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LONG-TERM TRENDS AND DRIVERS OF AQUATIC INSECTS IN THE NETHERLANDS

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CASPAR A. HALLMANN
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Water beetle - *Cybister lateralimarginalis* - tuimelaar
Photo: Bureau Biota

COLOPHON

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Left: bush-tailed caddisfly - *Sericostoma personatum* - kokerjuffer
Right: common darter - *Sympetrum striolatum* - bruinrode heidelibel

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Banded demoiselle - *Calopteryx splendens* - weidebeekjuffer
Photo: Wil Leurs

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Larva of cranefly - *Phalacrocera replicata* - buismug
Photo: Bureau Biota

SAMENVATTING

ECOLOGISCH HERSTEL NEDERLANDSE WATEREN LEIDT TOT GROTERE INSECTENDIVERSITEIT EN LAGERE AANTALLEN

Monitoring door de Nederlandse waterschappen biedt nieuwe inzichten. Ondanks dat het totale aantal individuen van aquatische insecten de afgelopen drie decennia halveerde, is de diversiteit juist toegenomen. De grote afname kan vooral toegeschreven worden aan bepaalde dansmuggen, die voorheen profiteerden van voedselrijke omstandigheden door vermesting en vervuiling.

Verontrusting over de afname van het aantal landinsecten roept de vraag op hoe het gaat met in het water levende insecten. Langer-lopende monitoring zoals de macrofauna-bemonstering van de Nederlandse waterschappen biedt de mogelijkheid om te achterhalen van welke groepen insecten het aantal individuen afneemt of juist toeneemt, en welke milieufactoren en beheermaatregelen daarbij het meest van invloed zijn. De onderzoeksresultaten zijn bemoedigend en ondersteunen het beheer van de waterschappen dat erop gericht is de waterkwaliteit en biodiversiteit verder te verhogen.

MONSTERS VERZAMELD DOOR WATERSCHAPPEN TUSSEN 1990 EN 2017

Om insectentrends in de Nederlandse oppervlaktewateren te bepalen, analyseerden we de langlopende, maar complexe, dataset van monsters verzameld door de waterschappen. De acht bestudeerde waterschappen lagen in het zuiden (Limburg en Oost-Brabant), oosten (Achterhoek en Twente), en westen (zuidwestelijke helft van Zuid-Holland). In onze analyses hebben we rekening gehouden met mogelijke verschillen tussen de waterschappen en de veldmedewerkers die de monsters verzameld hebben, als ook het totale aantal meters dat bemonsterd is. Weer- en waterkwaliteitsgegevens werden ruimtelijk geïnterpoleerd naar de 1.709 locaties waar in de bestudeerde periode 12.087 macrofauna-monsters verzameld zijn.

DANSMUGGEN EN HAFTEN NAMEN AF, KOKERJUFFERS EN LIBELLEN TOE

Het aantal aquatische insecten is in 27 jaar tijd met 53% afgenomen. Ni*et al*le insectengroepen droegen daar evenveel aan bij. Vooral de sterke afnames in de meest talrijke groepen waren bepalend: vooral dansmuggen, maar ook haften. Waterwantsen en -kevers namen ook af, maar waren sowieso al minder talrijk en hadden dus minder invloed op het totale aantal insecten. Dat laatste geldt ook voor kokerjuffers en libellen, die juist in aantal toenamen. Trendanalyses op het niveau van taxonomische geslachten laten een geheel andere patroon zien: van 66% van 213 insecten-geslachten nam het aantal juist toe. De meest talrijke geslachten kwamen minder voor, terwijl van de zeldzamere geslachten juist steeds meer individuen aangetroffen werden. Dit zorgde voor een steeds gelijkere verdeling van de aantallen individuen over de insecten-geslachten. Aangezien ook het aantal geslachten per monster toenam, leidt dat tot een grotere diversiteit, hetgeen gunstig is.

OVERHEERSENDE EFFECTEN VAN VERMINDERDE EUTROFIËRING EN VERMINDERDE TOXICITEIT PESTICIDEN

Bij het vergelijken met data van meerdere omgevingsvariabelen, zagen de onderzoekers dat de aantalstrends het best verklaard worden door de verbeterde waterkwaliteit. Halvering van de stikstof- en fosforconcentraties was positief gecorreleerd met het aantal individuen van de meeste groepen insecten, maar verlaagde juist het totale aantal dansmuggen en kriebelmuggen. De flinke afname in de toxische druk van pesticiden in het water (uitgedrukt in meer-stoffen PAF) over de drie decennia had een vergelijkbaar positief effect op de diversiteit aan waterinsecten.

HERSTEL VAN WATERINSECTEN-GEMEENSCHAPPEN

Dit onderzoek geeft duidelijke aanwijzingen dat de gemeenschappen van waterinsecten zich aan het herstellen zijn van de slechte waterkwaliteit in de jaren tachtig (o.a. door vervuiling, vermesting en organische belasting door overstorten en directe lozingen van huishoudens). Insecten die karakteristiek zijn voor gezonde rivier- en meerecosystemen namen toe, terwijl negatieve indicatorgroepen juist afnamen. Zo deden insecten die van helder en stromend water houden het beduidend beter dan insecten die stilstaand water en slibbodems prefereren.

Tegelijkertijd zijn op veel locaties de concentraties van nutriënten en toxische stoffen nog steeds boven de vastgestelde normen, terwijl de diversiteit aan insecten gedurende de hele studieperiode toenam. Onze resultaten bieden dus het perspectief dat verdergaande verbetering van de waterkwaliteit effectief kan zijn voor het herstel van waterinsecten-gemeenschappen. Helaas kon het effect van oever- en waterbeheer niet meegenomen worden in de analyses, maar de verwachting is dat natuurvriendelijk beheer een zeer positieve bijdrage kan leveren. Het is echter niet duidelijk hoever de waterinsecten-gemeenschappen nog afstaan van historische situaties, omdat referentie-datasets die goed inzicht geven in de aantallen ontbreken.

NEDERLANDSTALIGE BIJSCHRIFTEN VAN FIGUREN EN TABELLEN

De volgorde van onderstaande bijschriften is dezelfde als die van de figuren en tabellen in dit rapport.

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- Figuur 2: A. Kaart van de waterschappen waarvan data meegenomen zijn in deze studie. B. Aantal macrofauna-monsters in de gebruikte dataset per jaar. C. Aantal macrofauna-monsters per locatie. De acht waterschappen zijn HHD (Hoogheemraadschap van Delfland), WHD (Waterschap Hollandse Delta), WAM (Waterschap Aa en Maas), WD (Waterschap De Dommel), WPM (Waterschap Peel en Maasvallei, tegenwoordig onderdeel van Waterschap Limburg), WRD (Waterschap Regge en Dinkel, tegenwoordig onderdeel van Waterschap Vechtstromen), WRIJ (Waterschap Rijn en IJssel) en WRO (Waterschap Roer en Overmaas, tegenwoordig onderdeel van Waterschap Limburg).
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- Figuren 14, 15 en 16: Relaties tussen aantalstrends op genus-niveau en verschillende eigenschappen van die genera. Hier geplot staan de herverdelingen van de t-waardes van de mixed-effect modellen waarin steeds één van de eigenschappen als covariabele opgenomen zijn. Zwarte balken geven significante effecten aan (t-value > 1.96). De richting van de balken (positief of negatief) hangt af het teken van de correlatie tussen de aantalstrends en scores voor een eigenschap op het niveau van de genera. De geteste eigenschappen zijn in figuur 14 geprefereerd substraat, voortbeweging, kenmerkend nutriëntenniveau, stroomsnelheid, transversale verspreiding (gradiënt vanaf rivieren landinwaarts), en longitudinale verspreiding (van bovenstroom tot zee); in figuur 15 aquatische stadia, voedsel, wijze van eten, maximum grootte, levenscyclusduur, en saprobiteit (een maat van waterkwaliteit); in figuur 16 geprefereerde pH, reproductie, verbreiding, stadia waarin moeilijk omstandigheden overbrugd worden, ademhaling, hoogte, het aantal levenscycli per jaar, en temperatuur.
- Figuur 17: Relatie tussen aantalstrends op genus-niveau en verschillende indicator waarden van soorten in die genera. A: Er blijkt geen verschil in de gemiddelde aantalstrend van genera waar van de soorten wel, danwel niet, risico lopen door effecten van microverontreinigingen volgens de SPEAR methodiek. 31 genera waren 'at risk', 203 genera waren 'not at risk'. In panels B en C staat de gemiddelde aantalstrend uitgezet tegen indicatorklasse voor doeltypen van respectievelijk rivieren (B) en meren (C). De indicatorklasses zijn: N ("Negatief", soorten in een genus zijn voornamelijk negatieve indicatoren rivier- en meer-doeltypen), K ("Kenmerkend", soorten in een genus zijn voornamelijk karakteristieke taxa), P ("Positief", voornamelijk positieve indicatoren wanneer soorten dominant zijn), en 0 (genera zonder indicator soorten of met een mix van soorten met N, K en P indicaties). Zwarte balken geven significante verschillen aan.

Sewer overflow
Photo: Eelke Jongejans

ABSTRACT

World-wide concerns about declines in insect abundance have renewed appreciation for existing insect monitoring schemes. Longer-running schemes present the opportunity to learn which insect groups are doing better than others, and to look for environmental factors and management options that influence the abundance trends of insects.

To study insect trends in Dutch surface waters, we here analyse a long-running but complex dataset of insects in macroinvertebrate samples collected by Dutch Water Authorities (WAs). Prior quality checks resulted in a subset of one third of the WAs, covering parts of the south and east of the Netherlands as well as a large region in the

vicinity of Rotterdam. To arrive at a database based on a reasonably consistent sampling protocol, we used a subset of years: from 1990 until 2018. Identity of the person collecting the insects in a water body, the WAs, and the length of the sampling transect were included as factors in our analyses. Weather data and water quality samples were spatially interpolated to 1,709 macroinvertebrate sampling locations (with a total of 12,087 samples).

Our analyses show that taxonomic (genus level) richness and diversity increased over the course of 28 years, while the overall abundance of water insects declined by 53%. Although dragonflies and caddisflies increased in abundance, most other insect orders declined in abundance. In our final models of insect abundance, the negative effects of nitrogen, phosphorus and pesticides (i.e. there were fewer insects when nutrient concentrations or the combined toxicity of pesticides were high) were amongst the strongest effects in most groups. In contrast, non-biting midges (Chironomidae) and black flies (Simuliidae) showed positive responses to these factors. The presence of greenhouses and proximity of sewer outlets also had negative effects on the abundance of most groups. Other factors that showed up as important for the measured abundance of at least some groups included biochemical oxygen demand, wet natural areas, and growing degree days (a measure of accumulated heat).

We also quantified how much environmental changes over the course of the study period contributed to the abundance trend of various insect groups. Our models showed that the halving of N and P concentrations from 1990 to 2018, generally had a strongly positive effect on insect abundance trends, as had the threefold drop in combined toxicity of measured pesticide levels. Again, indicators of eutrophic and polluted conditions, Chironomidae and Simuliidae, showed opposing responses to the decreases in nutrients and pesticides. Land cover variables had not changed much over this time period and thus did not contribute much to abundance trends; nor did most weather variables. In 7 out of 10 insect groups, the abundance trends were mainly caused by unknown factors.

Seemingly in contrast with the observed overall decline in insect abundance, positive trends were found for 66% of the 213 insect genera for which we were able to determine abundance trends. Looking at the number of insect genera in the macroinvertebrate samples, and their evenness and diversity, we see clear increases over time. This increase in biodiversity is partly due to the decrease in abundance of the most abundant taxa, but also due to the increase of some of the previously more rare taxa.

Linking the genus-level trends to traits of insects in those groups showed that insects preferring clear, running water had more positive abundance trends than those preferring standing waters and sludge. This is confirmed by patterns among genera with indicator species. Taxa characteristic for healthy river and lake communities showed positive trends, while negative indicators declined in abundance.

The emerging pattern is that, in general, water insect communities are recovering from poor water conditions (high nutrient loads and pollutions) in the 1980s. As a result of the halving of the nitrogen and phosphorus concentrations and pesticide toxicity during the study period, groups like non-biting midges and black flies have decreased, which generally have opportunistic life history strategies and are indicators of poor (eutrophic) water quality. Overall, diversity indices continuously improved from 1990 till 2017. Since proper reference datasets are lacking, it remains unclear to what degree insect communities have recovered. However, management aimed at restoring water quality, which was the last phase of implementing sanitation measures (sewage systems), appears to have had a clear positive effect on the diversity of aquatic insects, favouring indicator species of healthy ecosystems. Seeing these encouraging developments, there is every reason to continue with the efforts to further improve water quality, as nutrient and toxicity levels are still above their maximally allowed concentrations in many water bodies. At the same time there is a need to research how management of water and vegetation can fully restore pristine insect communities, especially with respect to taxa-specific abundances.

Migrant hawker - *Aeschna mixta* - paardenbijter
Photo: Michel de Beer

INTRODUCTION

Recent reports in Europe and other parts of the world (Biesmeijer *et al.* 2006; Dirzo *et al.* 2014; Hallmann *et al.* 2017, 2019; Sánchez-Bayo and Wyckhuys 2019; Seibold *et al.* 2019; van Klink *et al.* 2020b; Zattara and Aizen 2021) suggest that terrestrial insects are declining at alarming rates, but knowledge on community-wide abundance trends of insects as well as on causal factors driving insect decline remain far from complete. Furthermore, how insect communities respond to changes in potential drivers remains uncertain, partially because of the large number of potential drivers (Wagner *et al.* 2021), and the different spatial and temporal scales at which they operate. Large-scale, fine-grained, multi-taxon and long-term standardized data are imperative to assess which groups of insects are in decline most, where such insect declines occur, and why.

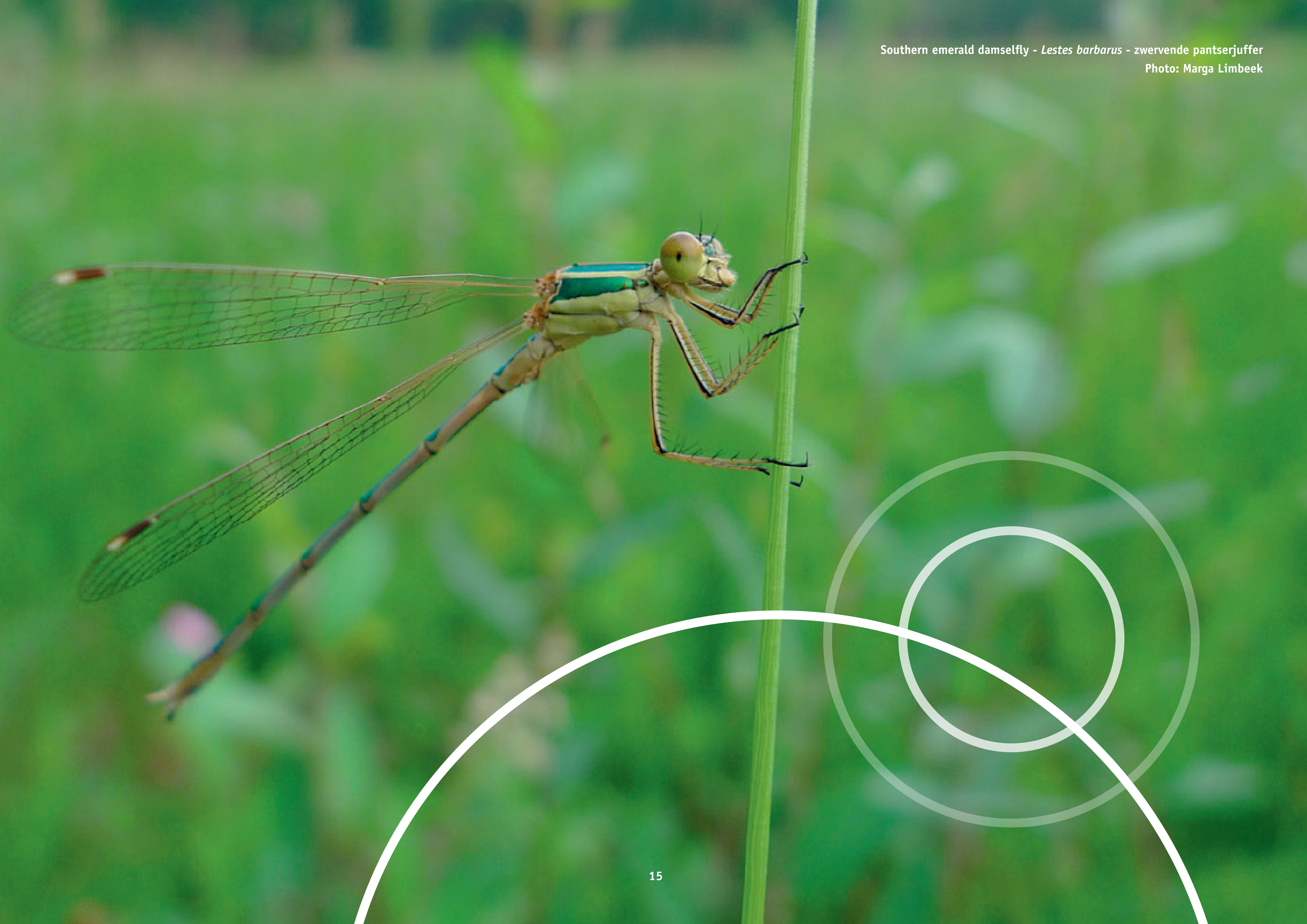
Freshwater invertebrates are known to be influenced by an array of chemical and physical water properties (Von Der Ohe and Liess 2004; Beketov *et al.* 2008, 2013; Posthuma *et al.* 2019; Birk *et al.* 2020; Lemm *et al.* 2021), pesticides (Liess *et al.* 2021), climate (Burgmer *et al.* 2007; Verberk *et al.* 2016; Jourdan *et al.* 2018; Baranov *et al.* 2020; Roth *et al.* 2020; Outhwaite *et al.* 2020) and land use (Richards and Host 1994; Weijters *et al.* 2009). Water quality was very poor in many (but not all) places in The Netherlands halfway the 20th century, but important measures were already taken in the 1970s and 1980s with the construction of new sewage systems and wastewater treatment plants. Also in other parts of Europe improvements in water quality have occurred (Vaughan and Gotelli 2019), further facilitated by the introduction of the EU Water Framework Directive in 2000 (Outhwaite *et al.* 2020). In the Netherlands, water quality has indeed improved in recent decades (van Gaalen *et al.* 2020), with water quality measurements indicating that emissions of industrial chemicals, nutrient emissions (N, P) and agricultural pesticides have been lowered, and that wastewater treatment has improved. Notwithstanding this marked decrease in total volume of application, the toxic pressure from new compounds may have increased at the same time, owing to a much higher relative toxicity of currently used pesticides and medicine residues. Additionally, increased summer droughts, earlier springs and other climatic changes are likely to induce variation in annual insect populations. These various developments may thus have both positive as well as negative effects on total insect abundance. Furthermore, factors like high nutrient concentrations and toxic compounds can have interactive effects, as has recently been shown in some empirical studies (Birk *et al.* 2020; Lemm *et al.* 2021). In order to disentangle potentially opposing or interacting effects, we require large-scale and long-term information not just on the abundance of macrofauna, but also on the state of potential drivers.

Designing a new macrofauna monitoring program that is large-scale enough to cover many regions and types of water bodies nation-wide is a daunting endeavour. This is particularly challenging as it is pivotal to also measure all environmental factors relevant now and in the future, and to continue the program consistently for a long enough time to detect macrofauna trends and effects of environmental changes. However, even when successful, new monitoring will obviously not uncover trends and effects of the past decades. Therefore, we take on an even bigger challenge in this report: we set out to integrate multiple, mostly disconnected datasets that have been constructed for different purposes than detecting and understanding abundance trends of insect macrofauna. The challenges for such an integration are multifold, even in the Netherlands where different monitoring schemes have high spatial resolution and are relatively long-running.

In the case of Dutch data on macrofauna, weather, land use, water quality and micropollutants, a major challenge is that these variables have been measured at different locations and at different points in time. Weather stations supply the most consistent, hourly data, but are relatively sparse in space. Water quality measurements (e.g. pH, nutrients) are repeatedly (generally several times per year) measured at the same locations, but these locations do not necessarily match the locations where macrofauna samples are taken. Interpolations to the macrofauna sampling locations are therefore required, taking into account temporal trends and spatiotemporal covariance structures of these environmental variables, while weighting by distance. Substances like pesticides, industrial compounds, and combustion products, also known as micropollutants, prove to be particularly challenging in that respect: due to their large variety and the relative high costs of tests, not all micropollutants are tested for, after a water sample is taken. Moreover, new agricultural and industrial compounds are being developed and taken into use regularly, while monitoring for new micropollutants in the environment generally starts only years after these compounds and their derivatives entered the environment. Also, many test results show that concentrations of specific micropollutants are below detection limits, which does not mean that the concentration is zero, nor that low concentrations are irrelevant for macrofauna. As interpolation of single micropollutants therefore prove too difficult, novel integration methods need to be developed to arrive at toxic pressure estimates for each of different groups of micropollutants (e.g. pesticides, industrial compounds, and combustion products).

The macrofauna data themselves pose challenges as well. Originally set up to assess the biodiversity and intactness of macroinvertebrate communities and their relationships with abiotic factors (van der Hammen 1992; Steenbergen 1993), the Dutch macrofauna monitoring scheme is accompanied by a clear sampling, processing and estimation protocol (Beers *et al.* 2014). Already before this protocol was published, macrofauna samples were collected and processed in reasonably standardized way. In practice, however, subtle but important differences have occurred between the different Water Authorities (currently there are 21 WAs in the Netherlands) and between individual field and lab workers. Proper analyses of macrofauna trends will have to accommodate all these aspects of sampling (e.g. how many meters were sampled) and processing (e.g. how larger numbers of individuals in a sample have been estimated) in a mechanistic way, rather than glossing over potential confounding factors.

In the present study we scrutinize and bring together long-term and large-scale datasets on i. freshwater insect macrofauna, ii. weather, iii. land use, iv. physical/chemical water properties, and v. toxicity. Our goal is to uncover the trend of freshwater insect-communities over the past three decades in the Netherlands, and to unveil the (potentially opposing) effects of multiple drivers. We integrate two powerful ecological assessment methods into the analysis, that of driver-change effect-on-trend interpretation, and that of trait-based interpretation of population trends, in order to characterize developments in freshwater insects along trait axes. Specifically, we study trends in abundance of various freshwater insects at two taxonomic levels: we analyse abundance trends at the level of genera, and for each of 10 insect groups defined at the order or family level. Furthermore, we also analyse the species richness diversity of the macrofauna samples. In order to do so we develop powerful statistical models that properly account for the spatiotemporal design of these measurements, i.e. disentangle ecological variation from sampling variation.



Southern emerald damselfly - *Lestes barbarus* - zwervende pantserjuffer
Photo: Marga Limbeek

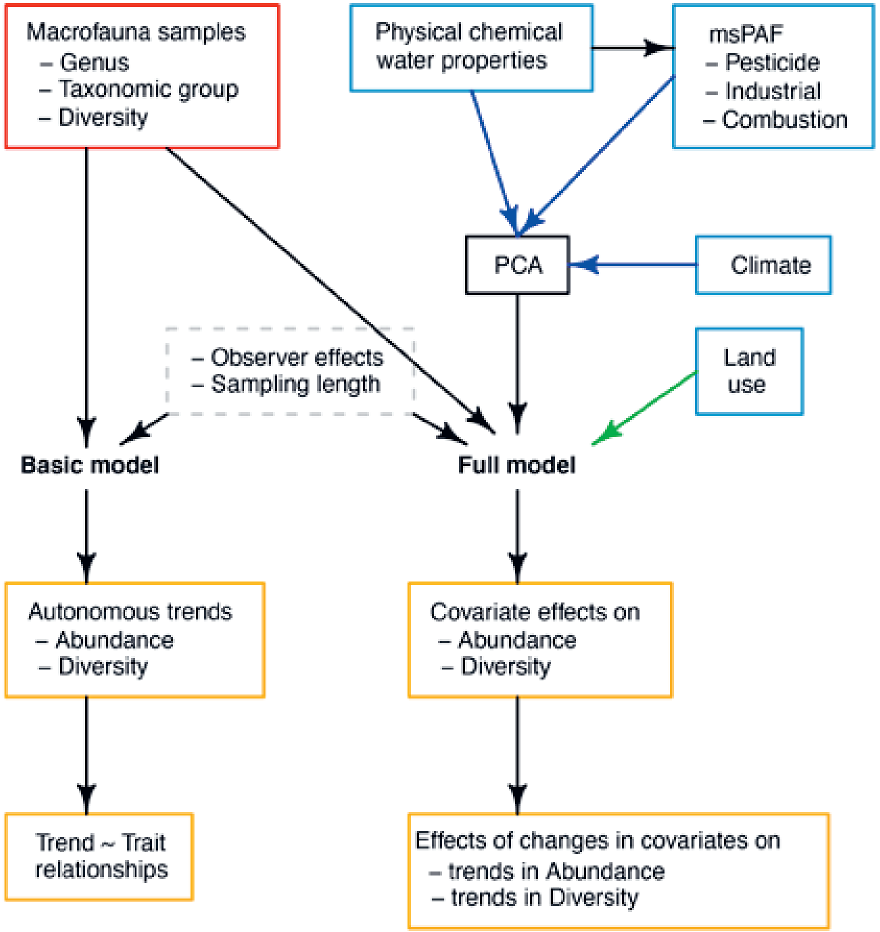
Taking macrofauna samples
Photo: Ilse van de Kraats

MATERIALS AND METHODS

The Materials and Methods section is organized as follows: We first introduce the macroinvertebrate dataset, including the monitoring and sample processing protocols used by the Water Authorities (WAs). We then describe models of insect abundance trends at two different taxonomic levels: i) per genus and ii) integrated over 10 taxonomic groups (e.g. insect orders, see Table S.1), as well as models of trends in diversity indices. Next, we explain our assessment of the effects of a large range of potential explanatory variables on the taxonomic-group abundances, genus-level diversity, and trends therein. Finally, we related abundance trends to characteristic traits of the investigated genera. A flow diagram of all analyses is given in Fig. 1.

FIGURE 1

Schematic diagram of analyses performed. Red box: response data. Blue boxes: Explanatory data. Orange boxes: results. Black arrows: Data link. Blue arrows: spatio-temporal interpolation. Green Arrow: Spatial weighting function.



FRESHWATER MACROINVERTEBRATE DATA

In the Netherlands, regional Water Authorities (also called Water Boards, and in Dutch: Waterschappen or Hoogheemraadschappen; hereafter: WAs) have amassed vast datasets on the abundance and distribution of macrofauna (i.e. small invertebrates still detectable without magnifier or microscope) in Dutch surface water bodies (brooks, ditches, ponds, lakes, canals, rivers, etc) since 1980. These data have previously been collated and archived under the name Limnodata Neerlandica, which are available at http://ipt.nlbif.nl/ipt/resource.do?r=stowa-limnodata. However, variable levels of data curating and metadata archiving, as well as methodological sampling differences between the WAs prohibit a straightforward trend analysis of species abundances with this national database (Netten *et al.* 2010; Verdonschot and van Oosten-Siedlecka 2010). Notwithstanding the protocol, field workers potentially differ in how exactly they collected macrofauna samples. In the lab, the taxonomic level at which specimens were identified varied somewhat between the people processing the samples, probably due to varying levels of experience and time available. Over the years, better identification guides became available, increasing the possibilities to accurately identify insects at the species level.

However, the developmental stage of the caught insects has a big effect on whether specimens are identified at the species level: in many cases it is not possible to identify species at larval or juvenile stages. Earlier attempts to aggregate the macrofauna data from all Water Authorities suffered from inconsistencies in the recorded abundances and metadata (Netten *et al.* 2010; Verdonschot and van Oosten-Siedlecka 2010). Therefore, it was necessary for us to go back to the original data of separate WAs and only select those datasets that were sufficiently well-organized and accompanied with necessary metadata (Koese and Zeegers 2018). To arrive at a subset of the macrofauna data with minimal issues, EIS Netherlands (Koese and Zeegers 2018) assessed the state of the macrofauna datasets of all WAs. These researchers checked whether for each macrofauna sample the transect length and the identity of the person who collected the sample in the field was known, whether the records were complete (i.e. no obvious groups of insects missing) and whether the sample processing protocols appeared to have been followed. They eventually selected eight WAs with the most complete and most consistently archived datasets, and identified which subsets of those datasets were of high-enough quality. Two of these eight WAs have recently (1 January 2017) merged, as has happened frequently in the rich history of Dutch WAs. The eight WAs selected based on the quality and availability of their data were:

- HHD: Hoogheemraadschap van Delfland
- WHD: Waterschap Hollandse Delta (also known as WSHD)
- WAM: Waterschap Aa en Maas
- WD: Waterschap De Dommel
- WPM: Waterschap Peel en Maasvallei (now part of Waterschap Limburg)
- WRD: Waterschap Regge en Dinkel (now part of Waterschap Vechtstromen)
- WRIJ: Waterschap Rijn en IJssel
- WRO: Waterschap Roer en Overmaas (now part of Waterschap Limburg)

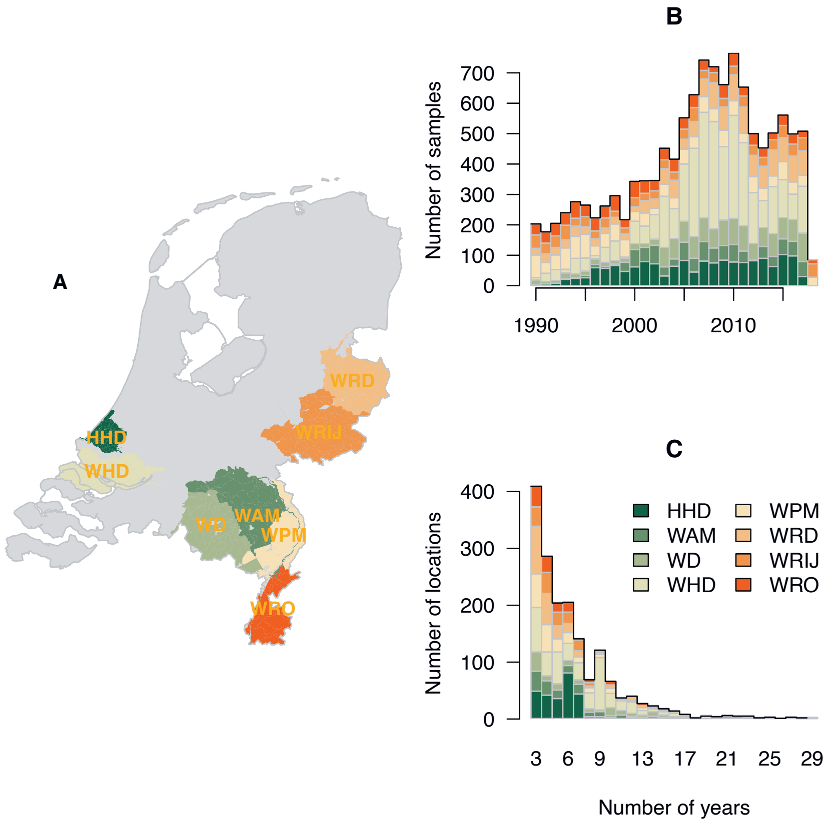
The spatial distribution of these WAs is given in Figure 2A. We here use a re-compiled database based on the original data of these eight WAs. The datasets were thoroughly examined by entomologists of EIS Netherlands (Koese and Zeegers 2018) for completeness, irregularities, and sufficient level of metadata archiving. Since then more data and metadata were made available by several WAs (HHD, WPM, WRO, WRIJ) and added to the dataset. These additions required new rounds of data checking to fix inconsistencies.

Sampling

Macrofauna samples were collected by field experts of the WAs following a prescribed protocol (Beers *et al.* 2014). To arrive at a multi-habitat sample, locally available microhabitats are sampled. The microhabitats can differ in vegetation, structure, soil properties and the transparency of the water. Often the total length over which samples are taken on a sampling location (i.e. summed over the sampled microhabitats) was five meters (5m in 47% of the cases, mean=6.3m, sd=2.6m). Earlier samples were largely collected with the same methodology as recorded in the protocol. Deviations in e.g. sampling length are recorded in

FIGURE 2

Map of Dutch Water Authorities (WAs) included in this study (A) along with the number of contributing samples per year (B) and the number of sampled years per monitoring location (C). Locations had to have samples from at least 3 years to be included in the study. The eight WAs are HHD (Hoogheemraadschap van Delfland), WHD (Waterschap Hollandse Delta), WAM (Waterschap Aa en Maas), WD (Waterschap De Dommel), WPM (Waterschap Peel en Maasvallei, nowadays part of Waterschap Limburg), WRD (Waterschap Regge en Dinkel, nowadays part of Waterschap Vechtstromen), WRIJ (Waterschap Rijn en IJssel) and WRO (Waterschap Roer en Overmaas, nowadays part of Waterschap Limburg).



the metadata. Standard macrofauna-nets, with a 20×30cm rectangle opening and 0.5-mm mesh size, were used for sampling the water column and substrate (Greijdanus-Klaas 1999). All macrofauna specimens caught were collected, sorted, preserved, and further processed in the laboratory, where they were identified at the lowest taxonomic level possible, and conserved.

For the trend analyses we selected all sites with at least 3 sampling years. In total, 12,087 unique sampling instances were used in our analysis, distributed over 1,709 unique monitoring locations, over the period 1990-2018. The number of samples have increased with time, from around 200 samples per year in the 1990s, to at least 500 samples per year after 2010 (Fig. 2B). Half of the locations (52%) were sampled in 3, 4 or 5 years, but the analysed database also included longer time series: 16% of the locations were sampled in at least ten years, while 2% of the locations was sampled 20 years or more (Fig. 2C). Macrofauna samples were mainly collected during spring, summer and early autumn, and rarely during winter.

Species identification

Sample processing was always done according to a nationally standardized protocol (Beers *et al.* 2014). Following this protocol, live macroinvertebrate samples were first divided into major groups, which were separately stored in appropriate preservation fluids. Next, specimens were identified at species level where possible, or at some higher taxonomic level if not. The percentage of specimens that were identified at species level is variable over time, with a slight increase in taxonomic depth: from approximately 65% identified at species level in 1990 to 70% in 2018 (Figure S1). Taxa with very high numbers were treated as follows: a random subset up to a taxon-specific maximum was identified at the lowest-possible taxonomic level. The abundance of the remaining individuals was counted or estimated, after which the abundances of the identified taxa are extrapolated proportionally. In many of the datasets these extrapolations are indicated specifically. For instance, for chironomids numbers surpassed the prescribed threshold in approximately 30% of the samples. Depending on the taxonomic group, the laboratory protocols (Beers *et al.* 2014) prescribe a minimum number of individuals required to be identified taxonomically. For example, for chironomids the minimum is set at 150 individuals, for Lepidoptera at 25 individuals. In practice, however, the number of individuals that are actually identified to species level varies somewhat between samples, but is dispersed regularly around the prescribed minimum number of individuals for each taxonomic group. Sub-sampling within abundant taxa affects the species richness, as this parameter scales non-linearly with sample size (i.e. number of individuals in a sample). A further complication that arises in 57% of the macroinvertebrate samples is that the number of individuals actually determined to species level is not archived, or is not certain. In such cases, only the extrapolated numbers abundance is archived, meaning that there is no way of telling whether or not species-level abundances have been partially extrapolated or by how much. Properly dealing with counted and extrapolated counts is also important for the certainty of the model parameters. In the present study, we take the approach of modelling these sampling processes explicitly, in order to propagate errors of uncertainty to the points of interest, such as the temporal dynamics of the focal species.

MACROINVERTEBRATE MODELS

Our modelling strategy to derive trends in abundance of insect macrofauna involved two sets of models: one set at the level of taxonomic groups (e.g. insect orders, see Supplementary Table S.1) and one set at the genus level. Additionally, we derived trend models for four genus-diversity indices. We first ran models on the sum of each taxonomic group per macroinvertebrate sample. These models were used to derive general trends in total abundance for each taxonomic group. Secondly, we developed and ran models per genus to assess spatio-temporal changes at that lower taxonomic level. We explain each of the two sets of models separately below.

Given the variation in how macroinvertebrate samples were collected and how samples were processed at the species level in the lab, we developed additive mixed-effects model to accommodate sources of variation in both ecological and sampling processes. Generally, there are five sources of sampling variation present in the data:

1. variation in taxonomic depth of species identification over time and between WAs,
2. variation due to sub-sampling for taxonomic identification,
3. variation in sampling-transect length,
4. variation between field experts who collected the macrofauna samples, and
5. slight variation in data processing protocols between WAs.

For each of these sources of variation, an attempt was made to ameliorate potential bias in our estimates. Sources of abovementioned variation 1 and 2 are not of influence on models at taxonomic group-level, but are of influence in models at the genus level, and in estimates of diversity. Sources 3-5 are of influence on all modelling estimates.

Taxonomic group models

We fitted two generalized additive models (GAMs, Wood 2017) to the summed counts for each taxonomic group. Preliminary results indicated that response data were overdispersed relative to a Poisson process, and hence we used a negative-binomial distribution in our GAMs, with a log link to the predictors. Our models (one for each of the 10 taxonomic groups), were of the form

$$\log(n_i) = \alpha + \sum b_{ij}x_{ij} + f(t|WA) + f_{cc}(s) + f_{sp}(x, y) + r_i + o_i \tag{1}$$

where i denotes sample, α a global intercept, x_{ij} covariate levels, along with the corresponding linear effects (b_{ij}). $f(t|WA)$ is a smooth annual trend function, conditioned on the identity of the Water Authority (WA), $f_{cc}(s)$ is a cubic spline function of day-number, $f_{sp}(x, y)$ denotes a spatial smooth spline of sampling location coordinates. r_i denotes a random effect for observer and finally o_i is the offset for log-sampling length. Our null model only included a single environmental variable: water type, while our full models involved a number of additional covariates, such as soil type, land use and cover variables, weather variables, chemical and physical water properties, and measures of total toxicity (see Table 1). Further details on covariates and pre-processing are given in section *Explanatory variables*.

Genus-level models

At genus level, we fitted a similar model to the null taxonomic-group models (i.e. including only water type as covariate; eq. 1). We did not fit genus-level trend models for genera for which less than 500 individuals were recorded in total, as there needs to be ample data to estimate reliable rates of increase. And even when overall numbers were above that threshold, still some models did not converge on reliable trend estimates for some genera. In those cases where a trend estimate had very wide confidence intervals, we excluded it from further analyses of the genus-level rate of increase.

Diversity models

We considered genus richness, Shannon's index of diversity, Simpson's index of diversity, and Shannon's evenness index, to describe trends in genus-level diversity over time and per WA. Unit of analysis was the index for each sampling instance, and as such we infer on the average diversity per sample. Shannon's diversity index scales between 0 (a single species) to log(S) and depends on both the number of genera and their relative abundances in a sample.

$$H = -\sum_{i}^{S} (p_i \log(p_i)) \tag{2}$$

The evenness is calculated as $H/\log(S)$. The Simpson's index of diversity is calculated as

$$J = 1 - \sum (p_i^2) \tag{3}$$

and reflects the probability that two independent draws of an individual in a sample belong to different genera.

Each of the four indices was modelled using generalized additive models. We assumed a negative-binomial error structure with a log link for richness. For other indices, we assumed Gaussian errors, but we used a log transformation of H, and a *logit* transformation of evenness and Simpsons index. Our models for each index *(k)* read as

$$l(\theta_k) = \alpha + \sum b_{ij}x_{ij} + f(t|WA) + f_{cc}(s) + f_{sp}(x,y) + f_r(\log(n)) + \gamma\log(p) + r_i + o_i \qquad (4)$$

where $l(.)$ is the link function specific to index k. Parameters and smooth functions b_{ij}, r_i, o_i, as well as smooth functions $f(.)$, $f_{cc}(.)$, $f_{sp}(.)$ are defined in this model as in equation 1, while $f_r(.)$ denotes a smooth function of total abundance (i.e. mimicking rarefaction) and γ measures a linear effect on the log-proportion of the sample identified to at least genus level.

EXPLANATORY VARIABLES

To uncover drivers of variation in abundance (at taxonomic group level) and diversity, as well as trends therein, we expanded the set of explanatory covariates (x_{ij}) in equations 1 and 4, by including land cover and land use, soil properties, physical/chemical water properties, toxicity, and weather variables. In doing so, we opted to obtain time-varying covariates (where necessary), which allows to examine, besides their relative effect on each response value, also how changes in each variable contributed to changes in abundance. Furthermore, we used a data-driven approach in determining the spatial scale at which land use and land-cover variables affected taxonomic group abundances. An overview of the explanatory variables is given in Table 1. Below we describe the source of each of these covariates and how we handled the data prior to the analyses.

TABLE 1

Overview of explanatory variables included in the analyses. The values of dynamic variables can change over the duration of the study period.

Parameter	Characterization	Years	Source
Water type	Static	-	WAs; CBS 2019
Protected areas	Static	-	Nationaal Georegister
Soil type	Static	-	Grondsoortenkaart
Land cover	Dynamic/Scale-dependent	1996-2019	CBS 2019; Kadaster 2019
Distance to sewage treatment plant	Dynamic	1990-2018	WAs
Weather variables	Dynamic	1990-2018	KNMI
Physical/Chemical properties	Dynamic	1990-2018	WAs
Toxicity	Dynamic	1990-2018	WAs

Water type

Owing to the large variety in types of surface water bodies, a water classification system was deemed necessary to characterize the sampled water bodies. Our classification was largely based on established Water Framework Directive definitions, which classifies water bodies according to major classes (e.g. lakes, ponds, rivers, canals, streams and ditches), with further subdivision depending on size, depth or flow-speed classes, or in some cases on soil type. A sizeable part of the macrofauna monitoring locations (24%) is not situated in a KRW-water body, and therefore have not been assigned a KRW-definition. For these locations we derived additional information from the national land cover map (TOP10NL: see www.pdok.nl), that classifies water bodies as ditch, waterways of 3-6 and 6-12 meter, rivers, and lakes. An overview of the number of macroinvertebrate sampling locations per water type is given in table 2. A full account of the relation between watertype-classification in this study with original water type classification from the two sources is given in table S.2.

TABLE 2

Number of macroinvertebrate sampling locations per water type and Water Authority (WA). Only locations with at least three sampling years were included in the study. WFD = Water Framework Directive.

	HHD	WAM	WD	WHD	WPM	WRD	WRIJ	WRO
Ditch	100	32	14	1	0	2	11	0
Slow running water	5	68	118	4	162	153	78	38
Fast running water	0	5	0	0	10	17	0	79
Canals	141	25	6	68	10	5	13	0
Ponds	0	6	0	0	25	36	0	16
Small shallow lakes	15	0	0	0	2	7	0	10
Large lakes	0	0	0	17	0	0	0	0
Brackish water	5	0	0	34	0	0	0	0
Ditch non-WFD	0	0	0	83	0	0	40	0
Waterway 3-6m non-WFD	0	0	0	57	0	0	1	0
Waterway 6-12m non-WFD	2	0	0	121	0	0	5	0
River non-WFD	0	0	0	46	0	0	1	0
Small lakes non-WFD	1	0	0	12	0	0	1	0

Land use and land cover

For each macrofauna sampling location, we extracted information on soil type, amount of protected area, proximity to sewage treatment plants, and land use. Information on soil type around monitoring locations was extracted from the Dutch Soil Map (WUR-Alterra 2006). We recorded which part of this surrounding area was protected (e.g. NATURA2000 or lower-level protection areas, nature parks, etc.) within a radius of 1km around each monitoring location. Data were obtained from the ’Nationaal Georegister’ (www.nationaalgeoregister.nl).

Sewage treatment plants are a source of water contamination in many parts of the world. In the Netherlands around 327 facilities exist, out of which 140 are located within the study area. To include possible effects of sewage pollution we used the proximity (i.e. the inverse of distance) of each measurement location to the nearest treatment plant. While doing so, the proximity was set to zero for all locations not likely to be influence by sewage water, such as for example sampling points in different water-catchment areas than that of the treatment plant, or sampling points clearly located upstream prior to sewage discharge points.

Information on land use was extracted from a vector-land use database (CBS 2019; Bestand Bodemgebruik), available for the years 1996, 2000, 2003, 2006, 2008, 2010, 2012 and 2015. Inevitably, we assumed no changes in landscape configuration in the period prior to 1996 and after 2015. Within a radius of 1km from the sampling location, we considered the area of the following land use categories: (i) agricultural land, subdivided into area for greenhouses, and remaining, (ii) forest area, (iii) dry natural areas, (iv) wet natural areas, and (v) build-up areas. Additional subdivision of remaining-agricultural land was achieved by integrating the Dutch Topographic Map (Top10NL, Kadaster 2012), which classifies land cover into grassland and cropped land, amongst other classes. For land use defined as remaining-agricultural land, we thus further subdivided it into grassland or cropped land. All computations were raster-based, at a resolution of 25m^2, implying that land use was described by over 5000 cells per sampling instance.

Coupling land use (surface data) to monitoring locations (point data) requires knowledge on the spatial scale at which potential land-cover attributes may affect insect macrofauna, in order to achieve maximum explanatory power of the models. Instead of relying on an a-priori defined spatial scale (e.g. percentage land-cover in a buffer of 500 meters), we developed a data-driven approach to identify the most appropriate scale at which to measure spatial covariate values for each taxonomic group. Additionally, one may envisage that the effect of a particular spatial covariate may diminish with distance to the focal point. To accommodate this in our models, we used a distance-weighted description of each variable, in a manner by which values of the covariate at close proximity to sampling points contribute more than values at increasing distance from measurement points.

The relative contributions are described by weights which themselves are structured by low-rank parametric kernels, that depend only on scale and shape parameters (two parameters for each category of each variable, see Figure S4). The scale parameters can be interpreted as a measure of the ”effective spatial scale” at which ecological processes are affected by a particular covariate, while the shape parameters as the steepness and tail length of the weighting function. More details on this approach are provided in Appendix C.

In practice, this approach is exhaustive (i.e. data-hungry), particularly with many covariates and covariate levels, increasing with spatial resolution and range of search. To reduce dimensionality of the computations, we first ran univariate models between response variables (i.e. sums per taxonomic groups) and each covariate, and identify the scale and shape of each spatial weighting function. We then fixed weighting parameters for each covariate in all subsequent analyses involving all (or subsets) of these covariates. Additionally, we used a 25m grid representation of land cover within a radius of 500 meters around each measurement location, with binary values for each category.

Weather variables

Weather data from 49 weather stations were obtained from the Royal Dutch Meteorological Institute (www.knmi.nl), where we extracted daily weather information for the period 1990-2018. We considered ambient temperature, precipitation and potential evapotranspiration. These three weather variables were interpolated to macrofauna monitoring locations using a combination of GAMs and spatial interpolation of the residuals (see Supplementary Information: Appendix A) for the full time period (i.e. daily, form 1-jan 1989 to 31-Dec-2018 and for each location). From the interpolated data, we derived multiple statistics, mean daily, weekly and monthly values of temperature as well as daily, weekly and monthly sum of precipitation and of evapotranspiration. Additionally, we considered growing degree days (i.e., the accumulated heat above 5°C), in order to account for potential phenological effects. To account for lagged effects, we considered the number of frost days in the preceding winter (Dec-Feb), as well as the sum of precipitation and mean temperature in the previous season (July-September).

Physical/chemical properties

Data on concentrations of chemical and organic compounds in the water have been collected by, or commissioned by, the WAs according to fixed protocols as part of regional water quality assessments (www.helpdeskwater.nl/onderwerpen/monitoring/toetsen-beoordelen). Raw data were checked for validity and completeness by RIVM in collaboration with Ecofide as part of a related project, and were made available to us by Jaap Slootweg. In total, between 12 thousand and 400 thousand samples were included in the present analysis (depending on variable), covering between 730 and 6884 unique locations (Table 3). As not all water quality variables were measured for each sample, the datasets for specific variables were considerably smaller. Despite the remarkably high density of spatio-temporal information, measurements of physical and chemical water properties are available at approximately 75% of the macrofauna sampling locations. Furthermore, water samples for chemical analyses often were not collected on the same day as the macrofauna sampling. Hence, there is only a partial spatiotemporal overlap between water quality samples and macrofauna samples. As the overlap between macrofauna and physical/chemical sampling locations was only partial, we require a means to arrive at estimates for each parameter at the location and date of each macrofauna sample (see Table 3). Expectations (usually concentrations, but also scales such as water temperature) at macrofauna sampling points were derived using a combination of Generalized linear models and residual spatio-temporal interpolations. Per WA, we modelled the quantities, e.g. log(concentration), as a function of water type, soil type, spatial coordinates, year and day-of-the-year, where the response variables were modelled as smooth-splines of the space and time variables (*x,y,t*). We used a tobit-1 link (Tobin 1958) to account for left censoring, where necessary. With this method we also took care of potential effects of changing detection limits over time.

TABLE 3

Overview of sample sizes for each of the physical/chemical variables included in the analysis.

Variable	Locations	Samples
NH_4	6495	325862
BOD	4582	204431
Cd	2647	90886
Ca	4331	70782
Cl	6682	436302
Chloro. alpha	3243	126713
Cr	2618	84761
PO_4	6495	306819
Sum P	6670	333341
Cu	3055	107218
Pb	2623	85778
Mg	3695	63941
Na	3534	52898
Ni	2728	92242
Suspended matter	4526	155328
NO_2+NO_3	5400	274879
Sum N	6256	296762
Water temperature	6796	381233
HCO_3	3917	52487
Zn	3052	107178
pH	6884	378892
O	6598	356967

Our models read as:

$$l(y) = \alpha + \sum b_{ij}x_{ij} + f(t|WA) + f_{cc}(s) + f_{sp}(x, y) + r_i \qquad (5)$$

where α an intercept, b_{ij} and x_{ij} coefficients and corresponding covariates (water type, soil type, precipitation in the previous week and month), $f(t|WA)$ a smooth function of year conditional on WA, $f(s)$ a seasonal smooth component, $f_{sp}(x, y)$ a spatial smooth component, and r_i a random intercept for watershed identity.

Model residuals were subsequently interpolated in both time and space to monitoring locations at measurement date. Further details of how we modelled and interpolated the physical/chemical water properties, as well as how we calculated residuals for measurements below the detection threshold, can be found in the Supplementary Information: Appendix B. Results of spatial and temporal correlation used in the interpolation of each compound, can be found in Table S.3.

Toxic pressure of mixtures of chemical pollutants

In order to include measures of toxicity of chemical substances originating from pesticide application, combustion or industry in our models, and at the same time, to deal with the multidimensionality problem of over 1400 measured substances in surface waters, we relied on multiple-substances Potential Affected Fraction (hereafter msPAF) for compound mixtures of chemicals (Posthuma *et al.* 2002; de Zwart and Posthuma 2005). The Potentially Affected Fraction (PAF value) of a compound is based on large numbers of lab experiments, and is a measure of ecotoxicological risk posed by a substance: it represents the proportion of species that is affected by a substance (Klepper *et al.* 1998). The combined impact of multiple substances (e.g., for each of the three groups of chemical pollutions assessed here) is expressed as msPAF, of which two variants exists, acute (EC50; half maximum effect concentration) and chronic (NOEC; No Observed Effect Concentration) (Posthuma and de Zwart 2012), of which we used the latter. Furthermore, only substances above detection/reporting limits were included in the calculations of mixtures toxic pressure.

A further problem in using mixture toxic pressure data, is that the calculation itself relies to a large extent on the number of substances analysed in the samples, with usually a strict increase in msPAF value with increasing number of substances measured. Additionally, the sets of substances that are tested for varied over time, among WAs, and even between samples. This renders direct comparison between samples problematic. As such, we used the maximum value of the msPAF values of measurements per location-year combinations, under the premise that toxicity is at least as high as the maximum msPAF value in a given year and at a given location.

To differentiate sources of toxicity, we categorized the large number of substances available in three groups (pesticides, industrial substances, and combustion by-products), while several substances belonging to either pharmaceutical or household-products were excluded due to insufficient samples sizes. As such, toxicity is described in our models as a three-component structure, differentiating sources of toxic pressure on insect macrofauna. An overview of the number of samples and locations used for modelling for the three groups of toxicants can be found in Table 4. Please note that we decided to leave heavy metals out of these toxic pressure estimates. Instead, heavy metals, whether occurring naturally or due to pollution, were treated like other water quality variable that were measured much more consistently than the chemical pollutants for which we expressed mixtures toxic pressure as msPAF.

We ran General Additive Models (GAMs), assuming a beta distribution, and a logit link, to derive mean trends per waterboard authority, and per chemical pollutant-group mixture toxic pressure subcategory, followed subsequently by residual interpolation on an annual basis. Our GAMs read, for each subcategory θ:

$$\mathrm{logit}(msPAF_{\theta}) = \alpha + \sum b_{ij}x_{ij} + f(t|WA) + f_{sp}(x,y) + r_i + \gamma \log(k) \qquad (6)$$

where alpha is an intercept, b_{ij} and x_{ij} coefficients and corresponding covariates (water-type,soil-type), $f(t|WA)$ a smooth function of year conditional on WA, $f_{sp}(x, y)$ a spatial smooth component, and r_i a random intercept effect for watershed identity. Finally, to correct for the number of substances (k) measured at the maximum per year-location measurement, we included a linear effect of γ in the model. This was necessary because water samples for which more chemical pollutants are tested, tended to have somewhat higher msPAF values.

Furthermore, when macrofauna sampling locations and toxicity measurement locations did not match, we spatially interpolated the annual-maximum msPAF values to those macrofauna sampling locations using similar methods as with the physical/chemical variables (see Table 4 and Supplementary Information: Appendix B), where serial covariation at the daily level was substituted for serial correlation at the annual level.

TABLE 4

Overview of sample sizes for each of the toxic pressure variables (one for each of three subcategories of chemical pollutants) included in the analysis. Toxic pressure of mixtures is expressed as msPAF values.

Variable	Locations	Samples
msPAF-Combustion	730	12785
msPAF-Industrial	5688	250343
msPAF-Pesticide	1376	35324

Principal Component Analysis

To account for confounding among interpolated variables, we performed Principal Component Analyses (hereafter PCA) and decorrelated the variables using the rotation matrix of the PCA. Hence, coefficient b_{ij} in equations 1 and 4, refer to scaled and decorrelated variables x_{ij}. Moreover, in our full-models, we retained all 38 axes of the PCA losing no information by the decorrelation process. All variables were scaled to zero-mean and unit variance prior to PCA-transformation. For an overview of all axes and how variables correlated to PCA-axes see Fig. S5 and for a full correlation matrix see Fig. S6.

Estimated relative effects of explanatory variables on abundance, diversity and trends

To derive the relative effects of covariates x on abundance and diversity indices, we used the following equation

$$\mathbf{E_n} = \mathbf{Rz} \tag{7}$$

where **R** is the rotation matrix from the PCA analysis, and **z** the vector of z-scores of coefficients from our macrofauna abundance and diversity models.

To derive the effects of changes of covariates x on the trends of macrofauna groups and diversity, we used the following approach:

$$\mathbf{E}_\lambda = \log(\bar{\lambda}) - \log(\lambda'_\theta) \tag{8}$$

where λ'_θ is the expected population growth rate in abundance of each taxonomic group when only one covariate (x) changed from its mean value in 1990 to its mean value in 2018 (i.e., marginal temporal predictions), and $\bar{\lambda}$ the growth rate as calculated when all covariates change from their 1990 to their 2018 mean values (i.e. full temporal predictions).

TREND-TRAIT RELATIONSHIPS

Since we expect considerable variation in the abundance trends among insect groups, we also investigated whether some of that variation could be explained by differences in traits of the insect taxa. It could for instance be that environmental changes that took place during the study period have favoured species with certain traits over others. We considered a wide range of traits, from morphology (maximum size), life history (e.g. number of generations per year), to preferences (e.g. for temperature). We also tested whether insect taxa that are indicators for desired insect communities in rivers or lakes, had different abundance trends than taxa that indicate undesirable insect communities. The full list of investigated traits can be found below.

Ideally, we would relate the variation in trends to traits at the species level. However, for 30% of the sampled individuals, identification was not performed at the species level. Moreover, trait data were not always available at the species level either. Since several of the considered traits have similar values among species within the same genus, and since we know the genus of 90% of the sampled individuals, we decided to perform the trend-trait analyses at the genus level. Thus, we used the ln-lambda values resulting from the genus-level trend analyses as the response variable in the trend-trait analyses. Due to the high number of traits considered, we tested the explanatory power of each trait separately in a series of linear mixed-effect models with insect order as a random effect (to account for potential phylogenetic patterns) and weighted by the inverse of the standard error of the ln-lambda estimates. These inverse standard errors were rescaled to a maximum of 1 prior to the analyses.

Several sources were used for trait data: 21 traits were extracted from Tachet *et al.* (2010), 1 trait from the SPEARpesticides database (Liess and Von Der Ohe 2005), and 2 traits from van der Molen *et al.* (2018). Here we will describe each in turn, starting with the Tachet *et al.* (2010) database. This database contains fuzzy scores (0, 1, 2 or 3) for 21 traits, each with 2 or more categories. For instance, for the trait 'aquatic stages' the database contains four categories: egg, larva, nymph, and adult. For each taxon in the database fuzzy scores for each of these categories indicate the degree to which those life cycle components are aquatic. The following list shows the 21 traits and their categories:

- Maximal potential size: ≤.25cm; >.25-.5cm; >.5-1cm; >1-2cm; >2-4cm; >4-8cm; >8cm
- Life cycle duration: ≤1 year; >1 year
- Potential number of cycles per year: <1; 1; >1
- Aquatic stages: egg; larva; nymph; adult
- Reproduction: ovoviviparity; isolated eggs, free; isolated eggs, cemented; clutches, cemented or fixed; clutches, free; clutches, in vegetation; clutches, terrestrial; asexual reproduction
- Dispersal: aquatic passive; aquatic active; aerial passive; aerial active
- Resistance forms: eggs, statoblasts; cocoons; housings against desiccation; diapause or dor mancy; none
- Respiration: tegument; gill; plastron; spiracle; hydrostatic vesicle
- Locomotion and substrate relation: flier; surface swimmer; full water swimmer; crawler; burrower; interstitial; temporarily attached; permanently attached
- Food: microorganisms; detritus <1mm; dead plant ≥1mm; living microphytes; living macro phytes; dead animal ≥1mm; living microinvertebrates; living macroinvertebrates; vertebrates
- Feeding habits: absorber; deposit feeder; shredder; scraper; filter-feeder; piercer; predator; parasite
- Transversal distribution: river channel; banks, connected side-arms; ponds, pools, disconnected side-arms; marshes, peat bogs; temporary waters; lakes; groundwaters
- Longitudinal distribution: crenon; epirithron; metarithron; hyporithron; epipotamon; metapotamon; estuary; outside river system
- Altitude: lowlands; piedmont level; alpine level
- Substrate (preferendum): flags/boulders/cobbles/pebbles; gravel; sand; silt; macrophytes; microphytes; twigs/roots; organic detritus/litter; sludge
- Current velocity (preferendum): null; slow; medium; fast
- Trophic status (preferendum): oligotrophic; mesotrophic; eutrophic
- Salinity (preferendum): fresh water; brackish water
- Temperature: psychrophilic; thermophilic; eurythermic
- Saprobity: xenosaprobic; oligosaprobic; b-mesosaprobic; a-mesosaprobic; polysaprobic
- pH (preferendum): ≤4; >4-4.5; >4.5-5; >5-5.5; >5.5-6; >6

Some genera for which we had ln-lambda estimates did not occur in the Tachet *et al.* (2010) database. In those cases, we checked whether data were available for another genus in the same tribe. If not, we subsequently searched for genera in the same subfamily, family, or infraorder. However, in the end we decided to only include in the analyses those genera for which both the abundance trend and trait information was directly available at the genus level.

In order to be able to use the fuzzy scores for each of the categories of a specific trait for the analyses of variation in the genus-level abundance trend (ln-lambda), we had to account for the multivariate (i.e. multiple categories) and multinomial (i.e. fuzzy scores 0, 1, 2 or 4) nature of the trait data. We therefore used the 'ade4' R package (Dray and Dufour 2007) to perform a Fuzzy Principal Components Analysis, first using the function 'prep.fuzzy.var' and then 'dudi.fpca'.

The resulting, mutually-uncorrelated fpca axes were then used as explanatory variables in the linear mixed-effect models described above. Afterwards we translated t-values of the fpca axes of the fitted linear mixed-effect model (lme) to the original trait categories, in order to make the results easier to interpret. We used the loadings of the trait categories on the fpca axes: we rescaled the absolute values of the loadings of each fpca axis, so they each summed to 1. This way we redistributed the t-values of the fpca axes over the trait categories. t-values larger than 1.96 were deemed significant. Whenever ln-lambda correlated negatively with the fuzzy scores of a trait category, its estimated t-value was depicted as a negative value.

The second trait source was the SPEARpesticides (Species At Risk) database (Liess and Von Der Ohe 2005). We used their estimates of sensitivity to toxic compounds in the same way as Liess and Beketov (2011): if a taxon had a sensitivity value lower than -0.36 and had three characteristics (low generation time, no possibility to recolonize from a refugium, and exposed at crucial times), we classified that taxon as ’at risk’. All others were ’not at risk’. If the genera for which we had ln-lambda estimates did not occur in the Spear database, we attempted to make links at subsequently higher taxonomic levels. This was necessary because links at the genus level could only be made for a minority of the genera. The frequency of the taxonomic level of these links will be shown in the Results section. Whenever at-risk-or-not information was available for multiple species within a genus, we chose the most frequently occurring factor level. Afterwards we tested whether being at risk could explain variation in ln-lambda, using the lme structure detailed above.

The third and final source of trait data is the STOWA report by van der Molen *et al.* (2018). From the appendices of van der Molen *et al.* (2018), we extracted information about indicator taxa, separately for lakes and rivers. For lakes we included lake types M12, M14, M20, M21 and M23, thus excluding brackish lakes of types M27 and M31. For rivers we included R5, R6, R7, R12, R13, R14, R15, R16, R17 and R18. Each of the species (or their absence) is an indicator of one or more of the river and lake types. The appendix tables in Altenburg *et al.* (2018) thus contain for each water type by species combination either a P (positive indicator when dominant), N (negative indicator when dominant), K (characteristic taxon) or nothing. As multiple species could be listed within a genus, we tallied all the P, N, and K within that genus. Whenever one of the letters dominated the tallies (i.e. more than 75% of all tallies), the genus was described as being predominantly in that category. If none of them dominated, or if no species of a genus occurred in the tables, that genus was noted as being not a strong indicator. For clarity: we performed this classification twice, once for rivers, once for lakes. Afterwards we performed linear mixed-effect models with either the lake-indicator factor or the river-indicator factor.

Larva of mayfly - *Ephemera vulgata* - gewone haft
Photo: Bureau Biota

Variable damselfly - *Coenagrion pulchellum* - variabele waterjuffer
Photo: Michel de Beer

RESULTS

ABUNDANCE TRENDS OF FRESHWATER MACROFAUNA INSECTS

Trends were calculated based on a series of Generalized Linear Additive models for each of the taxonomic groups. For all insects combined, the trend in total abundance was found to be negative: total insect abundance declined by 52.78% from 1990 till 2017 (Fig. 3A). This corresponds to, on average, a 2.7% decline per year (note that in order to retain a proportion of 0.4722 of the initial abundance after 27 years, you need an annual rate of $0.4722^{(1/27)}$, which gives 0.9725918 and thus a 2.74082% decline per year).

However, there are noticeable differences in the average rate of change among the 8 WAs (Fig. 3B), with WRIJ, HHD, WD, and WRD experiencing much larger decline rates (between -5% and -3% per year; see also Fig. S7) than other WAs, while only WPM had a moderate average increase over time. Some of the fluctuations in the trend in overall insect abundance (Fig. 3A) are related to fluctuations in specific WAs: for instance, the temporary peak in 2000 coincides with a peak in WRD (Fig. S7).

At the level of the different taxonomic groups, opposing trends over time can be discerned. While most groups declined in density over the study period (i.e., over 27 years from 1990 till 2017), increases were observed too (Fig. 4). Positive trends were found for Trichoptera, Odonata, and Lepidoptera (the latter being a low-abundance group in the studied database). The other seven insect groups had negative trends, including the most abundant groups: Chironomidae, Ephemeroptera, Heteroptera, and ’other’ Diptera (i.e., flies and mosquitoes not in the Chironomidae or Simuliidae families).

To see whether the abundance trends of the ten taxonomic groups were consistent across the spatial range of the WAs, we also summarized the trends for each of the three regions (West, South, and East) (Fig. 5). At this regional scale, Trichoptera, and Lepidoptera show consistently positive trends, while in the other cases the sign and magnitude of the trends were less consistent. For instance, Ephemeroptera decreased strongly in abundance in the eastern region (WRD & WRIJ), but increased in the western WAs (HHD & WHD) (Fig. 5). What is important to keep in mind though, is that Ephemeroptera abundance is about five times higher in ’East’ than in the other two regions (Fig. S9). The decline in the eastern region is thus dominating the overall decline shown in Fig. 4.

A similar spatial pattern is seen for Chironomidae, with strong declines in the South and East, but an increase in the West, where abundances were the lowest. Heteroptera decreased considerably in West, where it used to be more abundant than the other regions, and showed an increase in the South (Fig. S9). Keep in mind though that different species and genera could be involved in such spatial variation in overall increases and decreases in abundance. Paradoxically, Sim-

FIGURE 3

Trends in total abundance. A: Total insect-macrofauna abundance (pooled over groups and WAs) along with 95% confidence intervals over 28 years. B: Average annual percentage change per WA.

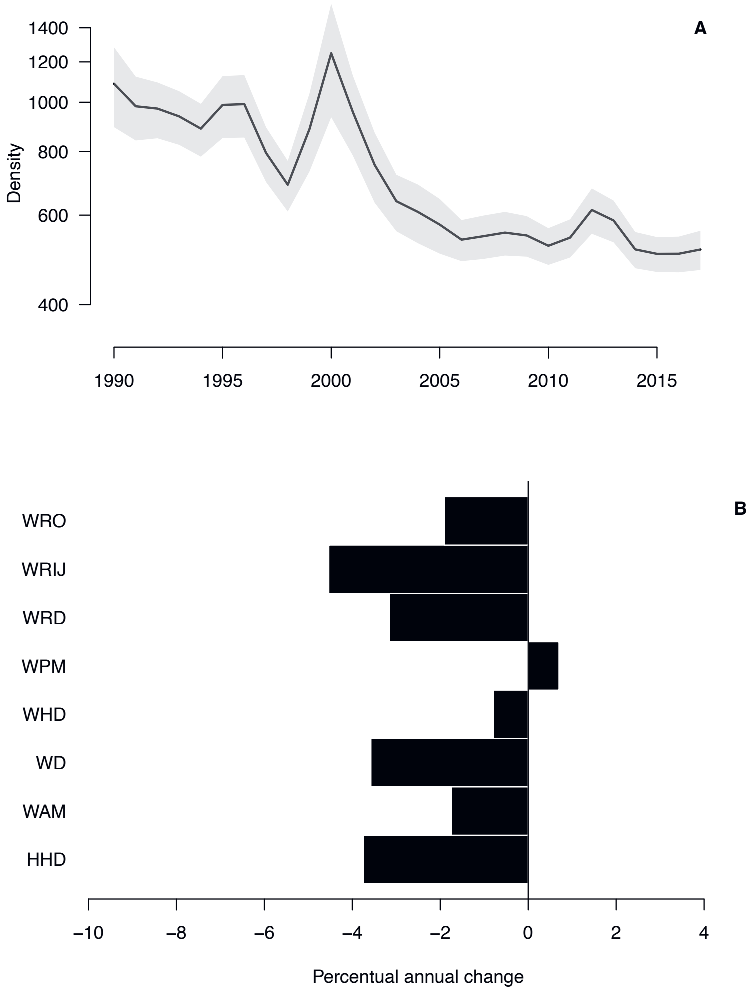


FIGURE 4

Trends in total insect-macrofauna abundance for each of the taxonomic groups. The blue dots depicts the mean trend as predicted by the models given default settings. In the bottom right average annual changes (percentages) are shown for each of the taxonomic groups. Trends per WA can be found in the appendix figures S8 and S9.

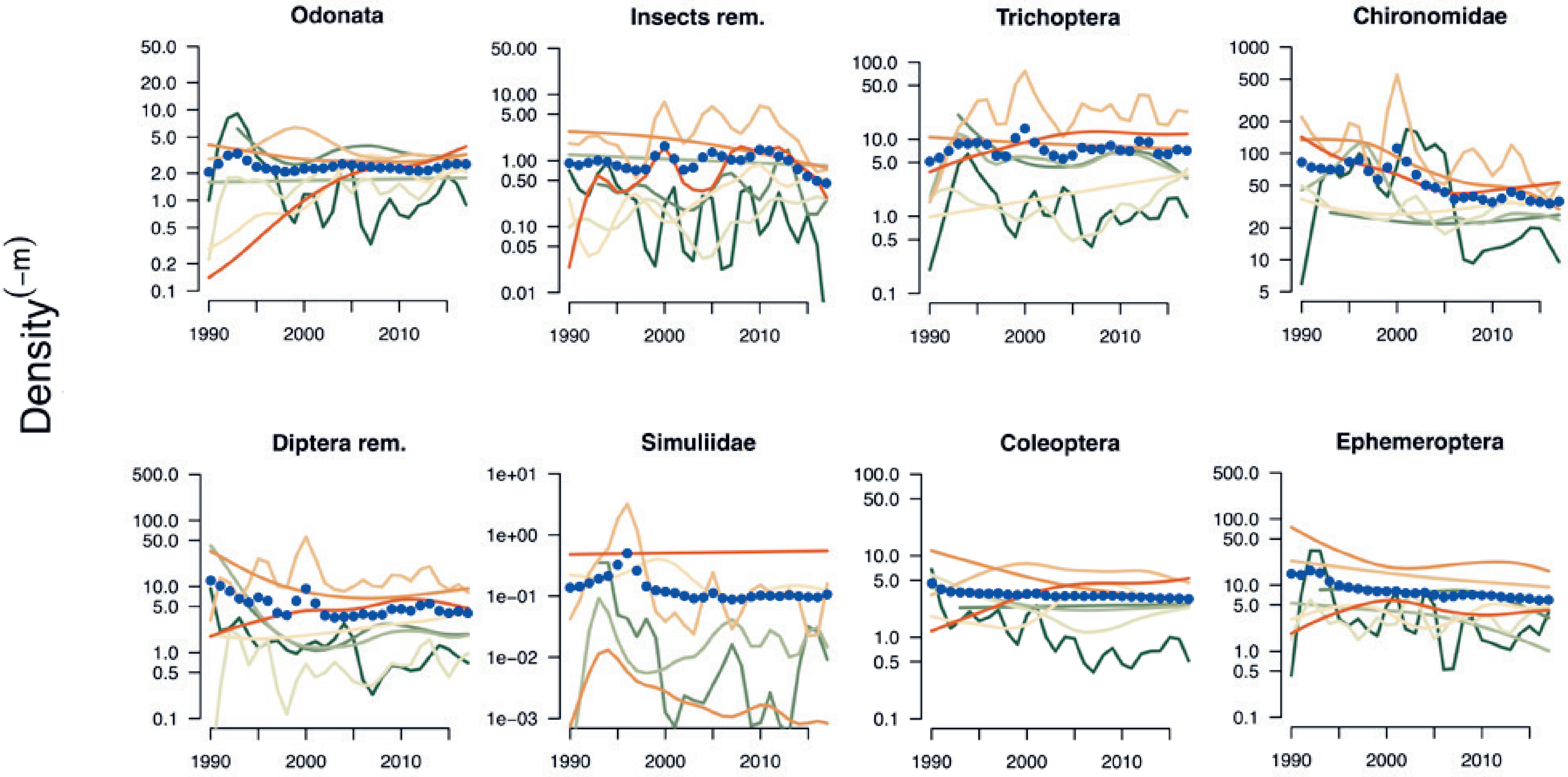
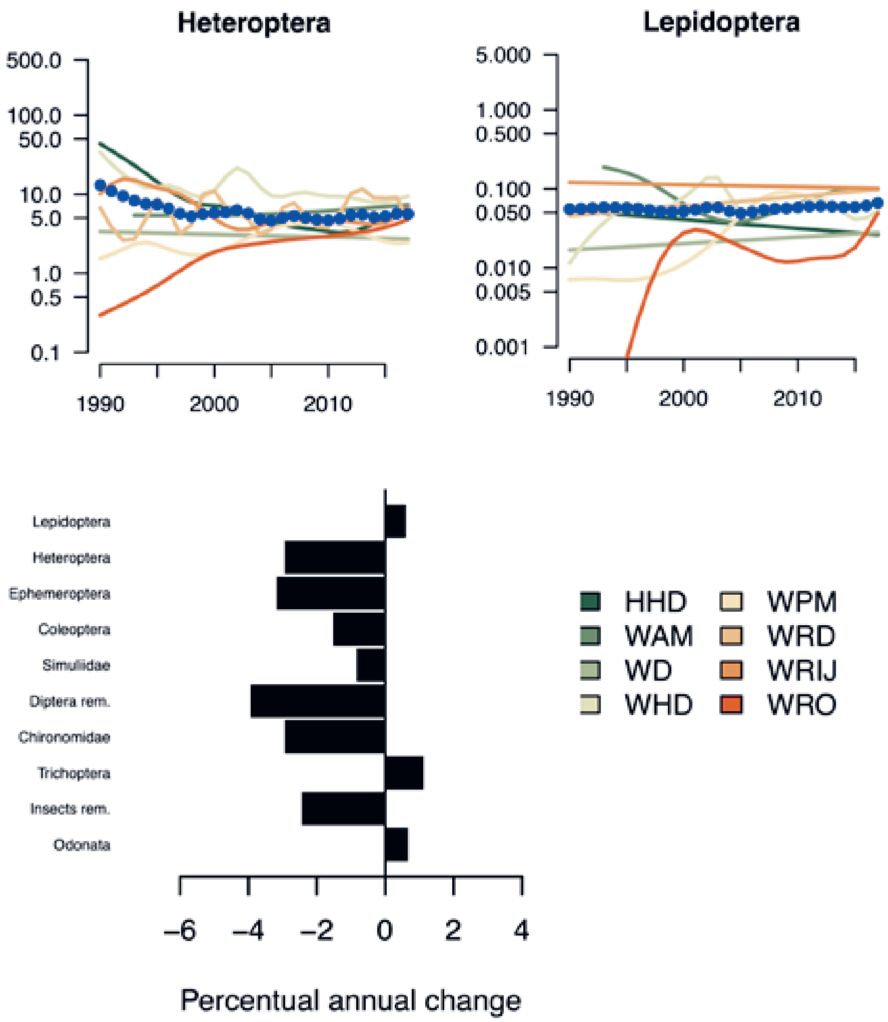


FIGURE 4

Continued from previous page.



uliidae showed positive trends in each of the three regions, but a negative trend overall. For this taxon it is important to notice that the average number of black flies (Simuliidae) per macroinvertebrates sample is low, but also highly variable. Abundance peaks in samples in the 1990s in some WAs are therefore likely responsible for inconsistent overall and regional trend estimates.

Among WAs, the increases in Odonata, Trichoptera and Lepidoptera were mostly pronounced in WHD and the south-eastern WAs (WPM and WRO; Fig. S8). Marked declines in chironomids were observed mainly in WRO, WRIJ, and WRD.

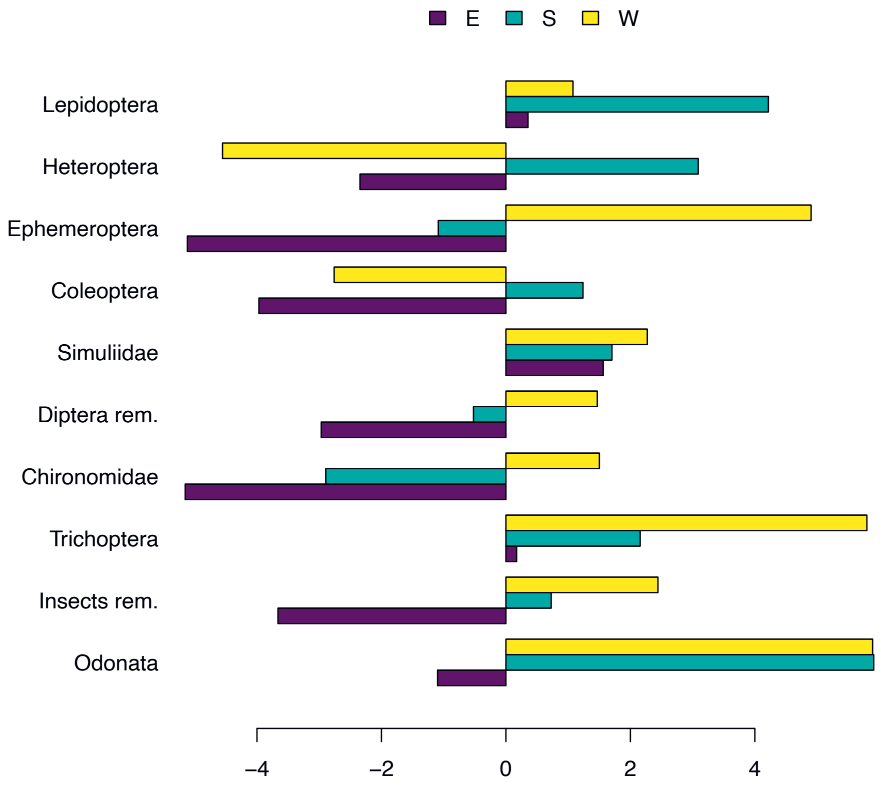
GENUS-LEVEL TRENDS

For 213 insect genera we estimated abundance trends. The rates of increase (i.e., the log-transformed per-capita growth rates; ln-lambda) of these genera were in majority (140) above 0, indicating positive trends. The median ln-lambda value was 0.0314, representing 3% annual growth. Due to the somewhat right-skewed distribution (Fig. 6A), the mean ln-lambda is higher than the median: 0.0431. The mean ln-lambda is significantly ($p<0.001$) higher than 0, mainly due to the high number of ln-lambda values, while the standard deviation (0.1132) is considerably larger than the mean.

Since Chironomidae decreased as a group and had the highest densities per sampled meter (Fig. 4), we also looked at the 68 genera within the Chironomidae family separately (Fig. 6A). Their mean ln-lambda was lower (0.0218 vs 0.0531; $t=2.565$, $P=0.03878$) than that of the 145 non-Chironomidae genera. As the mean ln-lambda of the Chironomidae genera was positive, indicating that the abundances of individuals had increased in most genera, one might wonder how the total abundance of Chironomidae could have decreased strongly (Fig. 4). The solution to this apparent paradox is explained in the following paragraph.
We related the genus-level ln-lambda values to the initial abundance of these genera, to see if abundant genera had different trends than rare genera (Fig. 6B). As a proxy for initial abundance we simply used the sums of all counts of individuals of a genus during the decade (1980-1989) before the start of our study period

FIGURE 5

Average percentual increase or decline among ten insect-macrofauna groups for each of the three WA groups (different colors). West (W): HHD & WHD, South (S): WAM, WD, WPM & WRO, East (E): WRD & WRIJ.



(1990). As figure 6B shows, there is an overall negative relationship: the regression line (fitted to the ln-lambda values) decreased significantly with increasing log-transformed initial abundance estimates, and was below zero for the 17% most abundant genera. The Chirono midae genera showed the same pattern, and did not differ from the other genera in that respect. However, the abundance of the average Chironomidae genus was significantly higher than that of the other genera (t=2.654, p<0.01, based on log-transformed abundance estimates).

This also means that the large, negative contribution of the non-biting midges (Chironomidae) to the overall trend in aquatic insect abundance (Fig. 3A), is mainly due to negative trends of a considerable number of formerly highly abundant genera (e.g. *Microsepta, Glyptotendipes* and *Procladius*). This is also true for all insect genera combined: even though the median and mean of the abundance trends is positive, the overall trend in insect abundance is negative, mainly because the most common genera decreased the most, on average. Considerable declines of formerly abundant species clearly has a stronger effect on the overall insect trend than the net increase of low-abundance genera.

At the same time, it is good to keep in mind that there might be a slight bias, as it is likely that a genus that occurred at low densities in the 1980s and then decreased in abundance, did not meet our a-priori thresholds for determining a reliable trend, or could have resulted in non-converging models (in which case we did not include it in the analyses of the genus-levels trends). This bias could have contributed to the on-average positive genus-level trends in genera with low initial abundances (i.e., the left side of Fig. 6B). However, the slope of the fitted regression line remains significantly negative when genera are omitted with less than 200 individuals counted in the 1980s, indicating that the pattern observed is not solely a statistical artefact.

The pattern of net declines of abundant genera and net increase of less-abundant genera (Fig. 6B) might suggest that the diversity of the macrofauna has increased over time. We analyse diversity parameters in the next section.

FIGURE 6

*Genus-level abundance trends. A) Histogram of 213 genus-level ln-lambda estimates. Abundance trends above 0 indicate increasing numbers of individuals within a certain genus over the years. Reddish color represents 68 genera in the Chironomidae family (i.e., non-biting midges), bluish color the 145 other insect genera for which we estimated abundance trends. B) Relationship between the genus-level ln-lambda values and the abundance of individuals in these genera in the nineties. Abundance in the period 1980-1989 is simply approximated by summing all counted individuals recorded in the dataset during that time period. The slope of the fitted regression line is highly significant: trend=0.11650-0.01377*ln(abundance), p<0.001. Whether or not a genus belonged to the Chironomidae did not significantly affect the slope or intercept of the regression line.*

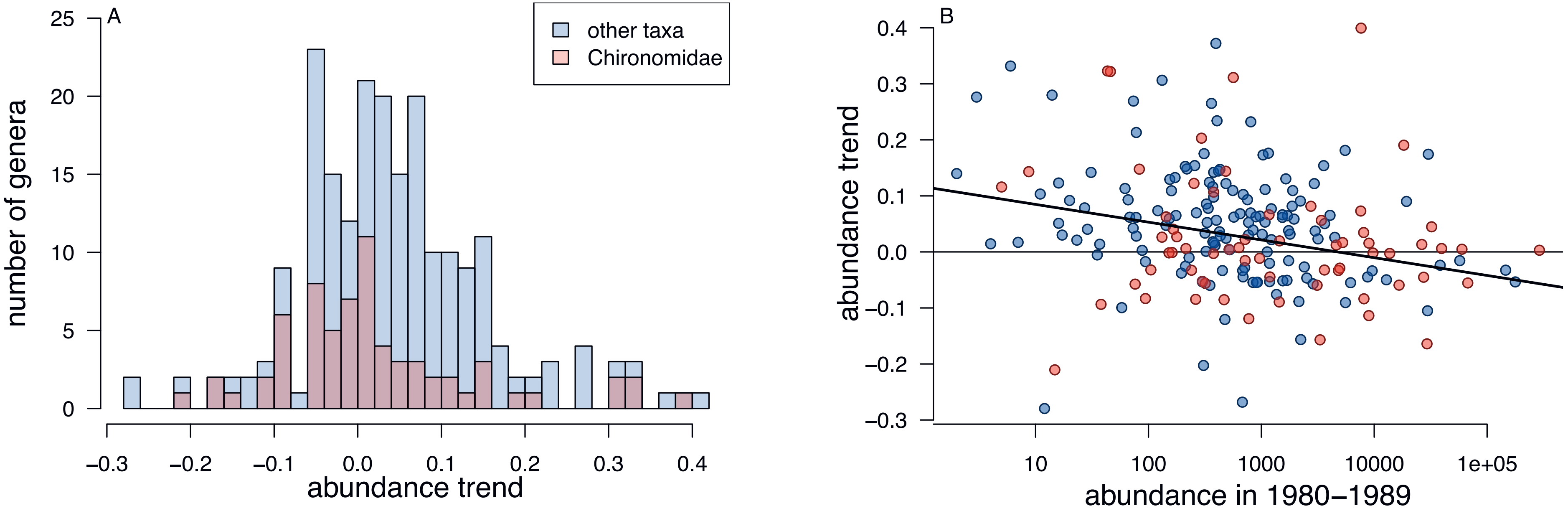


FIGURE 7

Trends in insect-macrofauna diversity. A Average taxonomic richness (i.e., number of genera per macroinvertebrate sample). B Shannon index of diversity. C Simpson's index of diversity. D Shannon evenness. B, C and D are also based on abundances per genus. For each index, blue lines depict average over the 8 WAs, while the thinner lines show variation in each index for each of the WAs. Please note that the number of macrofauna samples was low in some WAs in the first few years, resulting in more variation among the WA in the early years.

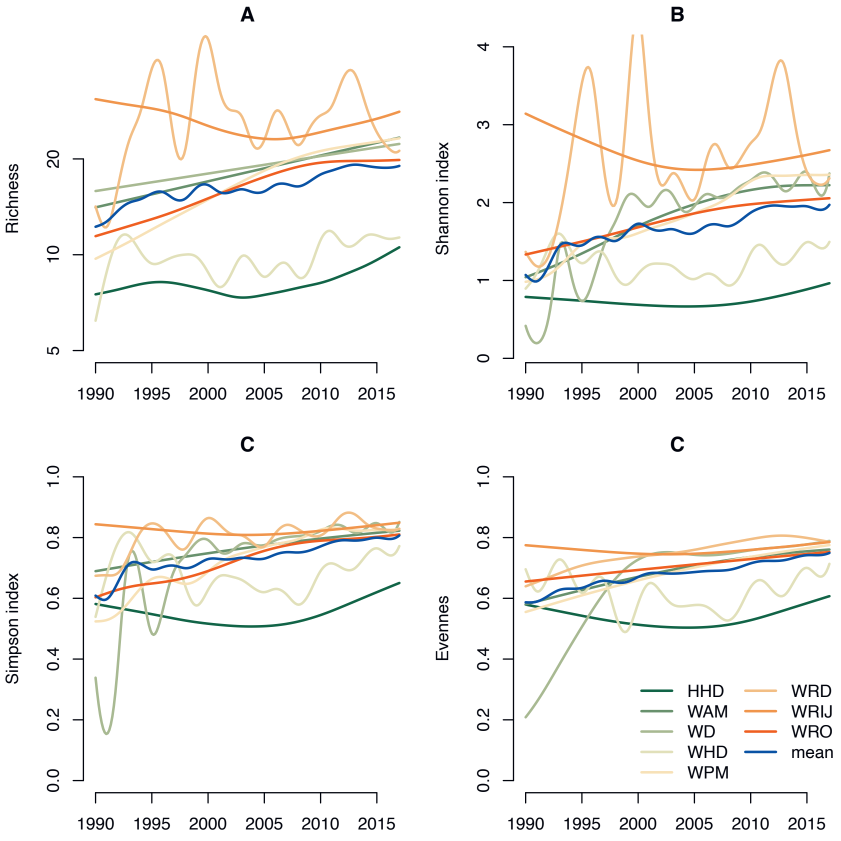
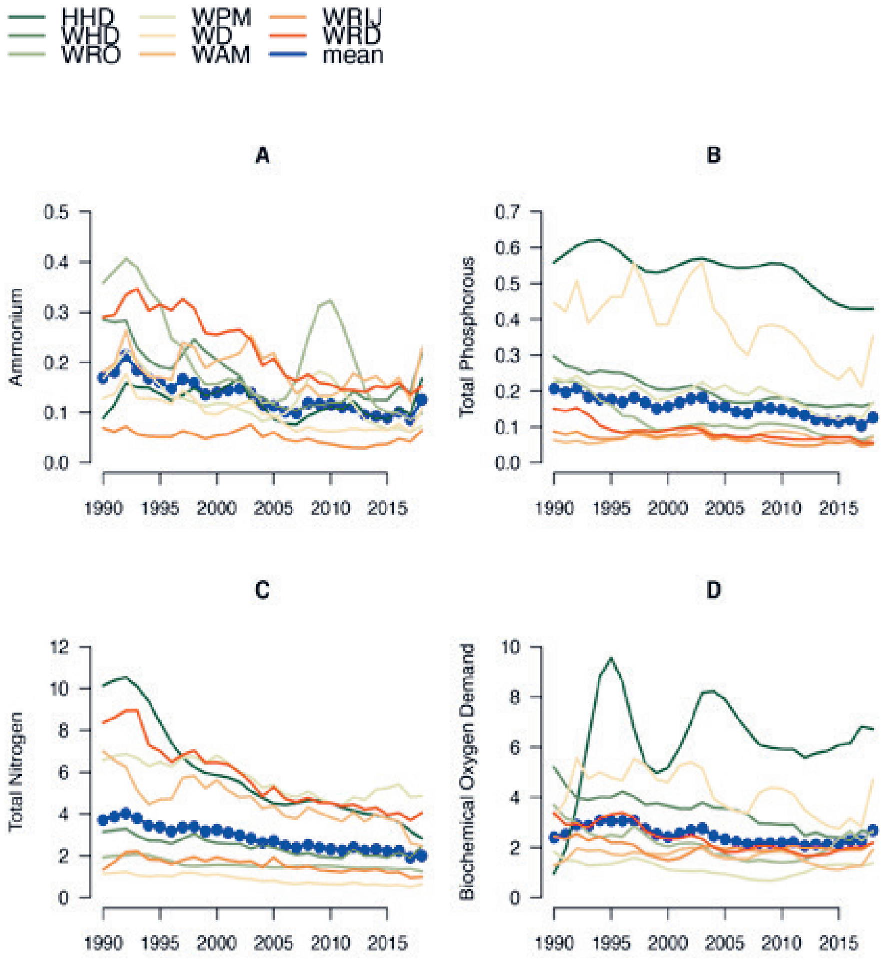


FIGURE 8

Trends in nutrient concentrations: A Ammonium, B Total phosphorus, C Total nitrogen, and D Biochemical oxygen demand. Blue dots indicate the average trend, while the uninterrupted lines show trends for each WA.



TRENDS IN DIVERSITY

All four of the calculated diversity indices showed positive trends over the period 1990 to 2017 (Fig. 7). While variation exists in the trends between the WAs (Fig. 7 and also Figs S10-S13), overall a consistent pattern emerges of increasing diversity of macroinvertebrate insect genera over the duration of the study period. The average number of insect genera in the macrofauna samples increased from 13 to 19 over the 27 years (Fig. 7A). Notwithstanding some fluctuations, taxonomic richness continued to increase over the whole period.

The Shannon index of diversity has increased in a similar way as taxonomic richness (Fig. 7B). The mean Shannon index of genus diversity was about 1 in 1990 and 2 in 2017. Please note that this index increases when more genera are found as well as when individuals are distributed more equally across the genera. Here, both mechanisms are at play: the number of genera has increased and the most abundant genera were not as dominant anymore at the end of the study period. The latter mechanism also resulted in an increase of the Shannon index of evenness, which takes on values between 0 to 1 (Fig. 7D). The Simpson index of diversity (Fig. 7C) showed the same patterns as the Shannon index of diversity. WA-specific trends in the Shannon an Simpson indices can also be found in figure 7D, and in more detail in appendix figures S11, S12 and S13.

TRENDS IN DRIVERS

Before we show how environmental variables explained variation in macrofauna abundance and trends across sampling locations and over the years, we first show how those environmental variables themselves have changed over the years. When a variable has a negative effect on macrofauna abundance, it is important to know whether that variable has decreased over the years, stayed stable, or increased. For instance, if a variable has a negative effect on abundance, but has decreased itself over the years, then macrofauna numbers actually go up due to the decrease of the variable.

Among the measured nutrients, ammonium, total nitrogen, and total phosphorus showed declining trends between 1990 and 2018 (Fig. 8ABC): ammonium and total phosphorus concentrations halved, while total nitrogen levels dropped from 4 to 2. There is variation among the WAs (see WA-specific lines in figure 8), but all WAs see a decline in these nutrient variables. At the same time, the biochemical oxygen demand (BOD) also declined, from about 3 to 2 (Fig. 8D). This decrease in BOD happened between 1990 and 2005, after which it was relatively stable.

Among land use variables, build up areas and forest increased between 1996 and 2015, while agricultural land (farmland, grassland and greenhouse) cover decreased (Fig. 9).

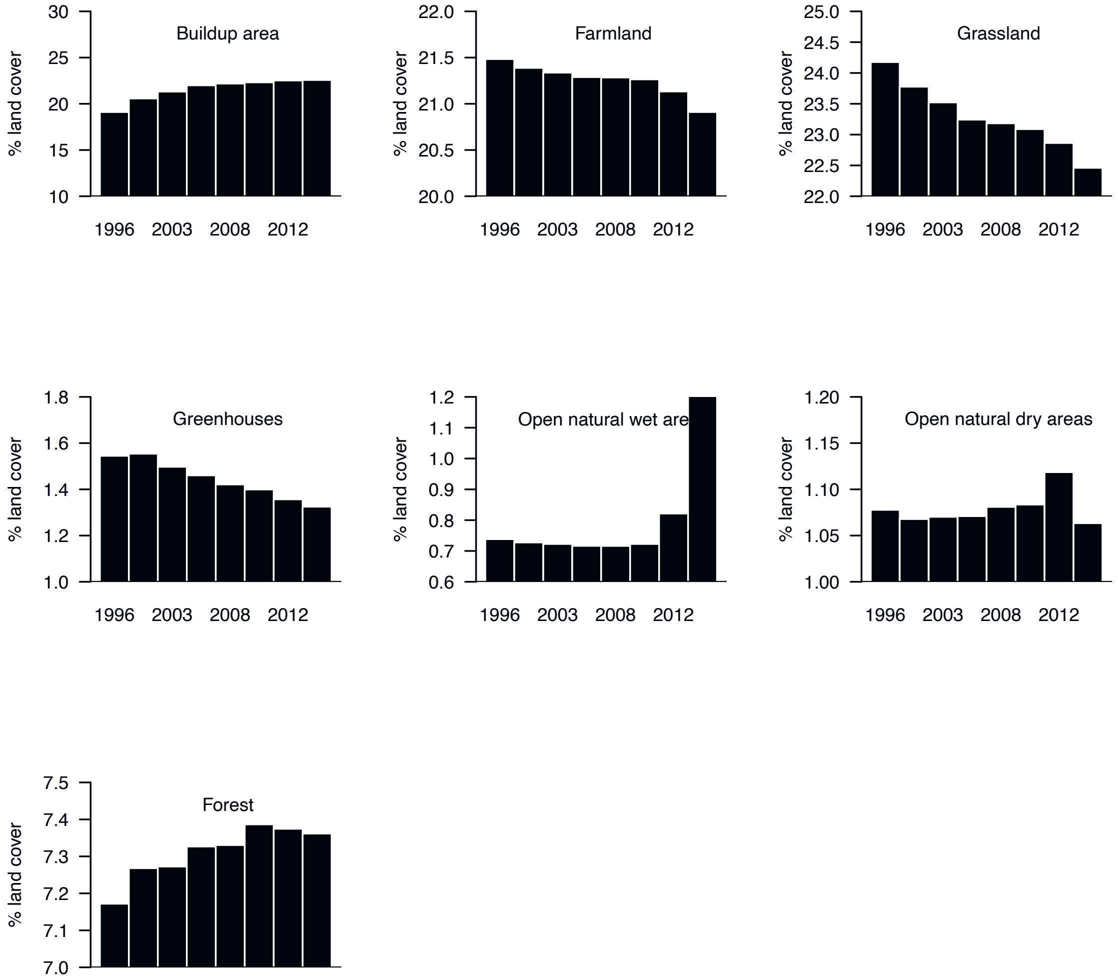
Toxicity in water, as measured by the annual maximum of the multiple-substance Potentially Affected Fraction indices (maxPAF-NOEC), showed stable trends over time between 1990 and 2018 for combustion compounds, slightly negative trend for industrial compounds, and a strongly negative trend for pesticide compounds (Fig. 10). Considerable variation existed between the WA-specific trends in these maxPAF-NOEC parameters.

EFFECTS OF POTENTIAL DRIVERS ON THE ABUNDANCE OF FRESHWATER INSECTS

Inferred from the global models (i.e., based on data from all eight WAs, separately per taxonomic group), covariate effects on the abundance appeared to be variable between the different insect groups (Fig. 11). Nevertheless, some general patterns could be observed. Among land use covariates, the cover of greenhouses was significantly negatively associated with insect abundance across all insect groups, except for Lepidoptera and Chironomidae (not significant; see Fig. 13 for effects per taxonomic group). The area of arable land near the macrofauna sampling points had a significantly negative effect on the abundance of Trichoptera, remaining Diptera and remaining insects (Fig. 13), but a significantly positive effect in Heteroptera (shown as a deviating point in the bottom row of figure 11. The amount of forest was generally significantly positively associated with

FIGURE 9

Average trends in land use variables between 1996 and 2015, within a radius of 1km centered around each of the monitoring locations. Please note the different scales on the y-axes.



abundance, except for Chironomidae, Coleoptera and Lepidoptera (not significant) and Ephemeroptera and Heteroptera (significantly negative). Wet natural areas had a significantly positive effect on the abundance of Heteroptera, Coleoptera, Simuliidae, Chironomidae, remaining Diptera, and the remaining insect group.

Effects of nutrients (particularly phosphorus- and nitrogen-related variables) appeared to be consistently and significantly negatively related to the abundance of all insect groups, with the exception for Chironomidae and Simuliidae (significantly positive). However, taxonomic groups differed in which variables showed the strongest effect: total nitrogen or NO_2+NO_3, total phosphorus or PO_4.

Of the three types of pollutants for which we had included msPAF-NOEC covariates in the global models, pesticides show the most consistent pattern (Fig. 13). In eight out of the ten taxonomic groups, pesticides had a significant effect on their abundance. The two deviating taxa are Chironomidae (no significant effect) and Simuliidae (significantly positive effect).

Compared to the other types of variables, weather variables showed relatively small effects on abundance. An exception was the growing degree days variable (a measure of accumulated heat, here above 5°C), which showed significantly positive effects on Odonata, Chironomidae, Lepidoptera, remaining Diptera, remaining insects, and especially on Ephemeroptera. On the other hand, higher GDD (Growing Degree Days) strongly decreased the abundance of Coleoptera.

Similar models for the three groups (East, South, West) of WAs, showed variation in effect sizes among the regions (Fig. 12). For instance, the negative effect of greenhouses was especially found for the West, while pesticides, nitrogen and phosphorus had consistently negative effects especially in the South and West.

EFFECTS OF POTENTIAL DRIVERS ON THE TRENDS OF MACROINVERTEBRATE INSECTS

Besides the abundance responses of taxonomic groups to variation in the considered potential drivers, we also studied how the abundance trends responded to the mean annual change in the values of each potential driver over the 1990-2017 period (Fig. 13). In these analyses we quantified how much the various environmental changes contributed to the trend coefficient according to our global models. With the exception of Odonata, Trichoptera and Heteroptera, the largest contribution to the average annual trend was provided by unknown factors. Otherwise, the most consistent contributions to the abundance trends are made by the decline in pesticides and by the decline in several nutrient concentrations.

FIGURE 10

Trends in toxic pressure of mixtures between 1990 and 2018. A Annual maximum of the Potentially Affected Fraction based on pesticide compounds (maxPAF-NOEC/pesticide). B Annual maximum of the Potentially Affected Fraction based on industrial compounds (maxPAFNOEC/industrial). C Annual maximum of the Potentially Affected Fraction based on combustion compounds (maxPAF-NOEC/combustion). Blue lines with dots represent the overall average, while the other lines show the trends per Water Authority (WA). The number of tested substances per water sample are taken into account.

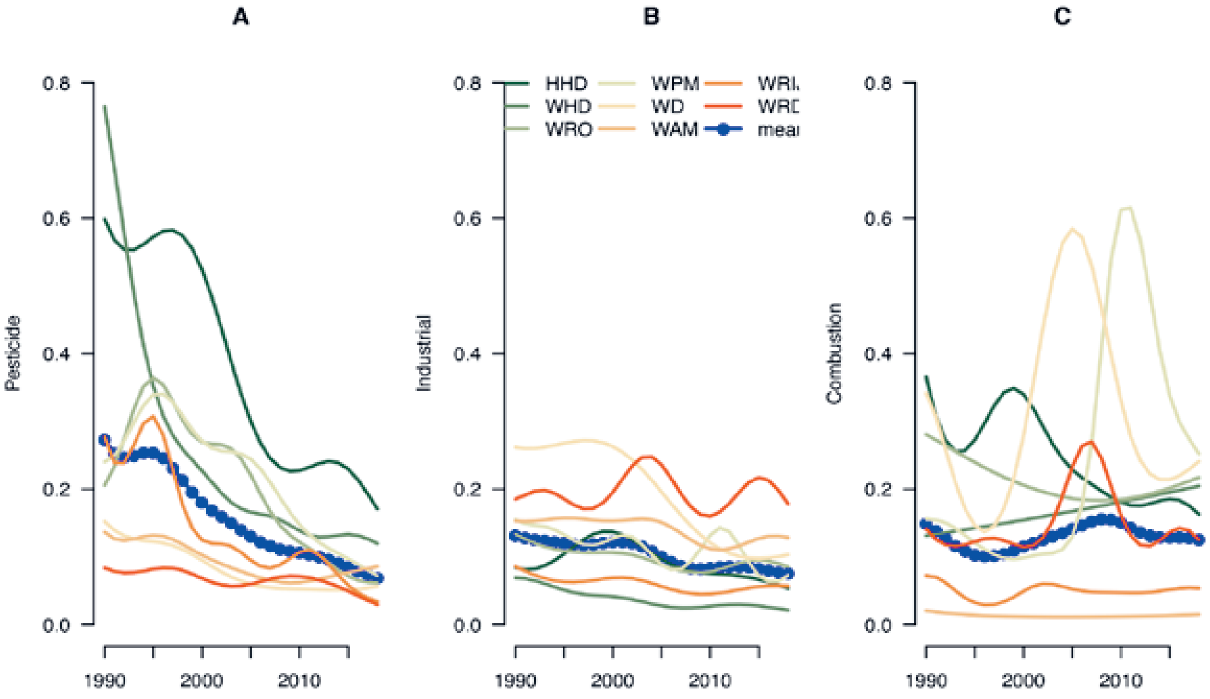
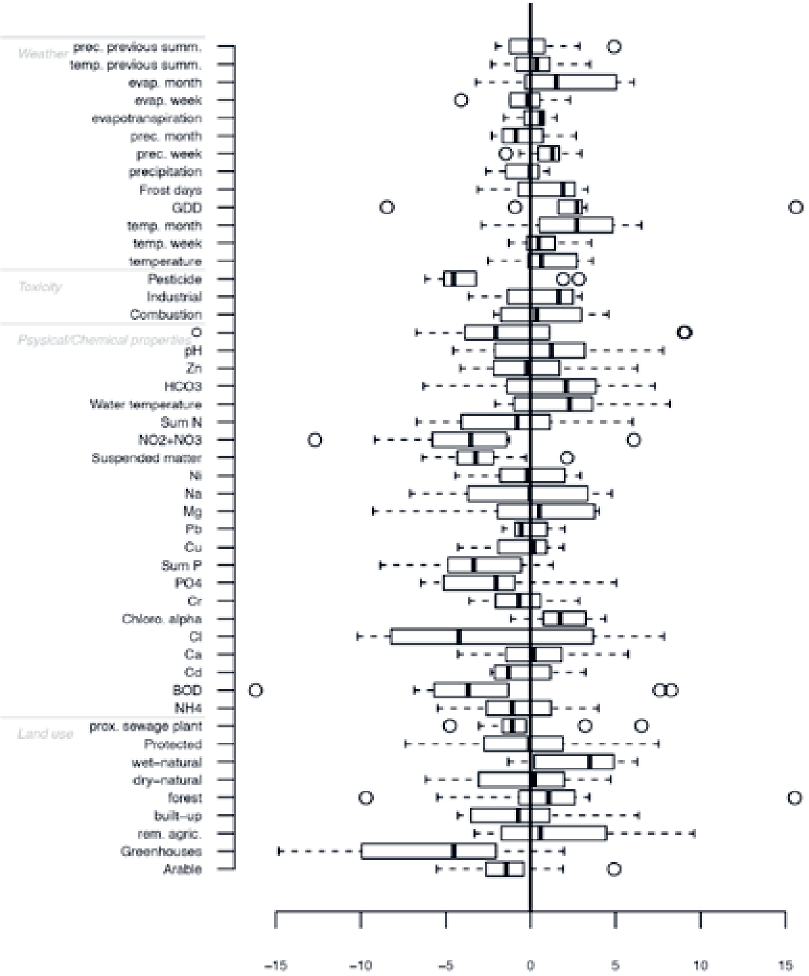


FIGURE 11

Back-transformed effects (z-scores) of covariate values on insect abundances. Effects are summarized over the ten taxonomic insect groups. Horizontal boxes therefore show the variation of effect sizes among the taxonomic groups, based on the global models (i.e., based on data of all eight WAs).



The decline in toxic pressure due to pesticides, as quantified by the negative trend in msPAF-NOEC values over the study period, had considerably positive effects on the abundance trend of eight of the ten insect groups. On the other hand, the decline in toxicity due to pesticides had a considerably negative effect on the abundance trend of Simuliidae (mainly occurring in streams), and a small negative effect on the trend of Chironomidae (occurring in a variety of water types).

Changes in mean concentrations of the various nutrients provided, together, the largest contribution to the average annual trends. Generally, changes in nutrient concentrations (mainly declining nutrient loads over the study period) had positive effects on the average trends of all taxonomic groups except Chironomidae and Simuliidae. For instance, the decline in total phosphorus ('Sum P') had a relatively large, positive effect on the abundance trend of Trichoptera. For Odonata, the decline in the nitrogen sum had a positive effect on abundance. The changes in nutrient concentrations also showed mixed effects on the trends of the taxonomic groups, with small positive and small negative effects partially compensating each other. For instance, in Ephemeroptera a dominant positive effect of the declining phosphorus sum is partly buffered by a smaller negative effect of the decrease in phosphate. Decreasing nutrient concentrations had net negative effects on the trends of Simuliidae and Chironomidae.

Changes in weather variables did not appear to contribute consistently to the trends of any of the insect groups. A few exceptions include a strongly positive contribution on the Simuliidae trend by the increase over time of the precipitation in the previous summer. The positive trend in the mean temperature in the month preceding a macroinvertebrate sample, had a positive effect on the trend of the 'remaining insects' group. Changes in land use parameter hardly contributed to the abundance trends. Changes in heavy metal concentrations generally had relatively small effects.

TREND-TRAIT RELATIONSHIPS

Of 213 genera for which we were able to derive abundance trends, 125 also occurred in the trait database of Tachet *et al.* (2010). For 33 of remaining genera a link could be made at the tribe level, for 40 at the subfamily level, for 14 at the family level and for 1 genus a link could only be made at the infraorder level. However, for the trend-trait analyses shown in figures 14, 15 and 16 we only used the subset of 125 genera for which both abundance trends and trait information were available.

The genus-level abundance trends (ln-lambda) were related to some of the traits considered, but certainly not to all. Please keep in mind that we conservatively included insect order as a random factor in our analyses to account for potential phylogenetic patterns.

One of the traits that did show statistically significant effects in the fitted linear mixed-effect models was the preferred substrate that species in a genus normally have. Genera with high fuzzy scores for the categories cobbles/pebbles, gravel, and sand had abundance trends that were significantly above average (Fig. 14A). Genera preferring sludge and macrophytes, on the other hand, performed below average, which is consistent with the reduced nutrient load that was observed in the studied water bodies in the period 1990-2017. The trait locomotion showed interesting relationships with the abundance trends (Fig. 14B), with genera with full-water swimmers performing below average. It might well be that this pattern is related to changes in the species compositions in lakes and rivers.

FIGURE 12

Back-transformed effects (z-scores) of covariate values on insect abundances. Effects are summarized over the ten taxonomic insect groups, separately for each of the three groups of Waterboard Authorities (A: East, B: South, C: West).

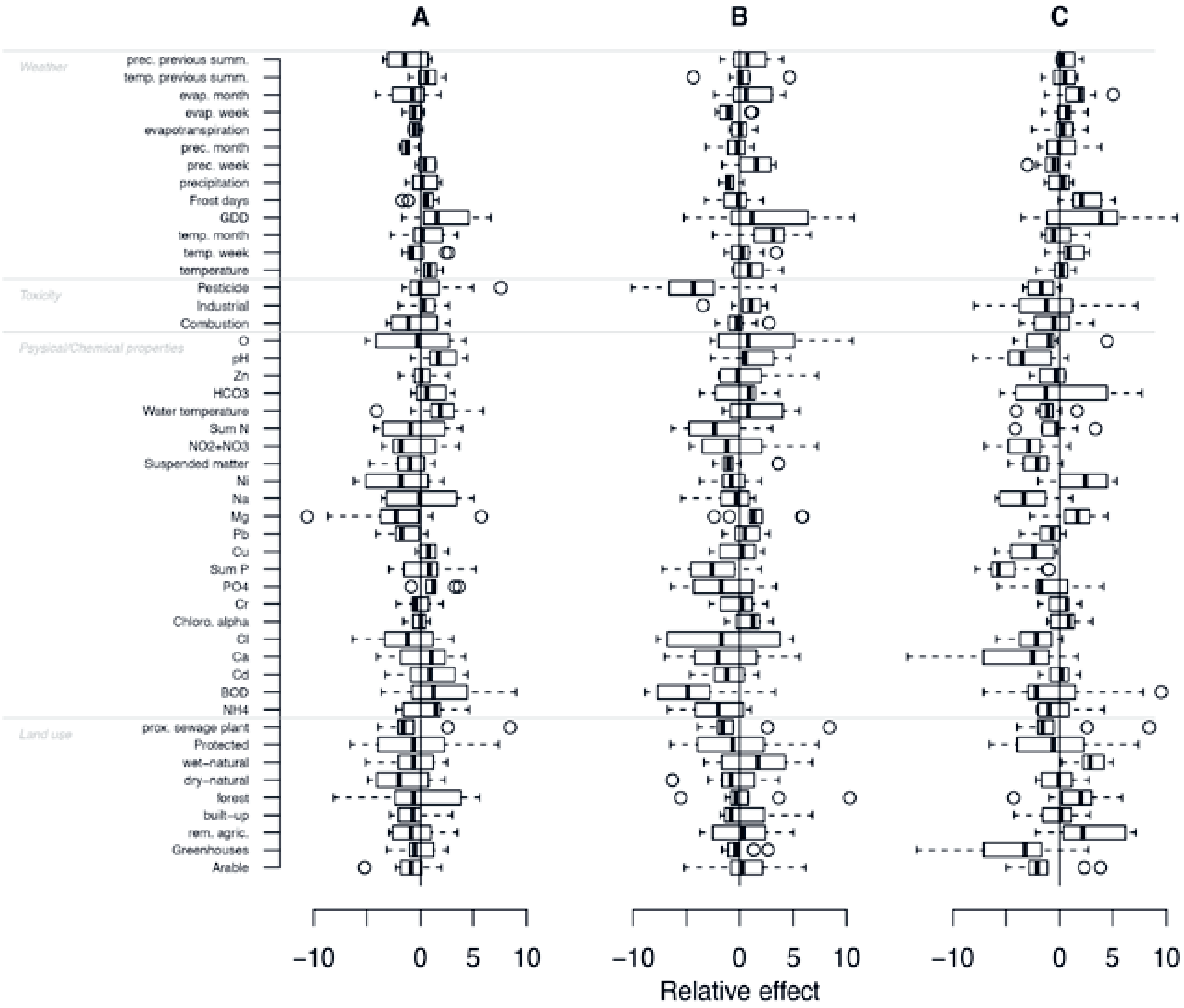


FIGURE 13

Effects of changes in covariate values on the abundance change of each of the ten taxonomic insect groups (see also the next two pages). Yellow arrows indicate the direction and relative magnitude of the change in each of the covariates from 1990 to 2018. The black bars then quantify the effect of that covariate change on the change in abundance. The blue interrupted line signals the sum of the black bars. Units are on the log- scale. In this graph the results of the 8 WAs are combined, unweighted by the relative abundances of insect groups in the separate WAs.

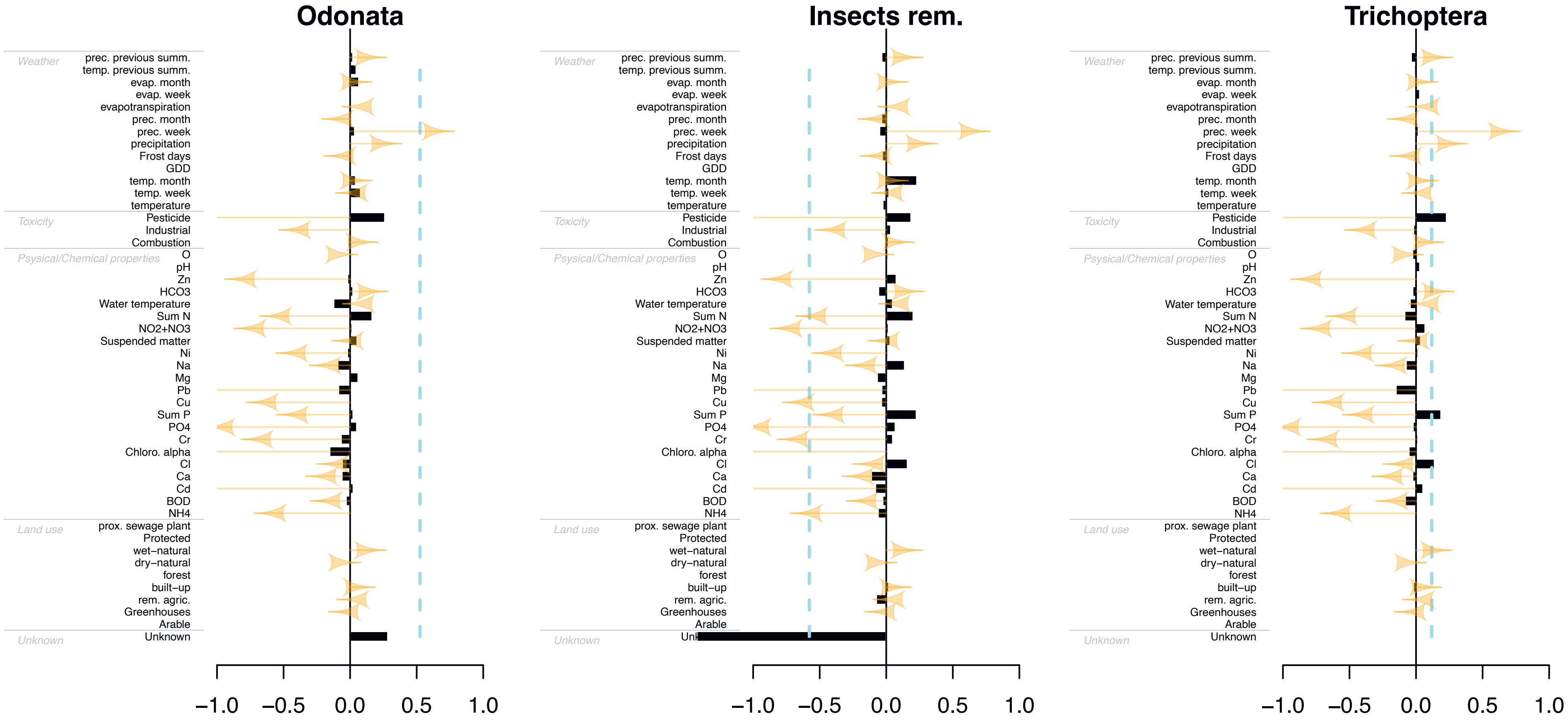


FIGURE 13

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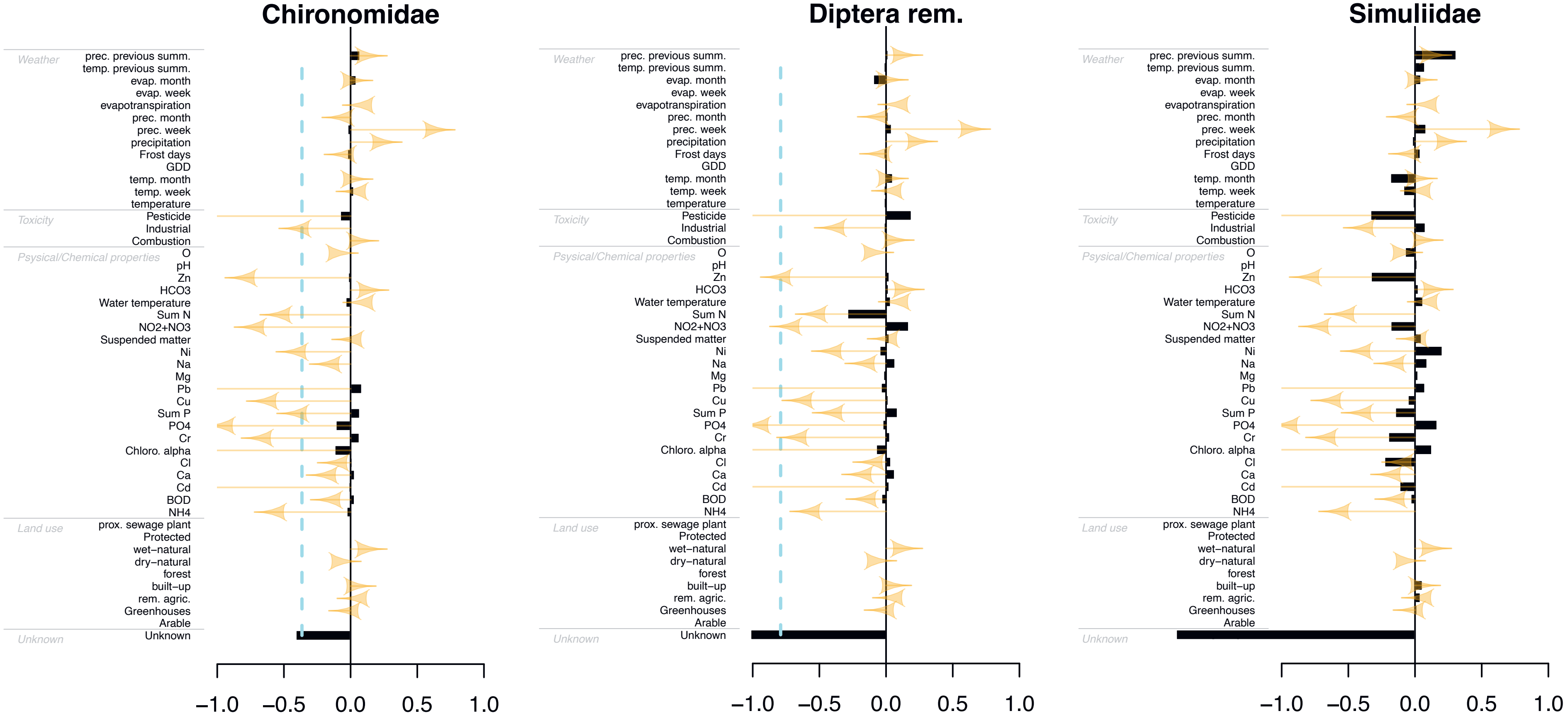

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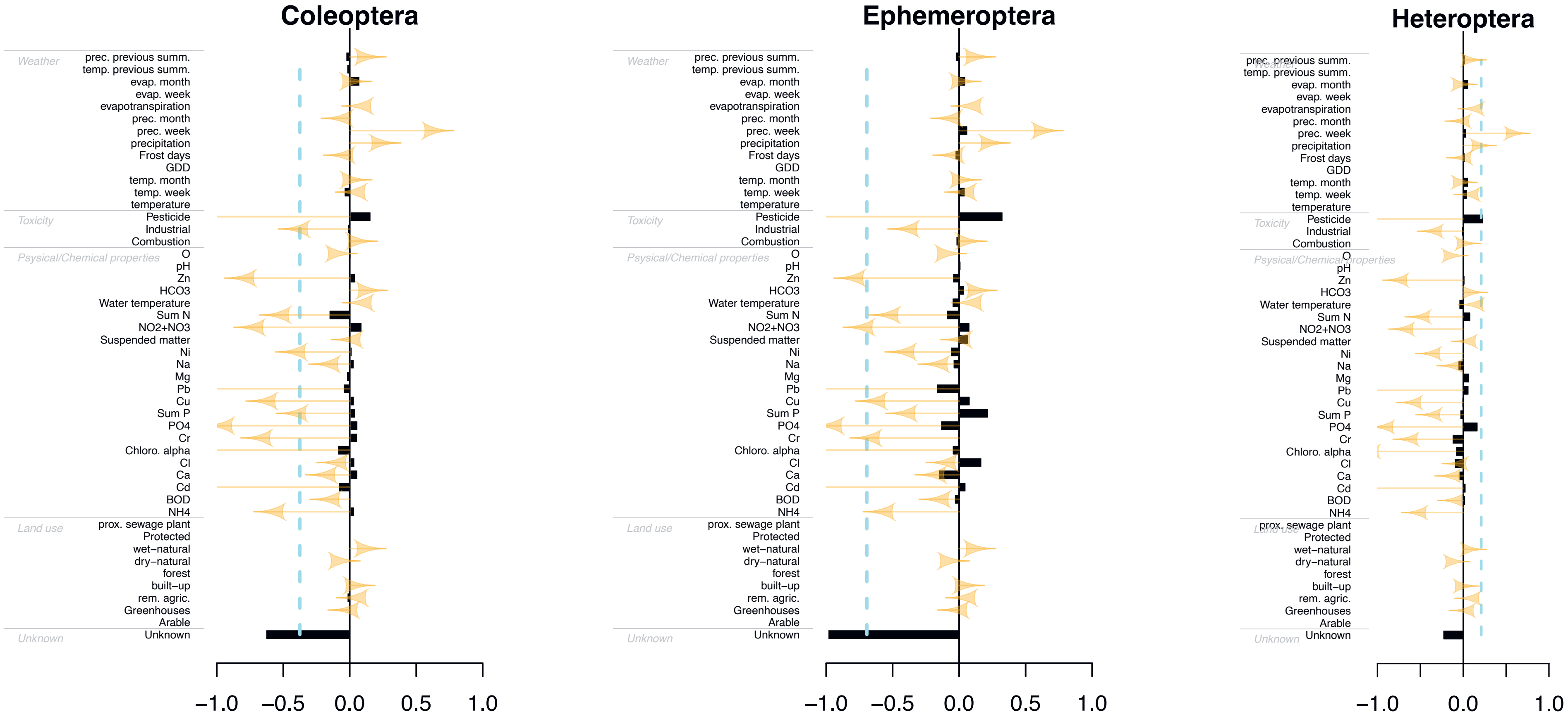

FIGURE 13

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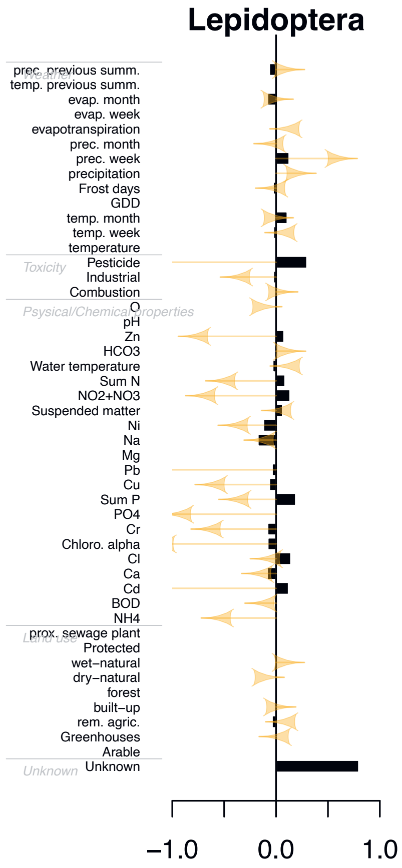


Genera with oligotrophic species increased in abundance (but not significantly so), while those with eutrophic species decreased (Fig. 14C). Again, this clearly is in line with reduced nutrient load over the study period.

The next three traits, current velocity, transversal and longitudinal distribution show related results (Fig. 14DEF). Genera associated with high fuzzy scores for respectively standing waters, lakes, and outside river system tended to have lower abundance trends. On the other hand, genera associated with slow and medium current velocity had significantly higher than average abundance trends, which is in agreement with the positive tendencies of the genera associated with river channels. It could well be that this pattern is related to improved oxygen availability.

Genera of which the adult stage lives in water performed significantly below average (Fig. 15A). The same is true for deposit feeders, while filter feeders had significantly higher than average abundance trends (Fig. 15C). Longer-lived species performed worse than shorter-lived species (Fig. 15E). Most of the other traits in the Tachet *et al.* (2010) database did not show significant relationships with the abundance trends (Figs. 15 and 16). For instance, genus-level abundance trends were not explained by the preferred temperature range of the genera (Fig. 16H).

We could not analyse potential effects of the trait preferred salinity in the same way. In the Tachet trait database salinity has two categories: fresh water and brackish water. Not surprisingly all genera found in the macrofauna samples of the WAs had high scores for fresh water. As only one category (brackish water) with variable fuzzy scores remained, we could not perform the same fuzzy principal component analyses as for the other traits in the Tachet *et al.* (2010) database. When regressing the genus-level rates of increase against the fuzzy scores for brackish water directly, a nearly-significant negative effect was found (ln-Lambda=0.0412-.0183*scoreBrackishWater; p-value of the slope=0.063), indicated that genera that are not only found in fresh water but also in brackish waters performed somewhat worse than genera affiliated with fresh water only.

The risk level derived from the SPEARpesticides database (Liess and Von Der Ohe 2005) did not explain variation in the genus-level rates of increase (Fig. 17A). The rate of increase did not differ (p=0.141) between the 26 genera at risk (mean ln-lambda=0.048) and the 175 genera not at risk (ln-lambda=0.041). Please note that there were also 12 genera for which we had an abundance trend but could not determine the risk level.

Analyses of the effects of the dominant KRW indicator scores per genera (van der Molen *et al.* 2018), roughly showed the same patterns for river systems and lake systems: genera indicating healthy rivers and lakes increased on average, while negative indicators declined in abundance. In the case of river indicators, most genera were classified as ’K’ (more than 75% of indicator scores being a characteristic taxon; n=96) or 0 (no indicator scores or none of the P, K or N scores having a majority; n=100).

The KRW lake indicator scores showed a somewhat similar but not significant pattern: lower rates of increase for the 9 ’N’ genera (i.e., when these genera occur at high abundance they are negative indicators of desired lake ecosystems) than of the 110 ’0’ genera (Fig. 17C). Those 9 ’N’ genera were, in alphabetical order: *Aedes, Anopheles, Callicorixa, Chironomus, Culex, Culiseta, Procladius, Psectrotanypus,* and *Tanypus*. Please keep in mind that we derived the abundance trends based on the entire macrofauna dataset, not separately for e.g. lake and river systems. That would have made the link with the KRW indicators more direct, but would have also meant smaller datasets and less-certain trend analyses for fewer genera.

Water strider - *Gerris lateralis* - rossige schaatsenrijder
Photo: Bureau Biota

Common backswimmer - *Notonecta glauca* - gewoon bootsmannetje
Photo: Bureau Biota

DISCUSSION

Our goal was to uncover how aquatic insect abundance has developed in the Netherlands over the past three decades, making use of the extensive datasets on aquatic stages of insects sampled by Dutch Water Authorities (WAs). Overall, insect abundance (expressed as densities) declined by approximately 53% over 27 years (from 1990 till 2017), which corresponds to a 2.7% decline per year, on average. However, trends differed considerably between and within insect orders. Declines were strongest in the most abundant orders and families (Heteroptera [typical bugs; wantsen], Chironomidae [non-biting midges; dansmuggen], other Diptera [other flies; andere muggen en vliegen]) and Ephemeroptera [mayflies; haften; especially the abundant *Cloeon* and *Caenis* genera], while trends in other insect orders varied from small decreases (e.g. Coleoptera [beetles; kevers]) to increases (e.g. Trichoptera [caddisflies; kokerjuffers/schietmotten] and Odonata [dragonflies and damselflies; libellen]). Trends also varied across WAs, indicating that regional differences in abiotic conditions and temporal changes therein likely matter.

The second objective of our study was to relate trends to potential drivers (changes in abiotic conditions) as well as relate trends of insect taxa to their traits. The combined toxicity of the observed pesticide concentrations dropped markedly over the study period. Similarly, nitrogen and phosphorus concentrations, which had strongly negative effects on most insect groups, halved during the study period, thus contributing positively to the abundance trends of these groups. On the other hand, the most abundant Chironomidae genera benefited significantly from eutrophic conditions, and consequently declined strongly. These patterns are also reflected by some of the traits of the genera for which we were able to determine trends: aquatic insects preferring sand, gravel, cobblestones or pebbles performed much better than those preferring sludge or macrophytes as a substrate to live in. And probably related to this: taxa preferring flowing water performed better than those preferring standing water. Indeed, filter feeders performed better than deposit feeders. The improvements in water quality are also reflected in the finding that taxa characteristic of healthy river and lake communities increased in abundance, while taxa that are negative indicators of healthy river and lake communities declined in abundance.

While total insect abundance declined, diversity in insect genera increased. The overall increase in diversity indices reflects both an increase in numbers of genera (higher richness) and a higher evenness, due to the decline in the abundance of individuals of the most abundant genera. It would certainly have been interesting to calculate and study species-level diversity, but we feel that that was not possible in general, as too many caught individuals were identified at only a taxonomic level higher than the species level. Furthermore, identification tools and expertise may have increased in the first decade of the study period, leading to more individuals being identified at the species level over time (from 65% to 70% of the sampled individuals).

It would also have been interesting to compare current diversity levels with those in undisturbed water bodies in the past (e.g. first half of the 20th century) or elsewhere within the same ecoregion, e.g. less disturbed streams in Germany, Sweden or Eastern Europe. However, such comparisons suffer from confounding differences in sampling methods and from the fact that many of the rare species found elsewhere do not occur in The Netherlands (Nijboer *et al.* 2004). In the current study we started our analyses with the starting year 1990, because sampling methods were much less consistent before that time. While these trends of improved water quality and increase of characteristic species is hopeful it is difficult to gauge how complete the current recovery is without proper data on reference community compositions prior to the strongly eutrophic and polluted water conditions of the second half of the previous century. It is possible that aquatic insect communities are steadily recovering towards historic levels, but it is also possible that recovery remains incomplete and stabilizes at a state impoverished relative to historic levels (but enriched relative to the situation in the 1980s). In other words, we have to keep in mind that we might be affected by shifting baselines (Soga and Gaston 2018; Didham *et al.* 2020) when interpreting these patterns of increasing biodiversity over a time period that started in a time with many disturbances.

WHAT CAUSED INSECT ABUNDANCE TO BE HALVED AND DIVERSITY TO INCREASE?

In their review paper, Jactel *et al.* (2020) expect land use change, pollution, climate change, invasive alien species, and interactions between these factors to be among the most likely causes of recent insect declines. Of the different sets of explanatory variables in our analyses, land use variables, pesticide and nutrients were generally the strongest explanatory variables to explain local insect abundance. Effects of land use included consistently negative effects of greenhouses on the abundance of ten taxonomic groups considered, mainly in the western WAs, where many greenhouse complexes are present. However, as only marginal changes in these variables occurred over the past three decades, changes in land use variables contributed little to the overall trend of insect groups. We have to acknowledge, however, that our analyses looked at relative changes in the area cover of land-use types, meaning that subtle changes like the disappearance of important small landscape elements like single trees at the water edge are not picked up.

Contrary to the lack of effect of land use changes, the overall declining trends in nutrient concentrations over time most consistently explained, statistically, temporal trends in the abundance of macrofauna insects, with positive effects on most species (but negative on the dominant Chironomidae), which ultimately resulted in increases in diversity indices over time. The found halving of the nitrogen and phosphorus concentrations is in line with calculations by Deltares, which show predominantly downward trends since 2010 in areas dominated by agriculture (Buijs *et al.* 2020a). Sewage water and poorly-functioning sewage sanitation systems used to cause high nutrient levels, as well as high organic load which causes oxygen shortage and sludge layers. It was probably this combination of nutrients and organic load that provided food for large densities of taxa preferring eutrophic conditions. While water quality has improved, in 2019, nitrogen levels were still higher than the system-specific water quality norm at 48% of the sampled locations, while phosphorus concentrations were still too high at 40% of the sampled locations (Buijs 2020).

In 81% of water samples from German lowland streams in agricultural areas (in 2018 and 2019) some pesticide concentrations exceeded regulatory acceptable concentrations (Liess *et al.* 2021). In the Netherlands, the proportion of cases where pesticide concentrations were found to be above their maximally allowed concentrations has decreased gradually in recent years (2014-2019) (Buijs *et al.* 2020b). Similar to the positive effects of the halving of the nitrogen and phosphorus concentrations, the decline in the toxicity of pesticides over the three decades of our study also improved the abundance trends of most of the insect groups, except for the groups indicative of eutrophic and polluted waters. Over those three decades new pesticides have been introduced in agricultural practice and domestic use (e.g. neonicotinoids), while other types of pesticides have been partly phased out. Given the patchy nature of the dataset on micropollutants (including pesticides), with different sets of substances being measured in different water quality samples, we found that it was not feasible to interpolate pesticide concentrations or their toxicity to the day of sampling, as we did do for the more standardly measured variables of water chemistry. Instead we used annual maxima of indices of the combined toxicity of multiple substances. However, using annual maxima of toxicity is preferential from ecotoxicological theory: peak toxicity (together with the duration and frequency of peaks) is expected to have lasting effects throughout the season. Using annual maxima is then a better proxy of ecotoxicological effects than daily measurements, as measured pesticide concentrations tend to fluctuate strongly over time: pesticide exposure tends to occur in ephemeral peaks (e.g. after rainfall resulting in run-off).

For most locations where macro-invertebrates were sampled, micropollutants were measured as well. However, for about one quarter of the locations we had to spatially interpolate the year-specific maximum-msPAF values. While this means that there is more uncertainty in those interpolated values than in the maximum-msPAF values that were measured at the same locations, we believe that that uncertainty is fairly small. Furthermore, we can argue that, if including these locations with interpolated maximum msPAF values had any effect, it would most likely result in more conservative conclusions: uncertainty in the values of an explanatory variable leads to weaker effect sizes. Ways to check this in future research is to also perform the analyses on the data from the locations where macro-invertebrate and micropollutant measurements intersected, or to gradually limit the addition of the remaining locations based on their proximity to the nearest location of micropollutant sampling. Despite the many abiotic conditions considered, we could not include all factors. For instance, management operations by the Waterboard Authorities (e.g. dredging and mowing), or local vege tation characteristics, are known to affect the abundance of the various insect species as well (Higler and Verdonschot 1989; Fairchild *et al.* 2003; Verberk *et al.* 2005; Verberk and Esselink 2007), but since proper data of the execution of these operations across the three decades of our study is lacking we could not include them in the analysis.

FIGURE 14

Relationship between genus-level abundance trends and various traits of those genera. Depicted here are the redistributed t-values of the linear-mixed effect models in which each of the traits was included one at a time. Black bars indicate significant relationships (t-value > 1.96). The direction of the bars (positive or negative) depends on the sign of correlation between the trends and the genus-level scores for each trait category.

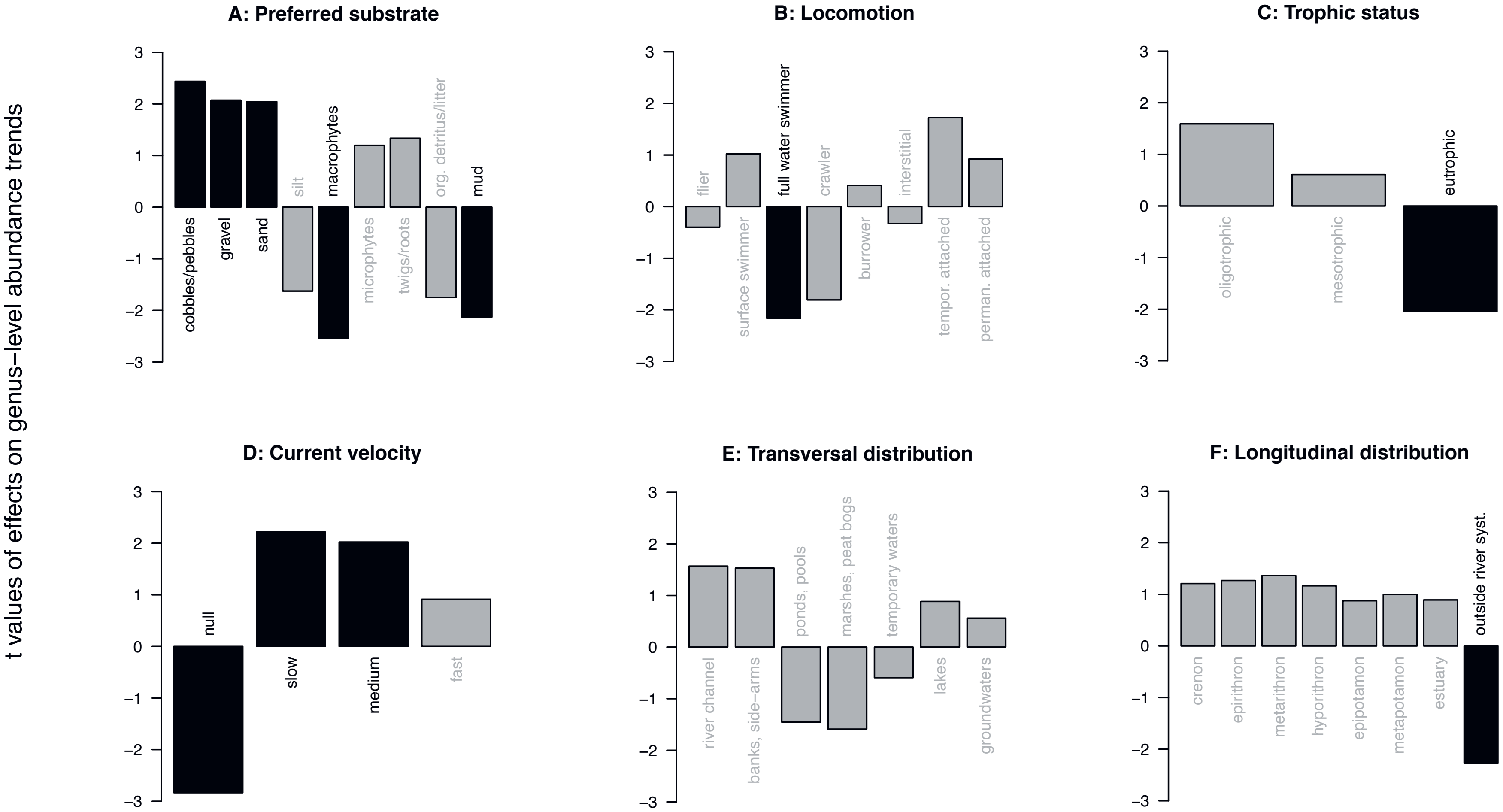
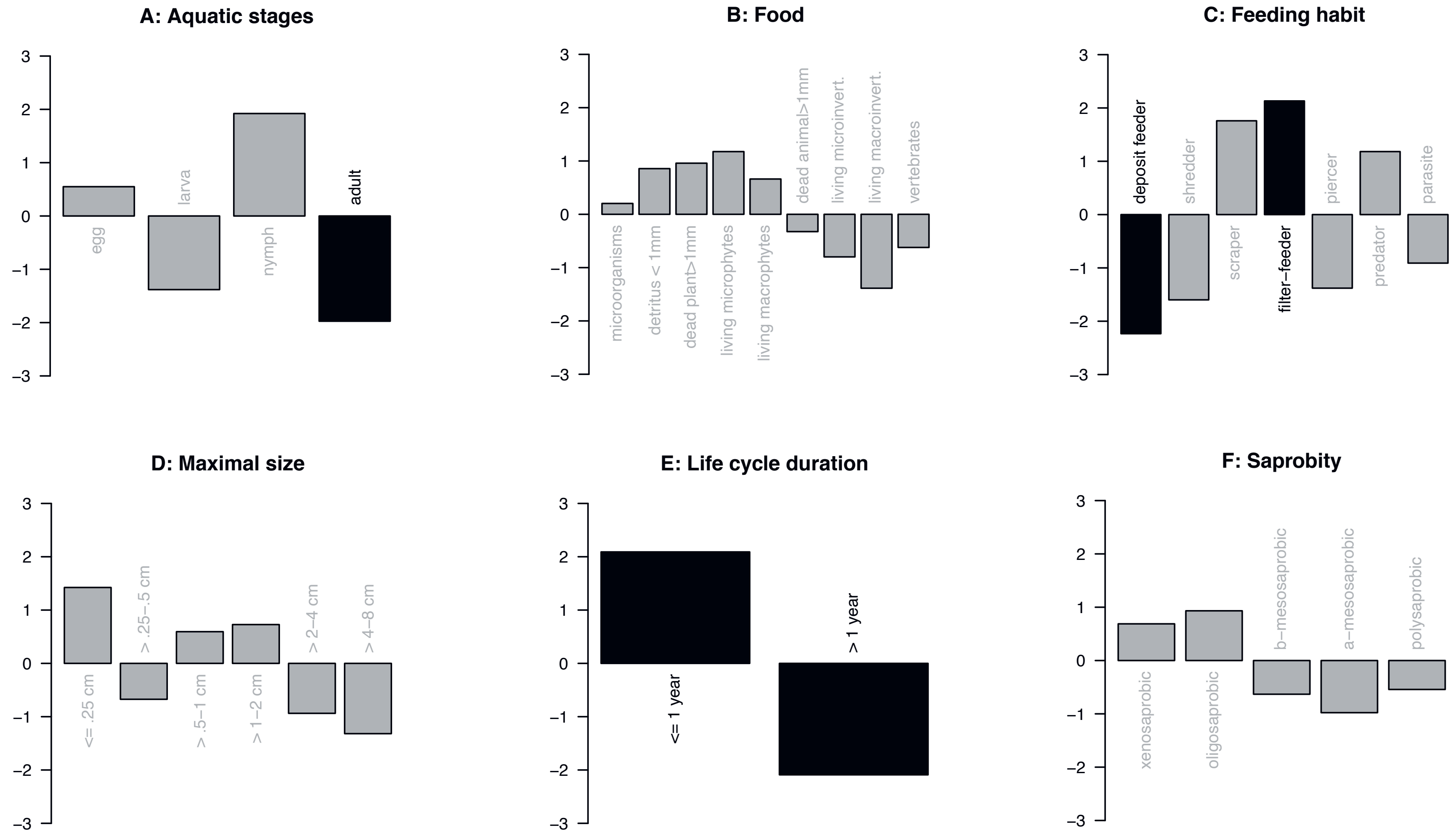


FIGURE 15

Relationship between genus-level abundance trends and various traits of those genera. Depicted here are the redistributed t-values of the linear-mixed effect models in which each of the traits was included one at a time.

Black bars indicate significant relationships (t-value > 1.96). The direction of the bars (positive or negative) depends on the sign of correlation between the trends and the genus-level scores for each trait category.



COMPARISON WITH AQUATIC AND TERRESTRIAL INSECT TRENDS ELSEWHERE

A recent meta-analysis (van Klink *et al.* 2020b) showed that while both rare and common taxa of terrestrial insects are declining in abundance conform earlier reports (Dirzo *et al.* 2014; Hallmann *et al.* 2017; Seibold *et al.* 2019), freshwater insects appeared to be on the rise in recent decades, likely recovering from low numbers related to strong pollution in the second half of the 20th century (Durance and Ormerod 2009). However, the selection of datasets going into this meta-analysis has recently been questioned: both their spatial coverage (Desquilbet *et al.* 2020; van Klink *et al.* 2020a) as their suitability for detecting trends (several monitoring schemes started after a major disturbances) (Jähnig *et al.* 2021; van Klink *et al.* 2021). In our analysis, we did find similar patterns of increase for some aquatic insect groups: Trichoptera and Odonata, for instance, increased over time as water conditions became less eutrophic and polluted, which are indications of improving water quality. The estimated trends for Odonata are consistent with published trends based on entirely independent data (Termaat *et al.* 2015), while for Trichoptera, the only published reference (Hallmann *et al.* 2019) showed opposed (declining) rates. However, the insect orders that contribute most to overall insect abundance (Ephemeroptera, Diptera (especially Chironomidae)) showed strong declines, leading to an overall 53% decline over 27 years. The decline in the total number of individuals of Ephemeroptera might be surprising, as this insect order is generally seen as an indicator of good water quality. Six of the ten Ephemeroptera-genera for which we calculated abundance trends increased in abundance. But the three most abundant Ephemeroptera genera (*Cloeon, Baetis* and *Caenis*), which contained more than 95% of the Ephemeroptera individuals, decreased in abundance. The most abundant species in those genera are more tolerant to poor water quality than other mayfly species. So, in spite of the abundance decline of all aquatic insects combined, richness and other diversity indices such as community evenness showed increases over time. Higher richness and diversity most likely reflect a combination of a) diminishing dominance of genera tolerant to high nutrient loads, and b) recovery of some genera indicative of improved water quality. In that respect our results on aquatic insect trends differ from recent findings from a terrestrial hoverfly community. Hallmann *et al.* (2021) found that while total insect biomass decreased by three quarters, species richness (accumulated over a season) decreased by 23% from 1989 to 2014, and even 82% on a daily basis. Clearly these synchronous declines in biomass, abundance and species richness in terrestrial habitats, contrast strongly with our current findings of decreasing abundance linked to increasing species richness and diversity. The combination of strongly eutrophic and polluted starting conditions and improving water quality during the study have resulted in this more promising development.

LIMNODATA NEERLANDICA: OPPORTUNITIES, LIMITATIONS AND RECOMMENDATIONS

After working with the data from the insect macro-invertebrate samples of the Water Authorities, we have several recommendations for improving existing datasets and future data. But first of all, we have to stress that these datasets constitute a wealth of data, providing a spatially dense and historically highly relevant coverage of how insect communities have developed in recent decades. The combined dataset on the abundance of aquatic insects allows for large-scale assessments of the effects of both environmental threats and conservation measures. At the same time, it is clear that there are important challenges with the current datasets. We have dealt with previously identified inconsistencies in the recorded abundances and metadata (Netten *et al.* 2010; Verdonschot and van Oosten-Siedlecka 2010) by selecting data from a third of the Dutch WAs for which datasets were sufficiently well-organized and accompanied with necessary metadata (Koese and Zeegers 2018). Furthermore, in our analyses we accounted for the ’noise’ caused by methodological differences across WAs, field workers who collected the samples, and the length of the sampled transect.

The eight WAs were selected based on how organized their data already were. It is likely that parts of the data of other WAs can also meet the criteria for trend analysis, requiring, however, that the original data sources are accessed to make the metadata as complete as possible. Information on who collected the sample, how many meters were sampled and in which microhabitats, and how the sample was processed, might still be available in the original forms and note books. In our analyses we accounted for the identity of the main person collecting a particular macrofauna sample in the field. Still, some difficulty arose, e.g. in the WPM data, where the team of field workers changed over the course of only a few years. Therefore, we cannot fully rule out that the deviating trend (WPM is the only WA with a positive trend) represents a true increase in total insect abundance, or partly also changing practices in the field.

The macrofauna monitoring scheme of the Dutch Water Authorities is accompanied by a clear sampling, processing and estimation protocol (Beers *et al.* 2014). Over the selected time period (1990-2018) and for the selected parts of the datasets of the eight WAs that were included in this study, the methods were consistent enough to analyse trends in individual abundance, species richness and diversity. Still, we believe that future analyses would highly benefit from further standardization of all aspects of sampling aquatic macro-invertebrates, processing the samples, and recording information about both the process (metadata) and the taxon-specific counts. To improve future analyses we therefore like to present to following recommendations:

- Continue to train field workers to collect macrofauna samples according to the protocol in a nationally coordinated course.
- Field workers should minutely document who took the sample, how many meters were sampled in total, how many meters in which microhabitat, concurrent water level, development stage of the vegetation, and other relevant aspects of the sampling conditions.
- Ideally water quality samples are collected at the same location and at the same time as the macro-invertebrate sample.
- While it might be important to retain flexibility with respect to new sampling locations, the importance of repeat-samples of aquatic insects on exactly the same location must also be stressed.
- Information about macroinvertebrate sample processing in the field should also be recorded.
- Key aspects of water and shore vegetation management should be recorded for the location and time of the macroinvertebrate samples. Ideally data on mowing frequencies and dates, dredging, etc. are recorded systematically for all (stretches of) water bodies. Pending such information system, management metadata should be recorded alongside each macroinvertebrate sample.
- In the lab, metadata about the processing of the samples is required as well: who (individual, not just a company name) processed the sample, how much time was spend on processing the sample and whether that restricted the identification process.
- As not only the level and field of expertise differs between people processing the samples, but also between the resources available to them to identify taxa, it would be useful to record which identification keys were available.
- If not all individuals in a sample are taxonomically identified and/or not all individuals are counted, the estimation and extrapolation process should be described in detail, allowing full reconstruction. Actually counted and additionally extrapolated numbers should be recorded separately.
- Database structures for organizing and collating the macrofauna data should not be hampering the documentation of metadata (e.g., due to restrictive data entry portals), but rather enable and steer proper documentation of all relevant aspects of the collection and processing of the macrofauna samples.
- It is also advisable to consider conserving and storing the actual samples in a standardised and coordinated way. That would allow checks of potential errors, revisiting samples in the future when new taxonomic insights could lead to different identifications, or future research on specific taxa or communities. A central, well-organized and accessible database of where these samples are is then a requirement, of course.

In addition to these recommendations for improving the quality and usefulness (value) of the macro-invertebrate sampling scheme of the WAs, we also want to briefly discuss alternatives. For instance, taxonomic sorting based on metabarcoding (Beentjes *et al.* 2019), environmental DNA (eDNA) (Goldberg *et al.* 2016; Valentini *et al.* 2016; van Bochove *et al.* 2020) or automated photography and image recognition (Hogeweg *et al.* 2019; Høye *et al.* 2021) might be suggested to

FIGURE 16

Relationship between genus-level abundance trends and various traits of those genera. Depicted here are the redistributed t-values of the linear-mixed effect models in which each of the traits was included one at a time. None of the trait categories had a significant t-value (< 1.96). The direction of the bars (positive or negative) depends on the sign of correlation between the trends and the genus-level scores for each trait category.

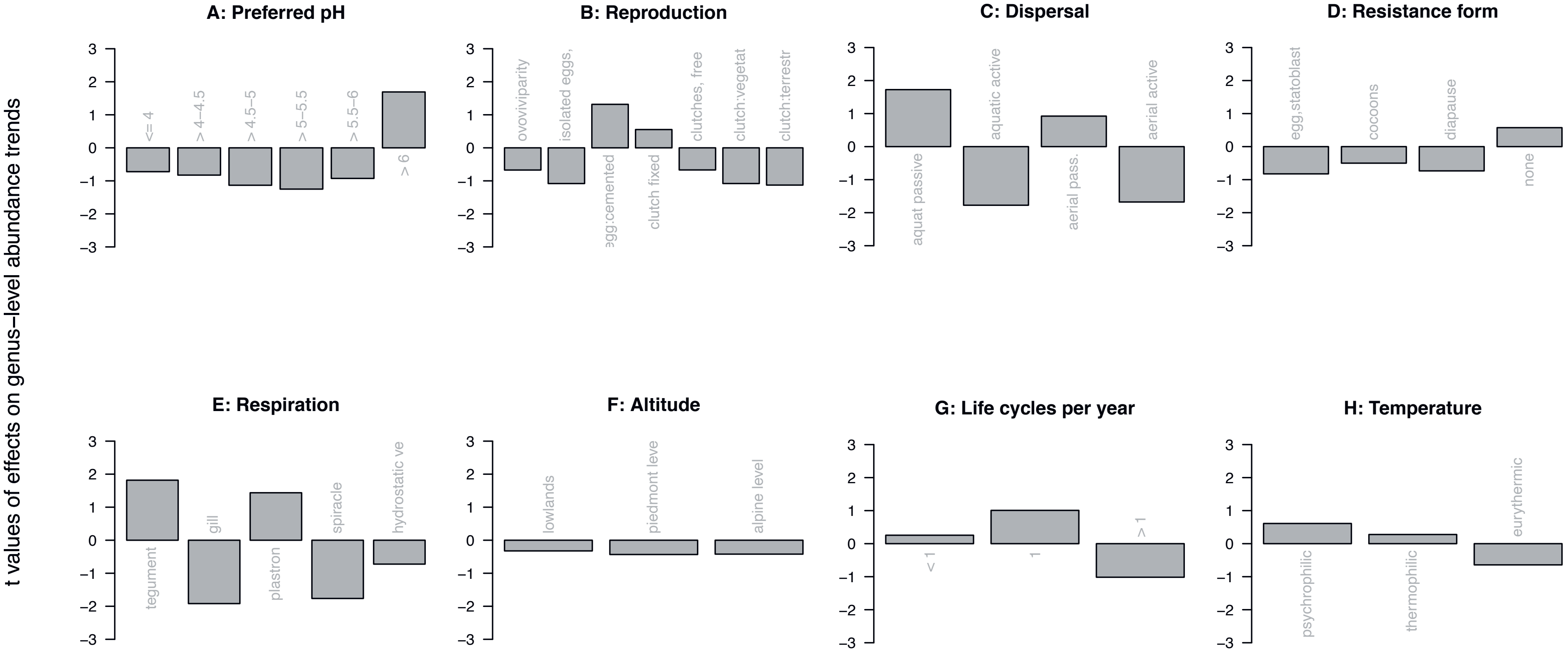
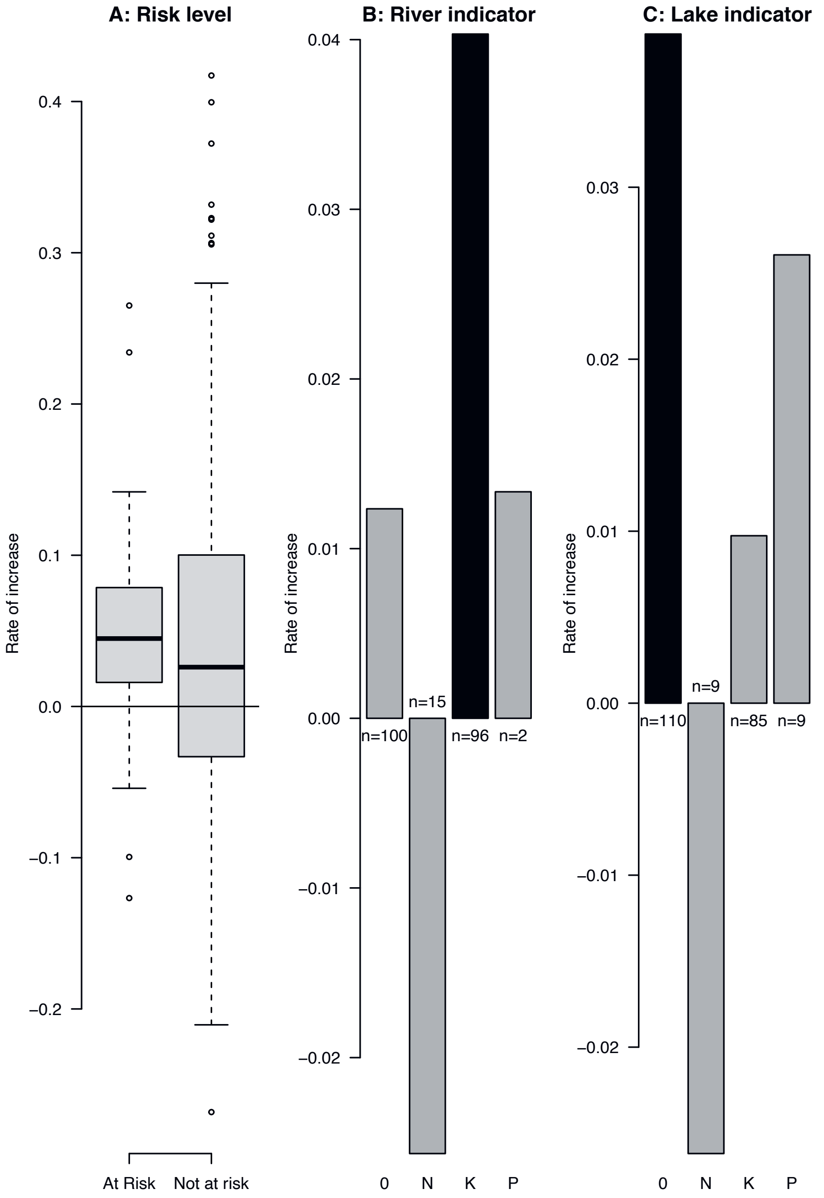


FIGURE 17

The abundance trend (rate of increase; ln-lambda) of genera as a function of certain traits: A. whether or not the species in a genus are at risk for the effects of micropollutants (n=26 and 175 genera, respectively), and indicator classes for rivers (B) and lakes (C). The indicator classes are: N (species in a genus are mainly negative indicators of typical river or lake systems), K (species in a genus are mainly characteristic taxa), P (mainly positive indicators when dominant), and 0 (genera with no indicator species or with a mixed bag of N, K and P). The black bars in B and C signal significant effects compared to the 0 class.



augment or even replace the current methodology of collecting aquatic insects from water bodies in the near future. While the technological possibilities are growing rapidly, the key aspect to evaluate these on is whether they can produce the same data as the current methods: counts per separate taxa. Translating metabarcoding data and eDNA samples to the number of individuals present is not (yet) straightforward (Beentjes *et al.* 2018). Even when new methods would produce reliable estimates of densities, very thorough, habitat-specific calibration would be needed to relate estimates of new and existing methods. As the value of the macroinvertebrate data of the WAs grows with the years in which the same protocol is followed, it is advisable to be very careful about changes in methodology, and when in doubt, to stick with the existing method with standard macrofauna-nets.

TOPICS FOR FUTURE RESEARCH

In this report we focus on the abundance and diversity trends of all aquatic insects found in the macro-invertebrate samples of the Dutch Water Authorities. For the abundance analyses we mainly focused on higher taxonomic levels in order to include all insects found. Already when analysing trends and trait-trend relationships at the genus level where we forced to leave out some of the counted individuals, because they were identified at a higher taxonomic level only, or because no reliable trends could be estimated for the genera due to lack of data. The diversity indices were calculated at the genus level. Estimating trends and trait-trend relationships at the species levels would have meant an even further subsetting of the data, and fell outside the scope of the research documented in this report.

There are good evidence- and hypothesis-driven reasons, however, to want to perform trait-trend analyses for subsets of genera or species in follow-up studies. For instance, one could imagine that whether or not the decline of genera is related to nutrient reductions is related to traits such as feeding on algae. Or that insects hunting by sight have different abundance trends than those that can rely on touch and do not require clear water. This kind of analysis, where trends are related to explanatory variables and traits can provide a lot of insight in the causality of the patterns found in this study.

Particular research questions and hypotheses which could be addressed in follow-up analyses include, amongst many others, and in no particular order:

- What are the relative contributions of terrestrial and aquatic environmental factors to long-term trends of various taxa?
- How much improvement in measures (e.g. species richness, evenness, Shannon index) of aquatic insect diversity is achievable, given assessments of natural/pristine aquatic communities?
- Is insect diversity higher in water bodies managed as nature reserves compared to other water bodies?
- Do wet nature reserves have a positive effect on insect diversity in surrounding, regular water bodies?
- Do algivores decline in those sites where nutrients have reduced and where there is not much forest cover?
- Are the negative trends in Simuliidae linked to positive trends of their predators (e.g. hydropsychid caddisflies)?
- Can it be shown that short-lived taxa respond stronger to the reduced nutrient concentrations than long-lived taxa? This would indicate that short-lived taxa no longer profit from a larger food base, or in the case of oxygen instead of nutrients: indicating that long-lived taxa are profiting from higher oxygen levels. Another hypothesis is that short-lived species can recover more quickly from disturbances like strong drops in oxygen availability and vegetation management, which are more frequent in nutrient-rich conditions.
- Are water quality improvements (nutrients, oxygen, pesticides), similar for isolated, standing water sites and connected, running water sites?
- One interesting comparison here could be to compare trends in running and standing waters: do we also see a decline of full-water swimmers in lake sites? Or is the pattern seen here driven by improvements in river habitats making them less suitable for lake specialists?
- Analyse the effects of greenhouses on abundances of species at risk, and compare them with the effect size of greenhouses for abundances of species not at risk?

- Are there interactive effects between oxygen parameters and temperature?
- How much of the increase in diversity is due to the found increase in species richness, and how much due to the increased evenness? One thing which may be informative is to calculate the expected shannon index if all individuals would be equally distributed across the species found. The difference between this max index and the observed index indicates the effect of skewed distribution and likely diminishes over time, indicating that as time progresses, diversity increases more and more because of species additions rather than increases in evenness.
- Can the observation of concurring decreases in nutrient concentrations and shift from shorter-lived to more longer-lived genera be explained solely by direct effects of food availability, or also through indirect effects of predators? It could well be that longer-lived predators are more sensitive to episodic conditions of poor water quality. Analysing patterns per feeding guild could perhaps shed light on this.
- Do species differ in their response to pesticides, and can those differences be related to traits determining how much they are in direct contact with these substances? Moths have a waxy cuticle and may therefore have a layer of air surrounding their body making them less prone to suffer effects of pesticides associated with greenhouses. One other group which are not in direct contact with water are true bugs (Hemiptera) that dwell at the water surface (e.g., Gerridae and Velidae). Beetles and heteropterans may be less prone to effects of pesticides but this only applies to the adult stage with sclerotized body parts; the situation is different for their juvenile stages (but not for the surface-dwelling true bugs, making this an interesting subgroup to look into).
- Ephemeroptera are particularly sensitive to salt, so although as a group we see a decline in abundance, individual genera may show positive trends, especially where salinity decreased?

CONCLUSIONS

We conclude that water quality improvements since 1990 (e.g. diminished nutrient concentrations and pesticide toxicity) in the Netherlands have positively affected insect-macrofauna communities so far. The most abundant groups, that were tolerant to high-nutrient conditions, have declined, while some indicator groups of healthy communities have increased in abundance. Trends in insect groups are, however, not fully explained by changing nutrient concentrations and toxicity of pesticides, but are explained to a considerable extent by unknown factors, not included in our analyses.

It is encouraging to see that the efforts of the WAs and other actors to improve water quality have had clear positive effects on the richness and diversity of water insects. However, given the abysmal water quality in the 1970s and 1980s, the conditions at the start of our study period should not be used as a baseline. Thus, the improvements observed reflect recoveries from a severely impoverished state regarding water quality. We therefore emphasize the need for taking further steps to assure the conservation and recovery of freshwater biodiversity. Follow-up research will have to show in which direction the composition of insect communities is developing and how WAs can steer that development in the desired direction. Our results therefore emphasize the importance of continuing to take conservation measures to maintain and restore aquatic biodiversity, and to standardize the sampling of insect macrofauna even more.

ACKNOWLEDGEMENTS

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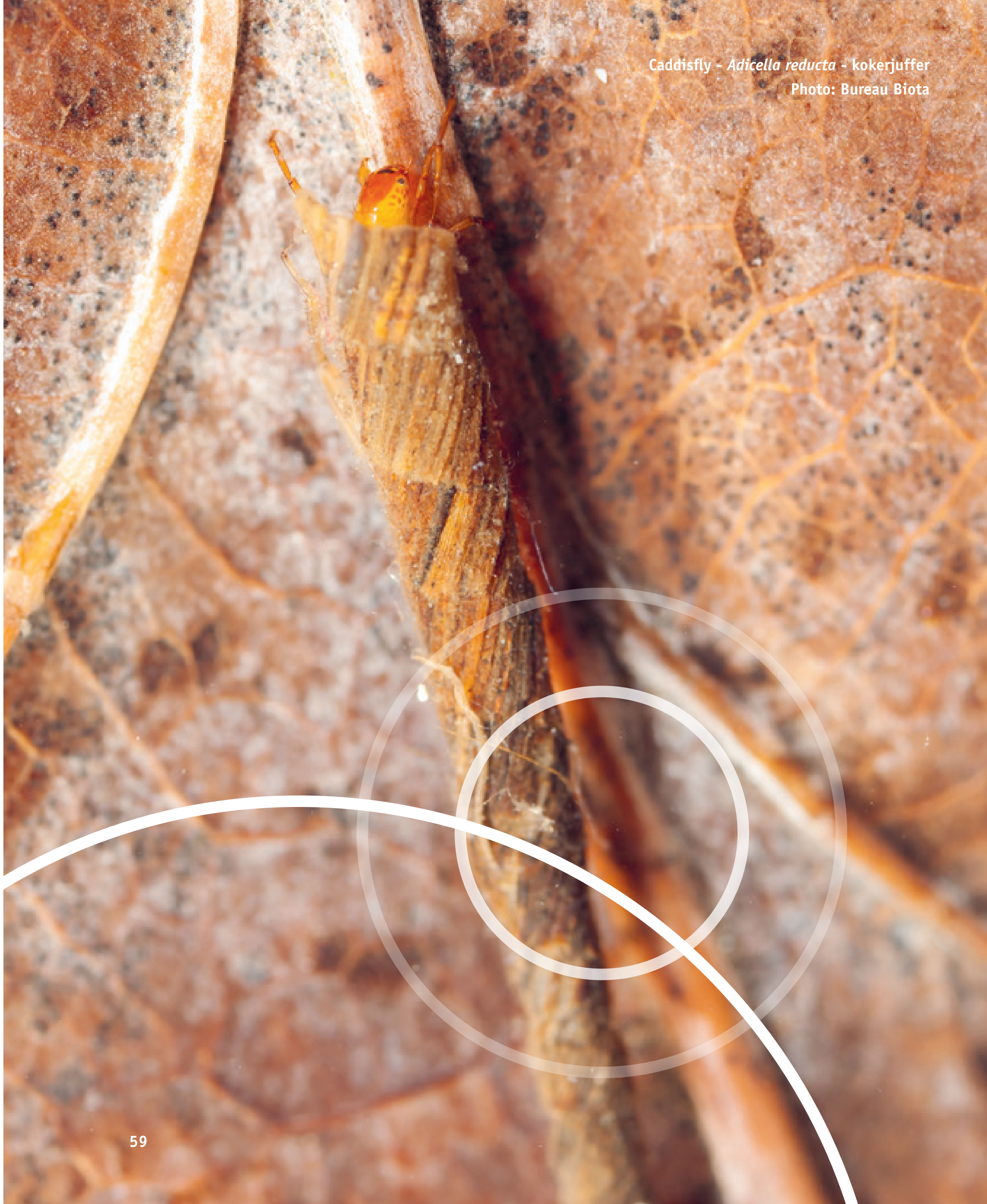

Caddisfly - *Adicella reducta* - kokerjuffer
Photo: Bureau Biota

Blue emperor - *Anax imperator* - grote keizerlibel
Photo: Wil Leurs

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Photo: Michel de Beer

SUPPLEMENTARY INFORMATION

APPENDIX A:

INTERPOLATION OF WEATHER DATA

We interpolated weather data from up to 49 weather stations to macrofauna monitoring locations for each particular sampling day and location. We considered temperature, precipitations and evapotranspiration. From the interpolated temperature variables we also extracted the average weekly and average monthly temperature prior to each sampling event, the growing degree-days (GDD: at 5°C). We decomposed each weather variable in a mean annual smooth trend, a mean smooth spatial trend, and a mean seasonal trend (see Supplementary Figure S2), using Generalized Additive Models assuming Gaussian errors. Using the residuals of the models, we derived the daily mean anomaly over all weather stations, and subtracted it from the model residuals, i.e. leaving only between-station daily spatial variation. From these residuals we calculated average daily-semivariance (γ_s) for spatial lags up to 300 km, and modelled these using an exponential variogram model:

$$\gamma_s = S \times e^{-\alpha * D} \tag{S.1}$$

where S is the model sill and α the steepness at which the semivariance reaches the sill over spatial lag D (See Supplementary Figure S3).

To interpolate to macrofauna locations (z), for a particular day (t), we predicted the means using each GAM-model ($\mu_{z,t}$), added the daily average residuals ($\hat{r}_t$), and finally interpolated the spatial residuals (r_z):

$$\hat{y}_{z,t} = \mu_{z,t} + \hat{r}_t + \mathbf{C_1 C_0^{-1} r_z} \tag{S.2}$$

where $\mathbf{C_0}$ is the covariance matrix between weather stations, and $\mathbf{C_1}$ the covariance matrix between weather and macrofauna locations. Both of these were obtained by

$$Cov(z_i, z_j) = S - S \times e^{-\alpha * D_{i,j}} \tag{S.3}$$

where $D_{i,j}$ is the pairwise distance between two locations z_i and z_j.

APPENDIX B:

SPATIO-TEMPORAL ESTIMATION AND INTERPOLATION OF CHEMICAL WATER PROPERTIES

Each of the Water Authorities (WAs) have collected measurements of chemical and physical water characteristics, covering 22 substances between 1990 and 2018 (see table 3). Approximately two thirds of the macrofauna monitoring locations overlap spatially with water quality measurement locations, but the two monitoring schemes rarely coincide in time. As such, we rely on modelling of the chemical and physical characteristics of water bodies, and deriving statistical predictions of water quality at the macrofauna locations and time of sampling. We used generalized additive models with Gaussian errors, where the (log of the) measured concentrations of each compound are modelled as a function of for example temporal (daily and yearly splines), spatial (nonlinear x- and y-coordinates, as well as a random effect for catchment area; see equation 5 in main text). An important aspect in the measurements of physical/chemical properties of the water, are the detection limits. Detection limits prohibit straightforward modelling, as they pose a left-truncated variable problem (e.g. below a given detection limit are essentially). Data that are above the limit provide robust information, while water-concentrations below the limit are left-censored: any positive value below detection limit is theoretically possible, but is essentially unobserved. Detection limits have been lowered by improved analytics over time, in all WAs and for most substances measured regularly. Ignoring the changing detection limits over time likely results in biased trends in space and time of the measured substances/parameters. Therefore, we used GAMs with a Tobit error (distribution), followed by spatio-temporal interpolation of model residuals to arrive at predictions for the macrofauna samples. Tobit regression (Tobin 1958) allows to deal with the problem of (variable) detection limits in a straightforward way, and recent developments (Fang 2017) have allowed to include this type of error distributions within the framework of generalized additive models (Wood 2017). Below, we explain how we interpolated residuals of the tobit-GAMs.

Let $\mathbf{z}(x, y, t)$ be the vector of observation of a given compound, indexed by space and time. L*et al*so $\mu(x, y, t) = 1(\ )$ be the corresponding vector of mean-predictions from equation 5, and let $\mu'(x', y', t')$ be the mean-fitted values at new locations ($\mathbf{z}'(x', y', t')$). To derive at full predictions (means + interpolated residuals) at new locations, we make use of predicted values μ' as well as model residuals $\mathbf{r} = \mathbf{z} - \mu$ (indexation dropped for simplicity) using

$$\hat{\mathbf{z}}' = \boldsymbol{\mu} + \mathbf{C}'\mathbf{C}^{-1}\mathbf{r} \tag{S.4}$$

where $\mathbf{C}$ the covariance matrix between measured concentrations $\mathbf{C} = Cov(\mathbf{z}, \mathbf{z})$ and $\mathbf{C}'$ the expected covariance matrix between measurement locations and prediction locations $\mathbf{C}' = Cov(\mathbf{z}, \mathbf{z}')$. Note that model residuals in the presence of censoring are not obvious for observations below detection limits, and as such we assume for the sake of explanation no censoring for now, but deal with this below.

Covariance matrices were specified by low-rank parametric covariance models, so that they can be represented as simple functions of distance in space *d* and time u, i.e. $Cov(z_i, z_j) = Cov(d_{ij}, u_{ij})$, for an arbitrary pair (*i* and *j*) of observations. We assumed a separable covariance structure in space and time (Pebesma and Heuvelink 2016). so that the full spatio-temporal covariance structure can be represented as a product of separate space- and time-covariance structures.

$$\mathbf{C}(\mathbf{d}, \mathbf{u}) = \sigma^2 - \gamma(d, u) \tag{S.5}$$

where σ^2 the estimated residual variance of the tobit-models, and $\gamma(d, u)$ the semivariance, which in turn depends on the semivariances in space ($\gamma_s(d)$) and time ($\gamma_t(u)$):

$$\gamma(d, u) = \sigma^2(\gamma_s(d) + \gamma_t(u) - \gamma_s(d)\gamma_t(u)) \tag{S.6}$$

(Pebesma and Heuvelink 2016). For each substance/parameter, two semvariance models were fitted: one for time (γ_t, using only within monitoring-location residuals), and one for space (γ_s, using only residuals of measurements within taken the same month at different locations). For each of the two components (spatial and temporal), two alternate semivariance functions were considered for modelling semivariance, the exponential:

$$\gamma(d) = v + \beta(1 - e^{-\alpha d}) \tag{S.7}$$

and the spherical

$$\gamma(d) = \begin{cases} v + \beta\left(\frac{3d}{2\alpha} - \frac{d^3}{(2\alpha^3)}\right) & \text{if } d < \alpha \\ v + \beta & \text{if } d \geq \alpha \end{cases} \tag{S.8}$$

, from which we selected the best fitting as judged by RMSE, for each substance and for each of the spatial and temporal dimensions. For an overview of estimated parameters see Table S.3.

Turning to the point of censoring, residuals of concentrations below detection limit are unobserved, just as the actual values of the substance are. Here, we used the expectations of the residuals for below-detection measurements, and the calculated residuals for non-censored values:

$$r_i = \begin{cases} z_i - \mu_i & \text{if } s_i = 0 \\ E[z_i|s_i = 0] - \mu_i & s_i = 1 \end{cases} \tag{S.9}$$

where s_i denotes a binary variable indexing censoring ($s_i = 1$) or not ($s_i = 0$), and the expected residual is given by

$$E[z_i|s_i = 0] = \frac{1}{p}\int_{-\infty}^{d} (z \times g(z)) \mathrm{d}z \tag{S.10}$$

APPENDIX C:

SCALE-DEPENDENT ANALYSIS

For a given response variable $z_{x0,y0}$ at location $\{x_0, y_0\}$, and a given set of covariate values $p_{x,y}$ at points $\{x, y\}$, where $\{x, y\} \in \Re$ we define the effect relationship to be:

$$z_{x_0,y_0} = \beta \sum_{x,y} (w_{x,y} p_{x,y}) \tag{S.11}$$

where β defines the sign and magnitude of the effect on the response, $w_{x,y}$ a spatial weight-vector depending on distance to focal measurement point, and itself. The weights are obtained from a distance-weighting function, which in the present study is defined as a two-dimensional parametric kernel. The kernel is depending on displacement distance ($d = \sqrt{(x-x_0)^2 + (y-y_0)^2}$, $d \geq 0$), a scale (σ) and a shape parameter (γ):

$$w(d; \sigma, \gamma) = Ce^{-\left(\frac{d}{\sigma}\right)^{\gamma})} \tag{S.12}$$

where C is a constant ensuring that $\sum w(d, \sigma, \gamma) = 1$. Parameter σ can be interpreted as the average distance to the measurement point over which the weighted covariate values are summed (see Figure S4a). Parameter γ defines the shape of the declining function. For $\gamma = 1$, the shape is a exponential function, for $\gamma = 2$ a half-gaussian, and further with $\gamma \to \infty$ result in a uniform distribution (Figure S4b).

SUPPLEMENTARY TABLES

TABLE S.1

Taxonomic groups used in classifying insect macrofauna by the Dutch Water Authorities. Of some groups only larval stages are found when sampling water bodies.

Code	Taxonomic group	Dutch names	English names
IDCHI	Insecta/Diptera - Chironomidae	dansmuggen	nonbiting midges
IDSIM	Insecta/Diptera - Simuliidae	kriebelmuggen	black flies
IDREM	Insecta/Diptera - remaining	overige vliegen	remaining flies
INCOL	Insecta - Coleoptera	kevers	beetles
INEPH	Insecta - Ephemeroptera	haften	mayflies
INHET	Insecta - Heteroptera	wantsen	true/typical bugs
INLEP	Insecta - Lepidoptera	motten	moths
INODO	Insecta - Odonata	libellen	dragonflies/damselflies
INTRI	Insecta - Trichoptera	schietmotten/kokerjuffers	caddisflies
INREM	Insecta - remaining	overige insecten	remaining insects

TABLE S.2

Categorization of water types as used in this study in relation to the original watercategorization of the Water Framework Directive (WFD) as well as the Dutch Topographic map (TOP10NL). Numbers denote number of locations.

Original variable	Source	Ditch	Slow running water	Fast running water	Canals	Ponds	Small shallow lakes	Large lakes	Brackish water	Ditch non-WFD	Waterway 3-6m non-WFD	Waterway 6-12m non-WFD	River non-WFD non-WFD	Small lakes non-WFD
M01	Water framework directive	136												
M02		2												
M03					161									
M06					74									
M07					4									
M08		22												
M10					28									
M11							25							
M12						57								
M13						18								
M14								3						
M20								14						
M25							9							
M26						8								
M30									32					
M31									7					
R01			3											
R02			10											
R03			29											
R04			229											
R05			270											
R06			77											
R07			1											
R08			3											
R11			4											
R13				26										
R14				15										
R15				5										
R17				50										
R18				15										
Ditch	Top 10									8				
Waterway <3m										115				
Waterway 3-6m											58			
Waterway 6-12m												128		
Waterway 12-50m													45	
Waterway >50m													2	
Small lake														14

TABLE S.3

Estimates of spatial and temporal semivariance parameters for each substance/variable, and per dimension of interpolation.

	Serial			Spatial		
Variable	model	parameter	Estimate(se)	model	parameter	Estimate(se)
NH_4	Exp	nugget	0.38(0.018)	Sph	nugget	0.943(0.016)
	Exp	part. sill	0.373(0.016)	Sph	part. sill	0.068(0.016)
	Exp	range	41.09(3.517)	Sph	range	10.817(1.364)
BOD	Exp	nugget	0.18(0.008)	Exp	nugget	0.282(0.003)
	Exp	part. sill	0.134(0.007)	Exp	part. sill	0.096(0.003)
	Exp	range	53.909(6.725)	Exp	range	4.727(0.224)
Cd	Exp	nugget	0.286(0.014)	Sph	nugget	0.319(0.016)
	Exp	part. sill	0.17(0.014)	Sph	part. sill	0.111(0.016)
	Exp	range	88.179(25.174)	Sph	range	18.404(1.904)
Ca	Exp	nugget	0.006(0.001)	Sph	nugget	0.108(0.007)
	Exp	part. sill	0.022(0.001)	Sph	part. sill	0.011(0.006)
	Exp	range	47.513(7.059)	Sph	range	22.997(29.071)
Cl	Sph	nugget	0.064(0.001)	Sph	nugget	0.213(0.004)
	Sph	part. sill	0.051(0.001)	Sph	part. sill	0.039(0.005)
	Sph	range	173.752(7.81)	Sph	range	19.659(4.372)
Chloro. alpha	Exp	nugget	0.417(0.07)	Sph	nugget	1.245(0.014)
	Exp	part. sill	0.846(0.067)	Sph	part. sill	0.229(0.116)
	Exp	range	38.607(6.544)	Sph	range	37.344(25.581)
Cr	Exp	nugget	0.218(0.021)	Sph	nugget	0.239(0.016)
	Exp	part. sill	0.131(0.018)	Sph	part. sill	0.078(0.016)
	Exp	range	52.933(17.081)	Sph	range	14.568(1.718)
Combustion	Exp	nugget	0.005(0.002)	Exp	nugget	0.002(0.002)
	Exp	part. sill	0.001(0.002)	Exp	part. sill	0.004(0.002)
	Exp	range	1.024(5.861)	Exp	range	2.092(2.004)
PO4	Exp	nugget	0.237(0.016)	Sph	nugget	0.845(0.01)
	Exp	part. sill	0.382(0.015)	Sph	part. sill	0.068(0.008)
	Exp	range	58.184(6.256)	Sph	range	22.624(5.724)
Sum P	Sph	nugget	0.161(0.003)	Sph	nugget	0.399(0.005)
	Sph	part. sill	0.127(0.003)	Sph	part. sill	0.055(0.043)
	Sph	range	160.503(6.582)	Sph	range	39.031(40.267)
Industrial	Sph	nugget	0.002(0.001)	Exp	nugget	0.006(0)
	Sph	part. sill	0.001(0.001)	Exp	part. sill	0.002(0.001)
	Sph	range	3.13(5.208)	Exp	range	10.127(10.965)
Cu	Exp	nugget	0.19(0.008)	Sph	nugget	0.249(0.006)
	Exp	part. sill	0.098(0.007)	Sph	part. sill	0.055(0.006)
	Exp	range	70.375(15.339)	Sph	range	20.648(2.87)
Pb	Sph	nugget	0.431(0.016)	Sph	nugget	0.421(0.014)
	Sph	part. sill	0.105(0.02)	Sph	part. sill	0.111(0.013)
	Sph	range	226.584(81.492)	Sph	range	19.741(2.063)
Mg	Exp	nugget	0.007(0.001)	Exp	nugget	0.057(0.007)
	Exp	part. sill	0.014(0.001)	Exp	part. sill	0.003(0.007)
	Exp	range	57.394(9.104)	Exp	range	2.292(6.215)
Na	Sph	nugget	0.022(0.002)	Sph	nugget	0.05(0.042)
	Sph	part. sill	0.042(0.002)	Sph	part. sill	0.059(0.042)
	Sph	range	152.756(10.061)	Sph	range	0.855(0.702)
Ni	Exp	nugget	0.034(0.01)	Sph	nugget	0.159(0.011)
	Exp	part. sill	0.118(0.01)	Sph	part. sill	0.032(0.011)
	Exp	range	30.038(5.341)	Sph	range	14.411(2.842)
Suspended matter	Exp	nugget	0.276(0.011)	Sph	nugget	0.466(0.008)
	Exp	part. sill	0.251(0.01)	Sph	part. sill	0.105(0.007)
	Exp	range	62.38(6.909)	Sph	range	21.297(2.159)
Pesticide	Sph	nugget	0.004(0)	Sph	nugget	0(0.013)
	Sph	part. sill	0(0.001)	Sph	part. sill	0.008(0.013)
	Sph	range	3.877(12.422)	Sph	range	0.791(1.289)
NO_2+NO_3	Exp	nugget	0.005(0.005)	Sph	nugget	1.179(0.366)
	Exp	part. sill	0.894(0.022)	Sph	part. sill	0.347(0.366)
	Exp	range	29.867(2.583)	Sph	range	2.71(1.399)
Sum N	Exp	nugget	0.04(0.006)	Sph	nugget	0.248(0.003)
	Exp	part. sill	0.143(0.005)	Sph	part. sill	0.025(0.003)
	Exp	range	49.578(5.073)	Sph	range	18.285(1.983)
Water temperature	Exp	nugget	1.555(0.147)	Exp	nugget	1.184(0.041)
	Exp	part. sill	1.038(0.134)	Exp	part. sill	1.05(0.037)
	Exp	range	55.063(18.327)	Exp	range	4.786(0.314)
HCO_3	Sph	nugget	0.019(0.002)	Sph	nugget	0.176(0.014)
	Sph	part. sill	0.034(0.002)	Sph	part. sill	0.047(0.014)
	Sph	range	143.066(13.151)	Sph	range	17.534(5.234)
Zn	Exp	nugget	0.144(0.013)	Sph	nugget	0.332(0.012)
	Exp	part. sill	0.211(0.012)	Sph	part. sill	0.04(0.012)
	Exp	range	59.648(9.746)	Sph	range	16.31(3.424)
pH	Sph	nugget	0.06(0.003)	Sph	nugget	0.131(0.003)
	Sph	part. sill	0.037(0.003)	Sph	part. sill	0.027(0.003)
	Sph	range	102.509(9.997)	Sph	range	24.402(5.31)
O	Exp	nugget	0.037(0.004)	Exp	nugget	0.098(0.011)
	Exp	part. sill	0.055(0.003)	Exp	part. sill	0.053(0.01)
	Exp	range	30.254(3.578)	Exp	range	1.171(0.258)

TABLE S.4

Estimated scale parameters for a range of land use categories used in weighting land cover in taxonomic group models. For each group and parameter, the fitted kernel (γ =1: exponential, γ = 2: gaussian) is given, along with estimated scale (or radius) parameter σ (in meters), and the sign of the effect of the weighted covariate on the response.

Group	Variable	γ	σ	Effect sign
INODO	Buildings	1	800	-1
INODO	Greenhouses	2	145	-1
INODO	Agricultural land	2	190	-1
INODO	Forest	2	763	1
INODO	Open wet natural area	1	433	1
INODO	Open dry natural area	1	87	1
INODO	Crop field	1	800	-1
INODO	Grassland	1	800	1
INREM	Buildings	1	196	-1
INREM	Greenhouses	2	44	-1
INREM	Agricultural land	2	230	-1
INREM	Forest	1	113	1
INREM	Open wet natural area	2	508	1
INREM	Open dry natural area	1	800	1
INREM	Crop field	1	800	-1
INREM	Grassland	2	800	1
INTRI	Buildings	1	675	-1
INTRI	Greenhouses	1	70	-1
INTRI	Agricultural land	1	800	-1
INTRI	Forest	1	225	1
INTRI	Open wet natural area	2	26	-1
INTRI	Open dry natural area	2	142	-1
INTRI	Crop field	1	800	-1
INTRI	Grassland	2	260	-1
IDCHI	Buildings	2	220	1
IDCHI	Greenhouses	2	202	-1
IDCHI	Agricultural land	2	557	-1
IDCHI	Forest	2	22	-1
IDCHI	Open wet natural area	2	113	1
IDCHI	Open dry natural area	2	252	-1
IDCHI	Crop field	1	800	1
IDCHI	Grassland	2	800	1
IDREM	Buildings	1	800	-1
IDREM	Greenhouses	2	219	-1
IDREM	Agricultural land	2	67	-1
IDREM	Forest	2	510	1
IDREM	Open wet natural area	1	60	1
IDREM	Open dry natural area	2	46	1
IDREM	Crop field	2	294	-1
IDREM	Grassland	1	800	1
IDSIM	Buildings	1	13	1
IDSIM	Greenhouses	2	371	-1
IDSIM	Agricultural land	1	800	-1
IDSIM	Forest	1	800	-1
IDSIM	Open wet natural area	2	135	-1
IDSIM	Open dry natural area	2	319	-1
IDSIM	Crop field	1	800	-1
IDSIM	Grassland	2	234	-1
INCOL	Buildings	1	219	-1
INCOL	Greenhouses	1	332	-1
INCOL	Agricultural land	1	148	1
INCOL	Forest	1	800	1
INCOL	Open wet natural area	2	585	1
INCOL	Open dry natural area	1	17	1
INCOL	Crop field	2	107	1
INCOL	Grassland	2	399	1
INEPH	Buildings	2	47	1
INEPH	Greenhouses	1	92	-1
INEPH	Agricultural land	2	30	1
INEPH	Forest	2	10	-1
INEPH	Open wet natural area	2	182	-1
INEPH	Open dry natural area	2	164	-1
INEPH	Crop field	1	39	-1
INEPH	Grassland	2	314	-1
INHET	Buildings	1	176	-1
INHET	Greenhouses	2	202	-1
INHET	Agricultural land	1	800	1
INHET	Forest	1	800	1
INHET	Open wet natural area	1	217	1
INHET	Open dry natural area	1	10	1
INHET	Crop field	1	800	1
INHET	Grassland	2	169	1
INLEP	Buildings	2	216	-1
INLEP	Greenhouses	2	126	1
INLEP	Agricultural land	1	63	1
INLEP	Forest	1	14	-1
INLEP	Open wet natural area	2	308	-1
INLEP	Open dry natural area	1	162	-1
INLEP	Crop field	1	162	-1
INLEP	Grassland	2	95	-1

TABLE S.5

Coefficients of full models per taxongroup.

	Odonata	Insects rem.	Trichoptera	Chironomidae	Diptera rem.
Intercept	**-1.22(0.15)**	**-2.91(0.26)**	**0.48(0.17)**	**3.12(0.11)**	**-0.73(0.14)**
Ditch	**1.14(0.14)**	**1.11(0.23)**	0.11(0.14)	**0.4(0.09)**	**0.94(0.13)**
Slow running water	**1.14(0.13)**	0.44(0.22)	**0.34(0.14)**	**0.37(0.09)**	**0.74(0.12)**
Fast running water	-0.32(0.16)	-0.2(0.26)	**0.52(0.17)**	**0.54(0.1)**	**0.84(0.15)**
Canals	**1.15(0.12)**	**0.68(0.2)**	**0.27(0.13)**	**0.2(0.08)**	**0.73(0.11)**
Ponds	**2.25(0.22)**	**-2.35(0.35)**	**-0.52(0.23)**	-0.28(0.14)	**1.23(0.2)**
Small shallow lakes	**2.2(0.2)**	0.46(0.31)	0.06(0.2)	**0.35(0.13)**	**1.41(0.18)**
Large lakes	0.37(0.23)	-0.27(0.35)	**-0.98(0.23)**	**0.32(0.15)**	**-0.49(0.22)**
Ditch non-WFD	**0.69(0.13)**	**0.51(0.21)**	-0.17(0.13)	**0.19(0.08)**	**1.13(0.12)**
Waterway 3-6m non-WFD	**0.94(0.14)**	**0.81(0.22)**	**0.71(0.14)**	0.02(0.09)	**1.01(0.13)**
Waterway 6-12m non-WFD	**1.22(0.12)**	**0.66(0.2)**	**0.26(0.13)**	**0.17(0.08)**	**0.98(0.11)**
River non-WFD	**0.89(0.14)**	0.38(0.23)	**0.53(0.14)**	0.08(0.09)	**0.87(0.13)**
Small lakes non-WFD	**0.51(0.21)**	**-1.17(0.46)**	0.22(0.22)	0.16(0.14)	**2.31(0.18)**
Prox. sewage plant	-0.01(0.02)	-0.05(0.04)	**-0.08(0.03)**	**0.05(0.02)**	**-0.12(0.03)**
Protected	0.04(0.04)	0.03(0.06)	**0.13(0.04)**	**-0.18(0.02)**	**-0.17(0.03)**
Built-up	**-0.38(0.15)**	**0.69(0.24)**	**-0.69(0.16)**	0.08(0.07)	-0.11(0.12)
Wet-natural	0.1(0.46)	**1.98(0.66)**	-0.22(0.18)	**0.93(0.15)**	**0.91(0.21)**
Dry-natural	0.22(0.29)	**-3.2(0.72)**	**-0.8(0.27)**	0.4(0.2)	0.12(0.18)
Forest	**0.44(0.17)**	**3.33(0.21)**	**0.53(0.15)**	-0.03(0.05)	**0.3(0.13)**
Arable	-0.23(0.14)	**-1.2(0.22)**	**-0.62(0.16)**	-0.04(0.1)	**-0.26(0.1)**
Rem. agric.	0.06(0.09)	**1.69(0.18)**	0.07(0.13)	-0.13(0.07)	**0.15(0.06)**
Greenhouses	**-1.31(0.23)**	**-2.85(0.77)**	**-3(0.26)**	-0.21(0.14)	**-0.84(0.2)**
Clay	**-0.52(0.17)**	**0.49(0.23)**	**0.4(0.15)**	0.01(0.1)	**0.89(0.12)**
Light clay	**0.37(0.08)**	-0.15(0.13)	**0.32(0.08)**	**0.2(0.05)**	**0.22(0.07)**
Light loam	**0.69(0.08)**	-0.02(0.12)	**0.4(0.08)**	**0.3(0.05)**	**0.63(0.07)**
Muddy on sand	**0.79(0.12)**	0.15(0.19)	**0.74(0.13)**	**0.3(0.08)**	**0.45(0.11)**
Bog	**0.59(0.1)**	**0.62(0.15)**	**0.68(0.1)**	**0.13(0.06)**	**0.3(0.09)**
Sand	**0.52(0.08)**	-0.06(0.12)	**0.31(0.08)**	**0.15(0.05)**	**0.4(0.07)**
Heavy clay	**0.48(0.1)**	**0.66(0.15)**	**0.47(0.1)**	**0.31(0.06)**	**0.38(0.09)**
Heavy loam	**0.52(0.07)**	0.21(0.12)	**0.51(0.07)**	**0.29(0.05)**	**0.38(0.07)**
PC1	**0.24(0.02)**	-0.04(0.04)	**-0.11(0.02)**	**0.05(0.02)**	0.02(0.02)
PC2	**0.08(0.02)**	**0.44(0.03)**	**0.26(0.02)**	-0.01(0.01)	**0.11(0.02)**
PC3	**0.05(0.02)**	0.07(0.04)	-0.04(0.02)	**0.1(0.02)**	-0.03(0.02)
PC4	**0.18(0.05)**	**0.2(0.07)**	0.03(0.05)	**0.07(0.03)**	**0.2(0.04)**
PC5	0.01(0.04)	**0.29(0.06)**	**-0.08(0.04)**	-0.02(0.02)	-0.02(0.03)
PC6	**-0.16(0.04)**	**-0.12(0.06)**	**-0.25(0.04)**	**0.1(0.03)**	**-0.11(0.04)**
PC7	-0.01(0.04)	**0.15(0.06)**	**0.2(0.04)**	0.02(0.03)	-0.03(0.04)
PC8	0.07(0.05)	**0.18(0.07)**	**-0.15(0.05)**	**0.12(0.03)**	**0.17(0.04)**
PC9	**0.07(0.03)**	**0.13(0.04)**	**-0.08(0.03)**	**0.05(0.02)**	**0.06(0.02)**
PC10	0(0.03)	-0.06(0.05)	**0.08(0.03)**	-0.01(0.02)	**0.06(0.03)**
PC11	**-0.1(0.03)**	-0.08(0.05)	**-0.18(0.03)**	**0.07(0.02)**	-0.01(0.03)
PC12	**-0.14(0.03)**	0.09(0.05)	0.03(0.03)	**0.07(0.02)**	0.03(0.03)
PC13	0(0.02)	**-0.12(0.03)**	**-0.05(0.02)**	**0.04(0.01)**	0.01(0.02)
PC14	0(0.04)	**0.15(0.06)**	**0.16(0.04)**	**-0.11(0.03)**	**0.11(0.04)**
PC15	**-0.16(0.03)**	**0.23(0.05)**	**-0.27(0.03)**	0.02(0.02)	-0.05(0.03)
PC16	**-0.18(0.04)**	-0.06(0.05)	**0.08(0.04)**	**-0.07(0.02)**	**0.09(0.03)**
PC17	0.06(0.04)	**0.2(0.06)**	**0.11(0.04)**	**-0.06(0.03)**	**0.11(0.04)**
PC18	**-0.08(0.03)**	-0.05(0.05)	**-0.19(0.03)**	-0.01(0.02)	0(0.03)
PC19	-0.02(0.03)	0.05(0.04)	**0.09(0.03)**	**-0.04(0.02)**	-0.04(0.03)
PC20	0.04(0.04)	-0.07(0.06)	**-0.09(0.04)**	0.02(0.03)	**-0.22(0.03)**
PC21	**-0.11(0.04)**	-0.05(0.05)	0.02(0.04)	**-0.15(0.02)**	**-0.21(0.03)**
PC22	0.05(0.04)	**-0.23(0.06)**	-0.04(0.04)	-0.04(0.02)	-0.01(0.03)
PC23	**-0.2(0.04)**	-0.12(0.06)	**-0.34(0.04)**	**0.1(0.03)**	-0.05(0.04)
PC24	**0.16(0.04)**	-0.06(0.06)	**0.23(0.04)**	0.02(0.02)	**-0.07(0.03)**
PC25	0.08(0.05)	**-0.15(0.07)**	-0.04(0.05)	-0.03(0.03)	**-0.14(0.04)**
PC26	0.05(0.04)	**-0.28(0.07)**	0.03(0.04)	-0.03(0.03)	-0.06(0.04)
PC27	**0.16(0.05)**	**0.2(0.08)**	0.08(0.05)	0.04(0.03)	-0.07(0.04)
PC28	-0.06(0.05)	**0.4(0.07)**	**0.5(0.05)**	**-0.15(0.03)**	**0.22(0.04)**
PC29	**0.16(0.05)**	-0.07(0.07)	-0.03(0.05)	-0.02(0.03)	**-0.26(0.04)**
PC30	0.04(0.06)	**0.2(0.09)**	**-0.21(0.06)**	0.01(0.04)	**-0.31(0.05)**
PC31	**0.48(0.06)**	**-0.42(0.09)**	**0.23(0.06)**	**-0.11(0.04)**	**0.16(0.05)**
PC32	**0.15(0.06)**	**-0.35(0.08)**	**-0.15(0.06)**	0.02(0.04)	**-0.24(0.05)**
PC33	-0.02(0.06)	-0.15(0.09)	**-0.12(0.06)**	-0.04(0.04)	**0.32(0.05)**
PC34	-0.08(0.06)	**-0.42(0.09)**	**-0.15(0.06)**	-0.03(0.04)	**0.12(0.05)**
PC35	-0.07(0.07)	0.02(0.1)	**-0.16(0.07)**	-0.02(0.04)	**0.13(0.06)**
PC36	0.1(0.08)	-0.12(0.12)	**-0.4(0.08)**	**-0.17(0.05)**	**-0.32(0.07)**
PC37	**0.47(0.1)**	-0.22(0.15)	0.1(0.1)	**0.25(0.06)**	**-0.18(0.09)**
PC38	-0.16(0.12)	-0.25(0.18)	0.05(0.12)	0.01(0.08)	-0.17(0.1)

	Simuliidae	Coleoptera	Ephemeroptera	Heteroptera	Lepidoptera
Intercept	-90.94(3474765.26)	**-0.36(0.13)**	0.26(0.16)	**0.88(0.14)**	**-6.44(0.36)**
Ditch	86.16(3474765.26)	**1.09(0.11)**	**1.41(0.15)**	**0.95(0.12)**	**2.4(0.34)**
Slow running water	86.3(3474765.26)	**0.9(0.11)**	**1.16(0.15)**	**0.61(0.12)**	**2.18(0.34)**
Fast running water	87.19(3474765.26)	**1.01(0.13)**	**1.25(0.18)**	-0.24(0.15)	0.25(0.46)
Canals	83.74(3474765.26)	**0.53(0.09)**	**0.97(0.13)**	**0.5(0.1)**	**2.02(0.32)**
Ponds	78.11(3474765.26)	**0.36(0.17)**	**1.34(0.25)**	**1.24(0.2)**	**3.08(0.48)**
Small shallow lakes	-8(6370069.75)	**0.98(0.15)**	**1.44(0.22)**	**1.07(0.18)**	**2.44(0.42)**
Large lakes	-2.15(8268554.23)	**-1.68(0.2)**	**-0.64(0.25)**	**-0.86(0.21)**	**1(0.5)**
Ditch non-WFD	88.49(3474765.26)	**0.99(0.1)**	**0.69(0.14)**	**0.35(0.11)**	**2.05(0.33)**
Waterway 3-6m non-WFD	85.01(3474765.26)	**0.91(0.11)**	**1.29(0.15)**	**0.43(0.12)**	**2.35(0.34)**
Waterway 6-12m non-WFD	86.36(3474765.26)	**0.72(0.1)**	**1.33(0.14)**	**0.45(0.11)**	**2.12(0.32)**
River non-WFD	-2.47(4972379.15)	**0.29(0.11)**	**1.13(0.15)**	0.03(0.12)	**1.82(0.34)**
Small lakes non-WFD	-3.07(7916832.49)	-0.15(0.17)	**1.23(0.23)**	**0.68(0.19)**	-0.49(0.61)
Prox. sewage plant	**0.51(0.08)**	-0.01(0.02)	-0.04(0.03)	-0.02(0.02)	-0.06(0.05)
Protected	**0.85(0.11)**	**-0.08(0.03)**	0.07(0.04)	**-0.07(0.03)**	-0.05(0.07)
Built-up	**1.96(0.31)**	**-0.4(0.11)**	-0.03(0.1)	**-0.52(0.12)**	-0.12(0.24)
Wet-natural	**3.06(0.62)**	**1.73(0.35)**	-0.39(0.3)	**1.37(0.35)**	0.4(0.67)
Dry-natural	-0.25(1.29)	**0.73(0.15)**	**-1.88(0.3)**	**0.5(0.16)**	**-2.41(0.79)**
Forest	0.75(0.38)	-0.08(0.14)	**-0.73(0.08)**	**-0.86(0.16)**	0.02(0.18)
Arable	-0.81(0.52)	0.15(0.08)	-0.12(0.11)	**0.69(0.14)**	-0.29(0.23)
Rem. agric.	**-0.92(0.33)**	**0.5(0.08)**	**-0.25(0.08)**	-0.09(0.11)	**0.88(0.2)**
Greenhouses	**-6.55(3.24)**	**-1.02(0.21)**	**-4.12(0.28)**	**-1.94(0.19)**	0.81(0.41)
Clay	-0.41(0.3)	**0.73(0.11)**	**0.48(0.16)**	0.08(0.14)	0.3(0.55)
Light clay	0.15(0.48)	**0.57(0.06)**	**0.53(0.09)**	**0.53(0.07)**	**0.7(0.15)**
Light loam	-0.35(0.23)	**0.52(0.06)**	**0.63(0.08)**	**0.57(0.07)**	**0.98(0.16)**
Muddy on sand	-0.06(0.31)	**0.62(0.1)**	**1.05(0.14)**	**0.71(0.11)**	0.52(0.27)
Bog	-0.5(0.29)	**0.74(0.08)**	**0.42(0.1)**	**0.39(0.09)**	**1.14(0.19)**
Sand	0.21(0.21)	**0.59(0.06)**	**0.65(0.08)**	**0.42(0.07)**	**0.63(0.16)**
Heavy clay	-0.89(0.87)	**0.46(0.08)**	**0.44(0.1)**	**0.55(0.09)**	**0.85(0.17)**
Heavy loam	-0.23(0.26)	**0.57(0.06)**	**0.7(0.08)**	**0.39(0.07)**	**0.6(0.15)**
PC1	**-0.18(0.07)**	**0.07(0.02)**	**0.13(0.03)**	**0.15(0.02)**	**0.2(0.05)**
PC2	**-0.24(0.05)**	**0.21(0.02)**	**0.06(0.02)**	-0.03(0.02)	**0.15(0.04)**
PC3	**-0.15(0.06)**	**0.06(0.02)**	-0.05(0.03)	-0.01(0.02)	-0.04(0.04)
PC4	**-0.37(0.12)**	0.02(0.04)	**0.31(0.05)**	0.03(0.04)	**0.21(0.09)**
PC5	**0.22(0.1)**	**-0.08(0.03)**	**0.18(0.04)**	-0.01(0.03)	0.13(0.07)
PC6	**-0.27(0.1)**	**0.08(0.03)**	**-0.45(0.05)**	**0.14(0.04)**	**-0.18(0.07)**
PC7	**0.25(0.1)**	**-0.11(0.03)**	**0.27(0.04)**	-0.05(0.04)	-0.01(0.08)
PC8	**-0.33(0.11)**	-0.03(0.03)	**0.22(0.05)**	0.03(0.04)	**0.29(0.08)**
PC9	**-0.55(0.07)**	**0.05(0.02)**	**0.14(0.03)**	0.03(0.02)	**0.25(0.05)**
PC10	-0.07(0.08)	**0.14(0.02)**	**-0.11(0.03)**	0.04(0.03)	0.08(0.06)
PC11	-0.08(0.09)	0.01(0.03)	**-0.09(0.04)**	**-0.06(0.03)**	**-0.22(0.06)**
PC12	**-0.31(0.08)**	0.05(0.03)	**-0.21(0.04)**	**-0.15(0.03)**	0.12(0.07)
PC13	**-0.12(0.05)**	-0.02(0.02)	**0.07(0.02)**	0.03(0.02)	-0.03(0.04)
PC14	-0.06(0.12)	**0.23(0.03)**	-0.04(0.05)	**-0.17(0.04)**	**0.18(0.08)**
PC15	-0.18(0.09)	0(0.03)	**-0.13(0.04)**	**0.14(0.03)**	-0.05(0.06)
PC16	0.07(0.09)	0.02(0.03)	0(0.04)	**-0.24(0.03)**	**-0.19(0.07)**
PC17	**0.44(0.11)**	-0.01(0.03)	**0.23(0.05)**	**-0.11(0.04)**	-0.12(0.08)
PC18	**-0.34(0.08)**	-0.01(0.03)	0.07(0.04)	**-0.12(0.03)**	-0.06(0.07)
PC19	-0.15(0.08)	0.02(0.02)	0.05(0.03)	**0.13(0.03)**	**0.18(0.05)**
PC20	**0.38(0.1)**	**-0.17(0.03)**	**-0.16(0.04)**	-0.04(0.04)	**-0.2(0.08)**
PC21	**0.39(0.09)**	**-0.2(0.03)**	**-0.33(0.04)**	-0.01(0.03)	**-0.22(0.07)**
PC22	-0.01(0.1)	**0.12(0.03)**	0.01(0.04)	**0.1(0.03)**	-0.05(0.07)
PC23	**-0.21(0.1)**	-0.01(0.03)	**-0.3(0.05)**	0.02(0.04)	-0.06(0.08)
PC24	**0.6(0.1)**	**-0.21(0.03)**	**0.18(0.04)**	**0.13(0.03)**	0.12(0.07)
PC25	0.02(0.12)	**0.11(0.04)**	**-0.26(0.05)**	**0.11(0.04)**	-0.04(0.09)
PC26	-0.04(0.14)	-0.05(0.03)	0.03(0.05)	**0.15(0.04)**	0.03(0.07)
PC27	-0.24(0.12)	**-0.12(0.04)**	**0.35(0.06)**	**0.24(0.04)**	0.12(0.09)
PC28	**-0.64(0.12)**	**0.16(0.04)**	**0.22(0.05)**	**-0.26(0.04)**	**0.26(0.1)**
PC29	**0.35(0.13)**	-0.03(0.04)	**-0.34(0.05)**	**0.22(0.04)**	**-0.34(0.1)**
PC30	0.01(0.15)	**-0.18(0.04)**	**-0.33(0.06)**	0(0.05)	-0.15(0.11)
PC31	-0.14(0.14)	-0.01(0.05)	0.07(0.07)	**0.46(0.05)**	**0.3(0.13)**
PC32	0.06(0.15)	**-0.17(0.04)**	**-0.16(0.06)**	0.06(0.05)	-0.09(0.11)
PC33	**0.43(0.15)**	**0.15(0.05)**	**-0.23(0.06)**	0.05(0.05)	**-0.41(0.12)**
PC34	**0.3(0.15)**	**-0.1(0.05)**	0.07(0.07)	-0.05(0.05)	0.08(0.12)
PC35	0.12(0.17)	-0.06(0.05)	**0.29(0.07)**	-0.07(0.06)	0.08(0.13)
PC36	**0.53(0.19)**	-0.12(0.06)	**-0.38(0.09)**	**0.2(0.07)**	**-0.37(0.16)**
PC37	-0.05(0.24)	-0.09(0.08)	**0.53(0.11)**	0.16(0.09)	-0.06(0.19)
PC38	0.54(0.29)	0.14(0.09)	0.08(0.13)	-0.1(0.1)	-0.24(0.23)

SUPPLEMENTARY FIGURES

FIGURE S1

Taxonomic identification depth of sampled individuals per year pooled over the eight Water Authorities. The four taxonomic levels that occurred most often are, from bottom to top: species, 'species combi', genus, and family. Most individuals (±65%) are identified to species level or below, followed by genus (17%) and family level (7%).

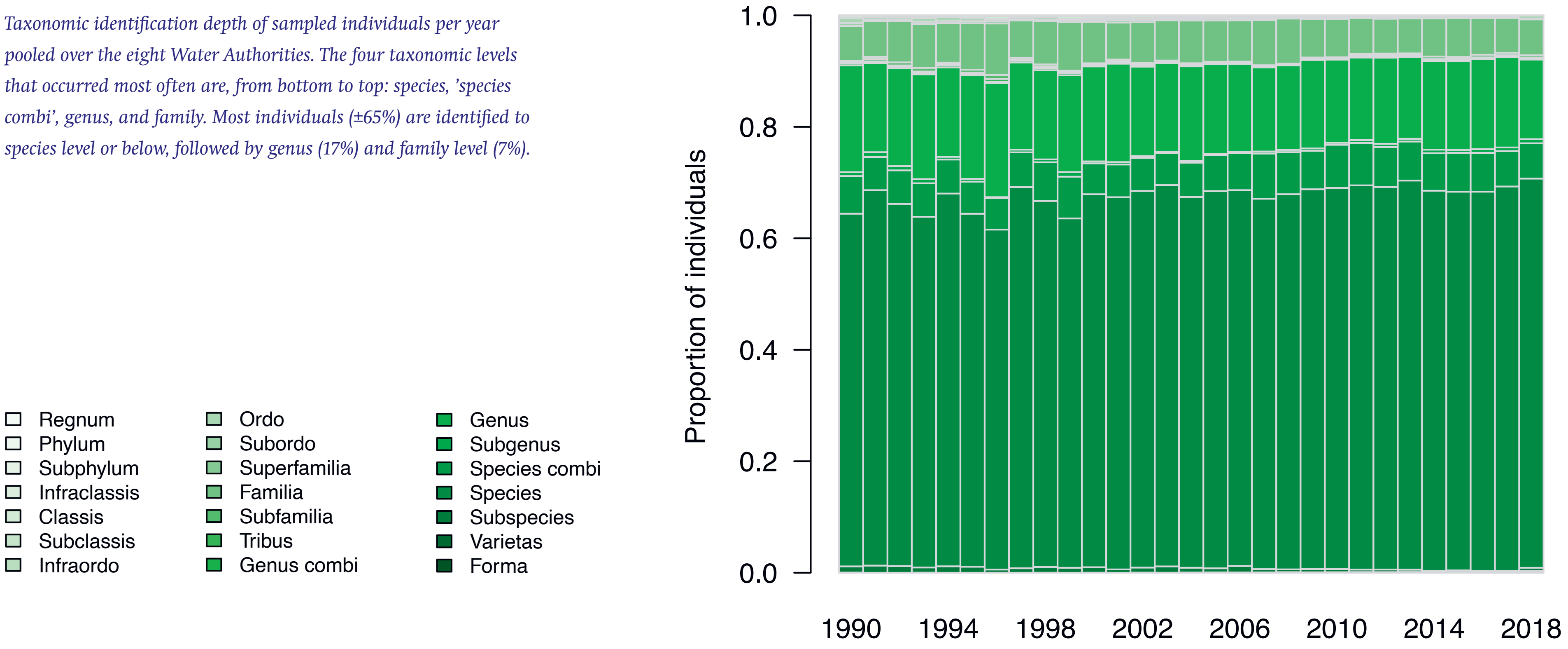

FIGURE S2

Weather decomposition for temperature (a:c), precipitation (d:f) and evapotranspiration (g:i), into a mean spatial, a mean annual, and a mean seasonal trend.

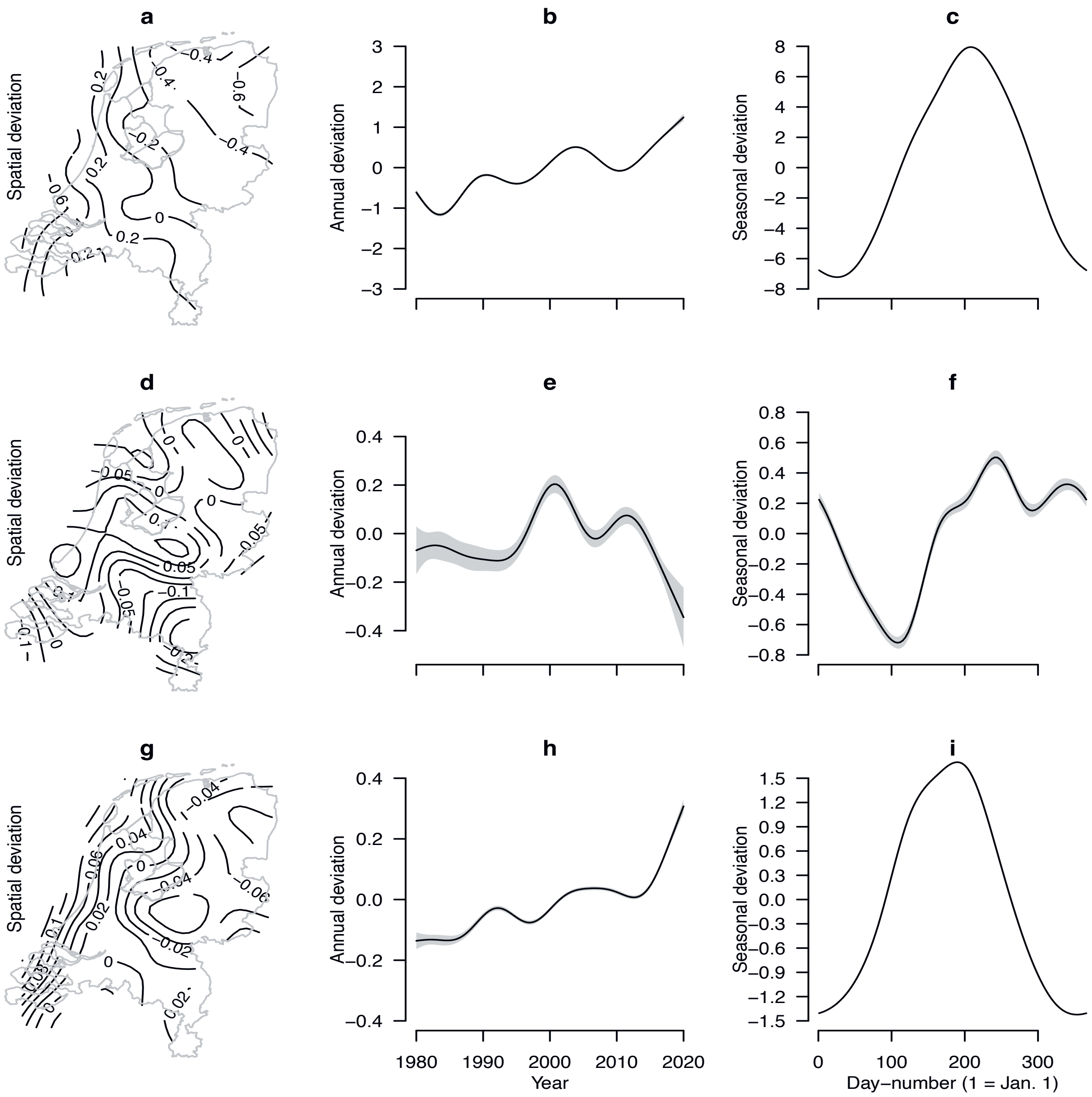

FIGURE S3

Semivariance plots of residual spatial variation in temperature (a), precipitation (b) and evapotranspiration (c).

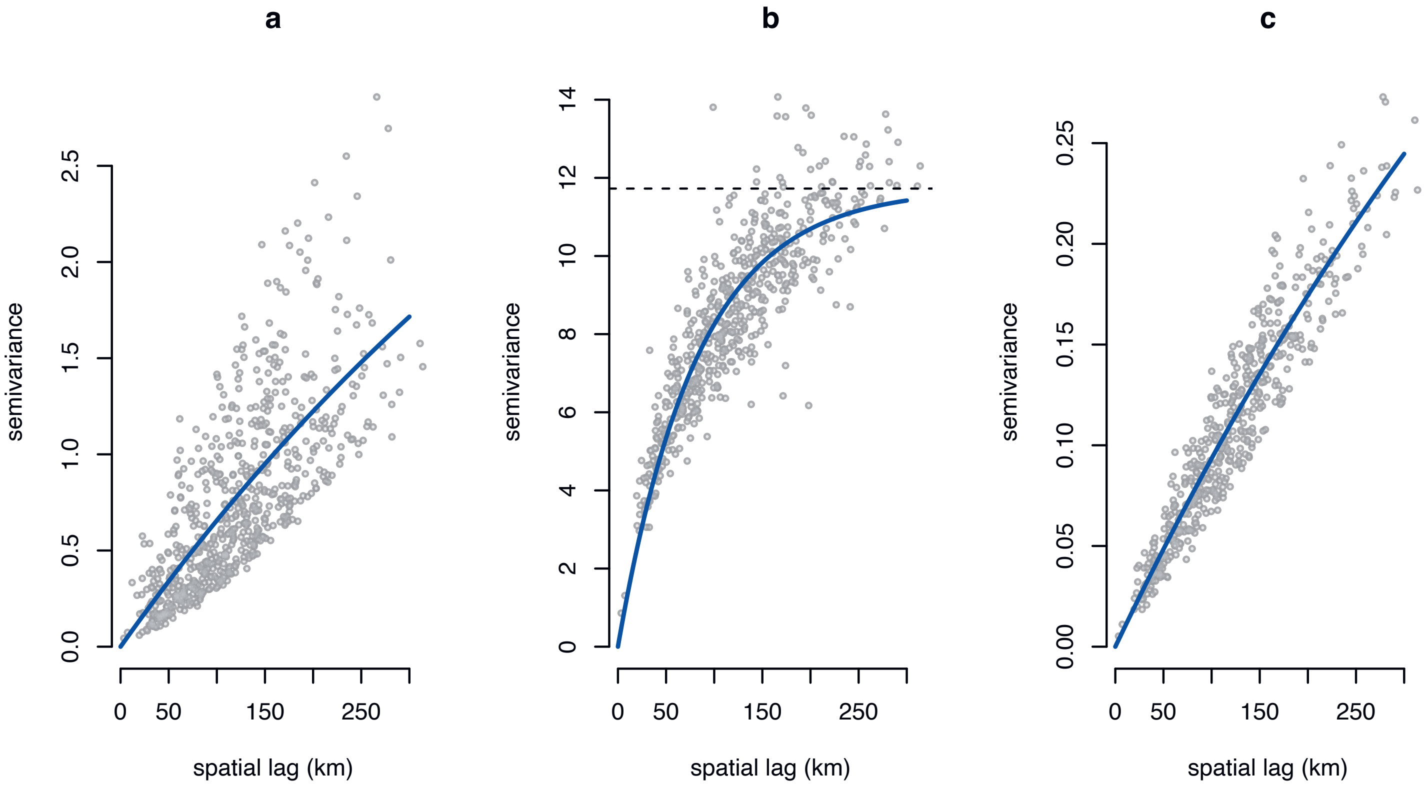

FIGURE S4

Example of weighting functions used in measuring land use covariates at certain distance form monitoring location. **a**: *different values of scale (σ) while keeping γ = 2 and C = 1, and* **b**: *different values of shape (γ) while keeping σ = 200 and C = 1, in equation S.12.*

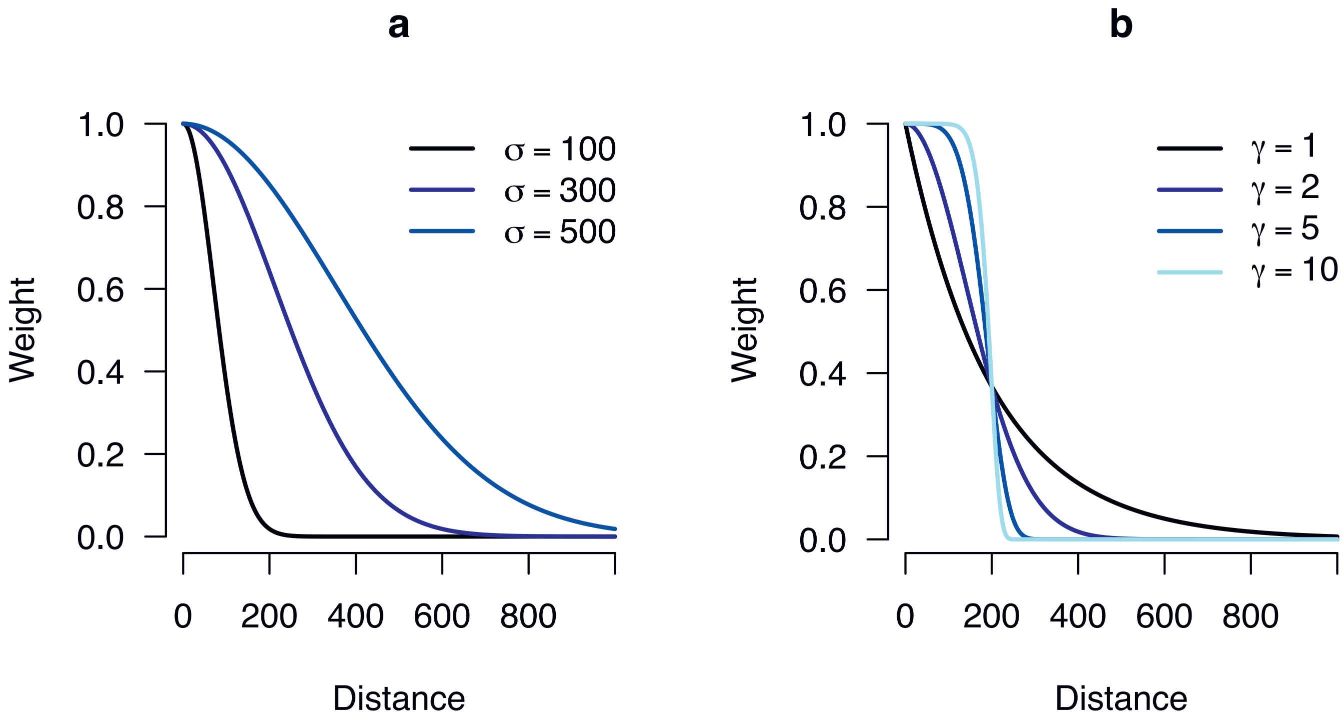

FIGURE S5

Principal component analysis of Explanatory covariates.

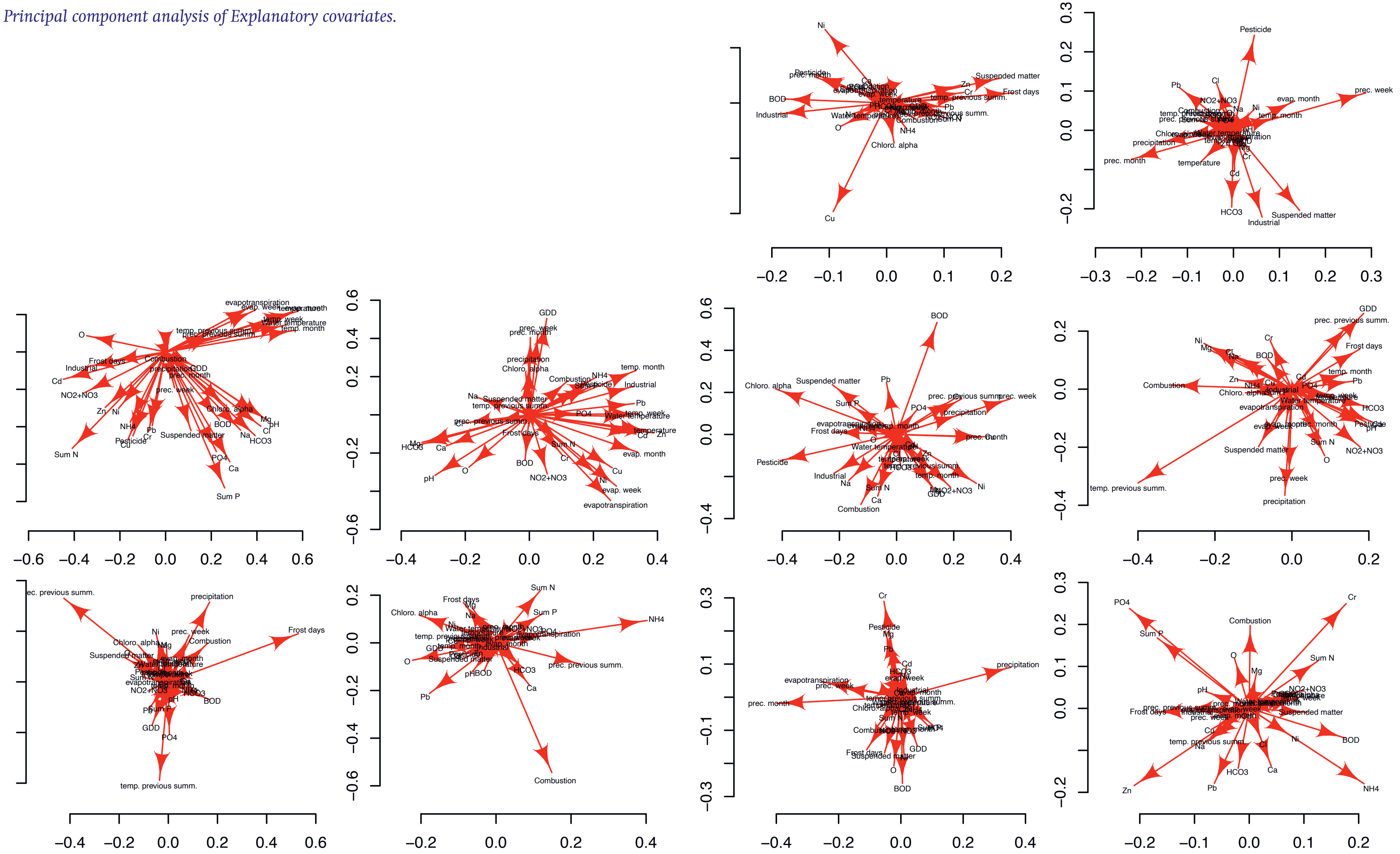

FIGURE S5

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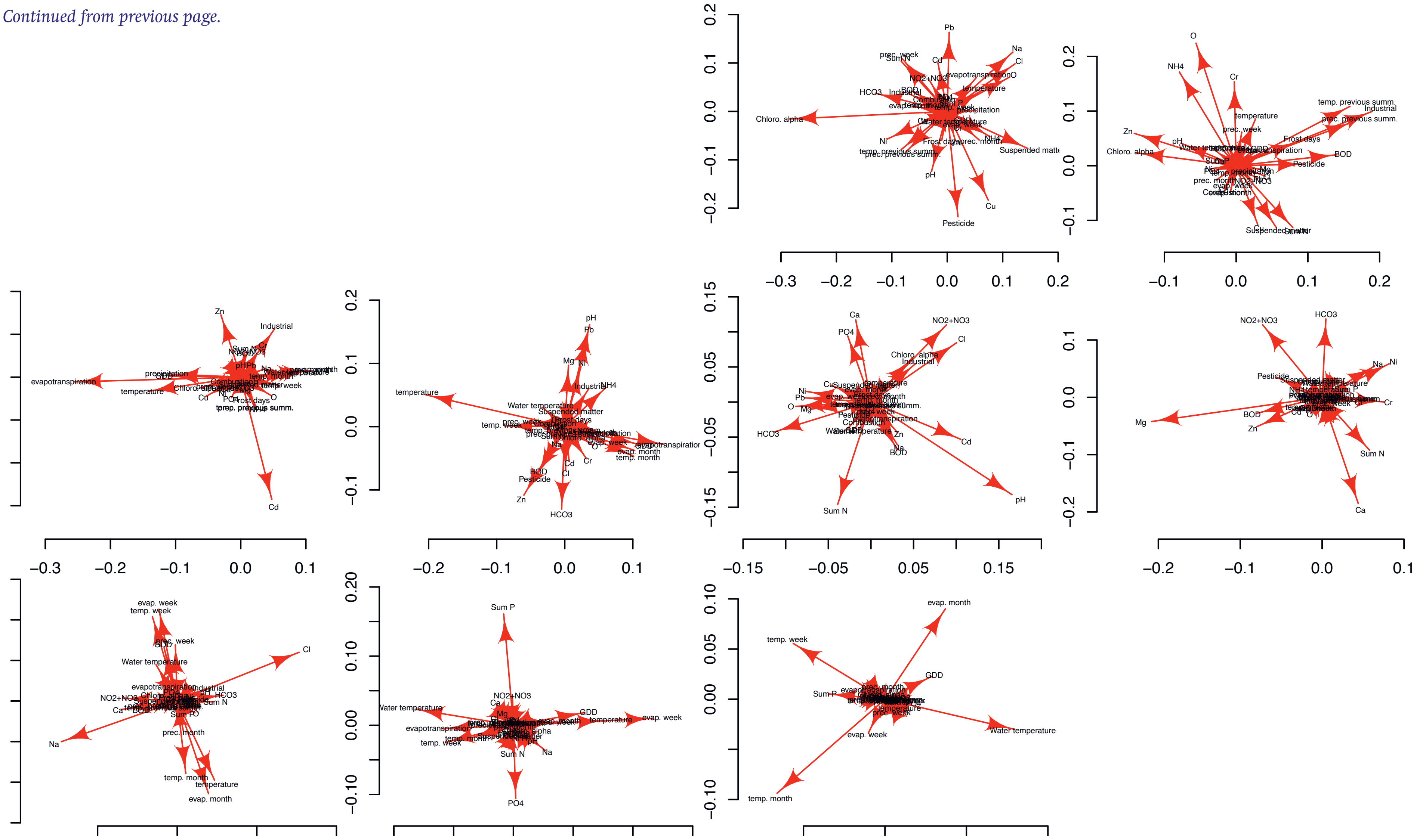

FIGURE S6

Correlation matrix between 38 explanatory variables used in PCA analysis and subsequent modelling. Green sub-boxes denote groups of covariates (Weather, Toxicity and physical/chemical water-properties).

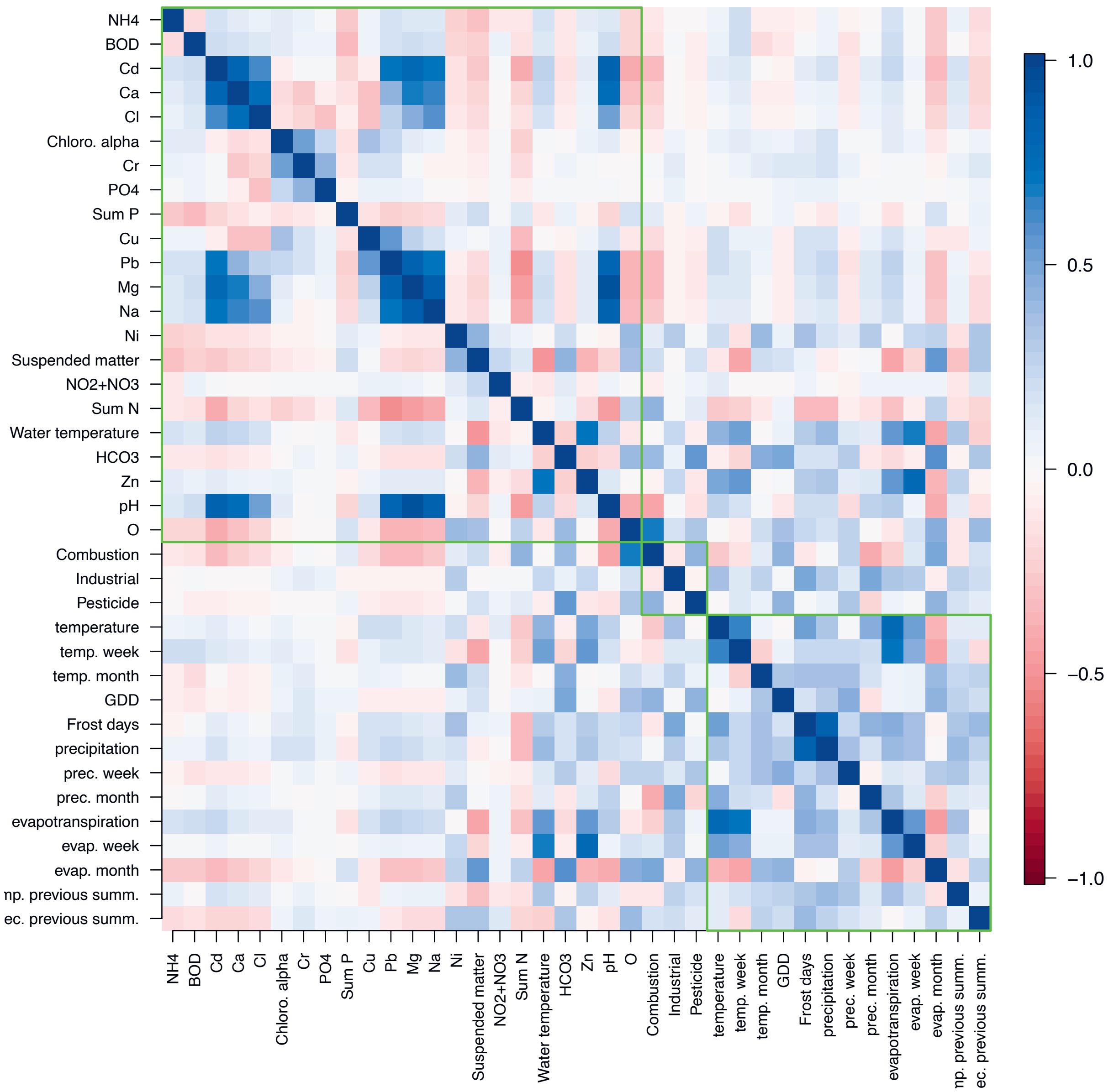

FIGURE S7

Total insect-macrofauna abundance (pooled over all taxonomic groups) per WA.

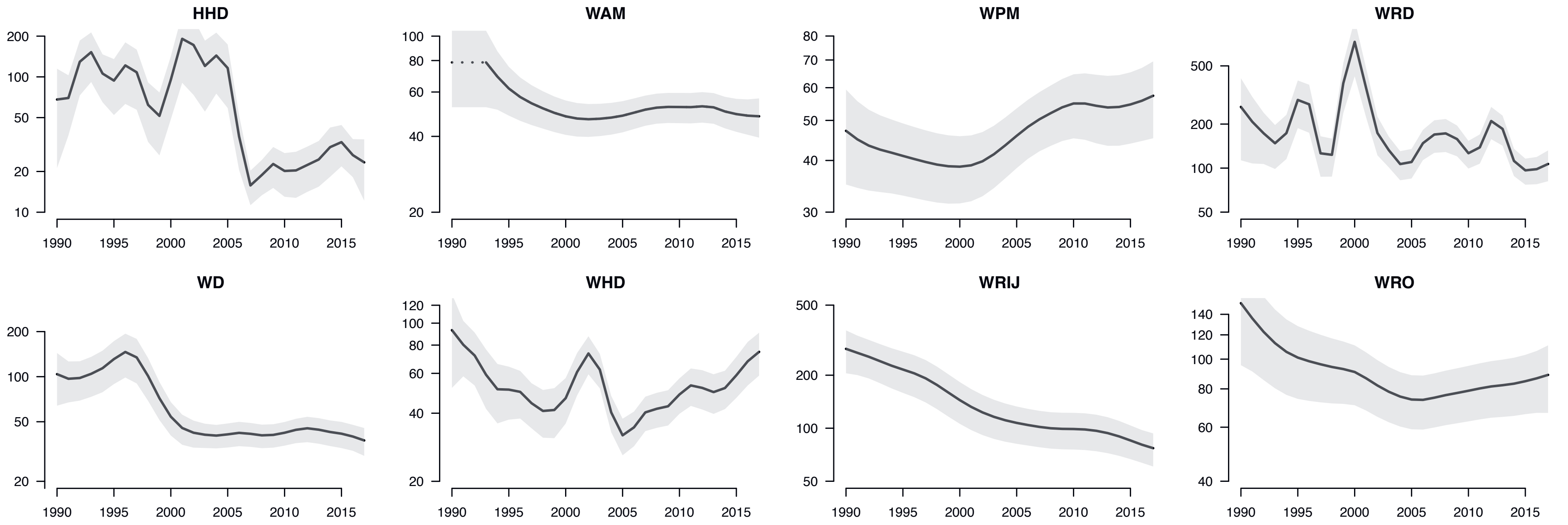

FIGURE S8

Average insect macrofauna trends for each of the taxonomic groups and each of the WAs.

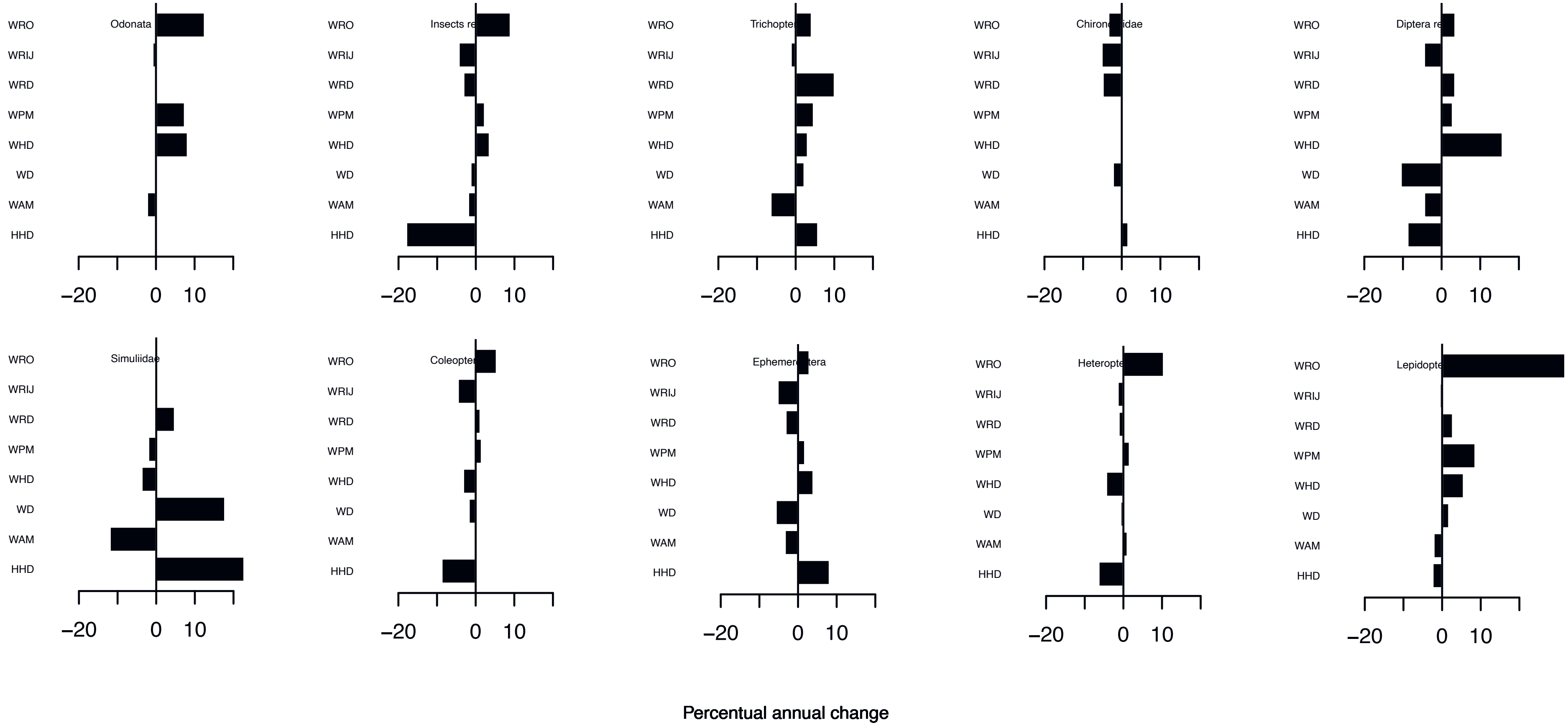

FIGURE S9

Trends among ten insect-macrofauna groups for each of the three WA groups. West (W): HHD & WHD, South (S): WAM, WD, WPM & WRO, East (E): WRD & WRIJ.

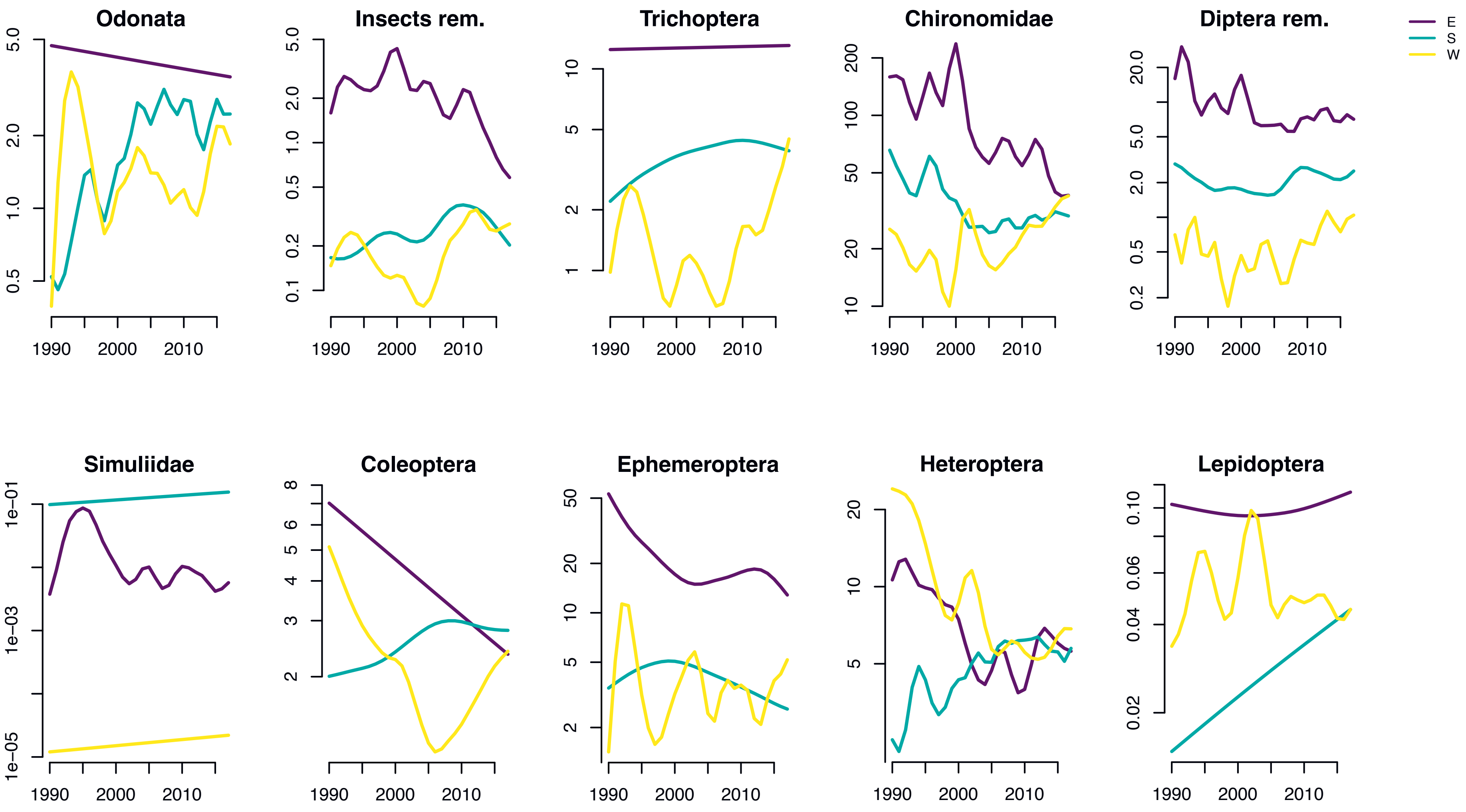

FIGURE S10

Trends in species richness for each of the Water Authorities (WA) in 1990-2017

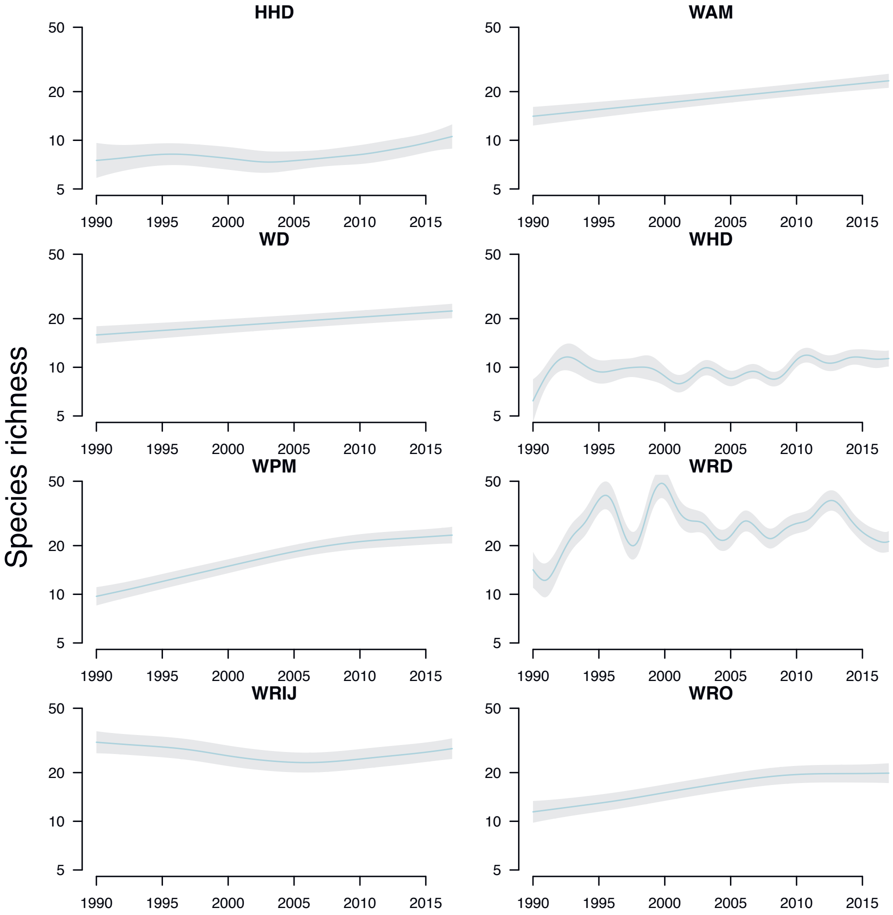

FIGURE S11

Trends in Shannon index of diversity for each of the Water Authorities in 1990-2017

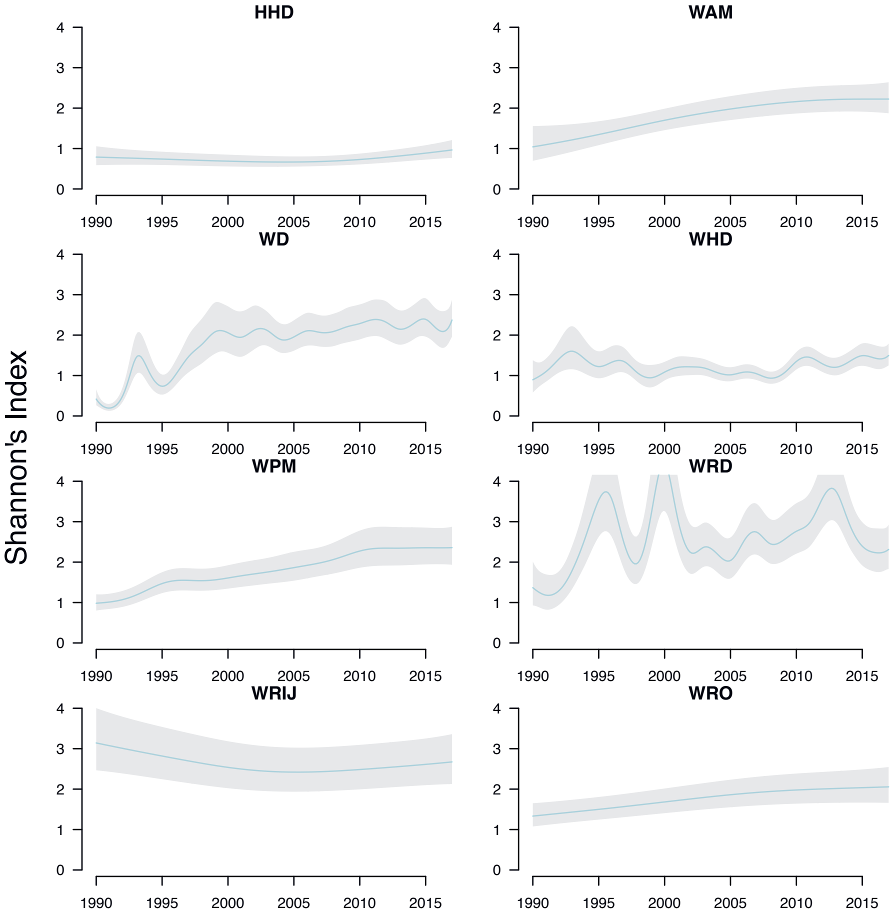

FIGURE S12

Trends in Simpson's index of diversity for each of the Water Authorities in 1990-2017

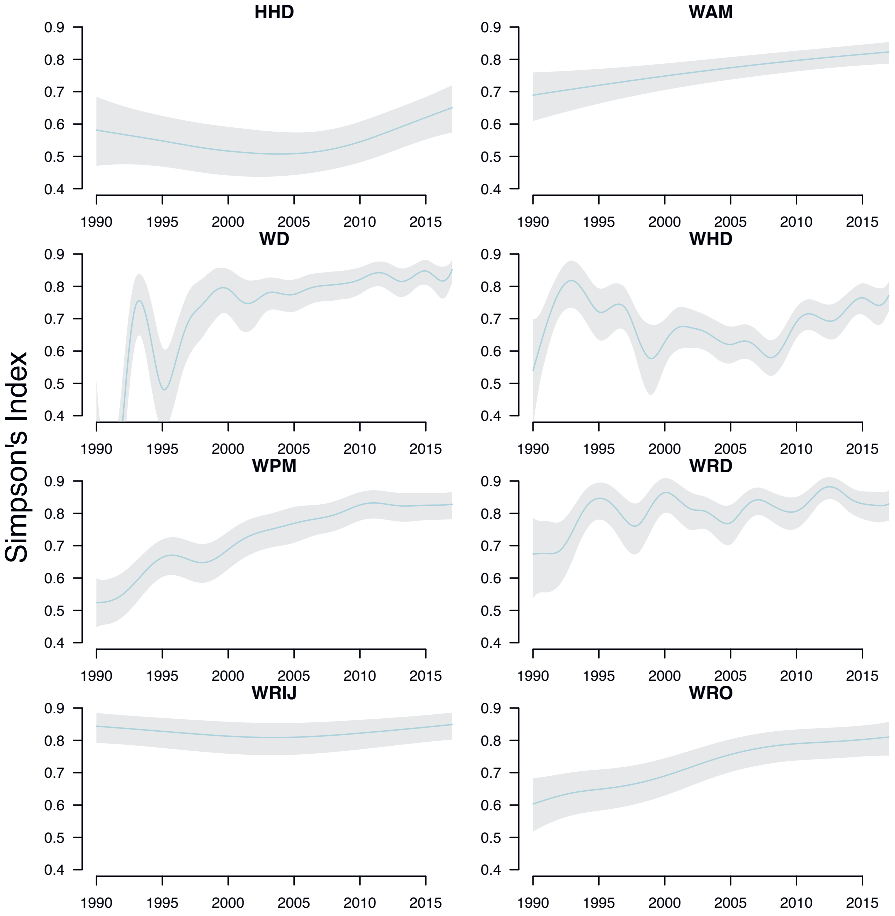

FIGURE S13

Trends in Shannon's index of evenness for each of the Water Authorities in 1990-2017

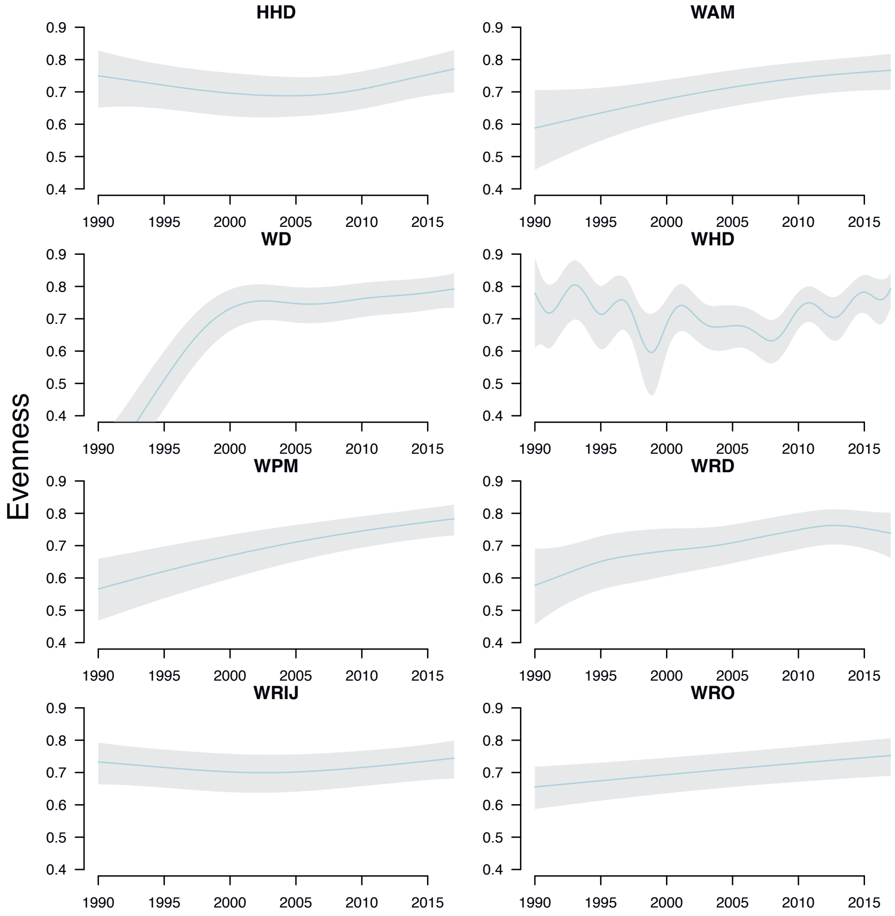

FIGURE S14

Back-transformed covariate effects (z-scores) for each of the ten taxonomic insect groups. Black bars indicate significant effects. COD = chemical oxygen demand, BOD = biochemical oxygen demand, maxHU = maximum hazard unit, msPAF = multiple-substance Potentially Affected Fraction.

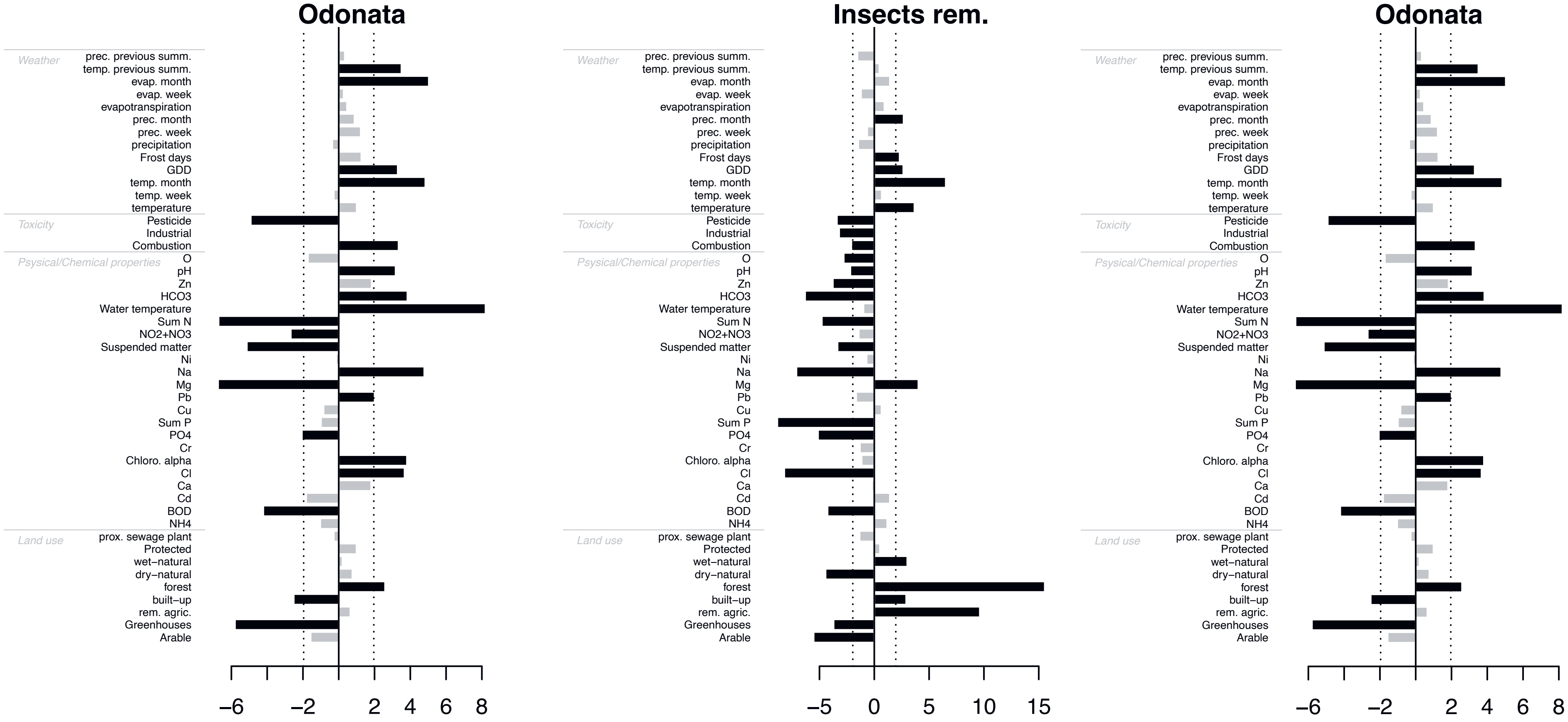

FIGURE S14

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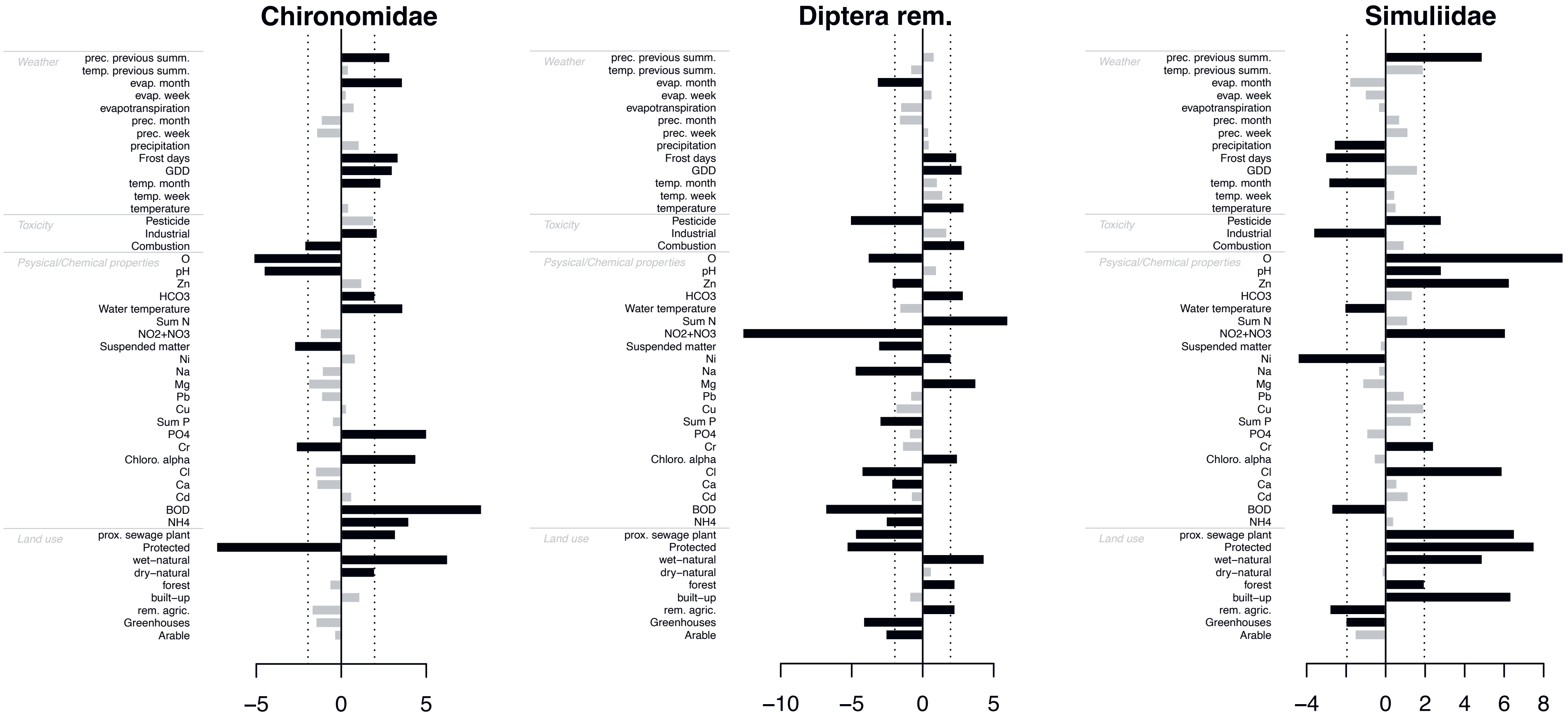

FIGURE S14

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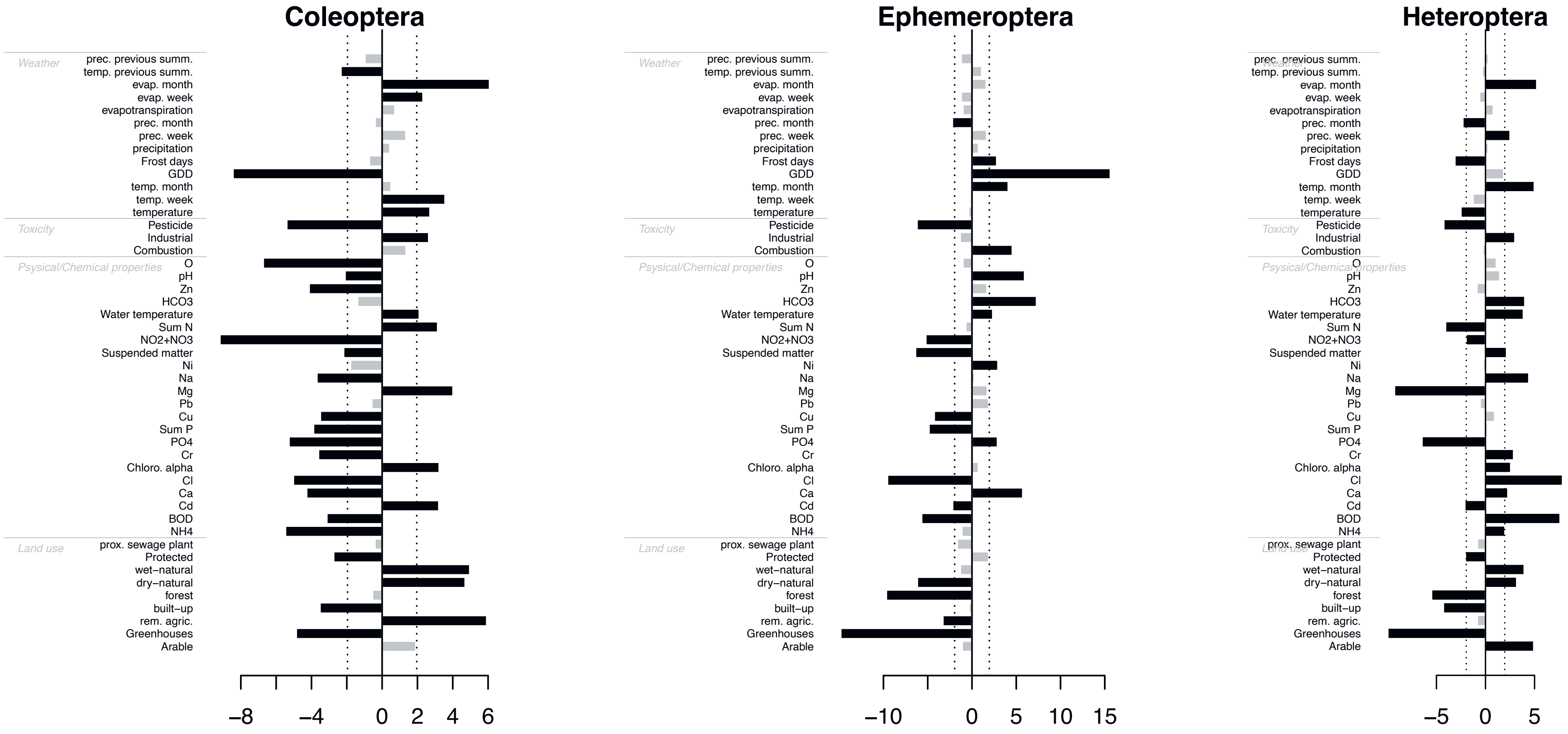

FIGURE S14

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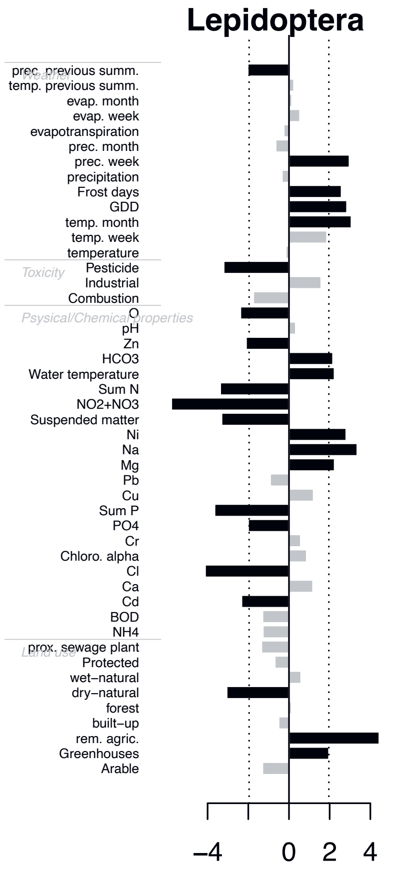

Larva of spring stonefly - *Nemoura cinerea* - beeksteenvlieg
Photo: Bureau Biota

ABOUT STOWA

STOWA (Acronym for Foundation for Applied Water Research) is the knowledge centre of the regional water managers (mostly the Dutch Water Authorities) in the Netherlands. Its mission is to develop, collect, distribute and implement applied knowledge, which the water managers need in order to adequately carry out the tasks that their work supports. This expertise can cover applied technical, scientific, administrative-legal or social science fields.

STOWA is a highly demand-driven operation. We carefully take stock of the knowledge requirements of the Water Authorities and ensure that these are placed with the correct knowledge providers. The initiative for this mainly lies with the users of this knowledge, the water managers, but sometimes also with knowledge institutes, business and industry. This two-way flow of knowledge promotes modernisation and innovation.

Demand-driven operation also means that we are constantly looking for the 'knowledge requirements of tomorrow' - requirements that we dearly want to put on the agenda before they become an issue - in order to ensure that we are optimally prepared for the future.

We ease the burden of the water managers by assuming the tasks of placing the invitation to tender and supervising the joint knowledge projects. STOWA ensures that water managers remain linked to these projects and also retain 'ownership' of them. In this way, we make sure that the correct knowledge requirements are met. The projects are supervised by committees, which also comprise regional water managers. The broad research lines are spread out per field of practice and accounted for by special programme committees. The water managers also have representatives on these committees.

STOWA is not only a link between the users of knowledge and knowledge providers, but also between the regional water managers. The collaboration of the water managers within STOWA ensures they are jointly responsible for the programming, that they set the course, that several Water Authorities are involved with one and the same project and that the results quickly benefit all Water Boards.

MISSION STATEMENT

STOWA's fundamental principles are set out in our mission:
Defining the knowledge needs in the field of water management and developing, collecting, making available, sharing, strengthening and implementing the required knowledge or arranging for this together with regional water managers.

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