EPT approached the plateau in the traps that ran for about ten years, while none of Diptera curves reached an asymptote, even in the traps running for 15–37 years. This is generally well aligned with the concept of "dark diversity" (Chimeno et al., 2022; Hebert et al., 2016; Meier et al., 2025). There is much higher undescribed diversity of Diptera than EPT or any other groups of insects except for (possibly) Hymenoptera (Forbes et al., 2018; Shirali et al., 2024; Srivathsan et al., 2023). In contrast, Diptera abundance strongly increased over the 37-year observation period, despite a marked drop in the "reference trap" B during the final two years of sampling (Figure 2). Thus, similarly to the EPT, abundance and turnover changes were strongly synchronized between traps, demonstrating that changes in the time-series of trap B is not an accidental fluctuation, but a broader trend reflected across the entire Breitenbach catchment.

Implications of including riparian and aquatic taxa in group comparisons

A caveat to our rarefaction analysis is that we compared the larvae of purely aquatic EPT taxa with families of Diptera that could contain a mix of larvae that occur in riparian, seepage, wet wood habitats as well as those that are fully aquatic (Wagner et al., 2011). Thus, it is worth justifying whether the central argument of the paper—that "dark taxa" remain undersampled compared to well-resolved taxonomic groups such as the EPT—is robust when only lotic Diptera are considered?

It is important to note that Diptera families with predominantly aquatic larvae (e.g., Ceratopogonidae, Chironomidae, Simuliidae) were consistently sampled in Breitenbach over the 37-year observation period, though these taxa were only identified sporadically (one to four years of data exists) from a limited array of traps. This was mainly due to the lack of taxonomic specialists for these groups among the staff of the Schlitz research station (Wagner, 1980; Wagner et al., 2011). Therefore, we decided to remove these data prior to our analysis. Non-biting midges (Chironomidae) are the most species-rich

aquatic Diptera family, with over 7800 species worldwide (Pape et al., 2011) and 781 recorded species in Germany (Schumann, 1999, 2002, 2004, 2010). The Chironomidae were sampled and identified from the Breitenbach in 1974, 1983 and 1987, recording 210 species (Wagner, 1980, Wagner et al., 2011). While Chironomidae were not consistently identified within the Breitenbach samples, they accounted for 40% of the Diptera species recorded in the stream and, depending on the time of sampling, 38%–73% of the Diptera biomass (Wagner, 1980). Previous research in Germany showed that the species richness of the Chironomidae tends to be drastically underestimated in most surveys, and therefore, this group can be classified as a "dark taxon" (Chimeno et al., 2022, Chimeno et al., 2023) with DNA-based analyses confirming their unresolved status (Macher et al., 2025). In Chimeno (2022), rarefaction of Chironomidae Barcode Index Numbers (BINs) from 14 malaise traps in three sites in Bavaria showed that while only 296 BINs were recorded, the estimated numbers of BINs at the collection sites ranged from 463 to 897. While BINs are no perfect analogues to species, these studies still suggest an underestimation of the Chironomidae species richness of 80–200% (Baloğlu et al., 2018, Chimeno et al., 2022, 2023). Likewise, 78 species within the Ceratopogonidae were detected (Havelka, 1975) in our study. With ca. 200 described species in Germany alone, and many more expected to be described in the future (Borkent et al., 2024; Borkent & Dominiak, 2020; Havelka & Aguilar, 1999), the Ceratopogonidae can also be considered a "dark taxon" and we expect that they too remain consistently undersampled in freshwater monitoring programmes. Thus, if more data were available, we would expect similar rarefaction patterns for Chironomidae, Ceratopogonidae and other lotic unresolved Diptera groups in the Breitenbach.

A total of 16 Simuliidae species were recorded in the Breitenbach between 1984 and 1988 (Reidelbach & Christl, 2002), whereas approximately 57 species are known from Germany as a whole (Seitz, 2022). As Simuliidae are human ectoparasites and vectors of some human diseases, they are relatively well studied and therefore do not meet the definition of "dark taxa" (Chimeno et al., 2022). Accordingly, we do not expect a large number of undetected taxa in this group within Breitenbach.

Thus, the estimated species richness of Diptera with purely aquatic larvae in Breitenbach greatly exceeds that of EPT species; we are confident that our finding of the slower saturation of the Diptera curve would still hold, even when considering only lotic Diptera taxa.

CONCLUSION

Species accumulation in "dark taxa" appears to be very slow, with no saturation even after 37 years of observation. This persistent undersampling of "dark taxa" highlights a critical blind spot in biodiversity monitoring: even extensive, high-resolution time-series may fail to capture their true diversity. As a result, biodiversity trend estimates, such as shifts in abundance or diversity, may go unnoticed, obscuring patterns of decline or increase. Their exclusion also introduces uncertainty in conservation planning and limits our understanding of ecosystem functioning, as their ecological roles are pivotal but often overlooked. While long-term datasets are essential, they must be complemented by targeted approaches—including combined molecular and morphological methods—tailored to the taxonomic complexity of the groups of interest.

AUTHOR CONTRIBUTIONS

Nathan Jay Baker: Conceptualization; data curation; formal analysis; investigation; writing – original draft; methodology; software; writing – review and editing; visualization. **Jonas Jourdan:** Conceptualization; formal analysis; writing – original draft; writing – review and editing. **Francesca Pilotto:** Conceptualization; methodology; writing – original draft; writing – review and editing. **Rüdiger Wagner:** Methodology; writing – review and editing; resources; funding acquisition; investigation. **Jessica Awad:** Data curation; validation; writing – review and editing; writing – original draft. **Valerio Caruso:** Data curation; validation; writing – review and editing. **Caroline Chimeno:** Data curation; writing – review and editing; validation. **Amelie Höcherl:** Data curation; writing – review and editing; validation. **Marija Ivković:** Validation; writing – review and editing. **Marina Moser:** Data curation; writing – review and editing; validation. **Viktor Baranov:** Conceptualization; methodology; software; data curation; investigation; formal analysis; project administration; writing – original draft; writing – review and editing; visualization; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Github at https://zenodo.org/records/19812269, reference number DOI 10.5281/zenodo.19812268.

ETHICS STATEMENT

All the insects, serving as a basis for this statement, were collected under the relevant permits of the Hesse local government (Germany) granted to the Max Planck research station in 1969–2006 (Schlitz).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Pairwise Pearson correlations between species richness (ΔS) and change in abundance (ΔN) for Diptera per trap.

Figure S2. Pairwise Pearson correlations between change in species richness (ΔS) and change in rarefied species richness (ΔS_n) for Diptera per trap.

Figure S3. Pairwise Pearson correlations between species richness (ΔS) and change in abundance (ΔN) for EPT per trap.

Figure S4. Pairwise Pearson correlations between change in species richness (ΔS) and change in rarefied species richness (ΔS_n) for EPT per trap.

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