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Original Articles

Land use contribution to spatiotemporal stream water and ecological quality: Implications for water resources management in *peri*-urban catchments

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A R T I C L E I N F O

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A B S T R A C T

Climate change and intensifying agricultural production and urbanization are central factors driving the global freshwater biodiversity decline. To design sustainable green transition schemes and support urban planning, a deeper understanding of the numerous interacting physicochemical and biogeochemical processes and their relation to ecological quality becomes essential. This study thus aims to explore links between hydrological regimes and patterns evident for key water quality parameters and benthic invertebrate indicators in a *peri*-urban catchment that has undergone several stream restoration projects. Results indicate significant seasonal variability in discharge and physico-chemical parameters confounding the identification of sources behind detrimental impacts on ecological quality, which may lead to the implementation of inappropriate mitigation strategies. Notably, sampling at the sub-catchment level underlined the dynamic contributions of both agricultural and urban-like areas for nitrogen and phosphorus, while non-volatile carbon was mainly exported from agricultural lands. Multivariate statistical methods were used to classify benthic macro- and meioinvertebrate (specifically nematode) taxa showing poor-to-moderate and poor-to-good ecological quality, respectively. Poor ecological quality was mostly found in the upstream part of the catchment, driven by a combination of low habitat quality and periodically impaired physico-chemical conditions (e.g. dissolved oxygen, temperature, and suspended solids). In addition, the nematode-based stress index NemaSPEAR[%] (expressing the proportion of species-at-risk within a sample and specifically sensitive to the chemical contamination), indicated a TSS-related transport of contaminants to the sediment. It could also reveal both the negative impacts of different urban features (low ecological quality just downstream of combined sewer overflows), as well as the potential benefits of wastewater effluents (i.e. good ecological quality, via well-treated flow contributions and limited fine sediment accumulation especially in summer) on the stream ecosystem. Our results highlight that the use of this indicator, in combination with high frequency monitoring are promising techniques to better link the dynamic impacts of land use and spatiotemporal changes in ecological quality.

1. Introduction

Rapid urbanization has fueled the development of *peri*-urban landscapes, which consist of heterogeneous patchworks of urban, rural and natural areas resulting in the eventual interconnection of initially distinct urban centers (Sonne et al. 2017; Lemaire et al., 2020; Piorr and

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Ravetz, 2011). The diverse human activities active in these systems can have profound impacts on the surrounding water resources, especially rivers and streams as evidenced by their high rates of biodiversity decline (Reid et al., 2018; Strayer and Dudgeon, 2010). These trends continuously accelerate, despite increasing focus on the need for systemic approaches for the sustainable management of water resources integrating human and natural dimensions (Carnohan et al., 2020; Voulvoulis et al., 2017).

Good hydromorphological characteristics (flow conditions and physical habitat quality) and the absence of multiple anthropogenic stressors, as typically indicated by traditional water quality parameters (e.g. biological oxygen demand (BOD), oxygen and ammonium; Rasmussen et al., 2013), are necessary conditions for a healthy and well-functioning freshwater ecosystem. Therefore, heavy legislative focus has been placed on evaluating these parameters in a first assessment of stream environmental conditions, together with determining the ecological state by Biological Quality Elements (BQE) targeting these specific aspects, e.g. with benthic flora, macroinvertebrate or fish-based metrics (Poikane et al., 2020).

However, these aspects of stream health may mask or be comingled with impacts from other stressors such as chemical contamination, so far understudied and relevant for impairments of ecological quality (Birk et al., 2020; Schäfer et al., 2016). Characterizing the impacts of chemicals on ecological quality is a challenging and expensive task, considering the high number of potential harmful substances released in the environment, and resulting range of complex mixture effects (Brack et al., 2017). Consequently, including additional ecological measures that can help to understand the link between the chemical and ecological status, or to act as a preliminary and integrative evaluation for xenobiotic chemical stressors is extremely relevant.

To this end, the NemaSPEAR[%]-index (nematode-based bioindicator; Brüchner-Hüttemann et al., 2021; Höss et al., 2011; Schenk et al., 2020) has already proven useful for identifying chemical impact zones across the stream-aquifer interface (Sonne et al., 2018) and is seen as a robust tool to assess chemically-induced changes in sediments. Indeed, the "good" status of a water body will be strongly dependent on the conditions in the sediment compartment (McKnight et al., 2015; Rasmussen et al., 2015; Schweizer et al., 2018), as both compartments are closely related through benthic-pelagic coupling (Baustian et al., 2014; Zhang et al., 2021). Thus, a sediment compartment in a poor condition will affect important ecosystem functions (e.g. nutrient cycling), and sediment-associated contaminants may be continuously transferred into the water phase either through remobilization processes or trophic transfer.

Along with the potential impairment of water quality parameters comes a dynamic component, whose effect on ecological quality is arduous to quantify (Birk et al., 2012; Ryo et al., 2019). Notably, water drained from *peri*-urban catchments exhibits large spatiotemporal variations in quality, stemming from their mixed land-use characteristics, associated pollution sources and multiple pathways (Ivanovsky et al., 2016; Lemaire et al., 2020; Sonne et al., 2017), in addition to local weather patterns and general catchment-specific attributes (e.g. topography or geology) (Guo et al., 2019; Lintern et al., 2018). While effects of different land-use types on hydrology and water quality are well documented in these catchments (e.g. Braud et al., 2013; Jankowfsky et al., 2014; Singh et al., 2020), as well as on water quality and ecology (e.g. Berger et al., 2017; Gücker et al., 2006; Jonsson et al., 2017), few studies focus on their co-mingled effects and specifically their dynamic aspects (but see e.g. Jackson et al., 2021). This may be due to the fact that water quality is sometimes used interchangeably with ecological quality (as pointed out in Heal et al. (2020)), leaving the larger interface spanning hydrology-water quality-ecology understudied, resulting in a more limited picture of the complex spatiotemporal dynamics at stake in these systems.

This paper explores the seasonal variability (from spring to winter) present in a stream system heavily influenced by anthropogenic modifications affecting the hydrological cycle, and its potential link to ecological quality. Notably, this catchment has been the focus of ecological restoration efforts spanning > 10 years as part of an early Nature-based Solution (NbS) strategy (Naumann and Davis, 2020; Nesshöver et al., 2017). It thus represents a relevant study in the growing body of NbS literature, with respect to documenting changes (or lack thereof) to ecological quality after NbS implementation that has also focused on promoting biodiversity-enhancing features.

The objectives of this study were thus to: (i) assess seasonal variation patterns for key physico-chemical parameters and macronutrients (C; N; P); (ii) examine the governing mechanisms affecting water quality considering both hydrological and physico-chemical properties and (iii) link ecological quality revealed by two ecological bioindicators (benthic macro- and meioinvertebrates) with the various land-use features and aforementioned variations. Finally, we want to outline future directions for monitoring based on the application of traditional and novel ecological indicators. This study aligns with the UN sustainability goals 6 (Sustainable management of water and sanitation for all) and 15 (Protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss) further emphasizing the importance of these topics.

2. Materials and methods

2.1. Catchment description

The *Usserød Stream* catchment is located on Sjaelland, Denmark, 25 km north of Copenhagen (Fig. 1). The catchment size is 120 km^2 and the geology in the area is representative for the region, i.e. a sequence of clay tills containing sand lenses, which lies on top of a Danien limestone aquifer currently used for water supply with abstraction wells located close to the watercourse (Supplementary Information (SI) Fig. S1). The climate can be described as warm-summer humid continental (Dfb, Köppen-Geiger classification) with an annual average temperature of 8.8 °C (extreme temperature range [-11.4 + 30.2] °C - Station sjælmark; DMI, 2022). The annual average precipitation in the area is 850 mm (Station 56.22; DMI, 2020). The mean imperviousness for the urban part of the catchment (defined as the sewer catchment areas of the 3 existing wastewater treatment plants (WWTP) is ca. 34 % and 8 % for the overall catchment (NOVAFOS, 2020; CLMS, 2022a).

The stream has its origin in *Sjael Lake*, where the inflow is controlled via an automatic sluice located on the northwest side, and flows ca. 8 km before merging with the *Nivå Stream* and then discharging into the *Baltic Sea*. It is typical for a *peri*-urban system, with a mix of different land-use activities (Fig. 1). The southern region of the catchment is mostly urban (22 % of the catchment land-use), whereas the northern region includes the two largest tributaries *(Donse Stream* and *Nivå Stream)* and is dominated by agricultural activities (57 % of land-use). The remaining land is mostly natural-like areas (21 % of land use), e.g. secondary forest or transitional woodlands, and will be designated as forest hereafter (CLMS, 2021; Fig. 1). The urban areas of this catchment are drained by both separate stormwater and combined sewer systems. Effluents from three WWTPs and treated groundwater from one water supply facility contribute to the flow, and two low-head dams break the stream continuity.

2.2. Ecological and chemical status of the stream system

Monitoring activities in the catchment have shown that some of the physico-chemical conditions with potential consequences for ecological quality were below the local target guideline values: high water temperature (>21.5 °C) during the summer season (e.g. June-August), and low dissolved oxygen (DO) concentrations (daily average < 6 mg/l) (Iversen et al., 2011; Krüger, 2011; Rudersdal, 2018). The source of the stream, the heavily eutrophic *Sjael Lake* exhibiting impaired physico-

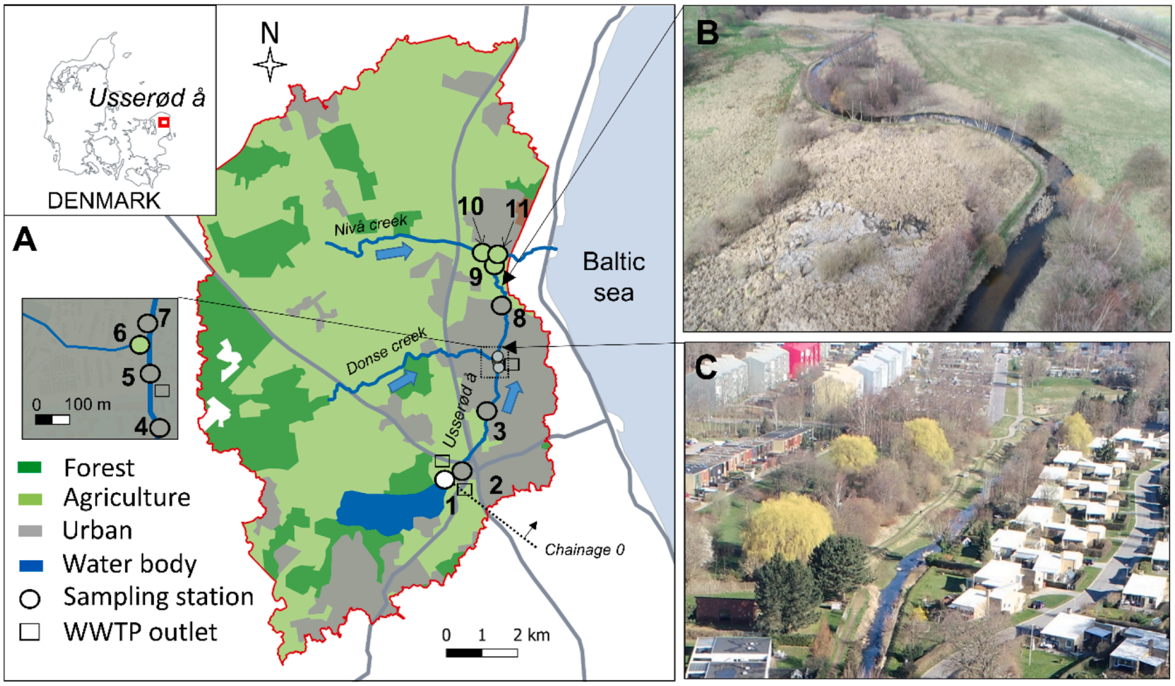

Fig. 1. (A) *Usserød Stream* catchment location in Denmark, including the locations for the 11 sampling stations (St.) and the key land-use features in the area (CLMS, 2021). Streamflow direction is from south to north. Note the sampling station markers are colored (green or gray circles) to denote their predominant land-use type in the corresponding sub-catchment (except station 1, white circle, with a relatively equal proportion of different land use types, has been considered as "mixed"), and stations 4 to 7 are visible in the insert. B-C: Aerial photos showing the stream flowing through the agricultural and urbanized areas, respectively (photos courtesy of F. Bandini).

chemical conditions, is also suspected to be an important degradation factor.

In terms of ecological quality, the *Usserød Stream* remains challenged to reach the required "good" ecological state, despite several restoration projects spanning > 10 years as part of a NbS solution (Hagerup and Pallesen, 2016; SI Table S1). These restorations focused on the physical conditions of the stream channel and urban flood reduction, with reach scale re-meandering for change in flow dynamics, dam removal for flow continuity, de-culverting and creation of double-profile sections. Using the benthic macroinvertebrate bioindicator (Danish Stream Fauna Index score (DSFI); Skriver et al., 2001), moderate ecological quality (DSFI = 4) was generally documented for 16 sampling stations spread along *Usserød Stream* (see Fig. 1, SI Table S2 for details). Notably, the DSFI has been capped at a moderate level for>10 years at most sampling stations along the stream (Iversen et al., 2011; Miljøstyrelsen, n.d.). Ecological quality based on fish (Danish Fish Index for Streams for community composition and juvenile density (DFIS a/t, respectively); Kristensen et al., 2014) ranged from bad (e.g. DFIS = 1, 7 stations, for data comparable to this study's sampling locations) to good (i.e. DFIS = 5, 1 station; see SI Table S2 and Gørtz & Schultz (2020) for details). The underlying causes hampering the attainment of a good ecological quality remain unresolved. The chemical status of the stream, as assessed using the legal framework defined by the European Water Framework Directive (WFD; EEA, 2018) is to this day still unknown (Miljøstyrelsen, n.d).

2.3. Sampling strategy

Measurements were carried out at 11 discrete sampling locations along the stream and two major tributaries from October 2018 to October 2019 to evaluate a general seasonal pattern (see sampling period, parameters and acronyms in SI Table S3). The mean streamflow for the overall measurement period is representative of the flow normally observed (mean flow [Oct 18 - Oct 19] = 532 L/s, mean flow [Oct 15 - Oct 18] = 590 L/s; Rudersdal, 2020) and 7 significant (>100 m^3/d) combined sewer overflow (CSO) discharges to the stream were registered during the one-year measurement period (SI Table 4). The locations for the sampling stations along *Usserød Stream* were chosen in order to capture potential or suspected impacts from key land-use features (see Fig. 1). These comprise the lake input (St. 1), three WWTP effluent outlets (two upstream of St. 2 and one upstream of St. 5), various land-use transitions and associated runoff (St. 3, 8, 9), and the (known) CSO outlet (flow diversion from the wastewater primary treatment stage, between bar screen and grit removal, outflow right upstream St. 4). Station 6 and St. 10 characterize the contributions of the *Donse Tributary* and *Nivå Tributary*, respectively. Finally, St. 11 was placed ca. 50 m downstream of the junction of *Usserød Stream* and *Nivå Tributary*. The sampling campaigns were performed in 6–8 week intervals and are seen as representative for the stream baseflow conditions (i.e. sustained flow between precipitation events in this study). Nevertheless, some of the samplings and investigated parameters may have been influenced by precipitation falling<24 h prior to sampling (Oct. 18: 18.2 mm, May 19: 0.8 mm, Aug 19: 2.2 mm, Sep. 19: 12.4 mm). Additional information regarding the sampling stations and related sections can be found in SI Table S5.

2.4. Streamflow and water quality

2.4.1. Data collection and analysis

Stream discharges were measured at all stations using an OTT MF PRO flow meter (mid-section method following the ISO 748 standard), except at St. 1 due to inadequate conditions for this type of measurement (regulated lake outflow). Instead, the flow from an existing monitoring station at the sluice was used (Rudersdal, 2020).

Stream water samples for chemical analyses were collected in duplicates - mid-stream, mid-water column - using a peristaltic pump and placed in 20 mL glass vials. Samples were stored on ice in the field, in the dark, and then at 4 °C prior to chemical analysis within 2 days. Field measurements of temperature, pH, electrical conductivity (EC, normalized to 25 °C; EC_{25}) and DO were taken *in situ* at the same locations using a flow cell and WTW3430 multiparameter probe, calibrated prior to each measurement campaign. Water turbidity was assessed by use of a portable turbidity meter (430 IR LED by WTW, mean value from 3 readings). A detailed description of the chemical analysis and concentration assessment can be found in SI Appendix S1.

2.4.2. Water quality data treatment

The concentrations of some chemical parameters were sometimes below detection values at specific stations and sampling periods and set equal to one-half the detection limit (DL/2, in total 6 values over the overall dataset). Some data for the sampling in October 2018 were missing (Flow, TN and Chl-a) and were therefore not included in this analysis. The various parameters were tested for variance homogeneity and normality by Levene's test and Shapiro-Wilk's method, respectively. EC, TSS, turbidity, NH_4-N, NO_3-N and Chl-a concentrations were consequently log-transformed prior to analysis of variance (one-way ANOVA) for temporal variations. Parameter correlations were investigated using Spearman's rank correlation ρ, both for the overall dataset and by sampling periods. Spatial correlation was suspected between sampling stations and examined by Moran'I test (inverse distance weighting). A cluster analysis (Affinity Propagation clustering; Frey and Dueck, 2007) was used to explore and facilitate the interpretation of some of the variations observed in this multivariable dataset (z-transform standardization of the dataset). The discussion on the parameter variations and possible influence of land-use was supported by an estimate of in-stream mass discharge (Appendix S2). All analyses were performed on the mean values of all duplicates using R software (v.4.1.1; R core team, 2021) and relevant packages (ape 5.6–1, lawstat 3.4, stats 4.1.1, apcluster 1.4.9).

2.5. Stream habitat quality and benthic invertebrate communities

2.5.1. Characterization of physical stream habitat quality

The physical habitat quality was surveyed for all stream stations according to the Danish technical guidance document for the Danish Habitat Index (DHI; Wiberg-Larsen & Kronvang, 2016), described in detail in SI Appendix S3. The DHI index score ranges from −12 to 63, where increasing index scores reflects increasing habitat quality (see SI Table S2).

2.5.2. Macroinvertebrate communities

Macroinvertebrates were sampled in August 2019 at all stations using a standard kick-sampling net (mesh size = 500 μm). Macroinvertebrates were collected using a standardized kick-sampling procedure (Skriver et al., 2001) with sub-samples collected at 25 %, 50 %, 75 %, and 100 % of the stream width along three equidistant transects positioned within the 50 m reach for each station. If riffle sequences were present within the reach, minimum one of the transects was positioned at the riffle. All sub-samples were pooled and conserved in 96 % ethanol in the field.

Subsequently, macroinvertebrates were collected from each sample and identified to species level (Trichoptera, Plecoptera, Ephemeroptera, Coleoptera, Zygoptera, Lamellibranchia, Gastropoda, Hirudinea and Malacostraca), genus (Heteroptera, Megaloptera, Neuroptera, and Lepidoptera), and family (Diptera). However, individuals of Chironomidae were identified to sub-family, and individuals of the genus *Chironomus* were identified to species level.

2.5.3. Nematodes

Sediment samples for nematode analysis were collected in triplicate in August 2019 at the same location where all physico-chemical parameters were sampled. Sampling locations were chosen where the streambed was dominated by fine sand and mud, and samples were taken using a piston drill (6 cm diameter) with an acrylic glass tube, according to Sonne et al. (2018). The upper 5 cm of each triplicate core were pooled together in a container and preserved using 4 % formalin.

After removing large stones and plant residues by rinsing the sediment through a 2-mm mesh, nematodes were separated from the sediment particles using flotation extraction with colloidal silica (Ludox TM50; diluted to 1.13 g/ml) according to Heininger et al. (2007). Nematodes were counted under a stereomicroscope at 20 to 40-fold magnification. For each sample, 100 nematodes were sorted out and prepared in glycerol for taxonomic identification according to Seinhorst (1959). In total, 1200 nematodes were identified under a microscope down to species level (1250-fold magnification).

The NemaSPEAR[%]-index was calculated based on the nematode species composition according to Höss et al. (2017). Classes of ecological status for NemaSPEAR[%]-values were defined in Höss et al. (2017) as: >54 = high; 30 – 53.9 = good; 20 – 29.9 = moderate; 10 – 19.9 = poor; 0 – 9.9 = bad.

2.5.4. Ecological data treatment

Based on the abundances of macroinvertebrate species in each sample, the DSFI score was calculated according to Skriver et al. (2001). The NemaSPEAR[%]-index was calculated based on the nematode species composition according to Höss et al. (2017). A redundancy analysis (RDA) was performed for both the nematode and macroinvertebrate data and selected water quality variables. The water quality dataset was first reduced using a PCA based on the data collected between October 2018 and October 2019 (data standardized to maximum; scaled between 0 and 1). Based on axis scores of plotted data within the first two dimensions of the PCA, the water quality parameters were reduced to two scores (PC1 and PC2; SI Table S6 and S7) for use in the RDA. To optimize the strength of the RDA, environmental data was limited to PC1, PC2, NH_4-N, BOD5, and DHI, where NH_4-N, BOD_5, and DHI were expected to be the single environmental parameters with the highest influence on the invertebrates (note that DHI is expected to exert low influence on the community structure of nematodes, however). Nematode and macroinvertebrate abundances were log10 transformed in order to increase the weight of rare species. In order to interpret RDA results correctly, a hierarchical cluster analysis (HCA) was performed for both the nematode and macroinvertebrate community data (Bray-Curtis similarities). PCA and RDA were all performed in R (v.4.1.1; R core team, 2021) with the relevant packages (stats 4.1.1, vegan 2.5–7), and the HCA was performed in PRIMER (version 6.1.5, PRIMER-E, Plymouth, UK).

3. Results

3.1. Variations in streamflow

Substantial temporal variations in streamflow were observed throughout the year: minimum measured flow of 173 L/s and maximum flow of 1170 L/s at the most downstream sampling station (St. 9) in June and October 2019, respectively. These were driven naturally by precipitation events and primarily by the variations of natural or anthropogenically controlled point inflows, e.g. tributaries, lake outflow and WWTP effluents. Indeed, these point inflows constituted an important part of the baseflow of the investigated stream system for almost all sampling periods, as seen in the steep increases in flow observed at St. 2 (downstream 2 WWTP outlets), St. 5 (downstream one WWTP outlet and St. 7 (Downstream a tributary; Fig. 2), and in their estimated relative contributions (SI Table S8). In June 2019, for instance, the contribution of these point inflows (lake WWTP outlets and tributaries) accounted for ca. 86 % of the overall flow observed at St. 9.

In return, these important contributions underlined that the overall groundwater contribution to the stream was rather limited. This can be seen in the relatively constant flow trend between St.2 and 4 for most of the sampling periods. For the northern region (from St. 7 and downstream), more variations were evident with alternation of losing and gaining sections at different times of the year, probably in connection with variations related to groundwater abstraction operations nearby in the deeper aquifer (SI Fig. S1) and possible modification of the shallow

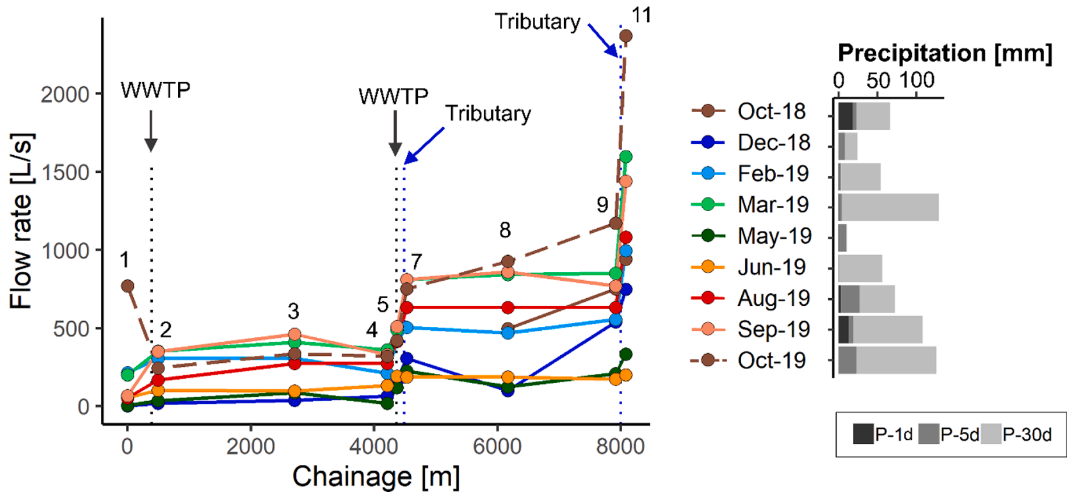

Fig. 2. Streamflow along *Usserød Stream* at the different stations for all nine sampling campaigns. WWTP outlets and tributary locations (inflow points) along the chainage are indicated by black and blue dotted lines, respectively. Accumulated precipitation during the day of the sampling campaign (P-1d), the 5 days prior to the sampling campaign (P-5d) and 30 days prior to the sampling campaign (P-30d) are also given.

groundwater flow field. Other shallow and dynamic inflows cannot be excluded: e.g. shallow terrain groundwater discharge, agricultural drainage or lag time for the samplings carried out after recent rains (e.g. Oct 18) or prolonged wet periods (in Sept - Oct 19).

3.2. Variations in physico-chemical parameters

Significant temporal variations were observed for all physico-chemical parameters monitored in this catchment (one-way ANOVA, $p < 0.05$; Fig. 3), with the exception of TSS. Some parameters (i.e. water temperature, DO, BOD_5, NH_3-N) were found to exceed the available thresholds defined for good ecological status in this catchment for some specific periods (Fig. 3a, b, c, k; Naturstyrelsen, 2014). The stream water temperature was subject to a large seasonal variation as well, with a maximum observed temperature difference close to 25 °C between winter and summer periods (Feb-June) in connection with the aforementioned limited groundwater inflows, but also shallow stream depth and resulting low thermal inertia. These variations directly affected DO saturation and concentration (corroborated by rank correlation $\rho = 0.7$, SI Fig. S2) that followed an inverse seasonal trend. The DO concentrations in the catchment were also affected by the relatively high values of BOD_5 (range [<0.1–10.2] mg/l, SI Table S9) driven by a combination of high NH_4-N concentrations ($\rho > 0.5$ in Feb 19, May 19; SI Fig. S3), settling/degradation of organic material and algae from the lake drifting downstream (see Aug 19, SI. Fig. S4p; $\rho > 0.5$ for 6 sampling periods, SI Fig. S3), low flow velocities (i.e. longer retention times) and higher water temperatures in the summer months.

In terms of macronutrients, PO_4-P concentrations presented a clear seasonal pattern, with significantly higher concentrations during the summer months (maximum value of ca. 0.5 mg/L in May 2019 all periods considered, and mean values > 0.2 mg/L in June and August 19, Fig. 3m). The highest concentrations could be connected to a reduced dilution in summer and significant contributions from the urban effluents (as seen in the concentration peaks often found at St. 2 and 5; SI Fig. S4o and S5). NO_3-N (mean = 1.69 mg/L) and NH_4-N concentrations followed a similar spatial trend, but opposite timewise. The highest concentrations were observed during the sampling in the winter period (e.g. mean NO_3-N concentration in Feb. 2019 of > 3 mg/L, Fig. 3j), possibly caused in this catchment by a reduced nutrient uptake in the stream and reduced removal efficiency from the WWTPs at low temperatures (data not shown). For NH_4-N, however, high concentrations above the defined quality threshold of 1 mg/L were also sporadically captured in the agricultural-dominated subcatchments (Fig. 3k, SI Appendix S2). These subcatchments constituted a major carbon input to this *peri*-urban stream for all sampling periods (Fig. 3h, SI Appendix S2), with maximal NVOC concentrations observed for the spring and autumn campaigns (Mar-Sept-Oct 2019).

Beyond the overall seasonal trend, the variations in flow contributions resulted in variable spatial patterns of water quality in the investigated *peri*-urban catchment (Fig. 4 and SI Fig. S5 and S6). For some periods, the physico-chemical conditions in the southern region were predominantly driven by the stream's source and controlled release from the lake (e.g. pH, DO or TSS for the red cluster; Fig. 4B-C) or by the two WWTP outlets at other periods (e.g. NH_4-N, PO_4-P, green cluster; Fig. 4D). In the northern region, the physico-chemical conditions were mainly driven by the third (and largest) WWTP effluent especially in the summertime (notably *N*-species as previously discussed, blue cluster in Fig. 4B − 4D; water temperature downstream st. 5, SI Fig. S4b), but at other periods by agricultural lands, as seen in the relatively higher concentrations of nutrients (N, P), carbon (NVOC) and TSS sporadically captured downstream these areas (blue cluster, Fig. 4A; Appendix S2 and Fig. S6), and possibly in connection with fertilizer application and recent precipitation events.

3.3. Ecological quality

3.3.1. Benthic macroinvertebrates

The macroinvertebrate community composition changed along the stream continuum from communities dominated by taxa with low sensitivity to low oxygen concentrations and standing water (e.g. *Asellus aquaticus*, *Erpobdella* sp., *Helobdella stagnalis*, and Tubificidae) closest to the outlet from *Sjæl Lake* towards higher frequencies and abundances of taxa with stronger preferences for flowing water and higher sensitivity towards low oxygen concentrations (e.g. *Gammarus pulex*, *Elmis aenea*, and *Baetis rhodani*) (SI Table S10) (Schmidt-Kloiber and Hering, 2015). This shift was also revealed from the HCA with stations 7–11 (furthest downstream) forming a disparate cluster (Fig. 5a). Stations 1 and especially 3 were characterized by low taxonomic richness and overall low abundance, which probably explains the formation of separate clusters for each of the stations (Fig. 5a).

Macroinvertebrate community structure was most strongly correlated with TSS, PC1 (representing mainly *ortho*-phosphate, EC, and pH), and DHI (Fig. 5c). TSS values were generally highest upstream of the first low-head dam (located just after St. 3) possibly due to a combination of limited dilution and load / settling of particles from the lake and overhanging vegetation ($\rho = 0.4$ between Chl-a and TSS, SI Fig. S2).

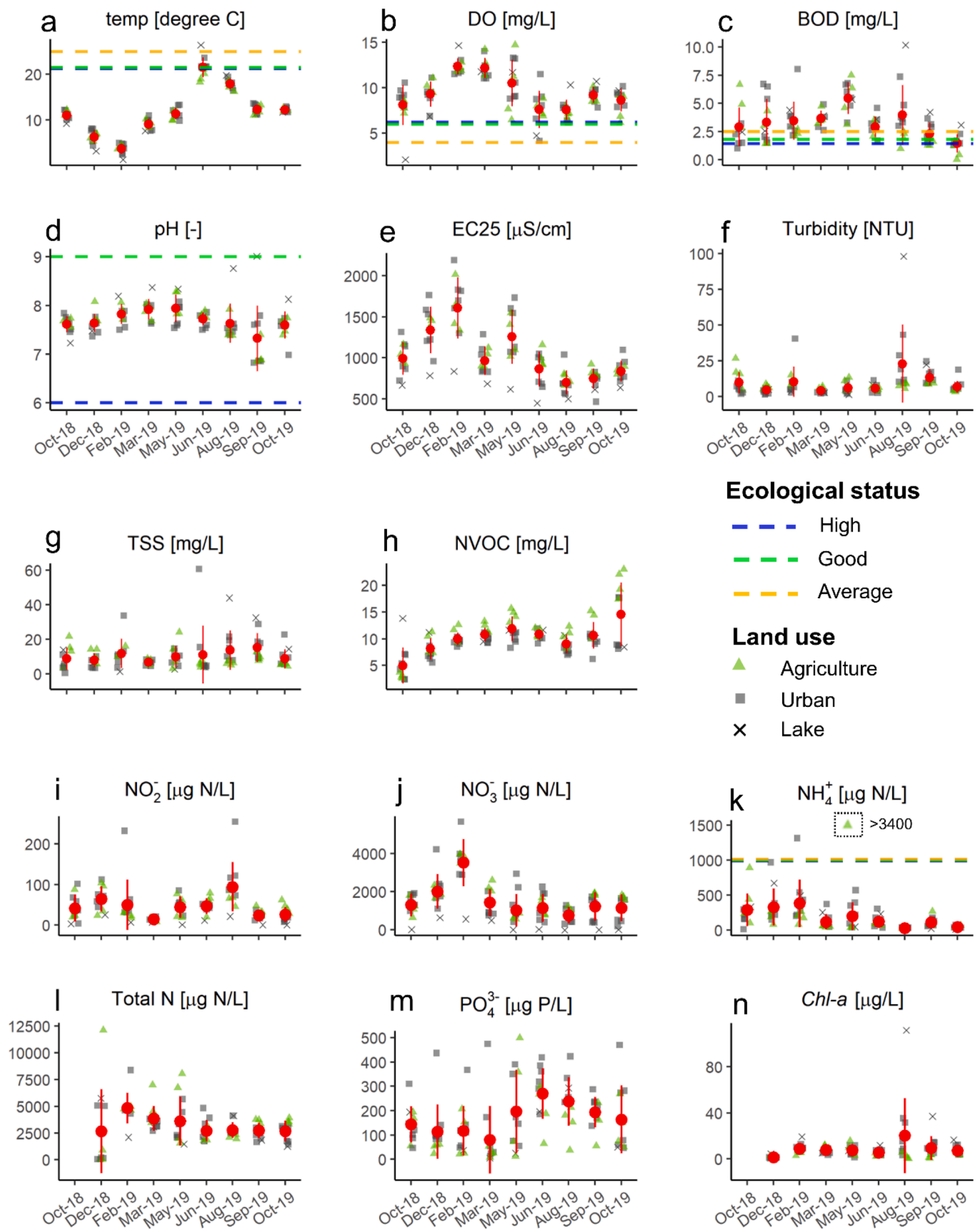

Fig. 3. Temporal variations of the general water chemistry parameters for all sampling stations (N = 11). The different markers represent the dominant land-use in the corresponding sub-catchment (St.1 corresponding to the outlet of the lake is considered as "mixed"). Red dots and vertical bars show the mean value and +/- SD between stations. The dashed lines correspond to available suggested limit values for high (blue), good (green) and moderate ecological status (Naturstyrelsen, 2014).

Considering the low variability in time and space of pH and EC, PC1 should probably reflect mainly *ortho*-phosphate. DHI mainly exerted influence along the first axis in the RDA (Fig. 5c) with highest values (highest habitat quality) towards the left in the ordination space. Stations 1 and 3 were characterized by high TSS and BOD concentrations and low DO (represented by PC2, SI Table S11) while strongly contrasting in habitat quality (SI Table S2), where St. 3 was straight, wide, shallow and dominated by sand and mud in the substrate, and St. 1 was narrow, meandering and with dominant coarse substrate types (data not shown).

Ecological quality mimicked the general differences in macroinvertebrate community composition with moderate-to-bad quality at stations 1–3 and with moderate-to-good ecological quality at the remaining stations (Fig. 5c, SI Table S2).

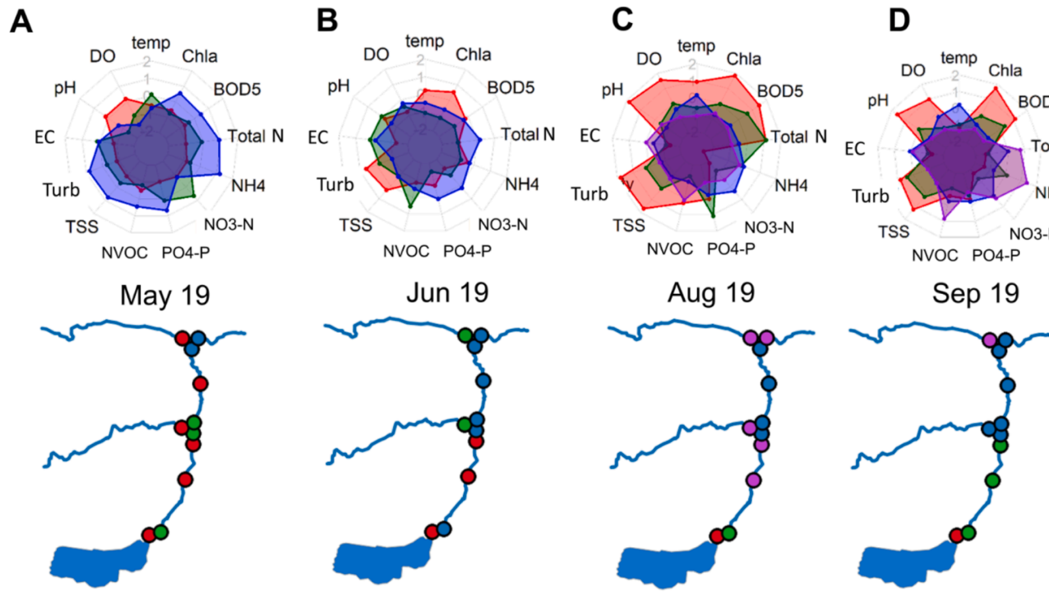

Fig. 4. Station clusters using all general water chemistry parameters (AP clustering, similarity measure r = 1 with number of clusters = 3 or 4). The radar plots show the standardized mean deviation of a specific cluster compared to the overall standardized mean of the dataset for all parameters. The selected periods illustrate the spatiotemporal variations along the stream: (A) strong nutrient (N,P) concentrations in the northern and agricultural part (blue cluster), (B) physico-chemical conditions driven by the lake contribution upstream (red cluster) and by the northern WWTP effluent downstream (blue cluster) during a low flow period, (C) important Chl-a discharge from the lake at its source (red cluster) and effluent-dominated flow downstream the northern WWTP (blue cluster), (D) physico-chemical conditions driven by the WWTPs in the southern part of the catchment (green cluster). Results for the other campaigns can be found in SI Fig. S7.

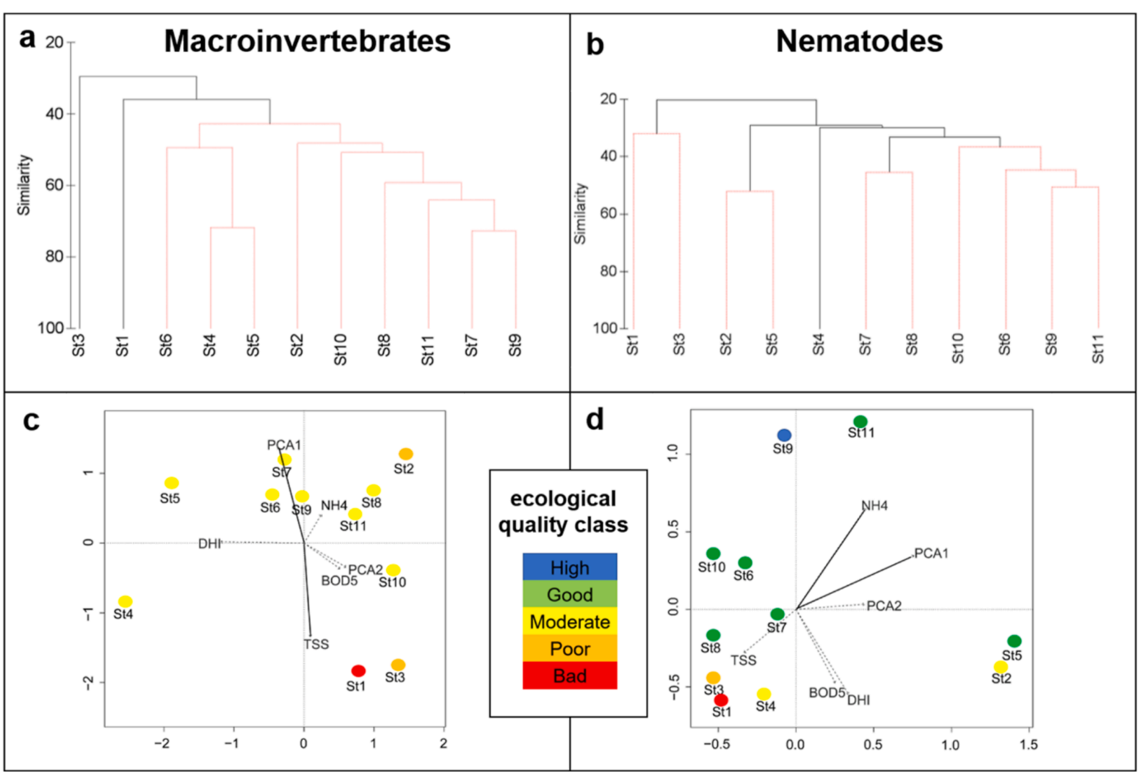

Fig. 5. Bray-Curtis similarities hierarchical clustering based on log(x + 1) transformed data for (a) benthic macroinvertebrates and (b) nematode communities sampled from all 11 stations ($p < 0.05$). (c) and (d) RDA for (c) benthic macroinvertebrates and (d) nematode communites, based on log-transformed absolute abundances of species; color-coding of station symbols corresponds to the ecological quality for the DSFI (c) and NemaSPEAR[%]-index (d); solid, bold vectors represent environmental parameters that were significantly related to species composition ($p < 0.05$; Monte Carlo permutation test).

3.3.2. Nematodes

Nematode communities sampled in August 2019 at the various sites varied considerably, both in terms of total abundances (from 39 individuals (ind.)/100 mL sediment at St. 8 to 907 ind./100 mL at St. 5) and number of species (from 17 at St.1 to 35 at St. 11). Nematode communities were mainly dominated by bacterial feeders (36–77 %), followed by algae feeders (1–44 %) and omnivores/predators (4–27 %). Detailed information on the nematode species composition at the various stations can be found in SI Table S12. The NemaSPEAR [%]-index ranked from 2.8 to 60.9 indicating bad ecological quality at St.1, poor ecological quality at St.3, moderate ecological quality at St.2 and 4, good ecological quality at St. 5–8 and 10–11, and high ecological quality at St. 9 (Höss et al., 2017; SI Table S2).

The cluster analysis according to species composition related well to the NemaSPEAR[%]-values, where stations with poor and bad ecological quality, stations with moderate ecological quality and stations with good and high ecological quality generally clustered together. In terms of species composition, St.1 and 3 were found to be significantly different from all other stations (Fig. 5b), which may be explained by the special environmental physical conditions discussed previously. Moreover, the NemaSPEAR[%] at these stations (2.8 and 16.7, respectively) points to a bad and poor ecological status, respectively. Within the second cluster, there were three significant groupings: St. 2 and 5 (WWTP effluent outlets), with St. 2 showing a moderate ecological status (NemaSPEAR[%]: 27.8). Although the ecological quality at St. 5 is classified as good (35.6), based on the NemaSPEAR[%]-index, the actual value was only slightly above the threshold to a moderate water quality classification ($\geq$30). The second sub-cluster in cluster 2 comprises only St. 4 (CSO outlet). These findings suggest that the NemaSPEAR [%]-index may be sensitive to capturing subtle (temporal) changes stemming from a variety of urban features, including wastewater treatment plant efficiency levels (compare i.e. St. 2 and 5 locations), as well as pulsed events from e.g. CSOs (compare St. 4). Within the cluster comprising St. 6–11 (all stations showing a good or high ecological status: NemaSPEAR[%]: 37–61), St. 7 and 8 (located at the down-gradient end of the urban area) can still be distinguished from the rest ($p < 0.05$). The RDA revealed that the nematode species composition was significantly related with PC1 and NH_4-N ($p < 0.05$; Monte-Carlo permutation test; Fig. 5d). Both parameters seemed to be positively related to the ecological quality (according to the NemaSPEAR [%]-index; as shown by color-coding in Fig. 5d), which might be indirectly driven by potential food-web effects. Indeed, water quality parameters that are dominant for PC1 (phosphate, EC) might have influenced potential food sources for the nematodes. It is known that the nematode species composition is not only shaped by direct effects of abiotic variables, but also by food-web interactions, such as competition and predation (Heininger et al., 2007). In the RDA, TSS and BOD_5 were not significantly related to the nematode community structure ($p > 0.05$; Monte-Carlo permutation test; Fig. 5d). However, the RDA shows that these two parameters, which indicate the transport of (contaminated) particles to the sediment (TSS) and organic pollution (BOD_5), point towards the stations showing a moderate-to-bad ecological quality, as represented by the NemaSPEAR[%] (Fig. 5d; SI Table S2). Notably, in August 2019 (when nematodes were sampled), the highest values for both TSS and BOD_5 were recorded at St. 1 (43.9 mg/L and 10.19 mg/L, respectively), and were influential in the correlations found for both of these parameters (and NOx) with the NemaSPEAR[%] (SI Fig. S8). TSS values were generally above 10 mg/L from St. 1–7, with the exception of St. 5 (7.86 mg/L, probably dilution due to WWTP outflow) and St. 6 (6.36 mg/L, *Donse Tributary* location). Interestingly, BOD_5 doesn't fall below a threshold value of 2.8 mg/L until St. 9 (only station documenting high ecological quality) along the main stem of the *Usserød Stream*. This value was suggested in Baattrup-Pedersen et al. (2016) as a threshold above which the probability for reaching good ecological state (DSFI $\geq$ 5) is estimated to be<20 %, in combination with NH_4-N > 1.25 mg/L). Otherwise, only the tributaries had low BOD_5 values (with St. 6 = 1.87 mg/L; St. 10 = 0.96 mg/L, for Aug. 2019).

4. Discussion

4.1. Hydrology, water quality and land-use influence

Our study shows important statistically significant variations for most of the physico-chemical parameters (except TSS, masked by important spatial disparities), stemming from the multiple flow contributions. Variations in streamflow are seen as a major factor in explaining surface water quality variations, both by activation and delivery of different constituents and by dilution effects (Guo et al., 2019). Notably, small streams are hydrologically more dependent on local and often variable discharges, compared to larger streams or rivers fed by more regional and stable groundwater inflows (Dahl et al., 2007). The investigated stream is characterized by a limited groundwater inflow (Fig. 2), and thus strongly influenced by both the contribution (quantity) and physico-chemical characteristics (quality) of its source (lake and controlled sluice), wastewater effluent outlets, tributaries and runoff from different land-use types. Such a stream configuration is now ubiquitous, with the increase in *peri*-urban landscapes (Allen, 2003).

Discharge and delivery of nutrients and potential eutrophication issues were seen as particularly dynamic (both temporally and spatially), and importantly, could not be explicitly related to one specific land-use. Indeed, nutrient inputs originating either from agricultural or urban subcatchments were found to be dependent on the sampling period. Such variations in water quality and change in land-use contributions have also been documented in e.g. Ivanovsky et al. (2016), facilitated by measurements at much higher frequency, and by Le Moal et al. (2019). WWTP effluents undoubtedly constitute a significant pathway for nutrient pollution (both NO_3-N and PO_4-P) in this *peri*-urban catchment for a high (7 out of 9) number of sampling periods. Wastewater impacts on water quality parameters were found in fact to extend far downstream, even dominating water column composition well after transitions to down-gradient agricultural lands (compare Fig. 4). Fones et al. (2020) and Jarvie et al. (2006) drew similar conclusions about the relative importance of urban areas in terms of nutrient export (at least for PO_4-P). However, more continuous monitoring campaigns should be implemented to fully capture the relative yearly contribution in this catchment. Notably, rain events will induce important flush, leaching and runoff episodes responsible for high loads of suspended solids (and potentially bound contaminants), or nutrients (phosphorus for instance; Fones et al., 2020; Lefrancq et al., 2017).

4.2. Learnings from ecological quality

The ecological quality (benthic macroinvertebrates) was generally below the target levels for good ecological status, despite a series of restoration projects carried out in this catchment (SI Table S1). Regional monitoring of fish communities at the sampling sites (SI Table S2) further supports our results. In a large study covering numerous stream stretches, Karlsen et al. (2019) suggested a minimum natural area requirement of ca. 20–30 % within a given catchment as necessary for reaching good ecological status, and even higher (40–55 %) when only a 10 m riparian buffer zone is considered. These findings could suggest the need for a holistic and catchment-based land-use change and restoration to reach good ecological status (sensu the EU Biodiversity strategy for 2030; European Commission, 2020). Currently, the studied stream system exhibits 21 % of natural-like areas at the catchment-scale, and 38 % within a 10 m riparian zone (the rest being urban areas (29 %), managed grassland / agricultural lands (31 %) and water (2 %); CLMS, 2022b), indicating a need for comprehensive and further restoration initiatives targeting the land–water ecotone along the riparian corridors.

Poor stream habitat quality most likely constitutes an important bottle-neck in terms of improving the current ecological status, while the temporal variations of DO, temperature and BOD_5 levels act as further reinforcing factors. Moreover, the regular and systematic removal of aquatic macrophytes (weed cutting) during the summer adversely affects habitat quality and ecological quality (Bach et al., 2016). Lastly, the entire stream system is generally heavily degraded with most likely no known remaining source populations of benthic macroinvertebrate species that could improve the ecological quality. In fact, the strongest predictor for local ecological quality is the distance to and frequency of significant source populations of sensitive macroinvertebrate species rather than local habitat quality (e.g. Stoll et al., 2016). Consequently, the lack of source populations of sensitive macroinvertebrate species in the catchment may further delay system recovery even in the case of land-use transformation towards higher shares of natural areas in the riparian zones and improved water and habitat quality (e.g. Stoll et al., 2016).

4.3. Meiofauna as an ecological indicator

The investigation of the meiofaunal organisms (nematodes) and associated NemaSPEAR[%]-index revealed that particulates (and transport to sediment) should be taken into consideration when evaluating impacts on ecological quality. The lowest index values were indeed observed for the monitoring stations with the highest TSS concentrations. Suspended particles can act as important transport vehicles for many priority pollutants towards sediments, resulting in increased exposure concentrations for benthic invertebrates. As the NemaSPEAR [%] is specifically sensitive to chemical pollution and not affected by particle grain size per se (Höss et al., 2017), the negative correlation of this index with TSS concentrations, indicate a TSS-related transport of contaminants to the sediment. Specifically, contaminants have been linked to suspended particles in other studies of the catchment (heavy metals, see Kramer, 2020; Ribaucourt, 2019), suggesting that sediments can act as a potential source of toxicants in certain regions of the watercourse. This adds an additional layer of complexity with respect to identifying the causes of unwanted impacts on stream environments, as sediments can provide an exposure pathway to high concentrations of contaminants with no current sources (Munn and Gruber, 1997; Rasmussen et al., 2015; Stackelberg, 1997), and nematodes exposed to this contamination could act as a vector to higher trophic levels (Ptatscheck et al., 2020).

It is notable that the NemaSPEAR[%]-index could separate out distinct urban features, including the two locations for WWTP outflows, a (known) CSO outlet, as well as urbanized land-use areas from agricultural ones. This suggests that NemaSPEAR[%] may supplement traditional ecological indicators in terms of identifying primary causes of degradation and impact. Furthermore, the use of this novel indicator may potentially be useful for not only understanding ecological impacts from key urban features. It also holds the potential of serving as a supporting indicator when assessing the possible benefits from anthropogenic controls in the system that may otherwise be overlooked: the transition towards a green economy advocates the use of centralized WWTPs for energy, efficiency and resource recovery purposes (see e.g., Danish Water Forum, 2016). This would translate practically in this catchment to potential flow diversions from the stream to a more efficient WWTP discharging directly into the *Baltic Sea* (Discussion with the municipalities and NOVAFOS, 2020). These changes will certainly reduce the direct discharge of potential micropollutants from the urban side (not investigated in this study) and nutrients, which will be beneficial for stream ecosystems. However, the nematode indicator additionally seems to highlight a potential trade-off, i.e. beneficial effect of the flow on dilution or limitation of sediment accumulation. These findings are in line with the work by Marttila et al. (2020), warning that the green transition carries risk of unintended consequences that may adversely affect water quality and aquatic ecosystems despite the best intentions.

We therefore consider that this indicator could be useful in developing a deeper conceptual understanding for land-use effects, as well as spatiotemporal changes in biodiversity patterns. Notably, nematode communities can be sampled year-round with the resulting nemaSPEAR index [%] being relatively unaffected by seasonal variations (Brüchner-Hüttemann et al., 2021), a limitation for the other indicators mentioned in this study (e.g. macrofauna; see Reinholdt Jensen et al., 2021). This is a critical gap identified in Rolls et al. (2018), emphasizing that more evidence is needed regarding the effects of hydrological regimes on freshwater biodiversity across multiple spatial and temporal components, to better predict the impact of direct (e.g. water resources development; water quality) and indirect (e.g. climate change) effects of humans on ecology. Moreover, as the NemaSPEAR[%]-index is representative for integrative ecosystem stress, we further propose this indicator could be a way to preliminarily assess the potential for impacts stemming from both sediment contamination (Höss et al., 2011; Schenk et al., 2020; Schenk et al., 2022), groundwater contamination (Sonne et al. 2018) and additional dissolved-phase chemicals (Bighiu et al., 2020) for prioritizing pollution sources that may be active in *peri*-urban catchments.

4.4. Implications for NbS solutions, investigations and monitoring

In light of these results, we recommend a more holistic consideration of the overall stream system (across the flood reduction—human well-being—ecological quality intersection) when designing restoration measures and implementing NbS-type strategies to avoid the potential need (and expenses) related to missed opportunities and thereby ensure the creation of multiple benefits across NbS domains (Viti et al., 2022). In terms of the riparian restoration itself, vegetated riparian areas could bring increased shading with positive effects in terms of water temperature, dissolved oxygen and biodiversity recovery, as well as other services such as nutrient removal (Atkinson and Lake, 2020; Dosskey et al., 2010), and thus care should be taken to ensure multipurpose objectives (human well-being—ecological quality) can be met. The ecological recovery may be inhibited by a lack of source populations, and therefore NbS solutions could potentially be combined with freshwater species reintroduction. Nevertheless, the success of such measures will only be achievable if the stressors causing the initial degradation are removed or mitigated, as highlighted by Jourdan et al. (2019).

It should be noted that we do not question the fundamental logic behind the implementation of nature-inspired and/or carbon-reducing strategies within an urban planning context, as it is expected to have high environmental and socio-economic benefits. However, negative impacts on freshwater ecosystems may arise as a result of these activities, and should therefore be considered during the development and implementation of sustainable green transition solutions and policies to ensure they will be protective also of freshwater ecology.

Moreover, sampling for pollutant and chemical stressors (dissolved or in sediment phase) should be considered further as a potential explanation factor for the plateauing of ecological status for benthic macroinvertebrates and fish. This sampling could be combined with nematode biomonitoring, as a promising indicator for the dynamic contribution stemming from urban pathways. Finally, holistic assessments, considering the hydrology and water quality, and specifically the sediments, should be initiated to ensure green transition modifications under evaluation (e.g. removal of WWTP effluent outflows) and potential trade-offs on freshwater ecology are accounted for.

Finally, the investigations and monitoring suggested can be supported by the deployment of versatile high frequency sensors. The latter can help to better understand the dynamic impacts of the different land-use types in terms of water quality (e.g. nutrient source and pathways in Rode et al. (2016), but also on ecological quality (e.g. potential impact of P-concentration spikes for benthic macrofauna; Fones et al., 2020).

5. Conclusions

In this study, we investigated the temporal and spatial variations of key water quality elements in a small *peri*-urban catchment (baseflow conditions), combined with an ecological "fingerprint" using traditional (macroinvertebrates) and more novel indicators (nematodes). The current study highlights the complexity of *peri*-urban catchments as observed through the spatiotemporal variability of flow and physico-chemical conditions induced by different land uses, as well as their specific impacts on ecological quality. More specifically, we showed that:

- The investigated *peri*-urban stream system is affected by significant seasonal variability in hydrology and water quality parameters, dependent on the dynamic physico-chemical characteristics of the different flow components. Limited groundwater discharge combined with highly controlled point inflows (lake outflow; WWTP effluents) and shallow stream depths caused significant seasonal temperature and oxygen saturation variations, while relatively high BOD_5 and photosynthesis/autotrophic respiration processes exacerbated the low oxygen conditions.
- These significant seasonal variations in the hydrology and water quality parameters confounded the identification of land-use and sources behind detrimental ecological impacts, which may possibly lead to inappropriate mitigation strategies. Our seasonal sampling pattern revealed that WWTP effluents were major contributors to the nutrient levels (N, P) discharged to the stream, sometimes exceeded by agricultural-dominated subcatchments that constituted a primary carbon input (NVOC).
- The poor-to-moderate ecological status for benthic macroinvertebrates can partly be connected to the dynamic land-use related impacts, as well as alteration of riparian corridors, habitat quality, and possibly significant seasonal variations of stream physico-chemical conditions at the catchment scale (especially temperature, DO and high BOD_5). Notably, these indicators stayed relatively unchanged despite restoration efforts and NbS implementation. Potential improvements and required time to document positive shifts in ecological status after implementation of such modifications are still unknown, or may simply be masked by overlooked stressors such as additional chemical compound groups not investigated in this study (e.g. metals; pesticides).
- The ecological assessment carried out using nematodes and the NemaSPEAR[%]-index not only brought light to the potential impact of chemicals, e.g. with contaminant-bound particles discharging from the stream's source (eutrophic lake) and urban features like CSOs, but also the potential beneficial effects from well-treated wastewater effluent flow contribution emanating from the urban sector on the stream ecosystem.

Overall, these results underline that the use of nematodes as an additional bioindicator of chemical pollution, potentially combined with a more systematic use of high-frequency sensors could contribute to a better understanding of the dynamic impacts of land use on *peri*-urban stream water quality and ecology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109360.

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