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Calibration of the SPEAR_{pesticides} bioindicator for cost-effective pesticide monitoring in East African streams

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Abstract

Background: Pesticides are washed from agricultural fields into adjacent streams, where even short-term exposure causes long-term ecological damage. Detecting pesticide pollution in streams thus requires the expensive monitoring of peak concentrations during run-off events. Alternatively, exposure and ecological effects can be assessed using the SPEAR_{pesticides} bioindicator that quantifies pesticide-related changes in the macroinvertebrate community composition. SPEAR_{pesticides} has been developed in Central Europe and validated in other parts of Europe, Australia and South America; here we investigated its performance in East African streams.

Results: With minimal adaptations of the SPEAR_{pesticides} index, we successfully characterized pesticide pollution in 13 streams located in Western Kenya. The East African SPEAR_{pesticides} index correlated well with the overall toxicity of 30 pesticides (maximum toxic unit = maximum environmental vs. median lethal concentration) measured in stream water ($R^2 = 0.53$). Similarly, the SPEAR_{pesticides} index correlated with the risk of surface run-off from agricultural fields (as identified based on ground slope in the catchment area and the width of protective riparian strips, $R^2 = 0.45$). Unlike other bioindicators designed to indicate general water pollution, SPEAR_{pesticides} was independent of organic pollution and highly specific to pesticides. In 23% of the streams, pesticides exceeded concentrations considered environmentally safe based on European first tiered risk assessment.

Conclusions: Increasing contamination was associated with considerable changes in the macroinvertebrate community composition. We conclude that pesticides need to be better regulated also in developing countries. SPEAR_{pesticides} provides a straightforward and cost-efficient tool for the required monitoring of pesticide exposure in small to medium streams.

Keywords: Ecotoxicology, Bio-indicator, Pesticide pollution

Background

In 2020, the worldwide application of agricultural pesticides is expected to increase from 2 million tonnes to 3.5 million tonnes annually [1]. Pesticide pollution is considered one of the main drivers for the global decline in the abundance and diversity of insects, plants

and birds [2–4]. There is increasing evidence that the pesticide-driven impairment of biocenoses also affects valuable ecosystem services ranging from pollination [5] to leaf-litter degradation [6] and to the biological control of agricultural pests [7, 8] and of pathogens in freshwater [9]. For the United States and the European Union, where pesticides are used on a large scale, extensive literature on exposure and effects in the environment is available from academic research and from regulatory risk assessment [1]. In developing countries,

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regulation of plant protection products is often poor and information on pesticide pollution is scarce, though recent evidence suggests that pesticide usage has been increasing [1]. As agricultural producers grow more conscious about the use of synthetic pesticides and their toxic effects [10], more effort in the assessment and mitigation of pesticide pollution is urgently needed.

Freshwater macroinvertebrates in small streams are at particular risk, since streams collect pesticide loads from agricultural fields in the catchment area [11]. Moreover, many freshwater arthropods are highly sensitive to insecticides and fungicides [12–14]. However, the detection and quantification of pesticides in streams is challenging, as exposure occurs typically in short pulses due to spray drift and particularly due to surface run-off from agricultural fields following heavy rainfall [15]. Such short-term exposure peaks (in the range of hours) drive long-term effects on the macroinvertebrate community for months [16–18]. Therefore, pesticide measurements from grab samples of stream water and sediment at random time points tend to considerably underestimate the magnitude of pesticide exposure. Realistic environmental monitoring in streams must capture the exposure peaks by run-off event-triggered sampling [15] or by continuous passive sampling over extended periods of time [11]. However, such studies are labor-intensive and expensive [19]. In addition to the chemical analysis of samples for many compounds, samplers need to be installed, protected and regularly accessed in remote areas. These challenges limit the feasibility of monitoring pesticide pollution based on chemical analyses, particularly in developing countries.

As an alternative approach, pesticide exposure can be indirectly derived from its observed effects on the macroinvertebrate community composition. The $SPEAR_{pesticides}$ (“SPECies At Risk”) bioindicator has been developed to quantify decreases in the proportion of those taxa considered to be vulnerable to pesticides, as compared to reference conditions [20, 21]. For this task, stream macroinvertebrates have been classified as vulnerable or non-vulnerable taxa based on ecotoxicological traits (see methods). Because the $SPEAR_{pesticides}$ index describes the proportion of vulnerable taxa (weighted by individual number), it has no unit and does not facilitate the identification of individual toxic compounds in the environment. However, $SPEAR_{pesticides}$ values can be translated to an estimated toxic pressure for macroinvertebrates in the more informative form of toxic units (see methods). Since the $SPEAR_{pesticides}$ value is driven by long-term effects, it can be derived from a single community sample per site. $SPEAR_{pesticides}$ thus offers a potential cost-effective technique for the monitoring of pesticide

pollution in small to medium streams that may be specifically valuable in developing countries.

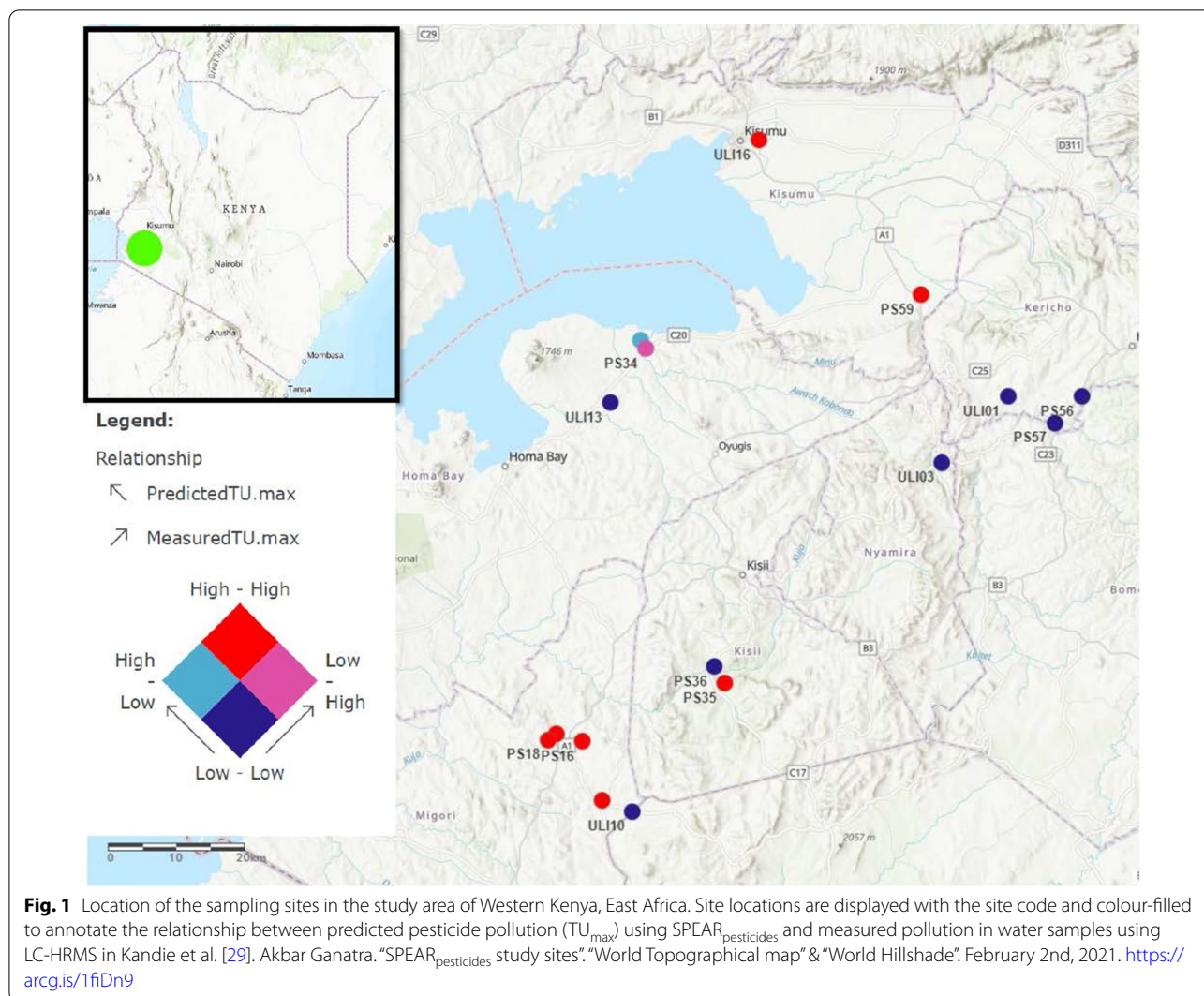
$SPEAR_{pesticides}$ has been developed for temperate streams in Central Europe. Thus, the studied species composition, pesticide exposure patterns and ecological conditions may differ from those in other continents such as in sub-Saharan Africa. Malherbe et al. [22] found only a non-significant response of $SPEAR_{pesticides}$ values to pesticide pollution in South African streams. In contrast, $SPEAR_{pesticides}$ has been successfully applied and validated to assess pesticide pollution in various streams ranging from Southern to Northern Europe [20], and (after minor modification) in Australia [23] and Argentina [24]. The $SPEAR_{pesticides}$ concept is based on ecotoxicological traits rather than taxonomic relations and thus provides a mechanistic linkage of pesticide stress and the community response; in contrast to classical taxonomy-based bioindicators, trait-based approaches can overcome issues with natural taxonomic variability and may thus be applicable even across different climatic regions [25]. For freshwater macroinvertebrates in the Southern hemisphere, information on ecotoxicological traits is very scarce [22, 23]. However, Wang et al. [26] found that related saltwater invertebrates from temperate and tropical regions differ only slightly in their acute sensitivity to toxicants. Moreover, $SPEAR_{pesticides}$ can be applied at the family taxonomic level [27], and palearctic and afro-tropical streams share most of their macroinvertebrate families [28]. Assuming that families of freshwater macroinvertebrates from temperate and tropical streams may generally share their vulnerability to pesticides, $SPEAR_{pesticides}$ may be, therefore, used also in sub-Saharan Africa.

Here we applied the $SPEAR_{pesticides}$ bioindicator to macroinvertebrate samples from 13 small to medium streams in Western Kenya. The $SPEAR_{pesticides}$ values were compared to the toxic pressure of 30 pesticides in water samples concurrently collected during the rainy season [29]. Additionally, we tested the specificity of the $SPEAR_{pesticides}$ index for pesticide effects and compared its performance to alternative bioindicators for environmental stressors.

Materials and methods

Study area

The Lake Victoria South Basin in the western part of Kenya (Fig. 1) is characterized by a tropical climate with a major rainy season between March and June and a minor rainy season between October and December. The rainy seasons are separated by dry months, particularly in the lowlands close to the shore of Lake Victoria. The area is densely populated, but poorly developed in terms of infrastructure and sanitation. It is dominated by food



production, mainly maize, for local markets with small scale fields that often range close to the unfortified banks of streams. However, large scale commercial farming also exists in the form of irrigated rice fields, sugarcane and particularly tea plantations [30]. During the rainy seasons, streams turn red from silt loads indicating heavy erosion and a high potential for surface run-off from agricultural fields.

Pesticide measurements

The sampling and analysis of pesticide residues has been described in detail in Kandie et al. [29]. In brief, 48 sites covering small to large streams, oxbow lakes, irrigation channels, reservoirs and rice fields were sampled during the rainy season in September–October 2017. A single 500 mL water sample per site was collected in a pre-cleaned glass. Solids were allowed to settle for 1 min,

before aliquots of 1 mL were transported to the laboratory in amber glass vials at −4 °C. Chemical analysis was performed by directly injecting 100 µL of the water sample into a high performance liquid chromatography system (Thermo Ultimate 3000 LC) coupled to a high resolution mass spectrometer (QExactive Plus, Thermo). The water samples were subjected to target screening for 428 chemicals and to suspect screening for 233 additional substances. This analysis included 162 hydrophilic pesticides (active substances and metabolites), but no highly hydrophobic compounds such as pyrethroid insecticides for which different sampling techniques are required. Raw data was processed using MZmine (Version 2.38) [31] and further confirmation and quantification done using TraceFinder 4.1 (Thermo) as detailed in [29]. Physicochemical water parameters were measured in situ (flow velocity, temperature, dissolved oxygen,

phosphates, pH, turbidity) and from additional water samples in the laboratory (nitrate, nitrite, ammonium, carbonate hardness).

In the present study, we only considered pesticides and converted the observed concentrations in the water samples to toxic units to quantify the toxic pressure for freshwater macroinvertebrates. The toxic unit (TU) [32] describes the environmental concentration *conc* of a pesticide *i* in relation to its median effective or lethal concentration (EC_{50} or LC_{50} , concentration that affects or kills 50% of individuals in an acute toxicity test):

$$TU_i = \frac{conc_i}{LC50_i} \quad (1)$$

For the TU calculation we generally referred to the LC_{50} for the water flea *Daphnia magna* after constant exposure for 48 h as reported in the Pesticides Properties Data Base [33]. *D. magna* was selected as a reference, because it belongs to the more sensitive freshwater macroinvertebrates and because most toxicological data are available for this species due to its use in regulatory risk assessment [34]. However, for some pesticides (particularly neonicotinoid insecticides), *D. magna* turned out to be considerably more tolerant than other macroinvertebrates [34]. Therefore, we additionally extracted the LC_{50} for the saltwater shrimp *Americamysis bahia* after 96 h exposure from the PPDB data base in case it was available. In cases, where *D. magna* was highly insensitive ($LC_{50} > 10$ mg/L), we additionally took data from the ECOTOX data base [35] for the standard test species *Chironomus riparius* and *Hyalella azteca*. If the LC_{50} of *D. magna* was > 10 times higher than that of an additional species observed after 48 h or > 100 times higher after 96 h, we used the most sensitive additional species as reference (mean LC_{50} from different studies in ECOTOX, if available). For metabolites we used the LC_{50} of the parental compound if no toxicity data was available or if the metabolite was less toxic than the parental compound.

The overall toxic pressure from pesticide mixtures in the water samples was summarized as the summed up (TU_{sum} , [32] and as the maximum (TU_{max} , [21]) toxic unit. TU_{max} and TU_{sum} were set to a minimum threshold of 10^{-4} for sites at which no potentially relevant concentrations were detected; this threshold is in the range of the limit of quantification for the most toxic pesticides in the chemical analysis (see Additional file 2: Table S6). Additionally, no effects of pesticides on freshwater macroinvertebrate communities have been generally observed in the field below a threshold range of TU_{max} between 10^{-3} and 10^{-4} [36].

The SPEAR_{pesticides} concept

The SPEAR_{pesticides} bioindicator has been developed to specifically quantify pesticide-driven deviations in the community structure from those observed under non-polluted reference conditions. Since its first publication in Liess and von der Ohe [21], SPEAR_{pesticides} has been constantly refined [20, 27]. Here we refer to the latest version 2019.11 that includes some major improvements described in Knillmann et al. [37].

SPEAR_{pesticides} is based on a classification of freshwater macroinvertebrates in "SPECies At Risk" and in taxa being not at risk based on four traits that describe their vulnerability to pesticides: (1) the average physiological sensitivity to various pesticides (*s* value, mean of $\log_{10}(LC_{50}$ relative to the LC_{50} of the reference species *D. magna* or *Chironomus* sp.)); (2) the ability of autochthonous population recovery from reproduction (generation time); (3) the ability of allochthonous recovery from recolonization (dispersal from non-polluted refuge areas); and (4) the probability of being actually exposed (e. g. aquatic life stages during the main insecticide application season). Taxa with high sensitivity, low ability of autochthonous and allochthonous recovery and high probability of being exposed have been classified as being at risk, others as not at risk. A trait data base for Central European taxa, linked to a program for the calculation of SPEAR_{pesticides} values is available with the software INDICATE (<https://www.systemecology.de/indicate>). The data base comprises entries for individual species, but also for higher taxa, where available data on lower taxonomic levels have been averaged.

The SPEAR_{pesticides} index relates to the proportion of taxa at risk within the macroinvertebrate community and decreases with increasing toxic pressure [37]:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^n \log_{10}(4x_i + 1) \cdot y}{\sum_{i=1}^n \log_{10}(4x_i + 1)} \quad (2)$$

with x_i being the observed number of individuals of taxon *i*, and *y* being 1 for those taxa classified at risk and 0 for taxa not at risk. In this formula, SPEAR_{pesticides} values can range between 0 (no species at risk, indicating high pesticide effects) and 1 (only species at risk). Abundant taxa are down weighted to limit the influence of populations with mass development so that the SPEAR_{pesticides} index increases rather equally with both the incidence and the population density of vulnerable taxa. To facilitate interpretation, since version 2019.11, "raw" SPEAR_{pesticides} values obtained from the equation above are divided by the average SPEAR_{pesticides} value observed in non-polluted reference sites (at measured $TU_{max} < 10^{-4}$) from Germany [37]:

$$\text{ScaledSPEAR}_{\text{pesticides}} = \frac{\text{rawSPEAR}_{\text{pesticides}}}{\text{referenceSPEAR}_{\text{pesticides}}} \quad (3)$$

With *reference* $\text{SPEAR}_{\text{pesticides}} = 0.27$. This scaled $\text{SPEAR}_{\text{pesticides}}$ index ranges from 0 (no species at risk) to 3.7 (only species at risk) and indicates no toxic pressure at values > 1 . For European streams, an empirical relationship between $\text{SPEAR}_{\text{pesticides}}$ and the measured \log_{10} -transformed TU_{max} during run-off events has been established to convert $\text{SPEAR}_{\text{pesticides}}$ values to the estimated toxic pressure [20, 37]. Because the community composition of Kenyan freshwater macroinvertebrates may differ from those in Germany, both the classification of individual taxa and the conversion to TU_{max} were subjected to revision in the present study (see results).

Application of $\text{SPEAR}_{\text{pesticides}}$ in the study area

We applied $\text{SPEAR}_{\text{pesticides}}$ to macroinvertebrate samples collected together with the water samples from Kandie et al. [29, see above]. The macroinvertebrate sampling has been described in detail in Becker et al. [9]. In brief, macroinvertebrates were collected at the same site and day as the water samples, following a standardised approach adapted from the South African Scoring System 5 (SASS5, [38]). At each stream site, a 50 m stretch was sampled in four quadrants by two persons in parallel. Each quadrant was sampled for 7 min using sweep nets for the water surface and littoral habitats, and kick sampling for the benthic macroinvertebrates. Additionally, gravel, soil and mud (GSM) habitats were sampled for 1 min per quadrant. Organisms were preserved in 70% ethanol and identified to the family level under a dissecting microscope in the laboratory. $\text{SPEAR}_{\text{pesticides}}$ v. 2019.11 and the associated estimated TU_{max} were calculated using the software INDICATE v. 1.2.0 (<https://www.systemecology.de/indicate>).

Application of other indicators of freshwater pollution

To compare the performance and specificity of $\text{SPEAR}_{\text{pesticides}}$ in the identification of pesticide pollution, we additionally applied the Biological Monitoring Working Party (BMWP) and the South African Scoring System (SASS5) bioindicators for freshwater quality. In contrast to $\text{SPEAR}_{\text{pesticides}}$, the BMWP and SASS5 indicators are based on a gradual instead of a binary classification system for the sensitivity of freshwater macroinvertebrates (mainly to low oxygen levels) ranging from 1 to 10. Like $\text{SPEAR}_{\text{pesticides}}$, the indicators have been established for freshwater families from a different region, but have been also applied in sub-Saharan Africa beyond their countries of origin [28, 39]

The BMWP score system was developed in the 1980s and refined later to assess organic pollution in British streams [40]. For a refined assessment according to the European Water Frame Work Directive, ecological quality indices are derived from the BMWP score by comparing observed vs. expected values based on an elaborate stream classification system. Such a classification system is not available for Kenyan streams; therefore, we simply calculated the average score per taxon (ASPT) using the software ASTERICS–AQUEM/STAR Ecological River Classification System v. 4.0.4, (https://www.gewasser-bewertung.de/index.php?article_id=419&clang=0). SASS5 was originally developed in 1994 and refined in 2002 to determine the condition or ‘health’ of rivers in South Africa [38]. As recommended, we calculated the Average Score Per Taxa (ASPT) using the score tables available in Dickens and Graham [38].

Finally, we calculated the proportion of ephemeropteran, plecopteran and trichopteran insects on the overall individual number (EPT) and the species diversity (Shannon index) as more general descriptors of the freshwater macroinvertebrate community. Both descriptors are considered to decrease with increasing levels of water pollution [37].

Additionally we estimated the run-off potential of a site, i. e. a predictor for the risk of pesticide exposure from surface run-off [41]. We used a highly simplified approach by classifying sites on an ordinal scale based on the average local ground slope and the average width of riparian strips up to 2 km upstream as visible from online satellite imagery. Slope was classified as flat, low, medium and high. The run-off potential was then classified as follows: 1 (none): ≥ 30 m buffer strip, or ≥ 20 m buffer strip and flat or low slope; 2 (low): 21–30 m buffer strip, or 11–20 m buffer strip and flat or low slope; 3 (moderate): 11–20 m buffer strip, or 6–10 m buffer strip and flat or low slope; 4 (high): ≤ 10 m buffer strip, or ≤ 5 m buffer strip and flat or low slope.

Data analysis

Data analysis was performed using R version 3.6.2. Because $\text{SPEAR}_{\text{pesticides}}$ has been developed for small and medium streams with flowing water, we limited our analysis to samples from natural streams (no artificial channels) with an average width of < 20 m and an estimated average flow velocity > 1 cm/s (based on the drift-body method). Two sites from a small river close to Kisii (PS42 and PS43, see Additional file 2: Table S4) were excluded, because biological sampling took part during high water. These samples were characterized by low numbers of taxa and individuals, as well as unusually high pesticide concentrations as compared to the $\text{SPEAR}_{\text{pesticides}}$ values, suggesting that sampling took place during a

run-off event. Therefore, we considered neither the $\text{SPEAR}_{\text{pesticides}}$ nor the pesticide data from this stream comparable to the other sites. Altogether, our analysis included 16 sites from 13 different streams. Three of the streams were sampled twice, with an average distance of ca. 2 km between both sites. Macroinvertebrate samples and toxic units from sites of the same stream were considerably more similar than those from different streams; to avoid pseudo-replication, we, therefore, aggregated data from different sites of the same stream using the mean values.

The original and the revised $\text{SPEAR}_{\text{pesticides}}$ index, as well as the associated estimated TU_{max} were compared to the TU_{max} from pesticide measurements using one-way linear regression; TU_{max} was \log_{10} -transformed prior to the analyses. To assess the potential disturbance of the sampling sites by additional stressors other than pesticides, we investigated the distribution of the measured TU_{max} and physicochemical water parameter values using violin plots; the data were compared to thresholds for a good ecological status from the literature. To assess the performance and the specificity of $\text{SPEAR}_{\text{pesticides}}$ for pesticides, we analyzed responses of the $\text{SPEAR}_{\text{pesticides}}$ index and of the additional bioindicators and descriptors to the physicochemical parameters using one-way linear regression. Additionally, relations of the $\text{SPEAR}_{\text{pesticides}}$ index and those physicochemical parameters that had a (marginally) significant effect on the $\text{SPEAR}_{\text{pesticides}}$, BMWP or SASS5 bioindicator were summarized using a principal component analysis (PCA). Finally, we performed pairwise correlations among the bioindicators and among those environmental variables that significantly affected these bioindicators to identify confounding factors.

Results

Pesticide pollution and effects on the macroinvertebrate community

We observed considerable pesticide toxicity to freshwater macroinvertebrates in our 16 study sites from 13 streams, quantified as the maximum toxic unit derived from chemical water analyses (TU_{max} , see methods). TU_{max} exceeded the threshold range for environmental effects of 10^{-4} to 10^{-3} [36] in nine and eight streams, respectively. The results indicate that pesticide pollution was a relevant environmental stressor in the study area. Pesticide toxicity was driven by the insecticides diazinon (7 sites, $\text{TU} = 10^{-2.36} - 10^{-1.81}$), pirimiphos-methyl (3 sites, $\text{TU} = 10^{-1.86} - 10^{-1.21}$), bendiocarb (1 site, $\text{TU} = 10^{-2.54}$) and imidacloprid (1 site, $\text{TU} = 10^{-3.47}$).

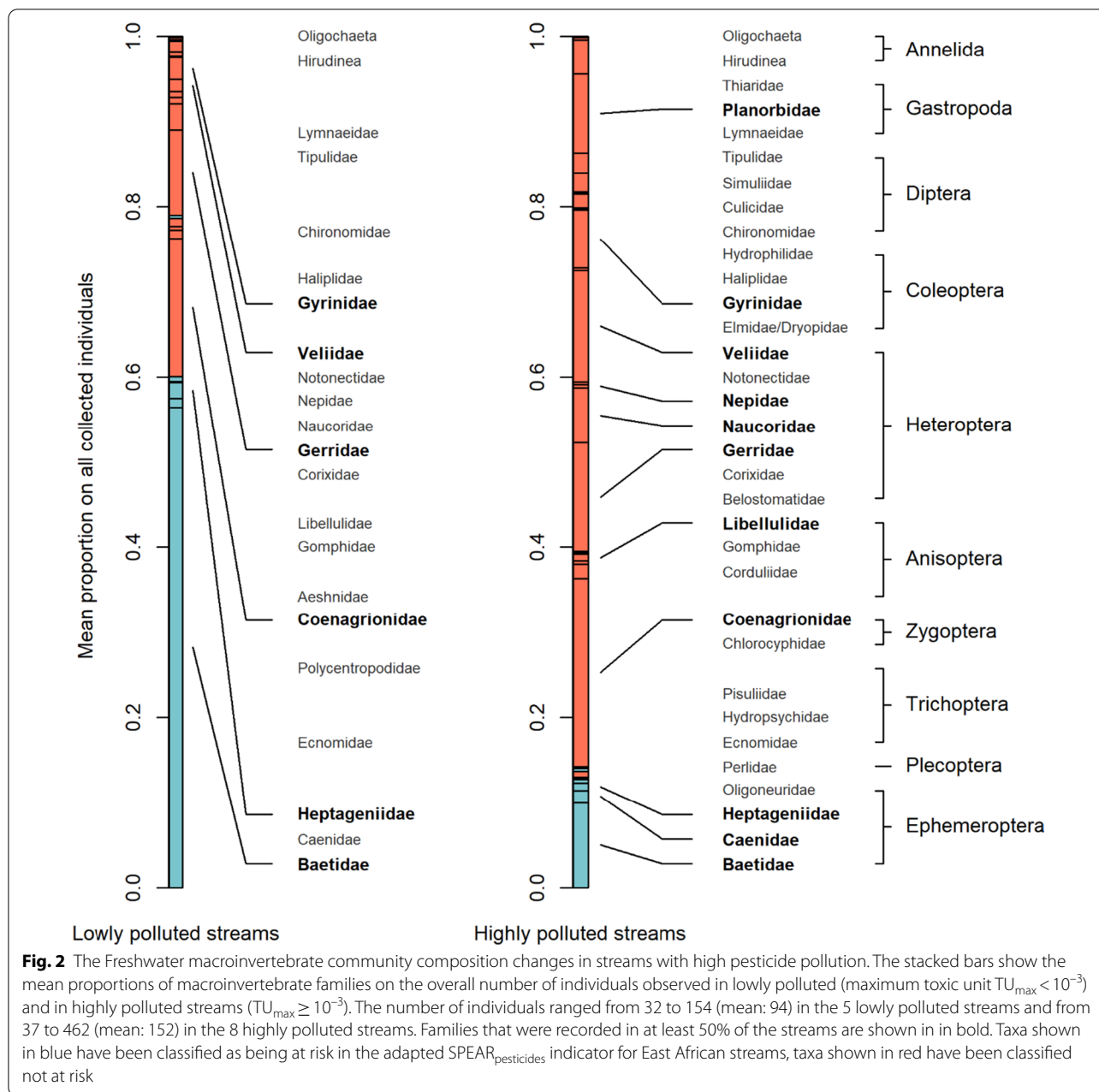
Overall, we identified 35 macroinvertebrate families in the streams. The proportion of taxonomic groups changed markedly with increasing pesticide

toxicity (Fig. 2). Non- or marginally polluted sites with $\text{TU}_{\text{max}} < 10^{-3}$ were dominated by the ephemeropteran families Baetidae and Heptageniidae, the zygopteran family Coenagrionidae, the heteropteran families Gerriidae and Veliidae, and by the coleopteran family Gyrinidae. These families occurred in at least half of the low-polluted streams and also showed the highest proportions on the mean number of individuals collected in a low-polluted stream (relative abundances). The same families occurred also in at least half of the highly polluted streams with $\text{TU}_{\text{max}} \geq 10^{-3}$, but additionally the ephemeropteran family Caenidae, the anisopteran family Libellulidae, the heteropteran families Naucoridae and Nepidae, and snails of the family Planorbidae were observed in the majority of these streams. As the most prominent changes in the community composition, the mean relative abundance of Baetidae decreased from 56% (range: 7–94%) in lowly polluted streams to 10% (range: 0.2–38%) in highly polluted streams ($n=5$ lowly and 8 highly polluted sites, $\chi^2 = 8.29$, $p = 0.004$ using a binomial generalized linear model). At the same time, Planorbidae increased from 0 to 9% (range: 0–36%, $\chi^2 = 7.95$, $p = 0.005$) in highly polluted streams. Therefore, changes in the community composition with pesticide pollution were driven by the response of frequently occurring taxa, suggesting that $\text{SPEAR}_{\text{pesticides}}$ may perform consistently across random samples from different streams. The results are consistent with Reiber et al. [42] who identified mayflies and snails among those macroinvertebrate taxa that most strongly decrease or increase with pesticide pollution in European streams, respectively.

Adaptation of $\text{SPEAR}_{\text{pesticides}}$ to East African streams

Most of the 35 macroinvertebrate families sampled were automatically linked to existing taxa in the trait database provided in the software INDICATE (see Additional file 2: Table S5). Only three families were not existing in the database, because they do not occur in Central European streams; these families were manually linked to higher taxa available in the database: *Pisuliidae* was linked to Trichoptera Gen. sp., *Oligoneuridae* was linked to Ephemeroptera Gen. sp., and *Chlorocyphidae* was linked to Zygoptera Gen. sp. *Culicidae* was automatically linked to Diptera Gen. sp., but traits are missing for this taxon (due to a lack of data, because dipteran species are too heterogeneous to be aggregated), and they were, therefore, automatically excluded from the calculation of $\text{SPEAR}_{\text{pesticides}}$.

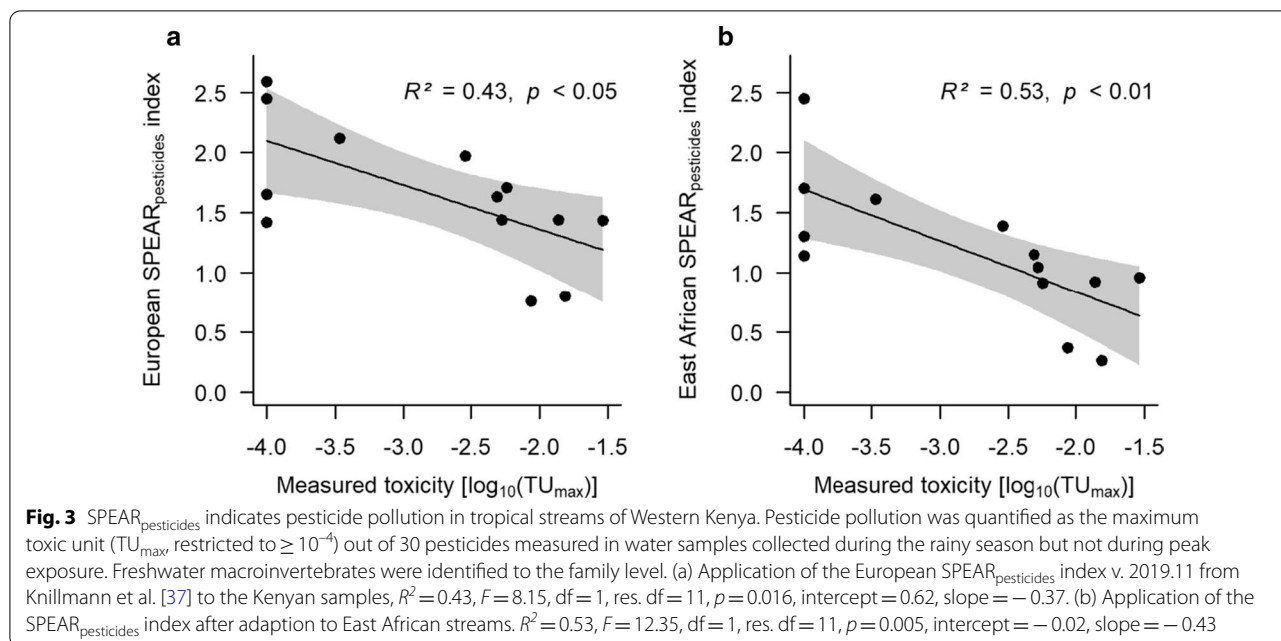
Though developed for European streams, the existing $\text{SPEAR}_{\text{pesticides}}$ index v. 2019.11 from Knillmann et al. [37] showed reasonable correlation with the measured pesticide toxicity (TU_{max}) from chemical water analysis when applied to the Kenyan macroinvertebrate



communities ($R^2 = 0.43$, Fig. 3a). The correlation of the $SPEAR_{pesticides}$ index with toxic pressure increased when we re-classified two taxa based on additional information for Kenyan taxa. First, the damselfly family of *Coenagrionidae* has been classified at risk in the European $SPEAR_{pesticides}$ index v. 2019–11, based on a relatively high physiological insecticide sensitivity (s value = -0.24). This value is only slightly above the threshold for sensitive taxa ($s \geq -0.36$). The classification was based on toxicity data for the European species *Eschnura elegans*. However, acute toxicity tests with the insecticides diazinon

and imidacloprid on freshwater macroinvertebrates collected in the study region of Western Kenya revealed that the tolerance of local coenagrionid species is comparable to those of other taxa such as *Notonectidae*, *Chironomidae* and *Dytiscidae* that have been classified as insensitive [9]. Accordingly, we re-classified *Coenagrionidae* as being insensitive (s value = -0.4); consequently, the taxon moved from the category “at risk” to “not at risk”.

Second, in $SPEAR_{pesticides}$ v. 2019.11, the heteropteran family *Corixidae* was considered as non-exposed due to a surrounding layer of air (physical lung) that potentially



protect individuals from pesticide exposure in the water. However, in acute toxicity tests we observed high insecticide sensitivity of *Corixidae* from the study region [9]. Thus, we re-classified *Corixidae* as being exposed, so that the taxon moved from the category “not at risk” to “at risk”. Results of toxicity tests with additional 13 taxa collected in the study region from Becker et al. [9] were in accordance with the existing classification in the $SPEAR_{pesticides}$ trait data base.

With these changes, we were able to adapt the $SPEAR_{pesticides}$ index to East African streams so that it explained the observed TU_{max} considerably better than the European $SPEAR_{pesticides}$ index v. 2019.11 ($R^2 = 0.53$, Fig. 3b). The East African $SPEAR_{pesticides}$ index decreased with increasing measured pesticide toxicity as follows:

$$SPEAR_{pesticides} = -0.43 * \log_{10}(\text{background } TU_{max}) - 0.02 \tag{4}$$

After solving Eq. 4 for TU_{max} , the East African $SPEAR_{pesticides}$ index may be used to predict background toxicity in streams from the observed macroinvertebrate community composition:

$$\text{Background } TU_{max} = 10^{\frac{SPEAR_{pesticides} + 0.02}{-0.43}} \tag{5}$$

The mean TU_{max} predicted from Eq. 5 and the mean observed background TU_{max} coincided well (difference by a factor of < 2.5 or of ≤ 0.4 orders of magnitude ($= \log_{10}$ -transformed TU_{max}) across the whole range of observed toxicity, Fig. 4a). Across all data points, the

ratio of estimated vs. measured TU_{max} ranged from 0.2 to 19.6, i. e. the predicted toxicity deviated from the measured toxicity by < 1.3 orders of magnitude in all investigated streams.

We related the East African $SPEAR_{pesticides}$ index to background pesticide toxicity measured in water from grab samples collected during the rainy season, but outside run-off events. In contrast, in Europe the $SPEAR_{pesticides}$ index has been related to the short-term peak toxicity measured during run-off events following heavy rainfall [20, 21, 37]. In temperate streams of Europe and North America, peak pesticide toxicity is approximately four times as high as the background toxicity during the season of main pesticide exposure in early summer [15, 43–46]. Assuming that the same ratio of peak vs. background toxicity may hold also in tropical streams, we applied a correction factor of 4 to relate the East African $SPEAR_{pesticides}$ index to an estimated peak exposure during run-off:

$$\text{Estimated peak } TU_{max} = 4 * 10^{\frac{SPEAR_{pesticides} + 0.02}{-0.43}} \tag{6}$$

The response of the East African $SPEAR_{pesticides}$ index to the estimated peak exposure in Kenya could then be compared to the response of the latest European $SPEAR_{pesticides}$ index v. 2019.11 to peak exposure in Germany [37]. The East African $SPEAR_{pesticides}$ index produced considerably higher numbers as compared to the European index, particularly when pesticide toxicity was low (Fig. 4b). The more pronounced increase in the East African $SPEAR_{pesticides}$ index with decreasing

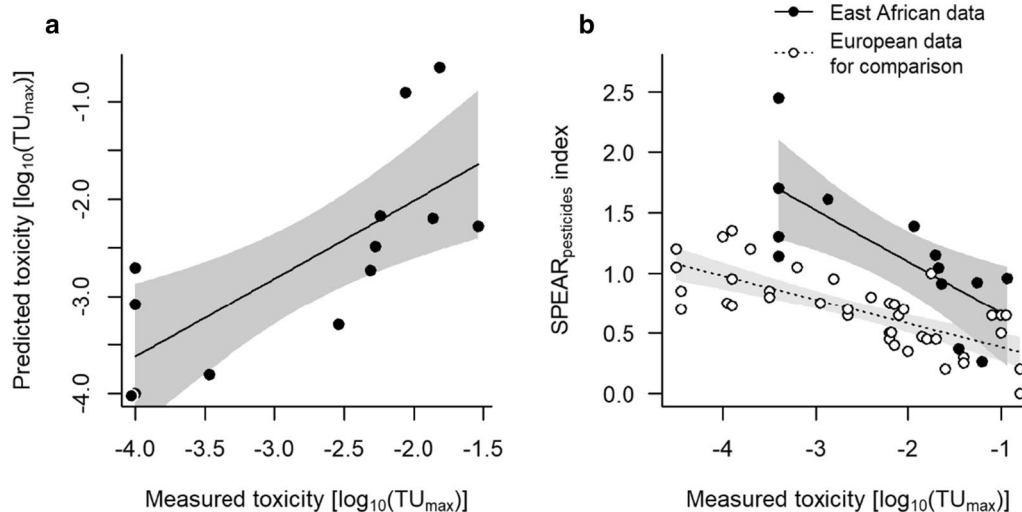


Fig. 4 Performance of the East African $\text{SPEAR}_{\text{pesticides}}$ index. (a) Background pesticide toxicity (maximum toxic unit, TU_{max}) predicted with the East African $\text{SPEAR}_{\text{pesticides}}$ index correlates with the measured background toxicity in Kenyan water samples. $R^2 = 0.54$, $F = 12.97$, $df = 1$, $\text{res. df} = 11$, $p = 0.004$, intercept = -0.41 , slope = 0.80 . TU_{max} was restricted to $\geq 10^{-4}$, because lower toxicity could not be measured due to detection limits for pesticides. TU_{max} has been \log_{10} -transformed, so that differences are presented in orders of magnitude. (b) Relation of the East African $\text{SPEAR}_{\text{pesticides}}$ index with the estimated peak pesticide toxicity during run-off in Kenyan streams, as compared to the relation of the European $\text{SPEAR}_{\text{pesticides}}$ index v. 219.11 with measured peak toxicity in German streams (data from [37]). Peak exposure in Kenyan streams was estimated by multiplying the measured background toxicity with a correction factor of 4 (see main text for justification)

pesticide exposure indicates differences in the composition of European and East African macroinvertebrate communities and in their response to pesticides. Therefore, the use of a separate conversion scheme from $\text{SPEAR}_{\text{pesticides}}$ to TU_{max} in afro-tropical streams, as established with Eqs. 5 and 6, is justified.

To assess the reproducibility and robustness of the East African $\text{SPEAR}_{\text{pesticides}}$ index, we extended our data set to all sites sampled in the study area of Western Kenya (see [9]). The 48 sites from 40 water bodies included habitats for which $\text{SPEAR}_{\text{pesticides}}$ was not designed such as artificial irrigation channels, rice fields, reservoirs, oxbow lakes and large rivers. We still observed a relation of the East African $\text{SPEAR}_{\text{pesticides}}$ index with TU_{max} that was very similar to those in Fig. 3b, but the non-explained variation increased considerably ($R^2 = 0.13$, $F = 5.55$, $df = 1$, $\text{res. df} = 38$, $p = 0.024$, intercept = 0.13 , slope = -0.31).

Specificity of the East African $\text{SPEAR}_{\text{pesticides}}$ index to pesticides

To assess the potential impact of confounding factors and the specificity in the response of the adapted East African $\text{SPEAR}_{\text{pesticides}}$ index to pesticide pollution, we first investigated the potential influence of additional physicochemical parameters on the macroinvertebrate community. A comparison of the observed physicochemical

water parameters with values recommended for good water quality from the literature revealed that pesticide pollution was indeed one of the dominant stressors at the sampling sites (Fig. 5). Additionally, in many streams we observed very high levels of phosphate and turbidity, as well as low levels of carbonate hardness and dissolved oxygen, which may have contributed in shaping the macroinvertebrate community.

Next, we assessed the sensitivity of the adapted $\text{SPEAR}_{\text{pesticides}}$ index for East Africa to such additional stressors. The $\text{SPEAR}_{\text{pesticides}}$ index decreased significantly not only with increasing pesticide toxicity (measured TU_{max} and TU_{sum}) and run-off potential, but also with carbonate hardness, conductivity and turbidity (Additional file 1: Table S1). However, pesticide toxicity was significantly correlated with each of these confounding factors (Additional file 1: Table S2). All these factors were associated with a dominant environmental gradient identified by the first principal component in a principal component analysis (PCA). This gradient explained 61% of the total variation and likely reflects a range of different stream types (Fig. 6). On one end, lowly polluted, fast-flowing mountain streams showing high $\text{SPEAR}_{\text{pesticides}}$ values (streams nr. 27, 28, 31 and 32) were running in comparably steep valleys through intensely cultivated tea plantations but were protected from run-off by wide buffer strips so that run-off potential and pesticide toxicity was low. On the other end, lowland streams were

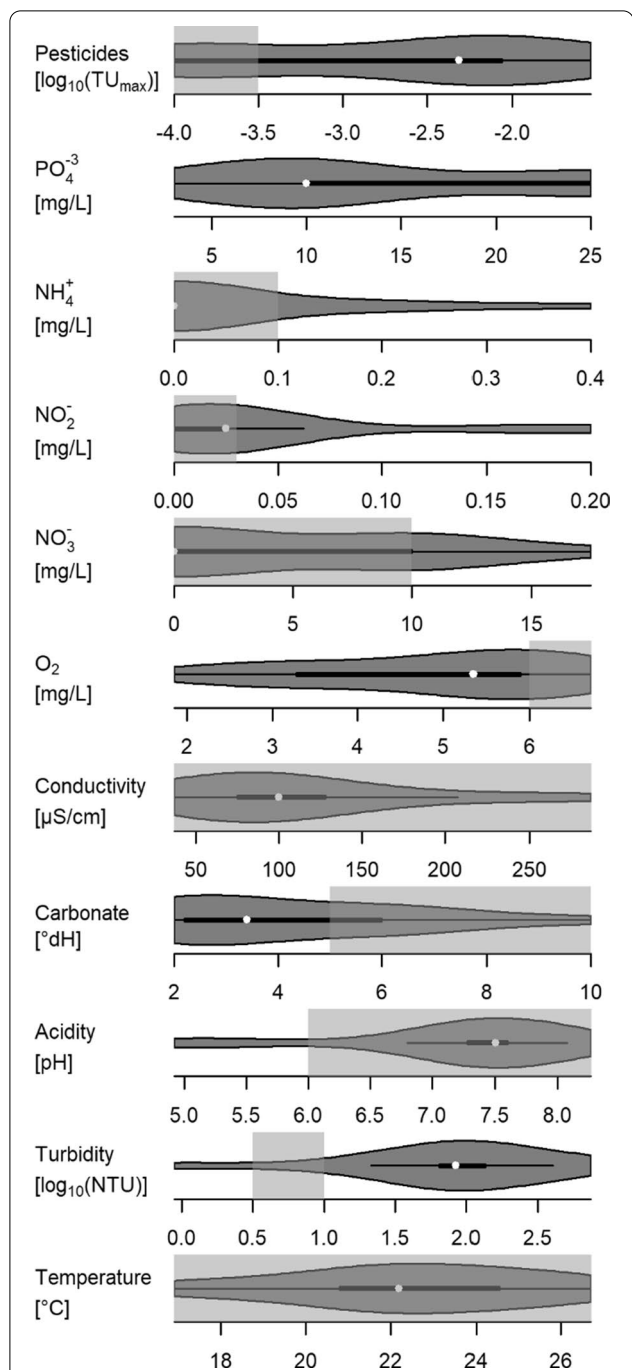


Fig. 5 Distribution of physicochemical parameter values at the study sites. The violin plot shows the kernel probability density of the data points across the parameter values. White points indicate the median, black boxes the interquartile range, and black lines 1.5 × the interquartile range. Light-grey boxes indicate the range of values considered typical or recommend for streams with good water quality

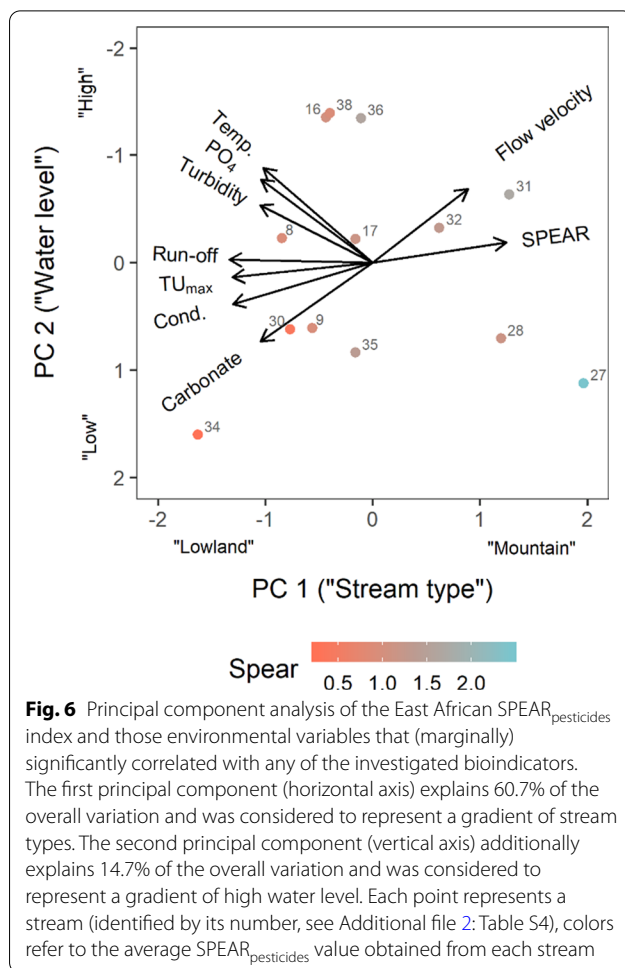


Fig. 6 Principal component analysis of the East African SPEAR_{pesticides} index and those environmental variables that (marginally) significantly correlated with any of the investigated bioindicators. The first principal component (horizontal axis) explains 60.7% of the overall variation and was considered to represent a gradient of stream types. The second principal component (vertical axis) additionally explains 14.7% of the overall variation and was considered to represent a gradient of high water level. Each point represents a stream (identified by its number, see Additional file 2: Table S4), colors refer to the average SPEAR_{pesticides} value obtained from each stream

slowly flowing and showed higher run-off potential due to surrounding agriculture with only small buffer strips. These streams showed high pesticide toxicity, as well as sediment (turbidity) and phosphate pollution that were associated with low SPEAR_{pesticides} values and high conductivity, respectively.

Though all these confounding stressors increased simultaneously with the first principal component (considered as stream type), they could be disentangled along the second principal component (Fig. 6): On one end, we observed slow flowing streams with clear water but high conductivity and carbonate hardness. Streams on the other end showed high flow velocity, turbidity and phosphate concentrations, but low conductivity and carbonate hardness. We consider the second principal component to represent a gradient of raised water level at the day of sampling. Raised water levels may have increased flow velocity, as well as turbidity and phosphate levels due to erosion, and decreased conductivity and carbonate levels due to dilution with rainwater. In contrast, neither run-off potential, nor pesticide toxicity (TU_{max})

or $\text{SPEAR}_{\text{pesticides}}$ responded to this potential gradient of water levels. First, run-off requires not only high water but also nearby agricultural fields. Second, raised water does not mean flooding in this context, which might have been associated with pesticide pollution from run-off (flooded sites were excluded from the analysis, see methods). The $\text{SPEAR}_{\text{pesticides}}$ index was thus most closely associated with pesticide toxicity and run-off potential, indicating indeed a high specificity of the adapted $\text{SPEAR}_{\text{pesticides}}$ indicator for pesticide pollution in Western Kenyan streams. Each of the higher principal components explained only $\leq 8\%$ of the total variance, and they were thus not further analyzed.

Finally, we compared the performance of $\text{SPEAR}_{\text{pesticides}}$ in indicating pesticide pollution to those of other bioindicators and commonly applied descriptors of the macroinvertebrate community. Pesticide toxicity most strongly correlated with the East African $\text{SPEAR}_{\text{pesticides}}$ index ($R^2=0.53$), followed by the European $\text{SPEAR}_{\text{pesticides}}$ index ($R^2=0.43$) and the average score per taxon (aspt) of the BMWP indicator ($R^2=0.33$, Additional file 1: Table S1). Correlation with the aspt of the SASS5 indicator was lower ($R^2=0.19$) and not significant. In contrast to $\text{SPEAR}_{\text{pesticides}}$ that was only related to pesticides and their associated stressors (see above), the BMWP and SASS5 indicators were additionally related to phosphate pollution and temperature but did not significantly correspond to run-off potential. The EPT index (cumulative proportion of *Ephemeroptera*, *Plecoptera* and *Trichoptera*) showed a significant but low negative response to pesticide pollution ($R^2=0.10$), turbidity and carbonate hardness, but increased with flow velocity. The Shannon index for species diversity increased with pesticide pollution, but the response was only marginally significant and may relate to the generally concurrent increase in species richness and pesticide pollution from the spring to more downstream sections [47].

The different bioindicators correlated with each other. The East African $\text{SPEAR}_{\text{pesticides}}$ index increased with the aspt of the BWMP and SASS5 indicator and with the EPT index, but decreased with species diversity (Additional file 1: Table S3). BMWP and SASS5 were clearly correlated with each other, but not with the EPT index and species diversity. The EPT index decreased with increasing species diversity.

Discussion

We adapted the $\text{SPEAR}_{\text{pesticides}}$ bioindicator for the quantification of pesticide exposure to afro-tropical conditions and demonstrated its use in thirteen Kenyan streams. A detailed step-by-step guidance for the application of the East African $\text{SPEAR}_{\text{pesticides}}$ index is provided in Additional file 1. In the following, we discuss the performed

modifications of $\text{SPEAR}_{\text{pesticides}}$ and the results from the present case study.

Pesticide pollution in Western Kenya

Pesticides are an important stressor to freshwater macroinvertebrates in small and medium streams of western Kenya. Our results confirm earlier conclusions from various freshwater habitats of the same study area Kandie et al. [29], in that study, the chemical freshwater pollution was assessed and the ecological risk (TU_{sum}) identified was highest for macroinvertebrates due to insecticide exposure. Pesticide toxicity observed in streams of the present study was comparable to those in European landscapes characterized by intensified agriculture [48]. In three out of 13 streams, we observed toxic units exceeding the threshold of 10^{-2} that is considered safe according to the first tier of governmental risk assessment in the European Union [49]. The results illustrate a need for the monitoring and regulation of pesticide application to reduce pesticide exposure in freshwater. In Kenya, plant protection products are sold at relatively low prices without the need for a certificate of competence from retailers, making them widely available to small farmers who are then not informed of the necessary precautions needed to comply to the proposed environmentally safe use. This includes products containing active substances that have been banned in many high income countries, such as the non-selective insecticide diazinon that can be applied on a wide range of crops (<https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/>) and was driving toxicity in most of our sampling sites.

Adaptation of $\text{SPEAR}_{\text{pesticides}}$ to East African streams

Effects on the macroinvertebrate community composition were successfully quantified even with the non-modified $\text{SPEAR}_{\text{pesticides}}$ indicator v. 2019.11 developed for Europe. $\text{SPEAR}_{\text{pesticides}}$ values decreased approximately log-linearly with increasing pesticide toxicity when the maximum toxic unit (TU_{max}) ranged from 10^{-4} to $10^{-1.5}$. At lower TU_{max} values $\text{SPEAR}_{\text{pesticides}}$ leveled off, confirming a threshold of 10^{-4} to $10^{-3} TU_{\text{max}}$ below which no effects on freshwater macroinvertebrates have been observed [36].

Reclassification of the families *Coenagrionidae* and *Corixidae* based on toxicity tests with Kenyan species [9] improved the correlation of $\text{SPEAR}_{\text{pesticides}}$ with TU_{max} . We suggest determining whether such a reclassification may increase the link between toxicity and invertebrate community composition also in European streams. $\text{SPEAR}_{\text{pesticides}}$ values from both the European and the adapted East African index were considerably higher in Kenya than those observed in Central European streams [37], particularly when pesticide toxicity was low. We

speculate that in East African streams, the natural proportion of non-vulnerable taxa may be lower. Indeed, macroinvertebrate communities in most Central European streams are dominated by amphipod crustaceans of the genus *Gammarus sp.*, and by mayflies of the *Baetis rhodani / vernus* group [50]. Both groups are considered non-vulnerable to pesticides and often contribute to more than 50% of individuals. In contrast, amphipod crustaceans were missing in Kenya, confirming previous observations e. g. from Elias et al. [51]. Additionally, we classified mayfly families observed in Kenya as vulnerable (confirmed by a strong decrease with increasing pesticide toxicity, and consistent with most other, less abundant European mayflies). Hence, $SPEAR_{pesticides}$ values in East Africa are associated to higher levels of pesticide toxicity than similar $SPEAR_{pesticides}$ values in Europe, and we adapted the link between the East African $SPEAR_{pesticides}$ index and TU_{max} accordingly.

Using the East African $SPEAR_{pesticides}$ index, the measured pesticide toxicity (TU_{max}) could be predicted with a precision of 1.3 orders of magnitude in each of the streams. This variation covered not only uncertainties in the $SPEAR_{pesticides}$ approach, but also in the measurement of pesticide concentrations and in their conversion to toxic units. Considering the generally high levels of variability and uncertainty in ecotoxicology, as reflected by an assessment factor of 100 (2 orders of magnitude) in first tier European risk assessment [49], results from chemical analyses and from the application of $SPEAR_{pesticides}$ coincided reasonably well.

Specificity of $SPEAR_{pesticides}$ for effects of pesticides

The overall variability in values of the East African $SPEAR_{pesticides}$ index was explained to 53% by the measured pesticide toxicity, and to 45% by the estimated run-off potential based on catchment slope and width of buffer strips. The estimated run-off potential was also closely associated with pesticide toxicity and thus provides a fast and simple method for the identification of potential sampling sites of interest, and for the verification of calculated $SPEAR_{pesticides}$ values: sites where the estimated pesticide toxicity based on $SPEAR_{pesticides}$ does not fit to the estimated run-off potential may be heavily affected by additional stressors and should be investigated further.

Additional stressors that affected the East African $SPEAR_{pesticides}$ index included carbonate hardness, conductivity and turbidity. All these confounding factors increased with pesticide toxicity along a gradient of different stream types but could be disentangled along a second, independent gradient of increasing water levels. While carbonate hardness, conductivity and turbidity varied with water levels presumably due to erosion

and dilution, the $SPEAR_{pesticides}$ index, pesticide pollution and run-off potential did not, because raised water levels alone do not increase run-off without nearby agricultural fields. Thus, our results show that indeed pesticide toxicity and not confounding factors is driving the $SPEAR_{pesticides}$ index.

In contrast to $SPEAR_{pesticides}$, other bioindicators for the assessment of freshwater quality have not been designed to specifically indicate effects of pesticides [38, 40]. As expected, the BMWP and SASS5 scoring system, therefore, responded to a broader range of stressors including phosphate and sediment pollution that may be associated with oxygen depletion. Similarly, the EPT index and the Shannon index for species diversity most strongly responded to stressors other than pesticides.

Our application of $SPEAR_{pesticides}$ is not the first attempt to establish or apply bioindicators for the assessment of freshwater quality in East Africa [28, 52, 53]. These case studies illustrate the growing interest in the use of bioindicators for freshwater monitoring but did not explicitly consider effects of a specific stressor such as pesticide pollution. Apart from our study, the only application of $SPEAR_{pesticides}$ in sub-Saharan Africa we are aware of has been described in Malherbe et al. [22]. The authors applied a previous version of the European $SPEAR_{pesticides}$ index and the Australian $SPEAR_{pesticides}$ index [23] to macroinvertebrate samples from the Crocodile River and the Harts River in South Africa. The sampling sites were located upstream, adjacent to and downstream of two large irrigation schemes. The $SPEAR_{pesticides}$ index decreased with increasing estimated pesticide toxicity ($R^2 = 0.26$) but the correlation was not significant.

It should be noted that Malherbe et al. [22] used a very coarse toxicity estimation based on the sampling site location, assuming that pesticide pollution increases from upstream to downstream of the irrigation system. Toxicity estimation thus did not consider pesticide input from outside the irrigation scheme, whereas our data show that subsistence farming may considerably contribute to pesticide pollution. Therefore, the poor performance of $SPEAR_{pesticides}$ in Malherbe et al. [22] may be partly related to uncertainties in the assessment of pesticide exposure. Additionally, the authors sampled macroinvertebrates from large rivers, whereas $SPEAR_{pesticides}$ has been developed for small to medium streams. When we tested the East African $SPEAR_{pesticides}$ index with a larger data set of unsuitable habitats, our conversion scheme from $SPEAR_{pesticides}$ values to pesticide toxicity turned out to be principally robust despite the small sample size used for development. However, the unexplained variance considerably increased, illustrating the importance to consider that the applicability of $SPEAR_{pesticides}$

is limited to small and medium streams with flowing water and no heavy streambed degradation [21].

Conclusions

As shown, high impact of pesticides on freshwater organisms is not limited to regions with intensified commercial agriculture. Widespread pesticide pollution in Western Kenyan streams and the associated decline in vulnerable macroinvertebrates indicate an ecological risk also in areas dominated by subsistence farming. Potential negative effects on species diversity and on ecosystem services such as leaf-litter degradation and biological pathogen control illustrate the need to improve the risk management of pesticides also in developing countries. Monitoring is essential in this respect to identify hot spots of pesticide pollution for the targeted development of mitigation measures, and to evaluate the effectiveness of actions that have been taken. We adapted the SPEAR_{pesticides} bioindicator for the quantification of pesticide exposure in streams of East Africa. This tool provides a cost-efficient alternative to the complex sampling and analysis of chemicals and thus may facilitate large scale monitoring with limited resources in developing countries.

Abbreviations

BMWP: Biological Monitoring Working Party index; SASS5: South African Scoring System 5; EPT: Ephemeroptera, Plecoptera & Trichoptera index; SPEAR_{pesticides}: Species at Risk index for pesticides; TU: Toxic Unit.

Supplementary Information

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Additional file 1. User guide and supplementary tables.

Additional file 2. Tables with raw data.

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Authors' contributions

ML conceived the approach; AG, FK, UF, FM, BT, WB, ML, HH & JMB designed the research; AG, FK and JMB conducted the research; JMB and AG analysed and interpreted the data; JMB and AG drafted the initial version; AG, FK, UF, FM, BT, WB, ML & JMB contributed to the final version—all approved the final version of the publication. AG: Akbar A. Ganatra; FK: Faith Jebiwot Kandje; UF: Ulrike Fillinger; FM: Francis McOdimba; BT: Baldwin Torto; WB: Werner Brack; ML: Matthias Liess; HH: Henner Hollert; JMB: Jeremias Becker. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its additional files].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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