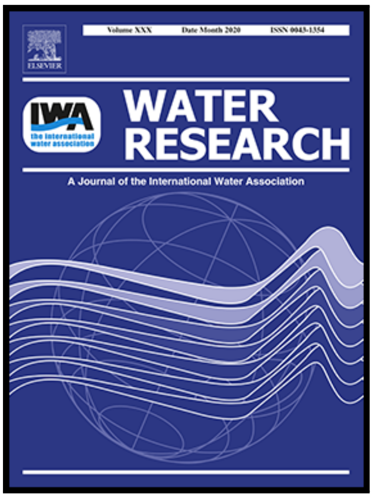

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Highlights

- First multi-taxon nationwide study of abundance-environment associations
- Ensemble models handle sparse data; validated by cross-validation and permutations
- Identifies key stressors (e.g., nutrients, metals) for biodiversity recovery
- Scalable method for bioassessment in data-limited regions worldwide
- Links long-term abundance trends to actionable water quality management goals

The relative importance of multiple environmental factors on river macroinvertebrate abundance across different groups based on a nationwide dataset from England

Authors

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Abstract Many countries are concerned by and wish to arrest or reverse what is termed a biodiversity crisis in invertebrates. To understand the issues facing riverine invertebrates in England, a fully integrated dataset where macroinvertebrate monitoring sites were aligned in space and time with physical, geographic, habitat, and chemical factors from 2003 to 2018 (quantitative abundance data being universally available from 2003) was brought together for statistical analysis. Over this per[illegible]m was to identify what the principal factors were that influenced *Ephemeroptera* (Mayflies), *Plecoptera* (Stoneflies), *Trichoptera* (Caddisflies), *Odonata* (Dragonflies and Damselflies), *Diptera* (True Flies), *Coleoptera* (Beetles), *Hemiptera* (True Bugs), and *Gastropoda* (Snails) abundance over this 16-year period. The dataset was examined using an ensemble framework within two modelling approaches: generalised linear mixed-effects models with permutation-based variable importance, as well as non-linear generalised additive mixed models to assess the percentage of deviance explained by each variable. The range of approaches aimed to offer different perspectives on variable importance, providing a more comprehensive understanding of the data and highlighting how model selection can influence ecological data interpretation. For most groups, physical factors, such as altitude, distance from source, slope, bed substrate and flow discharge, were strong predictors of abundance, likely reflecting natural habitat preferences shaped by evolutionary history. Land cover was also influential, with seminatural areas generally supporting higher abundances and urban land cover associated with lower abundances. Some chemical and ecological factors – such as wastewater and nutrient content, were particularly important for *Ephemeroptera, Plecoptera*, and *Trichoptera* abundance. For *Coleoptera*, *Hemiptera, Trichoptera, Diptera* and *Gastropoda*, metal levels played a role in their abundance, whilst for *Odonata,* mean temperature appeared to be important. *Diptera* appeared to be relatively insensitive to the factors examined. This statistical examination of large monitoring datasets, with no a priori assumptions, is vital in resolving a key challenge in bioassessment: identifying what influences invertebrate abundance when data are sparse. The results can provide policy options to improve ecological conditions, and the approach is transferable to other regions.

Introduction

There are now many international reports of increases in freshwater invertebrate richness and/or abundance that have occurred over the past 30 years (Hallmann and Jongejans, 2021; Haubrock *et a* e reports relate to rivers in industrialised regions of Europe and North America, multi-year increases have also been observed in other parts of the world, such as in Australian rivers associated with the ending of droughts (Paul et al., 2018). However, this pattern is not consistent, as other research has found stable communities or no clear temporal trend (e.g., Metzeling et al., 2002). The absence of a comprehensive systematic review means that the prevalence of these increasing trends remains uncertain and could be influenced by publication bias. Where increases have been detected, a common finding is that for many invertebrate groups, this increase in biodiversity, beginning in the early 1990s, has recently slowed or plateaued (Haase *et al.*, 2023; Pharaoh *et al.*, 2023; Qu *et al.*, 2023). There has been considerable speculation about what environmental changes led to these increases in biodiversity and what might now be preventing further recovery. These questions are not straightforward to answer, since geographic, landscape, habitat, physical and chemical factors will all play a role in the suitability of a waterbody to host assemblages of invertebrates. There are many candidates as to which stressors could have played or are still playing the leading role in controlling invertebrate diversity. These include temperature (Baranov *et al.*, 2020; Jourdan *et al.*, 2018), habitat/morphology (Gieswein *et al.*, 2017; Graeber *et al.*, 2017), nutrients (Pearson *et al.*, 2016), pesticides (Liess *et al.*, 2021), wastewater components such as Biochemical Oxygen Demand (BOD) and ammonia (Vaughan and Ormerod, 2012), metals (Johnson *et al.*, 2017), persistent organic pollutants (Malaj *et al.*, 2014; Posthuma *et al.*, 2020) or candidates from the long list of modern pharmaceuticals and care products (Groh *et al.*, 2022). In a recent analysis of 41 different variables, including physical factors, geography, landscape, habitat, and chemicals for a 30-year English national dataset, it was found that Zn, Cu, BOD, Ni and ammonia came out strongest as controlling variables for richness (Johnson *et al.*, 2025).

Globally, all these pressures are recognised as major drivers of biodiversity loss under international frameworks. Notably, the Convention on Biological Diversity (CBD) establishes a legally binding objective to halt biodiversity loss, with its subsequent Kunming-Montreal Global Biodiversity

Fra [illegible] is imperative is further reinforced by the UN Sustainable Development Goals, which explicitly call for improved water quality and the protection of water-related ecosystems (United Nations, 2015).

In the England Government's targets for biodiversity (UK Government, 2023), abundance is seen as a key metric, therefore, there is a particular policy interest in what controls freshwater invertebrate abundance. Abundance is widely embedded in regional and global IUCN conservation assessments and critical for understanding population collapse and recovery (Callaghan *et al.*, 2024). This focus is also mirrored by the international policy, with the Kunming–Montreal Global Biodiversity Framework establishing a specific goal to increase the abundance of native wild species to healthy and resilient levels by 2050 (CBD, 2022). This creates a direct and urgent policy interest in identifying the factors that control freshwater invertebrate abundance. Understanding how multiple stressors interact to drive these abundance trends is therefore a question of both national and global relevance.

A theoretical view on drivers of invertebrate richness versus abundance might be that richness (in effect, the presence/absence of different species) is determined by whether local conditions meet both the basic habitat requirements associated with the evolutionary preferences of the organisms and the essential environmental thresholds, such as sufficient oxygen levels and the absence of toxic concentrations of stressor chemicals.

In contrast, invertebrate abundance responds not only to the above, but also to long-term or short-term variations in food availability (which may be potentially influenced by nutrient levels, temperature or extreme flow events). It is also important to note that patterns in richness and abundance do not always align, a common finding in community ecology (Blowes et al., 2022). A change in richness, whether an increase or a decrease, may be attributed to specific factors, but overall abundance can still remain stable due to the presence of more resilient species within the community. Furthermore, the response of abundance may lag behind changes in richness. Thus, abundance may not reflect the same drivers as richness and understanding the key variables inf [illegible] s (invertebrates captured within a net) go back to the 1980s or even earlier, quantitative records for abundance across all regions in England only became commonplace from 2003.

This study investigated the key factors influencing the abundance in English rivers of eight invertebrate groups: *Ephemeroptera* (Mayflies), *Plecoptera* (Stoneflies), *Trichoptera* (Caddisflies), *Odonata* (Dragonflies and Damselflies), *Diptera* (True Flies), *Coleoptera* (Beetles), *Hemiptera* (True Bugs), and *Gastropoda* (Snails) over a 16-year period. We employed generalized linear mixed-effects models (GLMMs) with permutation variable importance, combined with a 5-fold cross-validation procedure and evaluated using mean absolute error (MAE) and root mean square error (RMSE). Additionally, non-linear generalized additive mixed models (GAMMs) were used to evaluate the percentage of deviance explained by each variable. A total of 43 key chemical, physical, habitat, and land-use variables were included in the analysis to explore their relative impact on invertebrate abundances. An additional objective was to explore how the application of two distinct modelling approaches offered different perspectives on the relative variable importance and provided a more comprehensive understanding of the data. This methodological approach not only sheds light on how model selection can influence the interpretation of ecological data, but also provides a practical framework that researchers can adapt to identify the most suitable modelling strategy for their specific datasets.

The Environment Agency monitoring network is designed for repeated sampling at fixed sentinel sites, reflecting its purpose as a long-term spatio-temporal surveillance programme. Accordingly, our objective is to identify environmental variables associated with invertebrate abundance across both space and time, explicitly modelling the structured dependence of samples within sites over time to draw inference on environmental effects. Although England represents a relatively small geographic area compared with continental or global studies (e.g. Feio et al., 2023; Haase et al., 2023; Kefford et al., 2023), the present analysis covers the entirety of the country, making it a truly national-scale assessment. The strength of this study lies not in its geographic extent but in its me [illegible] st spatially and temporally complete national monitoring programmes available globally. The approach developed here, integrating ensemble GLMMs and GAMMs, provides a scalable framework that can be readily applied to other regions or nations.

Data

We used an integrated dataset on macroinvertebrate abundance (Bachiller-Jareno *et al.*, 2025) comprising Environment Agency macroinvertebrate survey data, along with accompanying Environment Agency chemical and habitat data. Data on land cover were obtained from a 2015 land cover map (Rowland et al., 2017), river flow came from gauging stations curated by the National River Flow Archive, and wastewater exposure was derived from a water quality model. Air temperature was taken from CHESS-met (Robinson et al., 2023), a dataset providing daily meteorological variables at 1 km resolution. For each macroinvertebrate site, daily temperature values from the six months preceding the sampling date were extracted, and from these daily values, the minimum, maximum, and mean air temperatures were calculated (Table S2; Supplementary Materials). This dataset is available on the EIDC as “Macroinvertebrate taxonomic abundance, water quality, river flow, air temperature and environmental site descriptors from English rivers, 1965-2018” (Bachiller-Jareno *et al.*, 2025). Before 2003, macroinvertebrate abundance data were largely recorded in a semi-quantitative manner, whereas after 2003 quantitative recording became routine. Therefore, this study used data from 2003 onward from 1443 sites to ensure consistency and accuracy in our analysis. The sites were distributed across the seven regions of England and were originally selected by the Environment Agency to provide representative coverage across major catchments and river types, capturing a gradient of environmental conditions from relatively undisturbed to impacted locations (e.g. downstream of wastewater inputs or other potential stressors). Sites were included by Environment Agency if they had been monitored for at least 10 years, with approximately 200 sites per region. The same sites were sampled repeatedly, typically twice per year, in spring (March-May) and autumn (September-November). Because the monitoring des [illegible] d modelled explicitly rather than collapsed to a single observation per site. This approach maintains information on temporal variability in environmental variables. Repeated samples within site-years were treated as non-independent (Material and methods).

Further methodological details are described in Bachiller-Jareno et al. (2025).

This study focused on eight broad taxonomic groups: *Ephemeroptera* (Mayflies; 26 taxa), *Plecoptera* (Stoneflies; 14 taxa), *Trichoptera* (Caddisflies; 65 taxa), *Odonata* (Dragonflies and Damselflies; 9 taxa), *Diptera* (Flies; 25 taxa), *Coleoptera* (Beetles; 34 taxa), *Hemiptera* (True Bugs; 15 taxa), and *Gastropoda* (Snails; 23 taxa). We restricted our analysis to species included in Defra's D4 Indicator of Biological Diversity (Defra, 2024), which comprises only species with sufficient data to derive robust national abundance trends. Consequently, the dataset represents a policy-relevant subset of taxa (those contributing to England's legally binding species abundance target (The Environmental Improvement Plan, 2023), rather than the full diversity recorded across sites. All taxonomic names were standardised using a reference table based on Davies & Edwards (2011), ensuring consistency across time and EA regions. It should be noted that the dataset included both species- and genus-level identifications, which varied across taxonomic groups and spatial regions, reflecting the inherent diversity of the data and the varying levels of taxonomic resolution achieved during data collection. To ensure analytical consistency and comparability across all sites and years, we aggregated all records to the order level, thereby resolving any inconsistencies in lower-level taxonomic resolution.. The abundance data exhibited a large number of zeros, which is illustrated in Figure SM-1 (Supplementary Materials).

The dataset also contained extensive missing data in the explanatory variables, with up to 91% missingness for some variables (Table S1, Supplementary Materials). A total of 29,466 invertebrate monitoring records (observation data points) were available where at least two explanatory variables were recorded.

In this study, we [illegible] used a range of explanatory variables encompassing chemical, physical, and and habitat variables. These variables could be either spatiotemporal (varying across time and space) or spatial (varying across locations but not time) (Table S2; Supplementary Materials). With regard to the chemical data, we focused primarily on one metal type - specifically dissolved metal, with total concentrations considered only when dissolved concentrations were unavailable. Dissolved metals are more ecologically relevant for macroinvertebrates, as they represent the biologically available fraction of the metal that can directly interact with organisms. Given the broader ecological importance of temperature and flow in freshwater ecosystems – including evidence from studies on juvenile fish survival (Nunn *et al.*, 2003) – we included three different (air) temperature variables (minimum, maximum, and mean) and eight distinct flow variables (Table S2; Supplementary Materials) for the analysis. It is important to note that chemical, flow and temperature-related variables were representative of the six-month period leading up to the macroinvertebrate sampling date (Bachiller-Jareno *et al.*, 2025).

There is variation over time in the abundance of the taxonomic groups, with most showing stable or modestly increasing patterns on average over time (Figure 1). However, spatial variation in abundance was much greater than temporal change, with species showing differences of up to three orders of magnitude across sites (Figure 2). For example, *Odonata* were more abundant in southern regions, while *Plecoptera* were more abundant in the northern and south-western regions. This underscores the importance of more detailed temporal analysis to ensure that derived temporal trends have not been influenced by any changes in the spatial distribution of sampling over time. This is beyond the scope of this work and has been done by other authors (Boyd et al., 2022; Wilkes et al., 2025a; Wilkes et al., 2025b). We simply include these here to highlight the apparently much greater influence of spatial rather than temporal variation in abundance.

A complete list of variables, including their descriptions and units, is provided in Table S2 (Supplementary Materials).

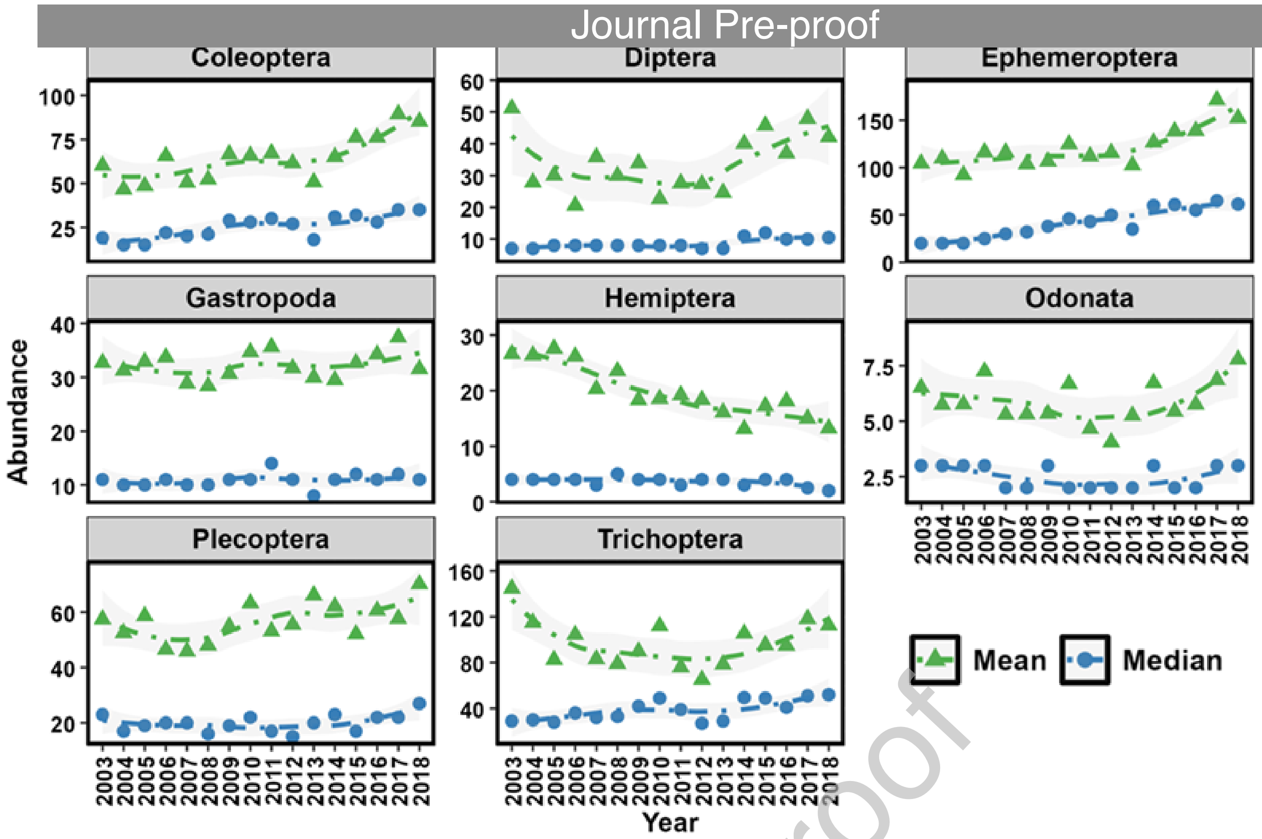

Figure 1. Temporal trends in mean and median abundance for eight taxonomic groups. Dashed lines show LOESS-smoothed trends fitted separately for each group. *Note: survey effort varied across years. Raw trends are shown, but the analysis accounted for spatial and temporal variation (see Methods).*

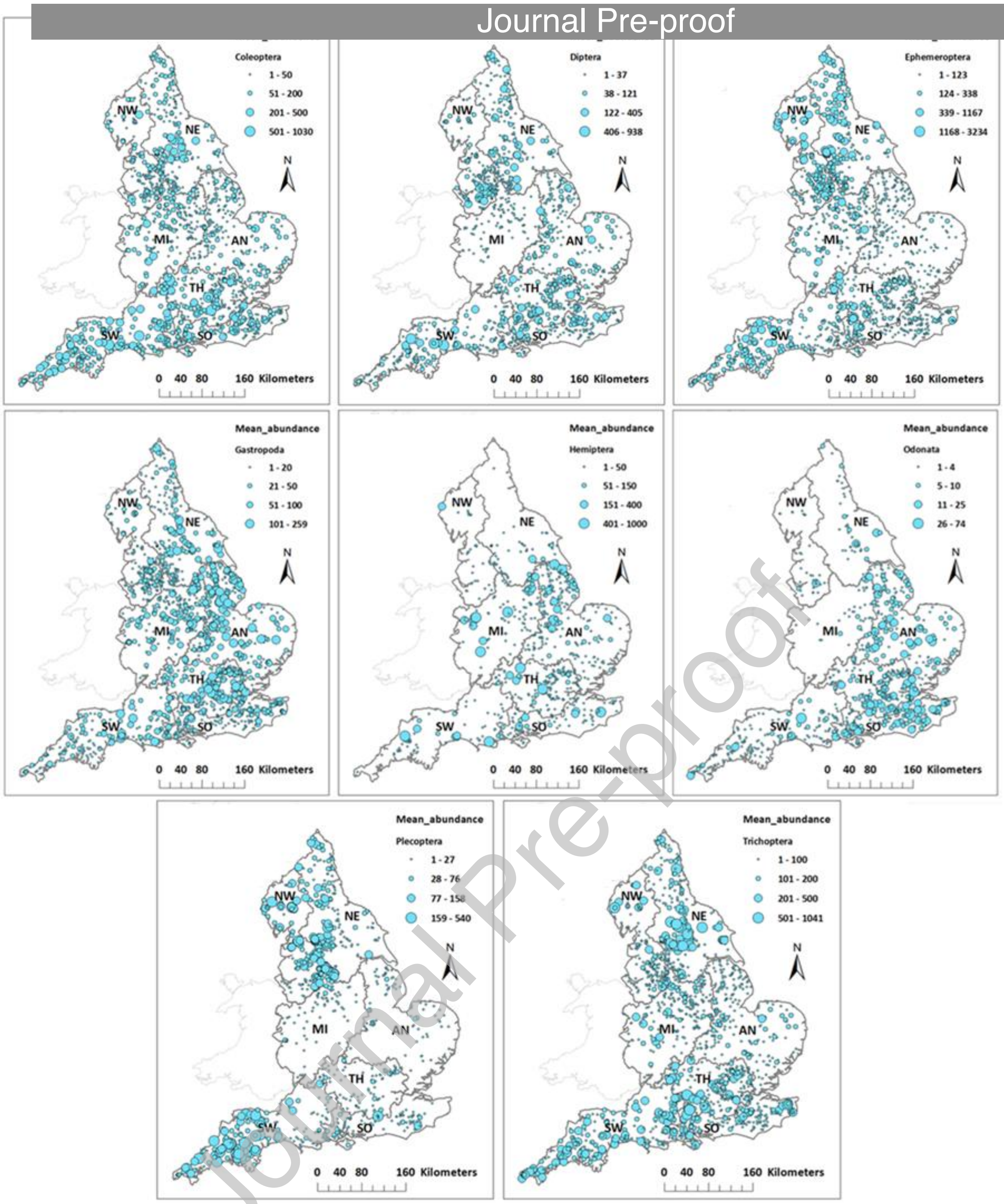

Figure 2. Spatial distribution of mean abundance of taxonomic groups across England. Regional acronyms: NW = North West, NE = North East, MI = Midlands, AN = Anglian, TH = Thames, SO = Southern, SW = South West.

Material and methods

We adapted the modelling approach developed by Johnson et al. (2025), but simplified it to suit our much smaller datasets. Full methodological details are provided in the Supplementary Materials.

All chemical, habitat, physical, land, and time variables were standardised (to mean of zero, standard deviation of one) using the “scale” function in R to ensure comparability across variables.

We used two complementary modelling approaches: Generalized Linear Mixed Models (GLMMs) with Template Model Builder (GLMM-TMB) (Brooks *et al.*, 2017; Kristensen *et al.*, 2016) and Generalized Additive Mixed Models (GAMMs) (Hastie & Tibshirani 1990; Wood, 2017; 2022).

For GLMMs, the abundance of each species group was assumed to follow a negative binomial distribution. We also tested other distributions (Supplementary Materials).

Each model included five variables of interest, modelled as linear effects: two chemical variables (to allow comparison between combined chemical effects), one habitat variable (habitat modification score, HMS), one physical variable, and one land-use variable. This structure was selected based on ecological rationale, following expert recommendations to represent a comprehensive set of pressures across key environmental domains. To ensure the robustness of the selected five-variable structure, we also tested models with 3 to 6 variables (Supplementary Materials).

We considered all possible combinations of five variables, which, after excluding models with highly correlated variables (≥0.7), or conceptual overlap (such as Ammoniacal Nitrogen and Ammonia), resulted in a total of ~25K unique models per taxonomic group. This approach, driven by the extensive missing data (up to 91% for some variables; Table S1, Supplementary Materials), allowed us to use every available observation where a small number of explanatory variables were recorded. Consequently, model sample sizes varied, but all were retained based on performance (Supplementary Materials).

To account for hierarchical spatial structure and the fact that some sites were sampled repeatedly across years, we included nested random effects for sites within regions. To evaluate whether varying within-site sampling intensity influenced inference, we conducted sensitivity analyses with reduced sampling intensity (Supplementary Materials). Temporal variation was addressed by incorporating both a linear year trend and a non-linear seasonal effect (modelled using natural spl [illegible] of overfitting.

This relatively simplified model structure was chosen to reduce overfitting risk. While residual temporal autocorrelation may remain, cross-validation indicated that it had minimal impact on predictive performance (Supplementary Materials).

Given the extensive number of models fitted across multiple taxonomic groups, this computational constraint motivated the use of broader taxonomic groups instead of species- or genus-level analyses. Modelling was conducted using the glmmTMB function in R (Brooks *et al.*, 2017; Kristensen *et al.*, 2016), chosen for its computational efficiency.

GAMMs similarly assumed a negative binomial distribution for the response variables. However, unlike the GLMMs where we considered five variables of interest, in the GAMMs, we used only two variables of interest per model to avoid overfitting and overparameterization, while allowing for nonlinear relationships between the explanatory variables and the species groups. While we also tested models with three explanatory variables, preliminary runs revealed frequent convergence issues, which limited their interpretability and practical use.

The explanatory variables selected for any given model could be from any category, such as chemical, habitat, physical, or land variables. For example, one model could include two chemical variables, another could combine a chemical and a land variable, etc. As in our GLMM approach, we excluded highly correlated (≥0.7) or similar variables (Ammoniacal Nitrogen and Ammonia, etc.) to avoid multicollinearity, resulting in a total of ~0.9K models per taxonomic group.

Finally, these GAMM models also incorporated nested random effects, along with a linear year trend and a non-linear seasonal trend, to account for temporal dependencies in the data.

As with the GLMMs, analyses were conducted at the level of broader taxonomic groups (rather than species or genus) due to computational constraints. Although GAMMs involved fewer models per group, the inclusion of nonlinear terms made them computationally intensive. Modelling was conducted using the gamm4 function in R (Wood, 2017; 2022).

Va

To assess the relative importance of explanatory variables, we used two complementary methods: permutation variable importance (applied to GLMMs) and deviance explained (applied to GAMMs). Permutation importance, combined with a 5-fold cross-validation procedure and evaluated using Root Mean Square Error (RMSE) and Mean Absolute Error (MAE), identified variables influencing predictive accuracy by quantifying both consistent baseline effects (via MAE) and outlier-driven impacts (via RMSE). We defined the impact score as the absolute percentage change between the original model's RMSE or MAE and the corresponding value after permutation (Supplementary Materials). Variables were considered important if they appeared as important in at least 90% of models (typically >95%) and had high impact scores (based on group-level sensitivity, typically >5%). We also note low-frequency, high-impact-scores variables, which may be important in certain contexts. Deviance explained quantified the proportion of variation in macroinvertebrate abundance attributable to each variable, offering a direct measure of explanatory power. For deviance explained, we emphasised top-ranking predictors or those explaining ≥10% deviance, although even the highest values were often modest (10-15%). A full definition of prediction power and explanation power is provided in the Supplementary Materials. Full methodological details are provided in the Supplementary Materials.

Because model types capture different aspects of variable importance, we do not rank predictors strictly but instead present them as important from complementary perspectives. Directionality is summarized across models as (positive), (negative), (direction varies), or (uncertain/near-zero), with categorical effects labelled accordingly (categorical, level-dependent effects). Detailed rankings are available in the Supplementary Materials (figures).

All relationships described in the following sections are statistical associations based on observational data. They should not be interpreted as evidence of causal mechanisms.

Results

Across all taxonomic groups, abundance patterns were closely linked to both land cover and water quality gradients. In general, seminatural land cover tended to support higher abundances, while urban land cover and wastewater exposure were associated with lower abundances - especially for sensitive groups such as *Plecoptera*, *Ephemeroptera* and *Trichoptera*. Nutrient levels, particularly orthophosphate and nitrate, also showed negative relationships with abundance, although the strength of these effects varied among taxa. Geographic and habitat features such as altitude, slope, distance from source, flow discharge and bed substrate were important as well, with upland areas and coarse substrates typically supporting more sensitive species. Metal contamination (notably copper and zinc) had clear negative effects on several groups, including *Coleoptera, Hemiptera, Trichoptera, Diptera* and *Gastropoda*, whereas temperature played a more variable role, being particularly relevant for *Odonata*. Variables related to basic water chemistry (e.g., hardness and alkalinity) showed more taxon-specific effects, being particularly influential for *Gastropoda* and *Plecoptera*. *Diptera* appeared to be relatively insensitive to the factors examined.

Overall, the findings suggest that both catchment-scale land use and local chemical conditions jointly shape macroinvertebrate abundance, but the strength and direction of these effects differ among groups.

Plecoptera (Stoneflies)

Plecoptera abundance was identified as the most sensitive to the variables available to be examined in this study. Both GLMM (based on RMSE and MAE) and GAMM methods indicated that *Plecoptera* abundance was strongly related to both land cover factors, such as seminatural (positive) and urban (negative) cover, as well as general water quality (negative) (Figure 3). Altitude (positive) and slope (positive), the latter referring to the gradient of the river at the biological monitoring point, also proved to be important. It is worth noting that slope had a much higher impact score in RMSE-based models compared to MAE-based ones, suggesting that this variable may be more influential in predicting extreme values or outliers.

Wa [illegible] d, on average, also explained a substantial proportion of the variability in abundance, highlighting the sensitivity of *Plecoptera* to organic rich water.

Identifiable chemical factors important for *Plecoptera* abundance, based on both GLMM and GAMM, included orthophosphate (PO_4) (negative), nitrate (NO_3) (negative), and alkalinity (negative). While these variables had relatively low impact scores, they were selected in a large proportion of models (either RMSE or MAE) and explained, on average, a substantial portion of the variability in abundance, indicating their relevance for understanding ecological patterns even when predictive influence is limited. Alkalinity or hardness may not be a direct influence on Plecoptera, rather that upland, high slope rivers, for which these invertebrates seem to have an evolutionary preference, are more acidic than the lowlands in England, which tend to be more basic in pH.

While GLMM results based on permutation importance were largely consistent with GAMM results using deviance explained, the latter also highlighted additional variables that explained a substantial amount of variability: arable land cover (direction varies), bed substrate (higher values = finer substrate, lower values = coarser substrate like gravel) (negative), and nitrite (NO_2) (negative).

Several other variables were consistently selected in a large proportion of models but had very low importance scores and deviance explained values, indicating weak associations with *Plecoptera* abundance (Figure 3).

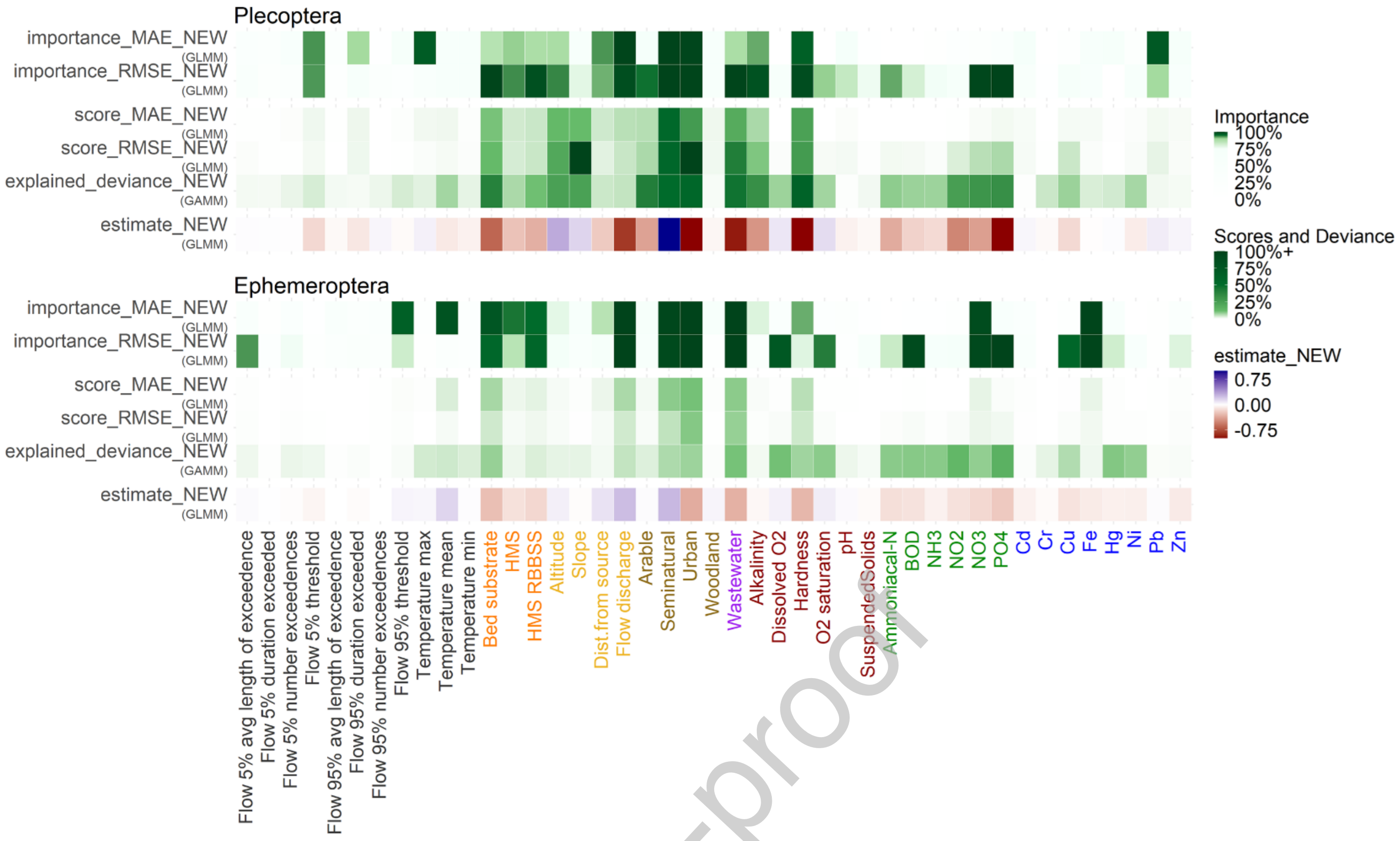

Figure 3. Heatmaps for Plecoptera (top) and Ephemeroptera (bottom) showing six metrics. Importance values (MAE/RMSE) indicate the percentage of models in which each variable was significant, with values >75% shaded green. Score values represent the mean absolute percentage change in MAE/RMSE between original and permuted models, capped at 100% (values >100% labelled "100%+"). Explained deviance reflects the percentage of deviance explained by GAMMs. Estimates are shown with both magnitude and direction: positive effects in dark blue and negative effects in dark red. For the categorical variable "discharge", estimates are averaged across factor levels. X-axis variables are color-coded by category: metals (blue), basic chemistry (rust red), nutrients & BOD (green), temperature & flow (grey), wastewater (purple), habitat (orange), geography (yellow), and land cover (brown).

Ep

Ephemeroptera abundance responses to the variables (Figure 3) appeared somewhat less sensitive than those of *Plecoptera*, as indicated by the smaller number of important variables identified and generally lower impact scores.

Similar to *Plecoptera*, *Ephemeroptera* abundance showed important positive relationships with seminatural land type and negative relationships with urban land type (both RMSE and MAE). However, these variables explained a relatively smaller portion of the variability in *Ephemeroptera* abundance compared to *Plecoptera*, suggesting weaker overall associations.

Both GLMM and GAMM methods indicated that *Ephemeroptera* abundance was sensitive to the presence of nutrients, particularly orthophosphate (PO_4) (negative), basic chemistry variables such as dissolved oxygen (positive), and wastewater (negative). Additionally, GAMM identified an association with nitrite (NO_2) (negative), while GLMM highlighted nitrate (NO_3) (negative). However, the impact scores for some of these variables were relatively low, indicating weaker predictive power, although they may still contribute to explaining variation in abundance.

A range of other variables were consistently selected in a large proportion of models, though they showed relatively low impact scores and explained variability (Figure 3).

Trichoptera (Caddisflies)

Trichoptera, the third group in the EPT indicator taxa commonly used to assess water quality, appeared less sensitive to environmental gradients than *Plecoptera*, similar to the pattern observed for *Ephemeroptera*. Higher abundance was associated with locations further downstream in the catchment (as indicated by a positive relationship with distance from source), in larger rivers (flow discharge; varied), and in areas with greater seminatural land cover (positive) (Figure 4). However, these variables explained only a limited proportion of the variability in abundance, indicating that their value for explaining past patterns in *Trichoptera* abundance was modest. Additionally, all of these variables were selected in a large proportion of MAE-based GLMMs, but not RMSE-based ones, suggesting they were more effective at predicting typical abundance patterns than at capturing extreme values.

As with *Plecoptera* and *Ephemeroptera*, *Trichoptera* abundance showed sensitivity to urban land cover (negative), wastewater exposure (negative), and orthophosphate (PO_4; negative) (Figure 4), with all three variables consistently selected in both RMSE- and MAE-based GLMM models. Among these, wastewater and PO_4 ranked highly in the GAMM results, indicating a relatively strong explanatory role, whereas the influence of urban cover appeared more modest. In contrast to the other EPT groups, *Trichoptera* were less responsive to nitrogen-based variables.

The GAMM results (non-linear relationships) differed from those of the GLMMs (linear relationships) for this group, highlighting a distinct set of variables with strong explanatory power. In particular, biochemical oxygen demand (BOD; negative), ammoniacal nitrogen (negative), and dissolved copper (Cu; negative) emerged as top explanatory variables in the GAMM models combined with deviance explained (i.e., informative for explaining past patterns). However, these variables had relatively low importance scores in the GLMMs, suggesting they may not be strong linear predictors of *Trichoptera* abundance. This difference likely reflects non-linear relationships between these variables and abundance, which are better captured by the flexible smoothing functions used in GAMMs.

Other variables were also selected across GLMM models, but showed modest impact scores and limited ability to explain variation (Figure 4).

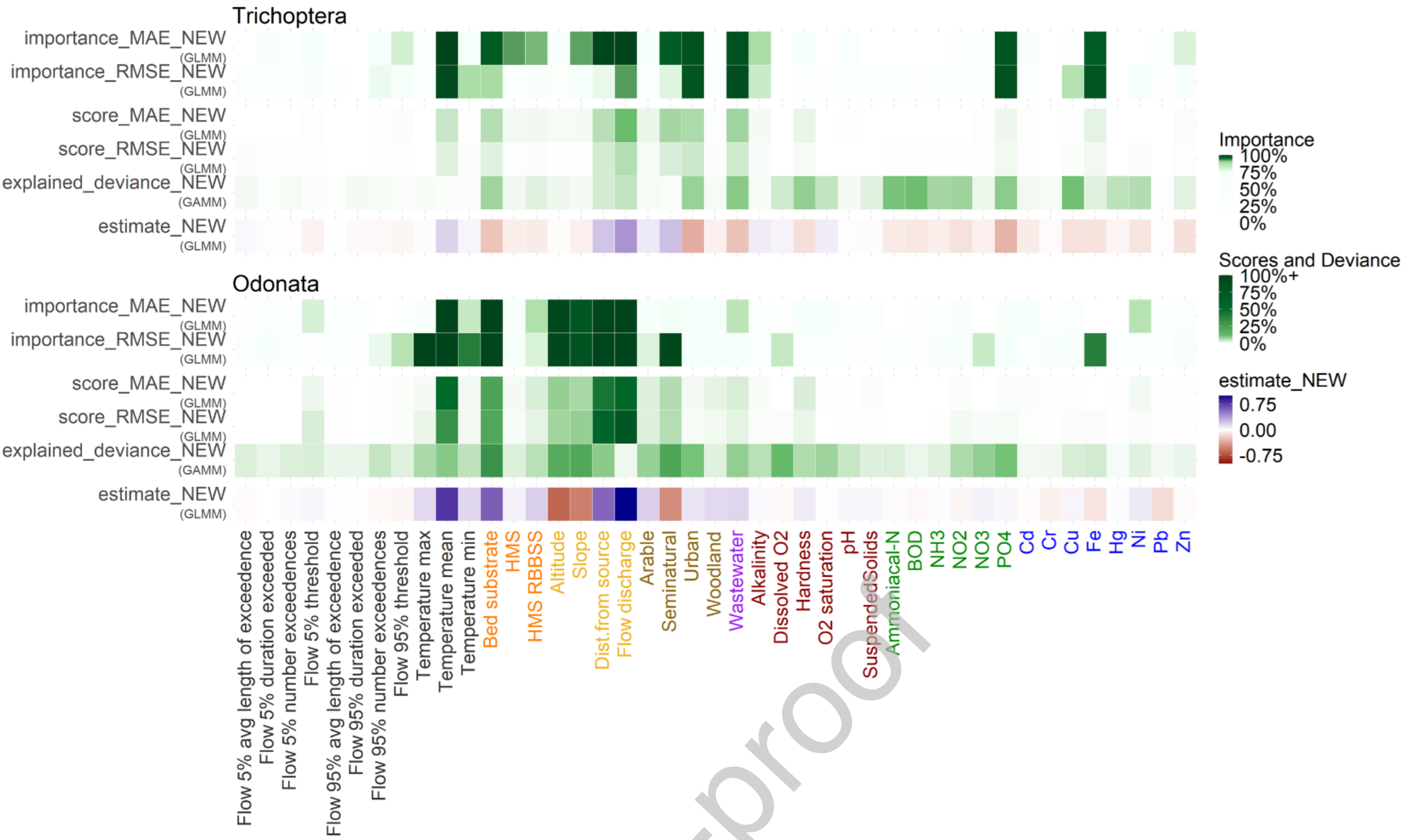

Figure 4. Heatmaps for Trichoptera (top) and Odonata (bottom) showing six metrics from GLMM and GAMM analyses. Metrics follow those in Figure 3.

Odonata (Dragonflies and damselflies)

Odonata abundance appeared to be most strongly associated with bed substrate (positive), which ranked highest in the GAMM models, indicating it explained a substantial proportion of variability, and also showed a high impact score in the GLMMs (both RMSE and MAE). Geographic factors also played a key role, including flow discharge (categorical, level-dependent effects), distance from source (positive), altitude and slope (both negative), with strong support across both RMSE- and MAE-based evaluations (Figure 4). Greater abundance was observed lower in the catchment (as indicated by distance from source) and in larger rivers (as indicated by flow discharge). While altitude and slope were selected in a large proportion of GLMM models and explained a substantial portion of the variability in GAMM results, their impact scores were lower than those of distance from source an [illegible] s, it explained little variability in the GAMMs.

Mean air temperature (positive) was another important factor for *Odonata*, with high impact scores, but it explained just under 10% of the deviance in GAMMs. Maximum air temperature was also consistently selected but had lower importance scores, suggesting a weaker yet stable association. Unlike the EPT group, *Odonata* abundance showed a negative relationship with seminatural land cover, which was selected in RMSE-based models (suggesting particular importance for predicting extreme values) and explained a substantial proportion of variability.

Among the variables that explained more than 10% of deviance were also urban land cover (negative), orthophosphate (PO_4; negative), and dissolved oxygen (direction varies), highlighting their potential importance for understanding ecological patterns, even if their relationships with abundance are not clearly directional.

Gastropoda (Snails)

Gastropoda were nearly as responsive as *Plecoptera* in terms of the number of important variables identified by the GLMMs; however, their impact scores were generally lower (Figure 5). Variables with relatively high impact scores included distance from source (positive), flow discharge (categorical, level-dependent effects), arable land cover (positive), alkalinity (positive), and hardness (direction varies), with strong support across both RMSE- and MAE-based evaluations. Both alkalinity and arable land were also among the top-ranking GAMM variables, which explained a substantial proportion of variability in abundance, along with slope (negative).

Among variables selected in a large proportion of models but with relatively low impact scores, many were metals, specifically zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd), and iron (Fe), all showing negative associations. These results were supported by the linear estimates, where Zn had the strongest significant negative effect on *Gastropoda* abundance, followed by Cu (Supplementary Materials). This indicates a particularly significant impact of metal contamination on the [illegible] n explaining just under 10% of the deviance and Fe just over 10%.

Among other variables, pH (positive, but near zero) from the basic chemistry group was identified in RMSE-based models and explained just below 10% of the deviance, but had a low estimate and small impact score.

Some additional variables were identified as significant in a large proportion of GLMM models but had low impact scores, small effect estimates, and minimal deviance explained (Figure 5).

Figure 5. Heatmaps for Gastropoda (top) and Coleoptera (bottom) showing six metrics from GLMM and GAMM analyses. Metrics follow those in Figure 3.

Coleoptera (Beetles)

Coleoptera abundance showed a strong positive relationship with seminatural land cover and a negative relationship with urban land cover and wastewater exposure (Figure 5). All three variables had [illegible]al abundance patterns and more extreme values.

Coleoptera tended to occur more frequently in larger rivers, as represented by the categorical flow discharge variable with level-specific effects.

Among the variables with the strongest explanatory power for past patterns were metals (Ni, Cu), nutrient-related variables (ammoniacal nitrogen, NH_3), BOD and hardness, all showing negative associations. However, GLMMs indicated that these variables had limited predictive power. This difference may be due to their non-linear effects, which are better captured by GAMMs than by GLMMs relying on linear relationships.

Some variables appeared consistently across GLMM models as significant but showed low impact scores, weak effect sizes, and explained little variance (Figure 5).

Hemiptera (True bugs)

For *Hemiptera*, there was strong agreement between the GLMM and GAMM models, and this group showed multiple key variables that demonstrated both strong predictive power (for predicting both typical abundance patterns and extreme values) and strong explanatory value (Figure 6). Among the most important variables identified by both modelling approaches were geographic factors (distance from source (positive), slope (negative), flow discharge (categorical, level-specific effects), and altitude (negative)), land cover variables (arable (positive) and seminatural (negative)), as well as the habitat variable clay/silty bed substrate (positive). However, slope appeared important in RMSE-based models but not MAE, indicating it may be particularly relevant for predicting extreme values. These patterns suggest that *Hemiptera* tend to be more abundant further downstream, in larger river systems, and in areas with fine-grained substrates such as clay or silt.

Wastewater (negative) emerged as an important predictor in MAE-based models, suggesting relevance for predicting baseline patterns. Meanwhile, HMS RBBS (mainly positive), and hardness (mainly positive) were selected in a smaller proportion of models (below 90%), yet showed large imp st regions, with some local variation in importance likely reflecting site-specific ecological differences. Several additional variables were commonly selected in GLMMs, yet their predictive and explanatory contributions were negligible.

Finally, Zn showed an extremely strong and statistically significant negative linear relationship ($p < 0.01$) in some models, as indicated by its linear estimate, which may suggest that zinc concentrations have a particularly strong influence on *Hemiptera* abundance in specific locations. However, it ranked lower in both predictive and explanatory power, indicating that its importance is more localized rather than widespread.

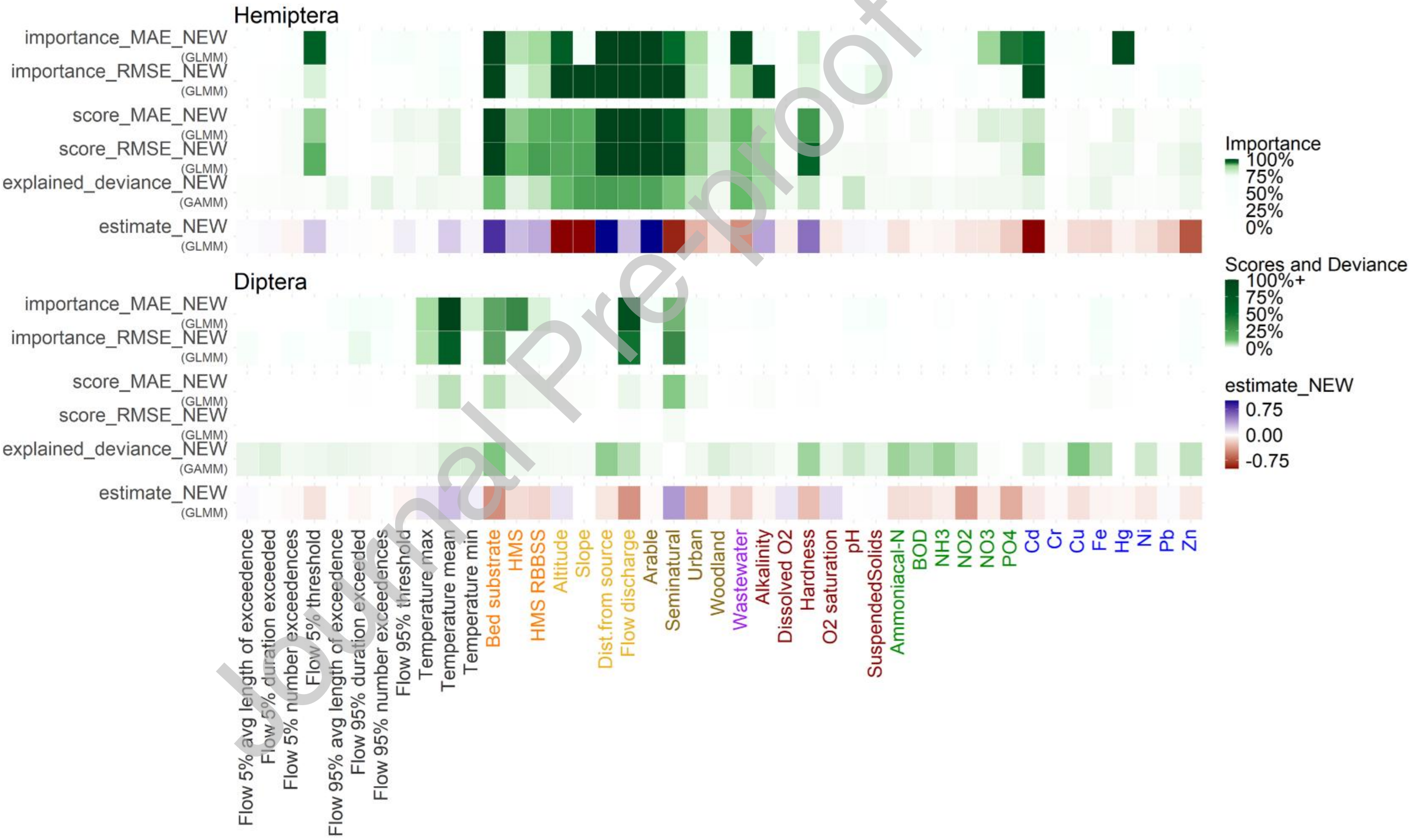

Figure 6. Heatmaps for Hemiptera (top) and Diptera (bottom) showing six metrics from GLMM and GAMM analyses. Metrics follow those in Figure 3.

Dip

Our analysis revealed that *Diptera* abundance was the least responsive to the environmental variables considered (Figure 6). No variable demonstrated both strong predictive power and explanatory power.

Only a few variables were selected in a relatively large proportion of models: mean air temperature (positive), seminatural land cover (positive), bed substrate (negative), and flow discharge (categorical). The first three ranked as the top MAE-based impact scores, though all remained very modest in magnitude, suggesting a limited role in predicting baseline abundance. Flow discharge had low impact scores, indicating a weak effect. Across all variables, RMSE-based impact scores were near zero, indicating that none had a strong role in predicting extreme abundance values.

In contrast, the variables with the strongest explanatory power (in GAMMs; defined as those explaining more than 10% of the deviance on average) were copper (Cu; negative) and bed substrate (negative). However, because Cu was selected in fewer GLMMs and had low impact scores, this suggests that while it may play a significant role in explaining abundance patterns, its overall predictive contribution (as a linear predictor) was limited. This appears to reflect a threshold-type response: *Diptera* abundance remained highly variable below ~7 µg/L but was significantly decreased at higher concentrations (Wilcoxon test on site-averaged data, $p = 0.01$), suggesting a suppressive effect of elevated copper levels on population size. A similar, even stronger pattern was observed in the full (non-averaged) dataset ($p < 2.2e-16$), though we prioritise the site-averaged result due to its better control for potential pseudoreplication. The effect remained significant when considering only sites where *Diptera* were present ($p = 0.01$, site-averaged data). Since concentrations ≥7 µg/L occurred at only ~2.6% of locations in the whole dataset (where Cu was recorded), Cu's overall predictive influence remained limited, despite its strong explanatory relevance in affected sites. Similar patterns were observed in other metal-sensitive taxa (data not shown).

Linear effects estimation highlighted, in addition to the variables above, NO_2 (negative effect), which showed strong and significant influence in some models. However, since it was important in only a small number of models and explained relatively little variability in abundance overall, it can be considered a variable of strong local importance in specific areas.

Discussion

Prediction vs explanation: a management dilemma

Predictive linear models are increasingly used in ecology to identify variables that forecast species responses (Tredennick, 2021). However, these models often prioritize broad-scale, static factors (e.g., slope, land cover) that generalize well but are not easily managed.

Explanatory non-linear models, by contrast, highlight dynamic variables (e.g., nutrients, metals) that show strong ecological relevance. For example, copper (Cu) was strongly linked to *Diptera* and *Trichoptera* abundance through threshold effects, but had limited predictive power, illustrating how some key stressors may be missed by models focused solely on linear prediction.

Some variables, such as bed substrate and alkalinity, showed both predictive and explanatory relevance.

It's important to clarify that a variable's role as "predictive" or "explanatory" in this context is defined by the modelling approach used in this study, not by any intrinsic property of the variable itself.

This dual-modelling approach has practical value. Predictive variables help with scenario planning, like forecasting land-use change effects, while explanatory variables guide interventions to reduce pressures such as nutrient or metal pollution. In practice, an integrated strategy might involve using high-explanatory-power variables (e.g., Cu, Zn, BOD, PO_4, etc.) to identify and monitor high-risk areas, such as catchments affected by industrial activity, while using stable, predictive variables (e.g., slope, altitude, land cover) to inform long-term conservation and land-use planning.

Us

This study assessed the value of order-level taxonomy for providing broad, management-ready insights into macroinvertebrate responses across England. We focused on this level because order-level data offer a consistent, nationally available metric for a first-pass, strategic assessment. This approach proved effective, revealing strong patterns for several key groups. For example, the consistent negative responses of Ephemeroptera and Plecoptera to nutrients provide clear, order-level signals of sensitivity, while the distinct profile of Odonata, driven primarily by physical habitat and temperature, offers complementary insights into different ecological pathways.

We acknowledge, however, that this coarse resolution may mask divergent responses within orders that include both sensitive and more tolerant species. The weaker signals observed for Diptera likely reflect this internal diversity, where opposing responses within the group can cancel each other out. This outcome is itself informative, as it immediately flags to managers which taxa would benefit from more nuanced, finer-scale investigation to understand underlying ecological dynamics.

In this way, our study provides both a validated template for large-scale assessment and a strategic roadmap for more detailed ecological analyses in the future.

Spatial and temporal dimensions

Our results consistently showed that spatially structured, temporally stable variables (e.g., geographic, habitat, and land cover features) were the strongest predictors of macroinvertebrate abundance. Abundance varied by up to three orders of magnitude across sites, indicating high spatial heterogeneity, while temporal changes were generally modest (Data section). The dominant role of spatial predictors explains why spatial variability in macroinvertebrate abundance outweighs temporal change within our 16-year study window.

The predictive strength of spatial variables likely reflects the evolutionary habitat preferences of macroinvertebrate groups, e.g., *Odonata* favouring warmer climates (Hickling *et al.*, 2005), *Ple*[illegible]th broad environmental gradients and local conditions.

Although overall abundance trends were generally stable (Figure 1), some groups such as the EPT group have increased in abundance over this time period. This implies that levels of some key stressors, whilst still important, are reducing.

Because variable importance was assessed across the full 16-year dataset, our analysis does not directly capture potential temporal shifts in the relative importance of factors. However, recent national-scale analyses of English rivers (Johnson et al., 2025) indicate that metal concentrations underwent major declines prior to 2000 and have since largely stabilised, which might explain their relatively low importance in our analysis for this more recent time period. Future analyses that explicitly partition the dataset by time period could help test these temporal patterns more directly.

Ecological mechanisms and trait-based interpretations

The contrasting responses among macroinvertebrate orders likely reflect fundamental differences in their ecological traits, including pollution tolerance, habitat specialisation, and life-history strategies.

The EPT taxa (*Ephemeroptera, Plecoptera, Trichoptera*) showed the strongest sensitivity to nutrient enrichment, wastewater exposure, and urban land cover, consistent with their well-known preference for cool, well-oxygenated, low-nutrient habitats (Haubrock et al., 2023). Perhaps more exuberant growth of some macrophyte and algal taxa in response to higher nutrients which in turn would produce more local leaf litter/detritus, is less suitable for case-building or habitat structure for these organisms (Tachet et al., 2010).

In contrast, *Odonata* showed weak responses to most chemical variables but were strongly influenced by temperature, flow discharge, and distance from source. These patterns are consistent with their relatively high dispersal capacity, thermophily, and reliance on emergent or slow-flowing habitats for reproduction. Their positive association with temperature likely reflects both direct effe [illegible] d prey availability.

Gastropoda showed strong relationships with hardness and alkalinity, as expected from their reliance on calcium for shell formation (Jordaens et al., 2006). Perhaps their greater sensitivity to Zn and Cu than many of the other invertebrate groups could be because one action of metal toxicity is interference with calcium metabolism and ion regulation. Similarly, *Hemiptera* and *Coleoptera* displayed variable responses to metals and wastewater exposure, reflecting their broad range of ecological niches and feeding modes, which confer intermediate tolerance levels (Iqbal et al., 2025). Finally*, Diptera* appeared to be relatively insensitive to most environmental gradients, showing only weak or localized relationships with chemical and physical variables. This likely reflects their short life cycles, high reproductive rates (as demonstrated in Zhai et al., 2025), and possibly wide habitat plasticity, traits that enable rapid recolonization and persistence under fluctuating conditions.

Taken together, these patterns demonstrate that variation in macroinvertebrate abundance across environmental gradients is largely structured by inherent ecological tolerances and life-history strategies, with sensitive taxa constrained by both catchment-scale and local-scale stressors.

Role of individual environmental variables

Altitude, slope, distance from source and flow discharge

For several groups, including EPT taxa, *Odonata, Gastropoda, Hemiptera*, and *Coleoptera*, at least one of these four physical geography descriptors emerged as an important predictor or explanatory variable for abundance. The results suggest varying habitat preferences, with some groups favouring headwater regions and others more downstream, larger river sections. The exception being *Diptera*, which seem to have no preference in terms of physical geography.

The importance of the distance from the source may relate to the drift behaviour of many groups, including both downstream drift and upstream movement, as well as their colonization cycle. Many stream insects engage in constant or catastrophic dispersal to downstream areas (Merritt & Cummins, 1996; Naman *et al.*, 2016), which likely explains the strong positive effects we observed for [illegible] g and colonizing patches where food resources are more abundant. For some species, this is further linked to the directional flight of females, particularly those that prefer to oviposit in headwater locations, such as shallow riffles with exposed boulders (Lancaster et al., 2020; Peckarsky et al., 2000). These areas, however, can lead to either increased abundance or higher egg mortality due to predator saturation (Hershey *et al.,* 2010).

<u>Land cover type</u>

Although the EPT group abundance is positively associated with seminatural land cover, this relationship may partly be due to the fact that such land cover often occur in upland, headwater areas where EPT taxa are naturally more abundant.

Similarly, there are positive relationships between *Odonata*, *Gastropoda* and *Hemiptera* and arable land cover, but that may be a reflection of a preference for lowland locations. This pattern could also be influenced by agricultural stressors not included in our dataset (e.g., pesticide use), which is often high in arable landscapes and is known to affect aquatic invertebrate communities (Liess *et al.,* 2021; von der Ohe & Goedkoop, 2013). The EPT group, *Coleoptera*, and *Hemiptera* showed a negative relationship with urban land cover. This may reflect a combination of stressors associated with urbanisation, such as transient acutely toxic events of organics and metals linked to road runoff. Routine monitoring is likely to miss such transitory, storm-generated events. As such, identifying the mechanisms involved is an important direction for future research.

<u>Habitat</u>

Upland sites are typically associated with coarser substrates such as gravels, whereas lowland rivers tend to accumulate finer silts and organic sediments. This may help explain why habitat variable bed substrate featured as an important variable for abundance, particularly for taxa that rely on coarse substrates for egg-laying (e.g., *Plecoptera*, many *Odonata*), case-building (e.g., many *Trichoptera*), or habitat structure (e.g., some *Diptera*) (Tachet *et al.*, 2010).

Wa

A range of organic contaminants are present in wastewater, but few are likely to be at acutely toxic levels for invertebrates in the receiving waters (Johnson *et al.*, 2017). Poorly treated wastewater typically contains high levels of organic matter, which increases biochemical oxygen demand (BOD), as well as elevated ammonia concentrations, both of which can be harmful to aquatic invertebrates (Bunzel *et al.,* 2013; Zhang *et al.,* 2023). In extreme cases, high BOD can lead to oxygen decline (Eriksen *et al.,* 2022). Wastewater exposure was negatively associated with the abundance of sensitive EPT taxa, consistent with expectations. For *Coleoptera*, wastewater, BOD, and ammonia were found to be important, indicating a broader sensitivity to multiple wastewater components. In contrast, while wastewater was associated with reduced abundance in *Hemiptera*, specific indicators such as BOD and ammonia did not appear to be significant contributors, suggesting that other constituents in wastewater may be more relevant for this group. No strong associations were found between wastewater, BOD, or ammonia and the abundance of *Odonata*, *Gastropoda*, or *Diptera*, indicating a higher tolerance or different response mechanisms in these taxa.

The nutrients

PO_4 was negatively associated with EPT abundance, particularly among upland-preferring *Plecoptera*, suggesting that phosphorus enrichment may alter habitat suitability for sensitive taxa.

PO_4 also emerged as an important explanatory variable for Odonata, indicating sensitivity to nutrient enrichment in this group - a pattern consistent with broader declines of aquatic insects under elevated nutrient conditions (Nessel *et al.,* 2023). In contrast, PO_4 appeared to have little or no influence on *Coleoptera, Gastropoda, Hemiptera,* or *Diptera*, reflecting a higher tolerance to nutrient enrichment among these taxa (Wagenhoff *et al.*, 2012).

Nitrate (NO_3) showed a weak negative association with *Plecoptera* and *Ephemeroptera*, while Nitrite (NO_2) had a stronger negative association with *Plecoptera, Ephemeroptera* (in explaining patterns), as well as with *Diptera* (at a more local scale). These results show that nutrient pollution disproportionately affects disturbance-intolerant taxa. Upland stream communities appear esp s and the presence of specialized taxa with narrow ecological tolerances.

Basic chemistry of alkalinity, hardness and pH

pH does not appear to be a strong driver of macroinvertebrate abundance overall, although it has some explanatory power in accounting for past patterns in *Gastropoda*. In England, many lowland rivers tend to have high alkalinity or hardness, reflecting the important contribution of aquifers to the river flow. This aquifer water brings with it high levels of Ca and Mg salts leading to the experience of hardness and alkalinity (Merrington *et al.*, 2016). Thus, relationships, positive or negative between freshwater macro-invertebrate abundance and these factors may be a geographic coincidence. For example, *Plecoptera* show a preference for upland regions, which are typically less influenced by hard groundwater inputs. This likely explains the observed negative association between *Plecoptera* abundance and hardness or alkalinity. But in the case of *Gastropoda* and *Hemiptera*, with their high sensitivity to metals, a positive relationship to hardness and alkalinity could be related to a reduction in free toxic metal ions with slightly higher pH as predicted in biotic ligand models (Meador, 1991; Stockdale et al., 2010).

Flow

It had been anticipated that flow, either high or low, could have played important transient roles in invertebrate abundance from year to year. Some low flow statistics are playing a role in *Plecoptera, an*d *Hemiptera* abundance, whilst *Gastropoda* and *Coleoptera* have a relationship to some high flow statistics. However, in our analysis, flow did not emerge as a strong influence. This could be due to our flow metrics representing 6-month averages and flow being more influential on shorter timescales.

(Ai

Abundance in most invertebrate groups was only weakly influenced by temperature, and this influence was generally positive. This is most likely related to the fact that these organisms are ectotherms, and warmer temperatures potentially supporting greater primary productivity and hence food sources.

However, temperature generally showed only weak associations with invertebrate abundance, with the exception of *Odonata* and *Diptera*, for which it emerged as one of the most important variables. This result may partly reflect a limitation of our dataset: we used mean air temperature over the past six months as a proxy for water temperature. Air temperature also may not accurately capture the thermal conditions experienced by aquatic organisms, especially in shaded or groundwater-fed streams. Additionally, different taxa may respond to temperature in taxon-specific ways, based on their life histories and feeding guilds, which can obscure consistent patterns at the community level. Furthermore, both hot summers and cold winters – rather than moderate, optimal conditions – can lead to extended periods of diapause or reduced activity, ultimately lowering observed abundance (Bowler *et al.*, 2017; Haase et al., 2019).

Metals

Whilst metals can be toxic at elevated concentrations, many – such as iron (Fe), zinc (Zn), and copper (Cu) – are also essential elements, as they play key roles in enzyme systems and other physiological functions in living organisms. As a result, nonlinear relationships with biological responses (e.g., invertebrate richness or abundance) are often observed. Previous research examining trends in invertebrate family richness from 1989 to 2018 found that declines in Zn, Cu, and nickel (Ni) were associated with ecological recovery and the return of sensitive invertebrate families (Supplementary Materials). Thus, it is possible that once metal concentrations fall below toxic thresholds, species (families) may recolonize a river. However, post-recolonization abundance may remain low due to factors such as interspecific competition, slow population growth, or other lim[illegible]m of “metal”-“invertebrate response” relationships may differ depending on whether richness or abundance is used as the response variable. In this study, we focused specifically on abundance-based responses aggregated at the order level, while recognising that richness-based metrics provide complementary insights into ecological condition.

Our analysis showed that metals played an important role, with notable negative associations observed in the following taxa: *Gastropoda* (Zn, Cu, Fe), *Coleoptera* (Ni, Cu), *Trichoptera* (Cu), *Diptera* (Cu), and *Hemiptera* (Zn).

Synthesis of ecological insights

Across environmental gradients, geographical and habitat variables (altitude, slope, bed substrate, and flow discharge) were important predictors of abundance for most groups, likely by determining habitat suitability. Coarse, upland environments tended to support sensitive taxa such as *Plecoptera* and *Trichoptera*, whereas finer, lowland habitats favoured more tolerant groups such as *Diptera* and *Gastropoda*.

Chemical stressors showed clear contrasts among taxa abundance: nutrients, BOD, and wastewater exposure reduced the abundance of sensitive EPT taxa, reflecting their low pollution tolerance, whereas tolerant groups such as *Diptera* showed weak or inconsistent responses. Metal concentrations, though generally lower than in past decades, still influenced several groups, particularly *Gastropoda*, possibly due to residual toxicity.

Together, these results reveal a broad ecological gradient from taxa adapted to clean, coarse, high-oxygen upland streams to those thriving in warmer, lowland, nutrient-enriched conditions. The consistency of these patterns across multiple stressors suggests that differences in pollution tolerance, habitat specialization, and dispersal strategies are key mechanisms shaping community responses.

Co

- Reduce orthophosphate (PO_4) levels, especially in upland areas where sensitive groups like *Plecoptera* thrive, assuming the metals are not already present at toxic concentrations. This can also benefit *Ephemeroptera* and *Odonata*, though effects on other groups may be weaker unless other stressors are addressed.
- Lower nitrogen compounds (nitrate, nitrite) to support *Plecoptera* and *Ephemeroptera*; impacts on other groups are less pronounced.
- Decrease metal pollution, notably copper (Cu), zinc (Zn), nickel (Ni), and iron (Fe), to improve abundance of *Gastropoda, Coleoptera, Trichoptera, Diptera*, and Hemiptera.
- Reduce biochemical oxygen demand (BOD) and ammoniacal nitrogen, indicators of organic pollution from wastewater, to benefit *Coleoptera, Trichoptera, Plecoptera*, and *Hemiptera*.
- Implement localized, catchment- or sub-national scale management to address varying sensitivities among taxa and ecological differences between upland and lowland areas.
- Adopt multi-metric monitoring and management, incorporating both species richness and abundance, to better capture ecosystem health and tailor conservation efforts effectively.

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