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Linking ecological health to co-occurring organic and inorganic chemical stressors in a groundwater-fed stream system

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Abstract Freshwaters are among the most endangered ecosystems worldwide, due predominantly to excessive anthropogenic practices compromising the future provisioning of ecosystem services. Despite increased awareness of the role of multiple stressors in accounting for ecological degradation in mixed land-use stream systems, risk assessment approaches applicable in field settings are still required. This study provides a first indication for ecological consequences of the interaction of organic and inorganic chemical stressors, not typically evaluated together, which may provide a missing link enabling the reconnection of chemical and ecological findings. Specifically, impaired ecological conditions – represented by lower abundance of meiobenthic individuals – were observed in the hyporheic zone where a contaminant groundwater plume discharged to the stream. These zones were characterized by high xenobiotic organic concentrations, and strongly reduced groundwater (e.g. elevated dissolved iron and arsenic) linked to the dissolution of iron hydroxides (iron reduction) caused by the degradation of xenobiotic compounds in the plume. Further research is still needed to separate whether impact is driven by a combined effect of organic and inorganic stressors impacting the ecological communities, or whether the conditions – when present simultaneously – are responsible for enabling a specific chemical stressor's availability (e.g. trace metals), and thus toxicity, along the study stream. Regardless, these findings suggest that benthic meioinvertebrates are promising indicators for supporting biological assessments of stream systems to sufficiently represent impacts resulting from the co-occurrence of stressors in different stream compartments. Importantly, identification of the governing circumstances is crucial for revealing key patterns and impact drivers that may be needed in correctly prioritizing stressor impacts in these systems. This study further highlights the importance of stream-aquifer interfaces for investigating chemical stressor effects in multiple stressor systems. This will require holistic approaches for linking contaminant hydrogeology and

eco(toxico)logy in order to positively influence the sustainable management of water resources globally.

1. Introduction

Freshwater ecosystems, integrating the multiple stressors related to urban and agricultural expansion, represent some of the most endangered ecosystems in the world (Strayer and Dudgeon, 2010; Vörösmarty et al., 2010). One of the main challenges has been to understand and identify the primary pressures (stressors) and responses impacting the structure and functioning of stream ecosystems for ensuring the (cost-) effective management of river basins (Feld et al., 2011; Navarro-Ortega et al., 2015). Stressors can be broken down into categories, such as habitat deterioration (poor hydromorphological conditions), invasive species and chemical impacts. Multiple stressors research has either focused on the interaction between constituents belonging to two (or more) of these groups (Townsend et al., 2008) or a larger numbers of individual stressors belonging to only one of these categories (Altenburger et al., 2015). The latter has especially been the case for xenobiotic pollutants e.g., pesticides (McKnight et al., 2015; Schäfer et al., 2012); groundwater contaminant plumes (McKnight et al., 2012; Rasmussen et al., 2016; Roy and Bickerton, 2012); and trace metal contamination (Malaj et al., 2012; Rainbow, 2002), where the goal has been to prioritize stressor impacts to better focus management efforts.

It has become clear that a better understanding of stressor interactions is critical for the evaluation of ecological status in stream systems subjected to multiple stressors (Haase et al., 2013; O'Hare et al., 2015; Schäfer et al., 2016). A review by Ward (2016) revealed that interdisciplinary hyporheic research has been successful in the study of individual processes, but our understanding of coupled, interacting processes – especially in the stream-aquifer interface – is still insufficient. A recent study by Schäfer and Piggott (2018) has shown that studies rarely exceed "two-stressor interactions", due to the need to otherwise control experimental conditions which are, however, far from the conditions in field-scale ecosystems (Heugens et al., 2001). So far, the translation of laboratory (ecotoxicology) results to real stream systems remains quite uncertain (Artigas et al., 2012; Stark et al., 2004), due in part to the complex interplay of hydrology and ecology found in the field (McKnight et al., 2015; Peralta-Maraver et al., 2018), and considering the number of confounding factors that may alter the toxicity of a chemical or mixture once it has been released into the environment (Heys et al., 2016). In any case, the crucial role xenobiotic organic chemicals play in defining ecological impairment is increasingly recognized at the local (Lopez-Doval et al., 2010), regional (Beketov et al., 2013) and global scales (Stehle and Schulz, 2015), and should therefore be weighted as a stressor as important for stream ecosystems as eutrophication and habitat degradation (Malaj et al., 2014; Palmer et al., 2010).

Mixed land use stream systems are commonly found in many industrialized countries with intensive agriculture (Ding et al., 2016; Stutter et al., 2007) and are thus a global phenomenon. The sources for chemicals entering these systems comprise both (i) diffuse pollution of the stream through e.g. surface run-off, tile drains or groundwater-stream water interaction; and (ii) point source discharge from e.g. wastewater outlets and polluted groundwater discharge from contaminated sites (Rasmussen et al., 2013; Sonne et al., 2017). To date, only few studies have addressed the potential for ecological impact from groundwater sources of chemical contamination (McKnight et al., 2010; Rasmussen et al., 2016; Roy et al., 2018). This despite the well-known role of groundwater exchange affecting surface water ecology by e.g. sustaining stream base-flows, moderating water-level fluctuations, providing stable temperature habitats, and supplying nutrients and inorganic ions (Hayashi and Rosenberry, 2002). Contaminated sites are potential sources of a large variety of compounds that can be found in groundwater, including chlorinated solvents, gasoline constituents, pharmaceuticals, inorganic macro-components and trace metals (Essaid et al., 2015). Notably, the level of metals in groundwater-fed streams can also vary significantly based on the local geology and active geogenic processes (Naik and Hammerschmidt, 2011), and presence of metals such as arsenic can be further induced by shifts in redox conditions that can occur due to degradation within a contaminant plume (Burgess and Pinto, 2005; Ghosh et al., 2006; Cozzarelli et al., 2016).

Assessment of stream water quality is generally evaluated by comparison to environmental quality standards (EQS) (Sonne et al., 2017) and supplemented by application of ecological indicator tools such as Toxic Units (Rasmussen et al., 2015; Wolfram et al., 2012). Especially macroinvertebrates have been used traditionally to quantify ecological impairment of streams through multiple decades (Rosenberg and Resh, 1993; Sandin and Hering, 2004), whereas studies using meiobenthic invertebrates as bioindicators are scarce (Höss et al., 2011; Höss et al., 2017). However, groundwater contaminants typically enter streams through the streambed, and many hydrophobic pesticides and polycyclic aromatic hydrocarbons (PAH) exhibiting potentially high toxicity to aquatic (non-target) organisms can be found bound to fine, adhesive sediments or particulate matter (McKnight et al., 2015; Stutter et al., 2007), where meiofauna are dominating the benthic invertebrates (Lopez-Doval et al., 2010; Patrício et al., 2012; Wolfram et al., 2010). Moreover, meiobenthic organisms, primarily residing in the upper sediment layers, may better reflect potential impacts of contaminated groundwater inflow or exposure to bound-phase chemicals than macrofauna and thus be a valuable supplement to the traditional bioindicators in providing a more robust overview of the ecological status (Wolfram et al., 2012). Although, to-date, much less experience exists for meiofauna regarding biomonitoring, these small organisms have proven to be suitable indicators for chemical stress (Hägerbäumer et al., 2015; Hägerbäumer et al., 2017; Höss et al., 2011; Höss et al., 2017).

In a previous ecological study of the stream, Rasmussen et al. (2016) showed that changes in macroinvertebrate community composition (i.e. abundance and taxonomic diversity) were strongest for sediment-dwelling taxa at sampling sites situated in the primary inflow zone of the contaminant plume compared to those at upstream control sites and sites situated along a downstream dilution gradient. This impairment could not be identified using any of the macroinvertebrate indices applied in their study, nor could the response patterns be related directly to the chemical contamination. It was therefore recommended to investigate benthic meioinvertebrates as the macroinvertebrate data was very sparse, and a more robust dataset using the more stationary communities in the stream could be beneficial in assessing impacts especially from contaminated groundwater impacting streams. Notably, their study focused solely on the contribution of groundwater contaminants originating from the factory site entering the stream (bed sediment was not included), and did not investigate other potential chemical interactions occurring in groundwater, or other contaminant sources and chemicals typically found in mixed land use stream systems. Moreover, Sonne et al. (2017) investigated the stream from a purely chemical point of view, assessing the chemical quality of the bed sediment in addition to the stream water and hyporheic zone throughout the catchment. Although this paper presented a holistic view of the chemical contamination dynamics, they did not directly link to ecological status. Instead, they applied a common approach (i.e. toxic units: TU) for predicting the toxic potential of known chemical data, but concluded that it was not possible to make a complete assessment as many of the detected chemicals (e.g. pharmaceuticals) did not have experimental toxicity data (e.g. LC50 values) from which TU can be calculated.

This study also takes a holistic approach towards evaluating the complex chemical contamination dynamics; however, this is carried out in conjunction with a direct assessment of the ecological quality along a mixed land use stream corridor. It contributes particularly to improving our understanding of coupled interacting processes that are related to the presence of multiple chemical stressors and their potential to impact ecological health. The purpose of this study is therefore to evaluate and where possible link the chemical and geochemical data collected in previous studies at this site, with impacts to the ecological health of the stream. The assessment of the stream in this study has, in addition to macroinvertebrates, been extended to include and assess the suitability of benthic meioinvertebrates as a bioindicator for chemical pollution in the urban part of the catchment. The specific objectives of this study are therefore to: (i) statistically group stream sampling stations – subdivided into the identified compartments according to their contaminant and biogeochemical similarities; and (ii) analyze linkages between macro- and meioinvertebrate community structure and supporting ecological indices to the chemical quality and biogeochemical conditions. We additionally examine the value of characterizing whole community assemblages on various taxonomic levels (meiofaunal groups; nematodes genera) compared to specific stress-indices based on single taxa (e.g. nematode community; NemaSPEAR[%]-index) for quantifying ecological effects of organic and inorganic stressors co-occurring in a mixed land use stream system.

2. Materials and methods

2.1 Study site and datasets

The investigated stream corridor (Fig. 1) represents a mixed land use catchment located in western Denmark where both agricultural and urban activities are present, including two large contaminated sites: an old chemical production facility (factory) and a landfill (Sonne et al., 2017). The study site represents a typical lowland groundwater-fed stream with a median flow of 2000 L/s, and depth and width ranging from 1-2.5 m and 8-12 m, respectively (Balbarini et al., 2017). An extensive chemical analysis of the water quality in the stream (SW), hyporheic zone (HZ) (as a proxy for groundwater contamination) and bed sediment (BS) was conducted in the period from 2012-15 (Rasmussen et al., 2016; Sonne et al., 2017).

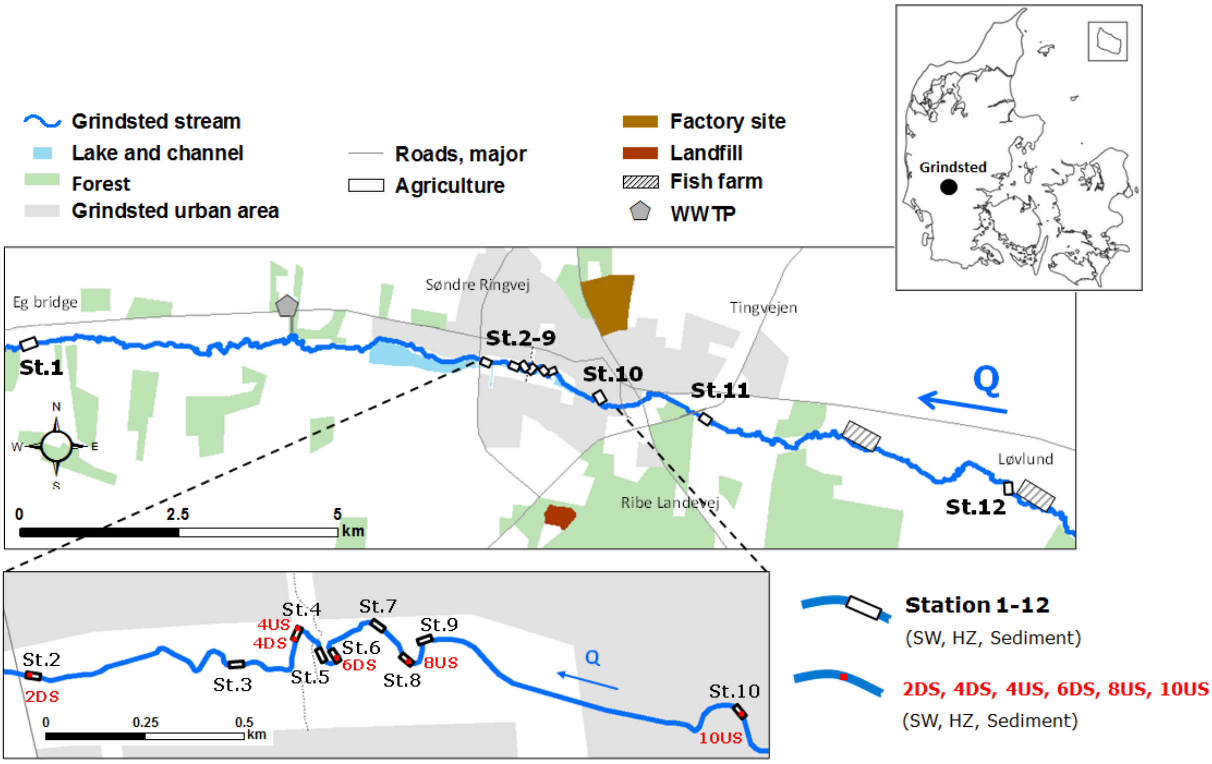


Fig 1. The investigated corridor along Grindsted stream indicating land use distribution, including Grindsted factory, Grindsted landfill, Waste Water Treatment Plant (WWTP), and two fish farms (note their sizes are magnified). The stream flow direction, Q, is illustrated with a blue arrow. Locations are also shown for the (i) twelve macroinvertebrate sampling stations, sampled in April 2014 (rectangles labelled St. 1-12), and the (ii) six meioinvertebrate stations (red rectangles labelled 2DS, 4DS, 4US, 6DS, 8US, 10US), sampled in August 2014. For further station descriptions, sampling and labelling see section 2 and SI Fig. S1. Figure modified from Sonne et al. (2017).

This study is based on data collected from twelve sampling stations covering a 16 km stream corridor. The density of sampling stations was higher in the central part of the stream corridor, where urban sources and inflow of contaminated groundwater from the factory site are expected. Overall, the datasets cover information comprising 167 stream water samples, 54 hyporheic zone water samples, 18 sediment samples, 12 macroinvertebrate stations and 6 meioinvertebrate stations, as described in more detail below.

2.2 Sampling of macro- and meioinvertebrates

Macroinvertebrate samples were collected in April 2014 at each of the twelve sampling stations (labelled 1-12 in Fig. 1) using a 500-cm^2 Surber sampler modified for sampling in larger streams (Rasmussen et al., 2016). Each station represented a 50 m reach, where twelve surber-samples for each station were collected as shown in SI Fig. S1B (green triangles). The samples were preserved in the field using 96% ethanol.

Meioinvertebrate samples were collected in triplicate in August 2014 at a subset of the macroinvertebrate stations (specifically 2, 4, 6, 8, 10). The sampling areas encompassed 5 x 5 m^2 positioned within the 50 m (macroinvertebrate) reach (see SI Fig. S1) where the streambed was dominated by sand and mud, as shown in SI Fig. S1C (green circles). A piston drill with an acrylic glass tube (6 cm diameter) was used, according to Statzner and Beche (2010). The upper 5 cm of each core was then placed in a container and preserved using 4% formalin.

To better account for the station size variability between the April (50 m reach) and August (5 x 5 m^2) sampling campaigns and the location restriction for meiofauna, we subdivided the 50 m reaches into an upstream (US) and downstream (DS) component when evaluating the August meioinvertebrate data, in order to enable a better overview of the extent of spatial variation present in the biogeochemical data. In total six meioinvertebrate sampling stations were investigated, as shown in red in Fig. 1 (lower inset): 2DS, 4DS, 4US, 6DS, 8US and 10US. Comparison between meio- and macroinvertebrate samples is justified through the low seasonal variability in community composition of groups of permanent meioinvertebrates (e.g. nematodes) (Traunspurger et al., 2015). For an overview of the taxon list for macro- and meioinvertebrates, see SI Tables S1-3 (note that data for the individual macroinvertebrate analysis (before pooling) is available upon request).

2.3 Sampling and chemical analyses of sediment and water

At each of the 12 (macroinvertebrate) sampling stations (50 m reaches), 36 streambed sediment sub-samples were collected in April using a kayak corer as shown in SI Fig. S1B (gray circles shown in triplicate), where the upper 2 cm of each core was then pooled into one sample for chemical analysis (SI Fig. S1B). The dominating substrate types (i.e. sand, gravel and organic matter) were then determined for each (sub-) station (see also SI Table S1). In addition, 12 sediment sub-samples were taken at each of the meioinvertebrate stations, evenly distributed across the 5 x 5 m^2 (12 gray circles, SI Fig. S1C), and pooled into one sample for chemical analysis.

Each sediment sample was analyzed for grain size distribution, total organic carbon (TOC), selected metals, and pesticides including relevant metabolites. Notably, the grain-size analysis of the streambed sediment collected from the meioinvertebrate stations resulted in a median grain size (at 50% cumulative mass) ranging from 0.35-0.42 mm equivalent spherical diameter (d) and a coefficient of skewness (d at 60% / d at 10%: d60/d10) of 1.6-2.3, indicating exceptionally similar habitats for each of the six meioinvertebrate stations.

Stream water samples were taken in well-mixed areas of the stream and from the hyporheic zone (40 cm sediment depth) according to McKnight et al. (2012), as shown in SI Fig. S1B-C (blue and red circles, respectively). All samples were analyzed for general water chemistry, trace metals and xenobiotic organic compounds according to McKnight et al. (2012) and Rasmussen et al. (2016). The xenobiotic compounds selected from previous studies included chlorinated ethenes, petroleum hydrocarbons (BTEX,N), pesticides and pharmaceuticals (see list of analytes in SI Table S2).

2.4 Geological and hydrogeological conditions at stream stations

The stream is well connected with the upper Quaternary, sandy aquifer indicated by similar variations in groundwater and stream level (Balbarini et al., 2017). Local inflow to the stream was investigated by use of head differences between stream and aquifer (measured in piezometers), hydraulic properties and temperature profiling along the stream reach (Rasmussen et al., 2016; Rønde et al., 2017; Sonne et al., 2017). The streambed consists of postglacial freshwater sand (medium to coarse), single gravel grains and stones, peat, plant remains and wood pieces. Thus, local variation of groundwater inflow could be documented within the 50 m long macroinvertebrate stations, comprising the observation of both upward and downward flows as well as variations in the flow itself. The streambed at the (smaller) meioinvertebrate stations consisted mainly of postglacial freshwater sand with very similar grain size distribution (as described in section 2.2). This indicated comparable hydraulic properties, which was supported by the flow rates during water sampling in the hyporheic zone. Furthermore, temperature profiles in the streambed indicated inflow in the vicinity of the stations such that, overall, similar hydraulic conditions are expected at the six meioinvertebrate stations.

2.5 Assessment of hydromorphological conditions at stream stations

The Danish Habitat Quality Index (DHQI) was used to assess the physical habitat quality for all twelve sampling stations (Pedersen et al., 2006). The DHQI assimilates quantitative assessments of 17 physical parameters, integrating measures for channel form and riparian characteristics (reach scale), and substrate characteristics, vegetation, and flow pattern (habitat scale). All habitat scale parameters are based on assessments conducted along 10 transects positioned with 5 m intervals along the 50 m stream reach. The DHQI index score represents the sum of scores for all variables and ranges from -12 to 63.

2.6 Characterization of macro- and meioinvertebrate communities

The invertebrates were sorted, counted and identified to the lowest possible taxonomic level, which was species/genus for most of the taxa of the macroinvertebrates, phylum/family for the meioinvertebrates and species/genus for the nematodes (SI Tables S3-5, respectively). Number of taxa (S), abundance (N: individuals per m^2), species richness (Margalef's richness: D_{Marg} = (S-1)/Ln(N)), Evenness (Pielou's Evenness: J' = H'/Ln(S) and Shannon (Shannon's Diversity Index: H' = $\Sigma(p_i$*Ln(p_i), p_i = fraction of species i) were calculated for each sampling station for both macroinvertebrates (SI Table S6) and meioinvertebrates (Table 1). The NemaSPEAR$_{genus}$[%]-index, a matrix based on the nematode community sensitivity to chemical-induced changes in meioinvertebrate communities, was calculated for the meioinvertebrate samples according to Höss et al. (2017) (SI Table S7).

2.7 Statistical methods and data treatment

Principal Component Analysis (PCA) was used to group and characterize sampling stations based on chemical composition of water from the stream and hyporheic zone (depicted as stations 1-12 for April data; note the addition of US/DS to the number for August data). All data were 4th root transformed and normalized (if needed) before the PCA and cluster analyses were performed. Ordination techniques, such as PCA, can be combined with cluster analyses to obtain a better interpretation of the ordination diagrams (Ramette, 2007). Here, hierarchical cluster analysis (HCA, SIMPROF $p<0.05$) was predominantly used to display linkage results and help identify stations belonging to the same clusters. Where HCA was not optimal, as the focus was on concentrations and not specific compounds, subjectively identified clusters were then compared using one-way ANOVA on PCA coordinates (Bonferroni t-test or Kruskal-Wallis on ranks, $p<0.05$) to test for significance between the station groupings. For both compartments, detected xenobiotic organic compounds were investigated; for the hyporheic zone, redox sensitive parameters, plume indicators and trace metals were also explored.

The taxonomic composition of macro- and meioinvertebrate communities was further analyzed using nonmetric multidimensional scaling (nMDS), in combination with HCA superimposed on the nMDS ordination (SIMPROF $p<0.05$). In order to emphasize the presence of rare taxa, abundance data was fourth-root transformed before similarity matrices were built using Bray-Curtis similarity and ordinations were run 100 times. For macroinvertebrate data, analyses were performed considering taxonomic groups for all sediment types, as well as for single sediment types (e.g. sand and organic matter) for the macroinvertebrates (SI Fig. S2). The individual groupings for the sediment-dwellers and for the insect orders Ephemeroptera, Plecoptera and Tricoptera (EPT) were also investigated (SI Fig. S3A-B). Meioinvertebrates and nematodes (genus composition) living on fine streambed sediment were analyzed separately. The statistical analyses were all performed in PRIMER (Clarke and Warwick, 2001).

3. Results and Discussion

Previous studies of Grindsted stream showed that the general water chemistry of the SW was minimally impacted by nutrients and had good stream water quality throughout the investigated stretch in all campaigns (Sonne et al., 2017; Rasmussen et al., 2016). In conjunction with limited pesticide findings in rural parts of the stream, this indicated that the agricultural impact was surprisingly small in this mixed land use system. On the other hand, trace metals of both geogenic and anthropogenic origin were found along the entire stream stretch (Sonne et al., 2017).

The urban part of the study area is impacted in both the SW and HZ compartments by several sources, including a contaminant plume with pharmaceuticals and chlorinated ethenes (CAH) originating from the former factory (Rønde et al., 2017; Sonne et al., 2017), which motivated us to focus a substantial part of the study along this part of the stream stretch. In this study, the physical habitat quality (DHQI) was found to be very homogenous ranging from moderate to good conditions (scores ranging from 15-43) for all sampling stations (1-12), except at station 11 where the stream was heavily channelized (DHQI score = 1; see SI Table S1). Thus, station 11 was excluded from all subsequent invertebrate community data analyses, as poor hydromorphology is known to mask impacts from other stressors, particularly in the case of chemical stressors (Rasmussen et al., 2011).

3.1 Evaluation of chemical and biogeochemical patterns along the stream corridor

Principal components 1 and 2 explained >95% of the data variance for organic xenobiotics detected in the HZ in April (Fig. 2A) and August (Fig. 2B) where concentrations generally decreased with increasing PC1 axis score, and two significantly different station groups were identified. One group contained those stations where no to very low concentrations of organic xenobiotics were detected (stations 1-3, 5, 7, 9-10, 12 in Fig. 2A; stations 2DS, 4DS, 6DS, 8US, 10US in Fig. 2B) in contrast to the other group where significantly higher concentrations were detected in the HZ (stations 4, 6, 8 in Fig. 2A; station 4US in Fig. 2B). For specific concentration ranges, see SI Table S8. Groundwater-born contaminants were found to be the primary drivers for the differences in chemical compositions between the two groups, as the same station groupings were found when all detected compounds were included (compare Fig. 2A with SI Fig. S4A, and Fig. 2B with SI Fig. S4B). Note that no significantly different groups were identified based on the trace metal concentrations alone (data not shown), as the levels were similarly high throughout the investigated stream corridor as reported in Sonne et al. (2017).

The clustering of sampling stations according to organic xenobiotics in the HZ differed slightly between investigations in April and August. Only station 4US remained highly contaminated (compare Figs. 2A-B), while stations 6 and 8 changed from a high to a low contamination level (see category ranges, SI Table S8 footnote) in August (6DS, 8US). These temporal changes may be influenced as a result of spatial variability in the inflow pattern of contaminated groundwater occurring at scales finer than the 50 m reach investigated in April, see section 2.4. The temporal variation related to the contaminant plume entering Grindsted stream, however, could also have affected the chemical contamination level at the site scale. The observed heterogeneity of xenobiotic concentrations in the hyporheic zone was further supported by the visualization of all HZ data collected from 2012-15 in Grindsted stream (see SI Fig. S5 for an overview of the spatial variation for vinyl chloride). In fact, each of the identified discharge zones for the contaminant plume(s) (comprising stations 4, 6 and 8) were found to be quite narrow (<50 m), with high spatial and temporal variations in the composition of chemical compounds.

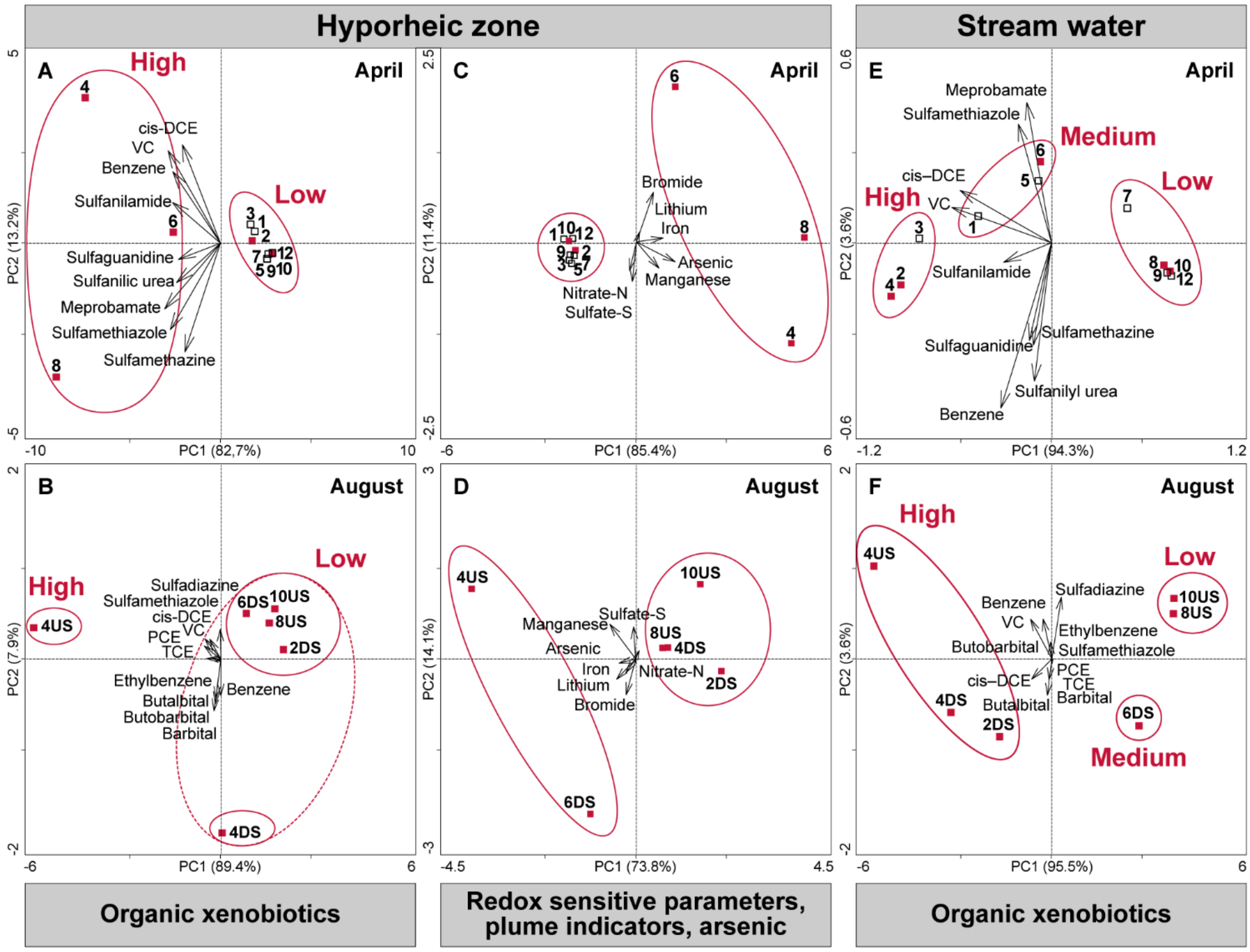


Fig. 2. PCA using the dissolved concentrations for the group of xenobiotic organic compounds (chlorinated ethenes, BTEX, pharmaceuticals) detected both in SW and HZ, and redox sensitive parameters (nitrate-N, manganese, iron and sulfate-S), including plume indicator compounds (bromide, lithium) and arsenic, detected in the HZ in April (A, C) and August (B, D) 2014. (E-F) PCA for dissolved organic xenobiotics in the SW for April and August 2014, respectively. Sampling stations are shown as squares. Stations investigated in April repeated in August are shown as filled (red) squares. Solid red circles indicate clusters that differ significantly from each other, where for sub-figures (B-D) and (F) the clustering was found by HCA (SIMPROF, $p<0.05$), while in (A) and (E) it was found using one-way ANOVA ($p<0.05$; F-value=33.8; degrees of freedom (DoF)=1 and

F-value=119.1; DoF=2, respectively) on PCA axis 1 scores. For organic xenobiotic concentration ranges (low, medium and high) in HZ and SW, see SI Table S8. Note that (B) additionally indicates the station grouping using the chemical concentration ranges (see footnote to SI Table S8) as a guideline (dashed red circle), which was determined by HCA using detected HZ concentrations in April 2014 (see Fig. 2A and SI Fig. S4A).

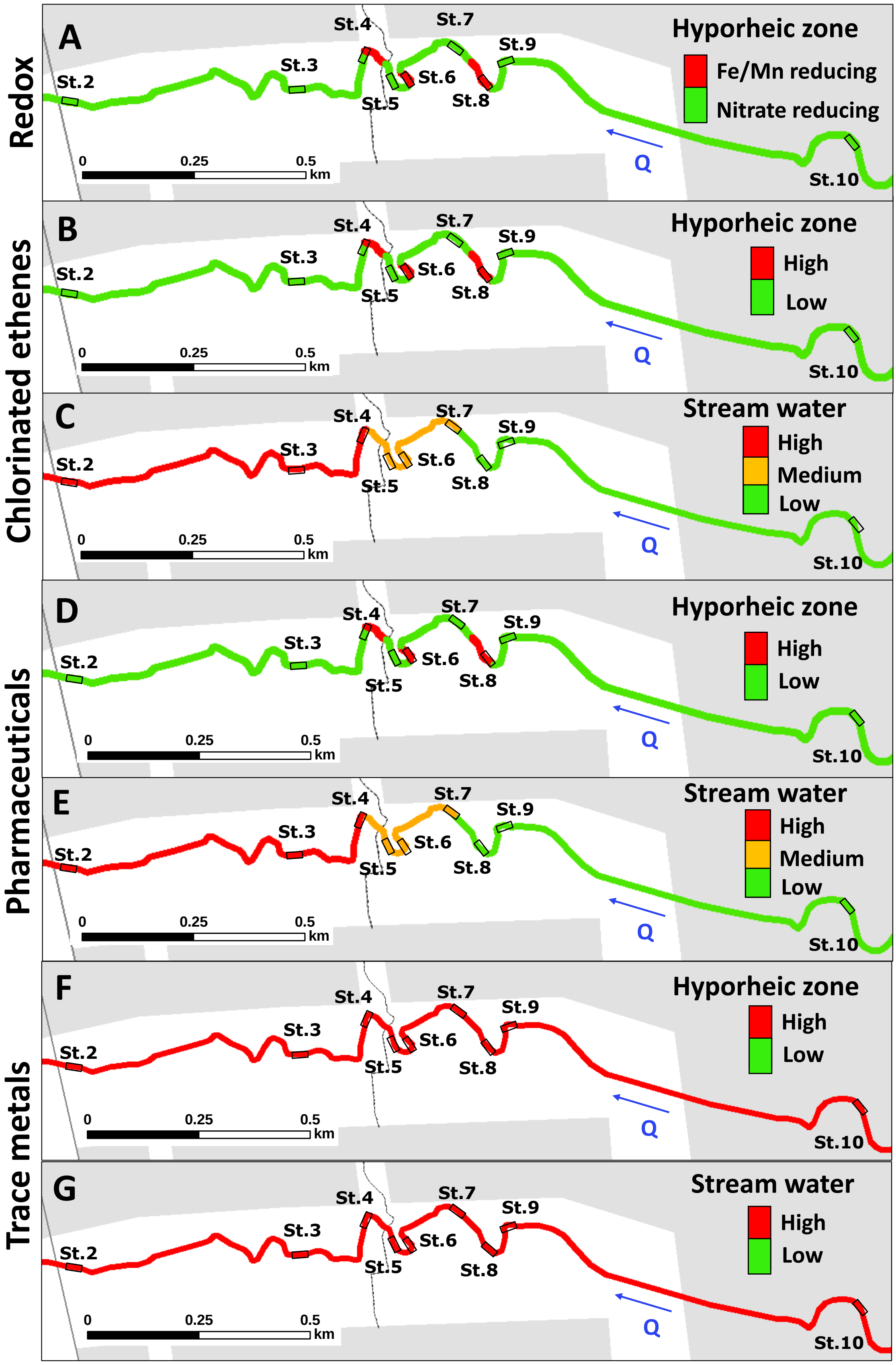
The PCA analysis based on redox-sensitive parameters, including specific factory plume indicators (i.e. bromide and lithium, pointing to the origin of the contaminants) and arsenic in the HZ explained 87-96% of the variance (Figs. 2C-D), with stations separating along PC1 according to the presence of specific compounds, where high concentrations of iron and manganese (representing strongly reduced conditions) grouped together with high concentrations for bromide, lithium and arsenic as opposed to high concentrations of nitrate-N and sulfate-S (less reduced conditions). Two statistically significant groups of sites could be identified based on HCA (SIMPROF, $P<0.05$).

Specifically, the HZ samples from stations 4US and 6DS showed a striking similarity in their strongly reduced redox conditions, characterized in particular by high concentrations of dissolved iron in conjunction with low dissolved nitrate-N and sulfate-S compared to the remaining four stations (SI Figs. S4-5). These two stations also had increased concentrations of bromide and lithium, as well as elevated concentrations of arsenic. Arsenic release might have been caused by the reduction of iron hydroxides, as shown for an active iron-reducing environment found in a hydrocarbon contaminated plume (Burgess and Pinto, 2005; Ghosh et al., 2006; Cozzarelli et al., 2016). Thus, prevailing redox conditions present in the HZ of 4US and 6DS (Fig. 2D) in August are not in accordance with the station clustering based on chemical composition (compare Fig. 2B), indicating the redox conditions resulting from the degradation of the plume constituents are more dynamically stable in the plume discharge than the specific contaminants (noting that stations 4US and 4DS are within the plume discharge zone). Similar differentiation of the discharge characteristics in the hyporheic zone was also found with the April data (data not shown). The areas in the stream characterized by a high probability of strongly reduced water in the HZ are shown in Fig. 3A. Note that average values were used in the PCA, shown in Fig. 2C, to enable a comparison with the large macroinvertebrate sampling stations, each representing a 50 m stream stretch.

In SW, the PCA analyses for organic xenobiotics could explain 97-99% of the variance for April (Fig. 2E) and August (Fig. 2F), where concentrations generally decreased with increasing PC1 axis score. Here, three significantly different groups (Low: 7-10, 12; Medium: 1, 5-6; High: 2-4 in Fig. 2E; Low: 8US, 10US, Medium: 6DS; High: 2DS, 4DS, 4US in Fig. 2F) were identified, except for when all compounds were included for August (SI Fig. S4D). For specific concentration ranges, see SI Table S8. Notably, this clustering of stations remained identical for both April and August despite the temporal variations present in the composition of the chemical mixture observed in both SW and the HZ (SI Table S9).

Overall we were able to statistically group stream sampling stations according to their contaminant and biogeochemical similarities. The significant PCA groupings of the stream stations showed a distinct spatial distribution for the xenobiotic organic groups, re-created in Figure 3 as concentration levels in stream sections for the CAHs, pharmaceuticals, and trace metals (see SI Table S8 footnote for the definition of the concentration level categories). For contaminants originating from the groundwater plume, i.e. CAHs and pharmaceuticals, concentrations changed from low (e.g. < 20 µg CAH/L) up to 4500 µg CAH/L over short distances in the hyporheic zone (see Fig. S2). Redox conditions in the HZ water of the groundwater discharge zones were also found to be strongly reduced, as reflected by the high dissolved iron and manganese concentrations (Figs. 3A, S2). This pattern in contaminant concentration changed in the stream water as a function of distance to the discharge zones of the groundwater plume (Figs. 3C, E; Figs. S6C-D). Although the trace metals did not show any significant grouping, the detected concentrations for Al, Cu, Pb, Ni and Zn all exceeded the 90% quantile for Danish stream water in SW and HZ (1.5 µg Al/L, 2.5

µg Cu/L, 0.63 µg Pb/L, 2.0 µg Ni/L, 14 µg Zn/L; (Boutrup et al., 2015; Sonne et al., 2017) and were therefore characterized as high (Fig. 3F-G; red).


Redox
Chlorinated ethenes
Pharmaceuticals
Trace metals
A
Hyporheic zone
Fe/Mn reducing
Nitrate reducing
B
Hyporheic zone
High
Low
C
Stream water
High
Medium
Low
D
Hyporheic zone
High
Low
E
Stream water
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0.5
km

Fig. 3. (A-G) Spatial biogeochemical quality and contaminant distribution along a 2.5 km enlargement (covering stations 2-10, where both macro- and meioinvertebrate stations are co-occurring) for detected chemical compound groups for both the hyporheic zone and stream water based on the PCA analyses. The data shows the trends along Grindsted stream for the (A) redox conditions, (B-C) chlorinated ethenes, (D-E) pharmaceutical compounds and (F-G) trace metals: Al, Cu, Pb, Ni and Zn.

3.2 Linking chemical quality to ecological impacts

The nMDS plot of the meioinvertebrate community revealed three significantly different groups of sampling stations (SIMPROF $p<0.05$) (Fig. 4A). Station 2 separated out on the basis of low diversity (Table 1); a trend also observed for the macroinvertebrates (SI Table S6) and nematodes (SI Table S7). This can be explained by the fact that this station was characterized by deviating in-stream parameters (e.g. absence of emergent/submergent vegetation and other physical variations of the streambed) compared to the other sites (SI Table S1). This is further supported by a high NemaSPEAR$_{genus}$-value (55.7%; SI Table S7) indicating no significant chemical stress (Höss et al., 2017). Similar results were found by Dunbar et al. (2010) on the macroinvertebrate communities, indicating that such in-stream parameters may be critical factors driving invertebrate diversity (Townsend and Hildrew, 1994).

Notably, the meioinvertebrate community structure was similar at stations 4US and 6DS due to a low overall individual density (Table 1). The same pattern was observed for the genus composition of nematodes, albeit the grouping was not statistically significant (SI Fig. S6). These results are consistent with the station grouping based on the redox sensitive parameters (and plume indicators and arsenic) in the HZ (compare Fig. 2D), but are not in accordance with the chemical grouping (Fig. 2C) where only 4US separated out from the remaining stations. Low NemaSPEAR$_{genus}$[%]-index values at station 4US (15.8%; SI Table S7) further indicates that the low individual (nematode) densities were caused by chemical stress. No apparent differences between the sampling stations were otherwise seen, i.e. for the species richness, evenness or diversity indices (Table 1).

As previously mentioned, the HZ dissolved iron concentrations were remarkably high (14-21 mg/L, SI Fig. S5) at stations 4US and 6DS compared to the other stations (0.1-6 mg/L). The stream was rich in oxygen and the upper streambed well aerated, expected due to the nature of the sandy bed sediments and high stream flow. Thus alterations in redox conditions and the presence of various iron species (solid, dissolved) with different redox states are likely in the interface between the HZ and the upper streambed (Linton et al., 2007). This is partly confirmed by increased levels of solid iron in the streambed at stations 8US, 6DS, 4US, 4DS compared to station 2 and 10 (Sonne et al. 2017).

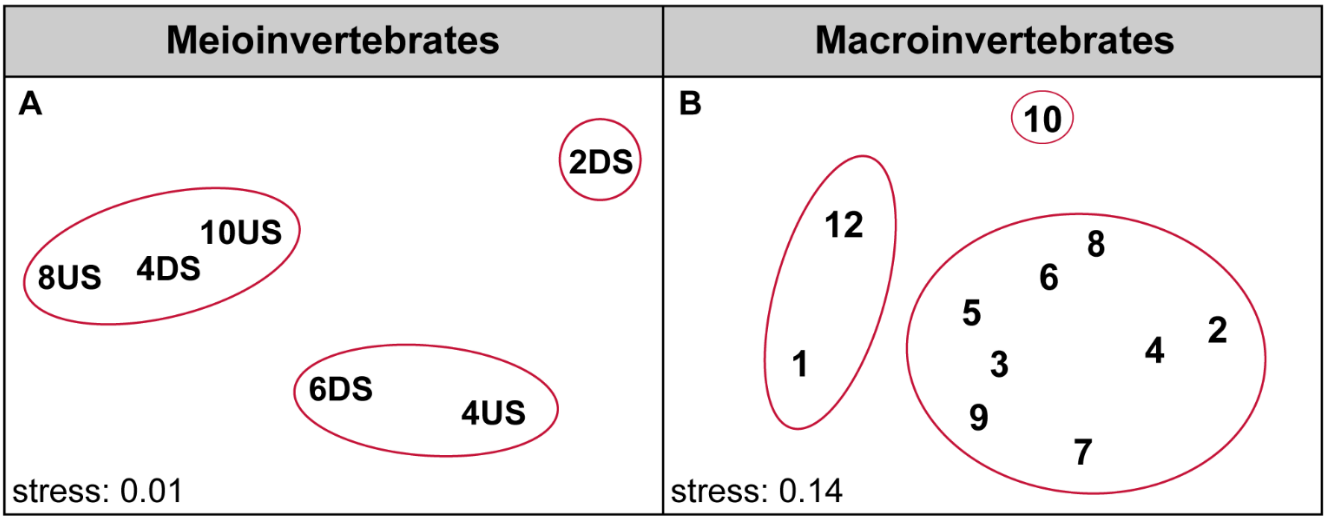

Fig. 4: nMDS plots based on the 2014 data of (A) all meiobenthic taxa at the stations sampled in August (2DS, 4DS, 4US, 6DS, 8US, 10US), and (B) all macroinvertebrate taxa identified for all habitat types (sand, organic matter, gravel) at the stations sampled in April. Circles indicate clusters that differ significantly from each other using HCA (SIMPROF, $p<0.05$). The following meiobenthic taxa were included: nematodes, rotifers, gastrotrichs, copepods, harpacticoids (adults), harpacticoids (nauplii), phyllopods, annelids, chironomids, tardigrades, mites, diptera, ciliates, ephemeroptera.

Table 1: Abundance (individuals/100 cm^2) and diversity indices for meiobenthic communities, including number of taxa, Margalef's richness (D_{Marg}), Pielou's Evenness (J') and Shannon's diversity index (H').

Station	Ind/100 cm^2	No. of taxa	D_{Marg}	J'	H'
10US	1891.2	9	1.14	0.64	1.41
8US	6391.8	12	1.34	0.60	1.50
6DS	529.7	10	1.57	0.62	1.43
4US	506.0	9	1.40	0.54	1.18
4DS	3278.2	9	1.06	0.69	1.52
2DS	2679.0	5	0.54	0.20	0.33

Three significantly different groups of stations could also be distinguished for the macroinvertebrate communities (SIMPROF, $p<0.05$) (Fig. 4B), but these groups were not comparable to groups formed on the basis of chemical or biogeochemical data (Fig. 2). The macroinvertebrate community structure appeared to be controlled more by physical habitat characteristics than chemical stress (compare SI Fig. S2) (Rasmussen et al., 2011). Our results are in contrast to the main findings of Rasmussen et al. (2016), which we believe to be due to the subtle differences in sampling locations between the two field campaigns in combination with the substantial spatio-temporal variability in chemical parameters within the groundwater discharge zone (see SI Fig. S5).

Species richness and diversity indices were yet again similar at all sampling stations (SI Table S6), also for the EPT insect orders and sediment dwellers (SI Table S10). However, substrate stability is most likely low in Grindsted stream due to fine and coarse sand dominating the bed sediment in combination with relatively high discharge, which can confound links to groundwater-born contamination and density/diversity of sediment-dwelling macroinvertebrate taxa (Townsend and Hildrew, 1994). In contrast, the meioinvertebrates are ubiquitous components in these rather unstable stream systems, as they reside in the deeper and more stable parts of the BS (Brunke and

Gonser, 1999). The higher mobility of most macroinvertebrate species and their low affiliation with the hyporheic environment compared to meioinvertebrates may therefore render macroinvertebrates less susceptible to contaminants entering streams systems from groundwater, since this reduces the risk of exposure to excessively high concentrations of chemicals in the hyporheic zone.

We therefore suggest that the observed impairment of meioinvertebrate communities – in particular reduced abundance of meioinvertebrates at stations 4US and 6DS – is a result of the groundwater plume contaminants, heavy metals of anthropogenic/geogenic origin and the strongly reduced redox conditions. In particular, the following mechanism for interaction seems to be crucial: xenobiotic-contaminated groundwater causes reduced conditions (iron-reducing conditions) in the subsurface; dissolution of iron containing minerals enhances dissolved iron (and other elements, e.g. arsenic); this leads to increased availability and thus higher exposure concentrations for benthic organisms. This is supported by studies of iron and toxicity properties in freshwater systems indicating that both precipitated and dissolved phases can have direct and indirect effects on invertebrate communities (Höss et al., 2015a; Höss et al., 2015b; Linton et al., 2007; Vuori, 1995; Wellnitz et al., 1994).

This highlights that local biogeochemical conditions in the hyporheic zone may change simple cause-effect relationships in such systems, where the ecotoxicological behavior of chemicals in a mixture is assumed to be predictable using e.g. the concepts of concentration addition (Heys et al., 2016). Hence, attempts to link chemical and ecological impacts stressor by stressor will most likely fail in this system, a finding that is further supported by the results of Höss et al. (2017) indicating no additional benefit could be gained by separating metal and organic pollution when these stressors occurred together. Further research is still needed to separate whether impact is driven by a combined (e.g. cumulative) effect of organic (Houde et al., 2015) and inorganic stressors (de Souza Beghelli et al., 2018) impacting the ecological communities, or whether the conditions – when present simultaneously – are responsible for enabling a specific chemical stressor's availability (e.g. trace metals), and thus toxicity, along the study stream.

4. Perspectives

The eco-response documented in this study strongly indicates that the hyporheic zone represents spatially diverse windows where high impact zones can be spatially quite small (<50 m), resulting here in a significant reduction in abundance of the meioinvertebrate community – an effect that potentially can propagate to higher trophic levels of the stream ecosystems. Thus, we suggest that the inflow of the groundwater plume, a continuous source for multiple organic and inorganic (including reduced redox sensitive species) chemical stressors, had a significant negative ecological impact on invertebrates residing in deeper layers of the streambed sediment. This study further underlines the importance of assessing all three compartments, i.e. stream water, hyporheic zone and bed sediment, combining aspects of the "Hyporheic Corridor Concept"; an approach which allows the integration of spatio-temporal data obtained from different disciplines in a more coherent way (Pacioglu, 2009; Stanford and Ward, 1993), in order to uncover essential patterns driving impacts to ecological health in mixed land use stream systems. It should be noted that the patchy nature of contaminants affecting the hyporheic zone is a challenge for assessing the ecological health of a stream system. Small windows of contaminant discharge can easily be overlooked in field investigations, so a good understanding of potential contaminant sources is crucial in planning and high resolution sampling in the hyporheic zone and stream bed may be needed.

This study furthermore points towards the applicability of meioinvertebrates for the chemical risk assessment of streams, especially in habitats such as soft sediments with high meioinvertebrate abundance. These organisms are important both in benthic and epilithic habitats (Majdi et al., 2017; Pacioglu, 2009; Peters et al., 2007) and therefore may be closely related and sensitive to the chemical status in both the sediment and water column. It should be further noted that our study in case of the meioinvertebrates was limited to the urban part of the stream impacted by the contaminant plume. The full potential of the approach in a mixed land-use systems requires additional work in other stream systems and a broader group of contaminants including agricultural pesticides. However, these organisms are important both in benthic and epilithic habitats (Majdi et al., 2017; Pacioglu, 2009; Peters et al., 2007) and therefore may be closely related and sensitive to the chemical status in both the sediment and water column. The incorporation of the meioinvertebrates could additionally be a way to support the harmonization of tools for the characterization of ecological health, as these taxa are ubiquitously distributed across all ecoregions (Finlay, 2002; Vanschoenwinkel et al., 2008).

This study further highlights the need for a holistic, transdisciplinary approach to evaluate the ecological impacts of chemicals in streams with multiple land use catchments, to help ensure that all co-occurring stressors are properly accounted for in the evaluation of ecological responses. There is a need to understand not only that numerous (individual xenobiotic) stressors are likely present especially in mixed land use stream systems, but that many (potentially competing) factors or circumstances may be at play leading to complex as opposed to simple responses as also discussed in Townsend et al. (2008). So although it could be as simple as needing to identify the "true" dominant stressor impacting aquatic ecosystems, this evaluation may need to come after an assessment of the governing context – revealing key patterns and impact drivers – in order to truly enable an effective prioritization of mitigation efforts. We therefore believe the holistic approach developed for assessing chemical contamination in mixed land use stream corridors by Sonne et al. (2017), and extended here for linking chemical contamination dynamics to ecosystem impacts can contribute to the further development of current management practices and policies.

5. Conclusion

The focus of this study was to investigate and improve the understanding of the presence of multiple chemical stressors and any related ecological impacts in the context of mixed land use stream systems. To determine potential impacts, the chemical quality of both organic (i.e. pharmaceuticals, gasoline constituents, chlorinated solvents and pesticides) and inorganic (i.e. metals, general water chemistry and macro-ions) compounds were assessed in all three stream compartments (SW, HZ and BS).

- Stream sampling stations could be statistically grouped according to both their contaminant and biogeochemical similarities.
- Significant changes in the meioinvertebrate communities were demonstrated, which likely resulted from the additional chemical stress due to the inflow of a contaminated groundwater plume in small windows in a stream corridor. We suggest that the mechanism behind this impact was in part driven by the strongly reduced redox conditions (e.g. high concentrations of dissolved iron and manganese) and associated secondary effects (e.g. increased concentrations of dissolved arsenic) as a result of the degradation of xenobiotic organic compounds occurring in the plume.
- The observed changes in the benthic meioinvertebrate community strongly imply that the continuous inflow from the contaminant plume signifies high impact zones occurring within the hyporheic zone.
- It was not possible to link the evenly distributed, high (> 90% quantile in Danish streams), toxic potentials of the metals in the SW and HZ throughout the investigated stream stretch to changes in community structure of meio- or macroinvertebrates.
- The meioinvertebrate community could be linked to the biogeochemical water quality in the hyporheic zone, which was not possible for the less prevalent macroinvertebrates, probably due to higher exposure concentrations in the deeper layers of the bed sediment. This study

thus provides field evidence emphasizing the importance of including meioinvertebrates to enable a holistic understanding of potential impacts of chemicals occurring in groundwater.

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