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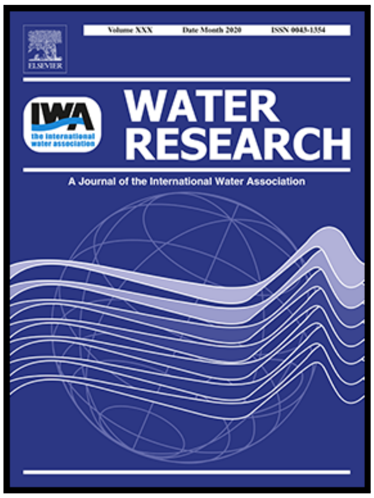

Associations of anthropogenic activity and tributaries with the physicochemical, nutrient and microbial composition of the Ganga (Ganges) River, India

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Highlights

- Physicochemical, nutrient & microbial water quality over 2700 km of the Ganga River
- Water quality shifts consistent with agriculture, industrial and sewage inputs
- Tributary confluences associated with pollution inputs and/or dilution effects
- Better understanding of water quality stressors and controls along the Ganga River

Associations of anthropogenic activity and tributaries with the physicochemical, nutrient and microbial composition of the Ganga (Ganges) River, India

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Abstract

The Ganga River (known internationally as the Ganges) is one of the world's most prominent rivers, running from the Himalayas to the Bay of Bengal and supporting the livelihoods of > 40 % of India's 1.4 billion population. The Ganga River is regionally and globally important, supporting agriculture and industry, yet faces potentially detrimental water quality challenges arising from runoff and discharge from increasing urbanization, industry and agriculture. A ~ 2700 km longitudinal survey of the nutrient and microbial water quality, including phytoplankton composition, of the Ganga River was undertaken in November 2019. The aim was to investigate if and how anthropogenic activities (*e.g.* urbanisation, industry, and agriculture) and tributary convergence (potentially reflecting both human activity and flow influences) affect and shift physicochemical, nutrient, and microbial water quality parameters along the river continuum. Segmented regression identified four zones of distinct nutrient/microbial characteristics along the Ganga River, with breakpoints located near Kanpur, Varanasi and downstream of the Farakka Barage, at distances of ~ 1020, ~ 1500 and ~ 2350 km downstream from the Himalayan Ganga source. Population density, land use and urban cover were associated with selected water quality parameters in parts of the catchment, with elevated nutrient, microbial and chemical concentrations likely associated with agriculture, industry, and sewage inputs. Some urban areas (*e.g.* Kanpur and Varanasi), converging tributaries (*e.g.* Yamuna and Varuna) and barrages (*e.g.* Farakka) were associated with changes in nutrient availability, microbial activity/abundance and modelled discharge, likely driving apparent water quality changes in the relevant locations. Downstream shifts in nutrient and microbial water quality parameters were observed throughout the ~ 2700 km Ganga River continuum. This information can help prioritize locations for targeted monitoring and/or remediation interventions and has illustrated an approach to quantify impacts of anthropogenic inputs on major river systems, such as the Ganga River.

Keywords

Ganga River basin, Nutrient availability, Microbial activity, Breakpoint analysis, Flow cytometry, Water quality.

Highlights

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1 Introduction

The Ganga River (known internationally as the Ganges) in northern India, supports the livelihoods of > 40% of India's population (Sharma et al., 2019), and is one of the world's major rivers, providing essential freshwater resources for approximately 600 million people (Bowes et al., 2020). The water quality of the Ganga River is increasingly threatened by extensive exploitation and a variety of potential pollution sources arising from increasing human activity and environmental stressors, including population growth, urbanisation, industrialisation, irrigation and climate change (Ansari et al., 1998; Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2013; Richards et al., 2023; Wilson et al., 2024). Globally, assessing and monitoring surface waters is complex due to the inherent dynamic and complex nature of river basins. However, there are national guidelines that determine expected water quality regarding geography, geology, uses/applications (i.e. bathing or recreational waters) or input (i.e. wastewaters) (ANZECC and ARMCANZ, 2000; Central Pollution Control Board, 2008; UK Government, 2015).

Evaluating and monitoring the impact of anthropogenic influences such as rapid urbanization (Gu et al., 2021) and agricultural activity (Santy et al., 2020) along the Ganga River and key tributaries is of global importance (United Nations Environment Programme, 2024). Rapid urbanization and industrialisation throughout the Ganga Basin have disrupted hydrological balances and, in some instances, have caused the degradation of wetlands and surface waters (Thambidurai and Dikshit, 2023). The significant role that freshwater systems play in global biogeochemical cycling is particularly important with the increasing challenges of climate change (Biddanda, 2017; Campeau and del Giorgio, 2014; Regnier et al., 2013).

Furthermore, ~ 1.4 million m^3 of untreated domestic wastewater and ~ 0.26 million m^3 of industrial sewage are estimated to be discharged into the Ganga River and its tributaries daily (Natarajan et al., 2016). It has been reported that 37.7 million of India's 1.4 billion population suffer from waterborne diseases such as diarrhoea, resulting in 1.5 million child mortalities annually (Khambete, 2019; Sharma et al., 2023), due to exposure to, or ingestion of, contaminated water sources (World Health Organization, 2022), including rivers such as the Ganga. The release of sewage or other pollutants such as microplastics,heavy metals and persistent organic pollutants from industrial waste has severely degraded the water quality of the Ganga River, causing sections of the river to be unsuitable for potable use (Satyanarayana et al., 2023; Siddiqui and Pandey, 2022; Singh et al., 2024).

Due to the immense scales associated with large river systems (> 2700 km long), effective, comprehensive assessment and monitoring of water quality (especially with regard to longitudinal and temporal variability) is very challenging (Kanuri et al., 2020; Singh et al., 2018; Zhu et al., 2022). Assessing the impact of anthropogenic activities on globally significant river systems often rely on models (e.g. geographic information systems (GIS)) that are developed with, informed by and improved with empirical data (Giri, 2021). Many current water quality monitoring methodologies (*e.g.* real time sensor networks, large-scale manual monitoring initiatives) are challenging to establish and maintain (particularly in a network with many nodes), time consuming, expensive and labour intensive (Patil et al., 2012; Postel, 2015; Santos et al., 2021). Click or tap here to enter text.In part because of these challenges, many previous studies of the Ganga River have been relatively small-scale and/or focus only on a limited list of physicochemical parameters or the Water Quality Index as specified by the Ganga Action Plan aiming to improve river water quality to "bathing class" (Alam, 2010; Central Pollution Control Board, 2013; Chandra et al., 2011; Khan et al., 2016; Sharma et al., 2003; Zafar and Kumari, 2024). Notwithstanding challenging implementation, comprehensive sampling over a range of sources water composition and geographical and geological features is essential in understanding the dynamics of large river systems, and in informing considerations of how vulnerable environmental resources may need to be protected in the face of increasing perturbations. A systematic approach for the first basin-wide longitudinal survey of the Ganga River was recently developed and implemented (Richards et al., 2022), however reporting of the nutrient and microbial composition of water along the river continuum remains an important scientific gap which is addressed in the current study.

A large-scale longitudinal survey along the > 2700 km of the Ganga River continuum, monitored the physicochemical, nutrient and microbial composition. The aim was to investigate how anthropogenic activities (*e.g.* urbanisation, agriculture and industry) and tributary convergence affect downstream patterns in physicochemical, nutrient, and microbial water quality parameters. Study objectives include (i) the investigation downstream trends of individual water quality parameters; (ii) the evaluation of associations with anthropogenic geochemical and microbial indicators; and (iii) determining locations where systematic shifts (referred to as breakpoints) in water quality occur.

2 **Materials and Methods**

2.1 Study area

All field sites were located within the Indian section of the Indo-Gangetic Plain, an area of > 2.5 million km^2 across Bangladesh, India, Nepal, and Pakistan which supports more than 750 million people (Fendorf and Benner, 2016; MacDonald et al., 2016; Mojid and Mainuddin, 2021). The sampling focussed on the main Ganga River and key tributaries in the Upper, Mid and Lower Gangetic Plain within India (Patil et al., 2014), an area hosting 40% of India's population (Bowes et al., 2020). The study area has been previously described in detail (Richards et al., 2022).

2.2 Longitudinal sampling survey

The longitudinal sampling spanned a distance of > 2500 km from the Himalayan foothills at Devprayag (Uttarakhand), at the convergence of rivers Alaknanda and Bhagirathi (where the river takes the name Ganga), downstream to estuarine Noorpur (West Bengal), just upstream of the Bay of Bengal (Richards et al., 2022). This longitudinal survey was conducted in the post-monsoon season where it is expected that there is relatively high connectivity between groundwater and surface water systems and the wider catchment, over a three-week period between 3 – 21 November 2019 (Richards et al., 2022). Sampling sites (n = 81; Figure 1) were located on the main Ganga and Hooghly rivers (n = 64) and key tributaries (n = 17). Sampling was undertaken by three coordinated teams, with overlapping transitional areas for cross-comparison. Sampling sites were chosen to ensure regular sampling intervals (mean interval ~ 30 km) and to capture a range of key potential inputs, whilst ensuring logistical feasibility including river access (Richards et al., 2022). Details regarding the representativeness of this longitudinal survey and sampling approach, relating to both spatial and temporal variations, are provided in (Richards et al., 2022). Sampling sites are named as "XYY" whereby "X" refers to the associated river or tributary (Ganga River (G), Hooghly River (H) or a tributary (T)), and "YY" refers to the sequential numbering system increasing with distance downstream.

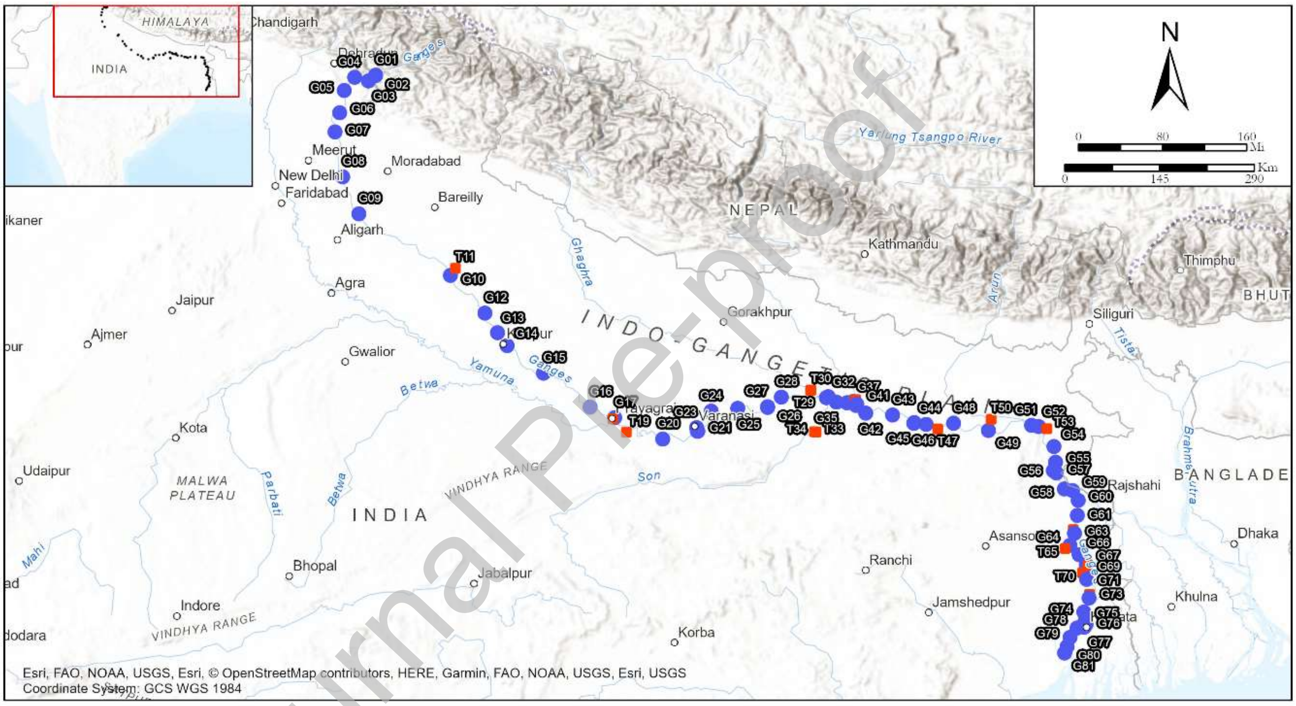

Figure 1: All sampling points (n=81) along the Ganga River, including tributaries. "GXX" in blue refer to sample point along the Ganga River or Hooghly River (n=64), noting the lower Ganga becomes known as the Hooghly downstream of site G55. "TXX" in red refer to tributaries (n=17).

2.3 Water sample collection

Samples were collected from accessible riverbank locations (often located nearby beaches, ghats or shallow sloping banks), using a ~ 20 L vessel. Unfiltered and filtered subsamples were collected for a range of laboratory analyses and stored in acid-washed (20% hydrochloric acid) Nalgene PTFE bottles. Subsamples were processed through 0.45 µm sterile cellulose nitrate membrane filters (Thermo Fisher Scientific, UK) within ~ 5 minutes of sample collection. Additional subsamples were collected and preserved for DNA sequencing by manual filtration into a 0.22 µm Sterivex™ enclosed filter (Millipore, UK), and addition of Zymo DNA/RNA Shield™ (Zymo, UK), as detailed in Bowes et al. (2020). Subsamples for flow cytometry analysis of phytoplankton, were collected as ~ 5 mL of unfiltered sample in a 7 mL Sterilin™ polystyrene bijou (Thermo Fisher Scientific, USA) and fixed in 0.25% glutaraldehyde. All samples were stored in cool and dark conditions (as practicable under Indian field conditions) and transported to the UK for laboratory analysis. All reusable sampling equipment was thoroughly sample rinsed before use (Fox et al., 2022; Richards et al., 2022).

2.4 *In-situ* measurements and optical sensing

Dissolved oxygen (DO) was measured *in-situ* using an optical DO meter (HQ10, Hach, CO, USA). Optical sensors were deployed in the bulk sample vessel and covered with dark, heavy-duty towels to exclude ambient light. The VLux TPro (Chelsea Technologies, UK), a prototype fluorimeter, was used to measure the fluorescence intensity of tryptophan-like fluorescence (TLF or Peak T; $\lambda ex/\lambda em$ 280/365 ± 25 nm; indicative of microbial activity) (Fox et al., 2022). Data was recorded via a handheld data logger (Hawk®; Chelsea Technologies), with a sampling time of 1 minute and integration time of 1 second (n = 60 per sample). Information regarding corrections and quality control can be found in Supplementary Information.

2.5 Laboratory analysis

Preserved water samples, see section 2.3, were analyzed at the UK Centre for Ecology & Hydrology (UKCEH) immediately upon return to the UK (2 to 12 days after sample collection) (Table S1). Information regarding quality assurance and quality control can be found in Supplementary Information.

2.5.1 Hydrochemical analysis

Nutrient concentrations (total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and ammonium) were determined by spectrophotometry, as described by (Bowes et al., 2020). Dissolved organic carbon (DOC) was analysed by thermal oxidation (Elementar Vario

Cube, Elementar Analysensysteme GmbH, Germany) (Bowes et al., 2020). Major dissolved anion concentrations (chloride, nitrate, nitrite and sulphate) were determined using ion chromatography (Dionex AS50, Thermo Fisher Scientific, USA) (Bowes et al., 2020). Aquacheck quality control standards (LGC Standards, UK) were carried out alongside all analyses.

2.5.2 Microbial analysis

DNA was extracted in line with Bowes et al. (2020) from Sterivex filters using the Qiagen PowerWater kit (Mo Bio Laboratories Inc., Carlsbad, CA) according to manufacturer instructions. DNA quantity and quality were assessed using the NanoDropTM 8000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the Qubit® dsDNA BR (broad range) Assay Kit and with Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). To determine 16S rRNA gene abundance (a proxy for bacterial abundance), a hydrolysis probe-based qPCR approach was used. Additional information can be found in Supplementary Information. (Suzuki et al., 2000)

Phytoplankton analysis on glutaraldehyde fixed samples was undertaken using a Gallios flow cytometer (Beckman-Coulter, UK), following the protocol outlined by (Read et al., 2014). The method was used to identify five broad groups: diatoms/large chlorophytes (5µm – 1.0 mm), nano-chlorophytes (0.2 – 20 mm), pico-chlorophytes (0.2 – 2.0 µm), cryptophytes (10 – 50 µm), and cyanobacteria (~ 10 µm).

2.6 Discharge data

Average monthly discharge data for November 2019 (based in daily simulations) was obtained from simulations with the integrated hydrological model PCR-GLOBWB 2.0 with a resolution of 10 km by 10 km (Sutanudjaja et al., 2018). Extraction of data specific to sample locations and, where necessary, corrections were carried out on QGIS 3.16. There is a gap in modelled discharge data between G56 and G61, where the Ganga River become the Hooghly River, as the feeder canal is not modelled by PCR-GLOBWB 2.0. It should also be noted that throughout the monitoring period (November 2019) it was very dry with little precipitation, resulting in no sudden changes to the rivers' flow conditions.

2.7 GIS data

A range of global gridded datasets at different resolutions were used to estimate spatial statistics for each of the sampling locations. Catchment area and flow paths to each sampling point were estimated using MERIT Hydro (Yamazaki et al., n.d.), a global flow direction map at 3-arc sec resolution (~ 90 m at the equator), within ArcGIS. Catchment areas were estimated by summing cells flowing to each sampling location. These maps were also used to estimate downstream distances in relation to the uppermost site (G01) and to derive the areas (5 km and 10 km) directly upstream of the sampling points. To estimate the 5 km and 10 km areas directly upstream of the sampling points, the main river was traced to 2.5 km and 5 km upstream, respectively, providing focal points of circles with diameters of 5 km and 10 km, respectively. Land use using ESA CCI Land Cover data (ESA) and population statistics using gridded population data (Doxsey-Whitfield et al., 2015) were determined within upstream and catchment areas, with spatial resolutions of ~ 300 m and ~ 1 km respectively.

2.8 Data analysis

Segmented regression was performed using R (version 4.2.2(R Core Team, 2021)) to test for threshold changes between water quality parameters and distance downstream. A Davies test was used to assess breakpoint significance, allowing segmented regression models to be fitted and ranked using Akaike Information Criterion (AIC), as detailed by (Richards et al., 2022). Breakpoints were estimated with a 95% confidence interval and extracted from the top-ranking model. Major and minor breakpoints were informed by Richards et al. (2022) and the type/number of parameters assessed throughout this study. A major breakpoint was operationally defined in this context as an approximate location where eight or more parameters significantly shifted, and a minor breakpoint was determined as a location where five or more parameters significantly shifted. Spearman correlations and resultant significant differences between water quality parameters, site numbers, and anthropogenic influences at a local/catchment scale were performed using GraphPad Prism 10.0.2 (GraphPad, CA, USA). A p-value of < 0.05 was considered significant. Non-metric Multidimensional Scaling (NMDS) was carried out on the full dataset (physicochemical and biological variables) and the physicochemical and biological datasets individually, using the metaMDS function in Vegan (Oksanen et al., 2024). ArcGIS (version 3.28, Open-Source Geospatial Foundation) was used to create maps, QGIS 3.16 was used to apply corrections to modelled discharge, and GraphPad Prism (Washington, USA) or OriginPro 2017 were used for other data visualisation.

3 Results and Discussion

Water quality parameters were analysed to determine downstream physicochemical, nutrient and microbial trends, including in relation to inorganic hydrogeochemical patterns (Richards et al., 2022). Interpreting water quality shifts alongside potential influencing factors such as modelled discharge and anthropogenic activity (urbanisation, agriculture and industry) allows for a better understanding of water quality stressors and controls along the Ganga River.

3.1 Downstream trends: *Physicochemical, nutrient and microbial parameters, and anthropogenic indicators*

The downstream trends in the physicochemical, nutrient and microbial parameter as well as land use and modelled river discharge, demonstrate systematic changes along the Ganga River (Figure 2). Physicochemical, nutrient and microbial parameters are listed in Table S1. Concentrations of silica ($r = 0.23$; $p = <0.0001$) and DOC ($r = 0.48$; $p = <0.0001$) significantly increase with downstream distance, as did population density ($r = 0.38$; $p = 0.0005$). Selected geochemical elements (Ca, Mg and Sr) were previously reported to increase downstream, largely attributed to regional hydrogeological controls including water-rock interactions (Richards et al., 2022). Conversely, ammonium ($r = -0.23$; $p = 0.03$), % DO ($r = -0.42$; $p = <0.0001$) and sulphate ($r = -0.54$; $p = <0.0001$) significantly decreased downstream. All other parameters considered here (SRP, TDP, TP, Cl, NO_2-N, NO_3-N, tryptophan-like fluorescence, green diatoms, nano-chlorophytes, pico-chlorophytes, 16S (rRNA copies/mL), % urban cover, % irrigated crop cover and modelled discharge) did not have statistically significant overall downstream trends. However, the processes that control these parameters are highly complex and therefore substantial local variability is expected (Lintern et al., 2018; Mainali and Chang, 2018).

Discharge has a major effect when assessing the downstream tends of parameter loading, see Figure S1. For example, between G01 and G55 (Ganga River, pre-Farakka Barrage) there were several significant positive correlations: TDP ($r = 0.52$; $p = 0.0004$), TP ($r = 0.40$; $p = 0.009$), Cl ($r = 0.35$; $p = 0.021$), NO_2-N ($r = 0.43$; $p = 0.006$), SO_4 ($r = 0.39$; $p = 0.011$), diatoms ($r = 0.45$; $p = 0.003$) and nano-chlorophytes (green meso: $r = 0.64$, $p < 0.0001$). However, post-Farakka Barrage (Hooghly River) microbial abundance (16S rRNA: $r = 0.68$; $p < 0.001$) was the only parameter to exhibit a significant positive downstream correlation. This indicates that for some parameters there is an apparent downstream accumulation that is then reset when the discharge is reduced, such as post Farakka Barrage a major barrage constructed to divert a substantial volume of water from the Ganga River to the Hooghly River and reduce sediment deposition in the Kolkata harbour.

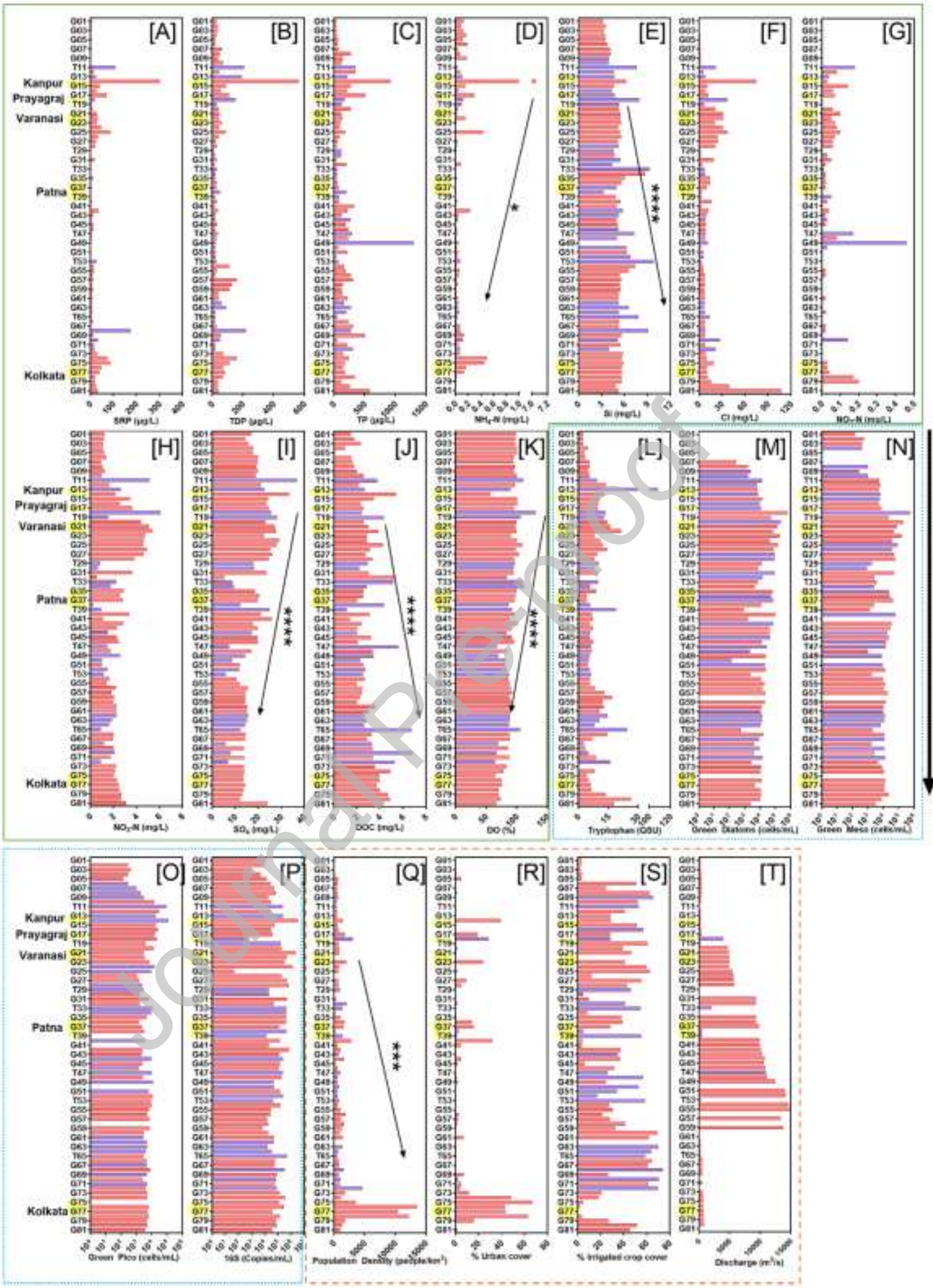

Figure 2: Downstream concentration trends for: physicochemical and nutrient parameters [A] soluble reactive phosphorus (SRP), [B] total dissolved phosphorus (TDP), [C] total phosphorus (TP), [D] ammonium (NH_4-N), [E] silica (Si), [F] chloride (Cl^-), [G] nitrite (NO_2^-N), [H] nitrate (NO_3^-N), [I] sulphate (SO_4), and [J] dissolved organic carbon (DOC) - within solid green box [—]; biological parameters [K] dissolved oxygen (DO), [L] Tryptophan-like fluorescence, [M] diatoms, [N] green nano-chlorophytes (0.2 – 20mm diameter), [O] green pico-chlorophytes

(<3µm diameter), and [P] 16S (rRNA) copy number – within dotted blue boxes [····]; and land use parameters [Q] population density, [R] percentage (%) urban cover, [S] percentage (%) crop cover and [T] discharge (m^3/s) – within orange dashed box [- - -]. Red bars refer to sample locations on the Ganga River or Hooghly River, blue bars refer to sample location on incoming tributaries. Yellow highlighted sample locations in Y axis refer to approximate location of major cities along the Ganga River: Kanpur, Prayagraj, Varanasi, Patna and Kolkata. Arrows indicate where significant positive or negative downstream correlations against distance downstream occur * = $p < 0.05$; *** = $p < 0.001$; **** = $p < 0.0001$.

3.2 Associations with Anthropogenic Indicators

Population density and urban cover had a greater impact on a greater number of water quality parameters at local scale (10 km upstream buffer zone) than at catchment scale (Table S3). Population density at the local (buffer) scale was significantly associated with 17 water quality parameters, with positive correlations including SRP, NO_3^-N, DO (mg/L), tryptophan-like fluorescence and green diatoms (Table S3), and negative correlations with SO_4 and DO (%). The difference in correlation between DO concentration (mg/L) and DO saturation is likely due to lower water temperatures in the upper stretch of the Ganga River, in comparison to the lower stretch of the Ganga River (Richards et al., 2022), as higher water temperatures reduce oxygen saturation level (Ice, 2008). In comparison, at the catchment scale 11 parameters were significantly positively associated including, diatoms and microbial abundance (16S rRNA), whilst TP, DO (mg/L) and pico-chlorophytes were all significantly negatively correlated. Population density and urban cover were strongly correlated with microbial indicators (*e.g.* tryptophan-like fluorescence) at local scale ($r = -0.38$; $p = 0.01$ and $r = -0.35$; $p = 0.01$, respectively) but not when the catchment area was considered ($r = -0.21$; $p > 0.05$ and $r = -0.15$; $p > 0.05$, respectively). Yet, for microbial abundance (16S rRNA) the influence of catchment scale differed, with significant correlations of 16S rRNA abundance only with total population density ($r = 0.28$; $p = 0.03$) and local urban cover ($r = 0.31$; $p = 0.01$) (Hosen et al., 2017; Yuan et al., 2019). Anthropogenic indicators (e.g. population density) have been associated with water quality parameters (e.g. total phosphorous) in other large river systems such as the Han River Basin, South Korea (Mainali and Chang, 2018).

3.3 Geochemical and Microbial Associations

Nutrient availability can impact microbial activity and abundance (Fox et al., 2022; Sigee, 2004) and this is reflected in correlations between physicochemical, nutrient and microbial parameters (Table S4). Tryptophan-like fluorescence, a proxy for microbial activity, exhibited significant positive correlation with many nutrient and microbial parameters including TDP ($r = 0.34$; $p = 0.01$), NO_2-N ($r = 0.7$; $p < 0.01$), NO_3-N ($r = 0.63$; $p < 0.01$), diatoms ($r = 0.42$; $p < 0.01$), and 16S rRNA ($r = 0.50$; $p <$

0.01). Microbial abundance (16S rRNA) also exhibited significant positive correlations between multiple nutrient and microbial parameters (Table S4), yet SRP was the only phosphorus species to have a significant positive correlation with both microbial activity and microbial abundance. This is likely due to SRP's increased bioavailability in comparison to TDP and TP (Calvo-López et al., 2021).

3.4 Breakpoint Zones

Distinct 'zones' within the Ganga/Hooghly were identified in this study through segmented regression analysis of shifts in physicochemical, nutrient and microbial parameters and modelled discharge. This study builds on previous work by Richards et al. (2022) where four major breakpoints were identified (i: ~ 1570 ± 70 km; ii: 1850 ± 50 km; iii: 2120 ± 80 km; and iv: 2700 ± 5 km) resulting in 5 hydrogeochemical zones. It should be noted that in the context of the previous study a major breakpoint was operationally defined where > 4 parameters shifted. However, four core zones (Figure 3) were newly identified in our current work on the basis of one major breakpoint (> 8 parameters shifted) and three minor breakpoints (≥ 5 parameters shifted), with transitions (± 95% confidence intervals) located at: (1) ~ 1018 ± 28 km (near G14); (2 A) ~ 1453 ± 42 km (near G23/G24); (2 B) ~ 1573 ± 42 km (near G26/G27); and (3) 2352 ± 54 km (near G61) downstream distance from Ganga source. Fitted segmented regression lines for individual water quality parameters are included in Supplementary Information (Figure S2 and Figure S3). Each zone will be discussed individually with regard to likely localised influences (e.g. tributary convergences, urban inputs).

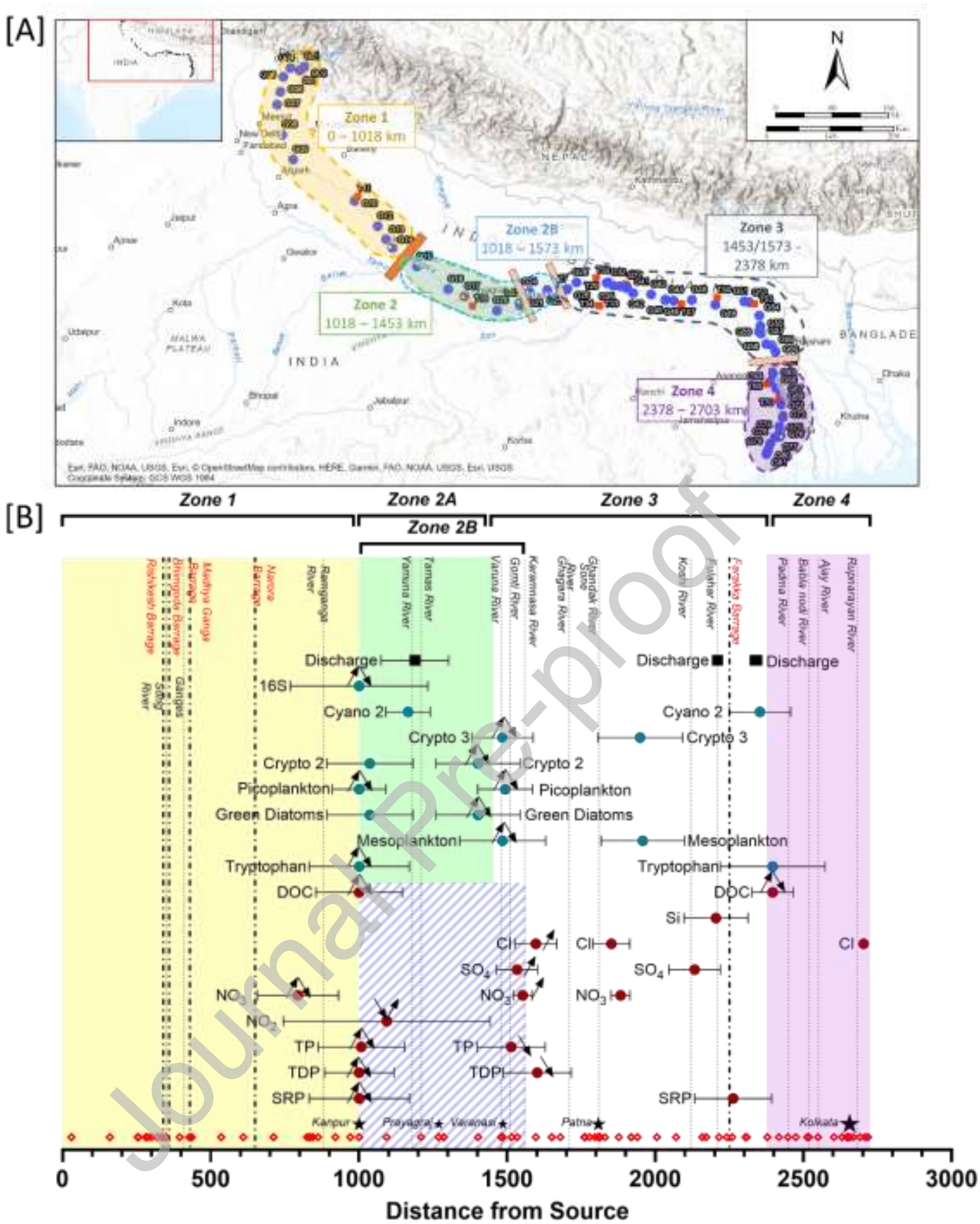

Figure 3: [A] Spatial overview of physicochemical, nutrient, and microbial breakpoints along the Ganga River resulting in four core zones (represented by dotted shapes). One major breakpoint (≥ 8 parameters) is represented by a solid orange rectangle, and three minor breakpoints (≥5 parameters) are represented by dashed orange rectangles. [B] Detailed overview of parameter specific breakpoints resulting in four core zones. Error bars represent ±1 standard deviation. Arrows refer to increases/decreases for each breakpoint. [-··-·-] indicate the location of barrages. [···] indicates incoming tributaries. Diamonds [◊] refer to sampling locations; and stars [★] refer to major cities along the Ganga River. Small stars refer to cities with populations between 1 – 2.5 million; medium stars refer to cities with populations between 2.5 – 5 million; and the large star refers to population ~15 million (Kolkata).

3.4.1 Zone 1

The first zone (Devprayag to ~ 1018 ± 28 km downstream) runs from the Himalayan foothills to approximately the city of Kanpur (G14). Although characterized by several contributing tributaries (Figure 3B), the modelled discharge remains relatively static (Figure 2T) due to four barrages (Rishikesh, Bhimgoda, Madhya Ganga and Narora) all within ~300 km (c. 350 – 650 km downstream) which redirect water for irrigation across northern India.

The shift between Zones 1 and 2 occurs approximately 100 km downstream of the Ramganga convergence near Kanpur, indicating a distinct shift in water quality also corroborated by NMDS analysis (Figure S4). This breakpoint sees a peak in SRP, TDP, TP, DOC, tryptophan-like fluorescence, pico-chlorophytes, diatoms, cryptophytes 2 and 16S rRNA, and a slight decline in NO_2-N. The peak in SRP, TDP and TP aligns with the peak in several phytoplankton species (pico-chlorophytes, diatoms, cryptophytes). The peak in phytoplankton also aligns with high silica concentrations in the Ramganga River (T11) and the Ganga River at Kanpur (G14), which is indicative of diatom breakdown (Tsunogai and Watanabe, 1983). Phosphorus is present in high concentrations in the Ramganga tributary (T11; Figure 2A-C), and the maximum concentration for SRP (300 µg/L) and TDP (560 µg/L) in the main Ganga body occur at Kanpur (G14) where the second highest concentration for TP (920 µg/L) is observed. Elevated nutrient loading in the Ramganga tributary has been previously reported (Bowes et al., 2020; Pathak et al., 2018). The high phosphorus concentrations present in the Ramganga does not appear to have an immediate effect on SRP or TDP on the Ganga River but increases around Bithoor (G13) and Kanpur (G14), suggesting that localised inputs are contributing to this increase. High nutrient concentrations plausibly impacted the microbial activity, with a peak in tryptophan (110 QSU; maximum in Ganga transect) and elevated bacterial numbers (3.38 million copies/mL) observed at Kanpur (Figure 2L & Q). Tryptophan has been demonstrated to be indicative of microbial activity in both laboratory (Fox et al., 2021; Perrin et al., 2022) and field settings (Fox et al., 2022).

The length of the Ganga River and distance between sampling locations may provide opportunity for nutrient consumption, biological processing, and particulate sequestration (Bowes et al., 2020; Paerl and Paul, 2012; Wetz et al., 2017). The downstream effect of the Ramganga is likely to affect microbiological activity (i.e. indicated by tryptophan-like fluorescence) and algal/bacterial numbers, noting the ± 28 km confidence interval on the breakpoint estimate includes the Ramganga junction (Figure 3B). Therefore, it can be postulated that the microbial community composition of the Ganga River was affected by Ramganga convergence (Bowes et al., 2020; Paerl et al., 2003; Pathak et al., 2018), but that these effects are observed further downstream from the initial convergence. For example, microbial abundance prior to the convergence of the Ramganga (G10) were 4.48 Log_{10} cells/mL, and an increase in microbial abundance (5.4 Log_{10} cells/mL) was first observed at G13, approximately 100 km downstream of the Ramganga tributary sampling location (T11; 5.35 Log_{10} cells/mL). However, additional microbial community composition and diversity analysis would be required to corroborate this due to other plausible influences, such as agricultural runoff and wastewater discharge, within this distance.

Wastewater quality around Kanpur has been reported to vary seasonally, with elevated NO_3-N and P in cooler autumn/winter months (Soni et al., 2022), e.g. November to February, when this sampling occurred. Maximum wastewater discharge concentrations for nitrate and phosphate have been previously reported ~ 10 mg/L and 5 mg/L, respectively (Schellenberg et al., 2020), yet in cases ^{NH4} in Ganga samples exceed this (G21 & G22; Zone 2 A/B). The increased phosphorus (SRP, TDP and TP) concentrations observed here are likely a result of the discharge of untreated domestic and industrial wastewater (Natarajan et al., 2016), as well as reduced efficiency of the wastewater treatment plant at Kanpur (Soni et al., 2022), relative to warmer months. This reduced activity is likely due to cooler temperatures, coupled with excess phosphorus in wastewater-derived inputs, becoming available to microorganisms, resulting in peaks in microbial activity and numbers (Figure 2L & P).

3.4.2 Zone 2

The transition between Zone 1 and Zone 2A occurs between G14 and G15 (1018 ± 28 km), approximately 20 km downstream of Kanpur, and ends at 1453 ± 32 km, approximately 20 km upstream of Varanasi (G21). The minor breakpoint for Zone 2B (1573 ± 42 km) ends midway between G24 (Gomti River convergence) and G25 (Ghazipur), approximately 80 km downstream of Varanasi. Zones 2A and 2B were distinct in terms of segmented regression analysis, with the microbial parameters clustered in one breakpoint (2A) and the physicochemical and nutrient parameters clustered separately (2B) and slightly downstream. Due to the relatively short distance between the two breakpoints (~120 km) and overlapping confidence intervals between microbial and physicochemical/nutrient parameters the two breakpoints were considered within the same zone, although it is interesting to note that the physicochemical/nutrient shifts followed changes in microbial parameters, suggesting these impacts may be causally associated (Sigee, 2004). The distinct differences in microbial water quality between Zones 1, 2A and 2B were also observed through NMDS analysis (Figure S4).

There are two major tributary convergences in Zone 2A: the Yamuna River (T18), passing through New Delhi, and the Tamas River (T19), near Prayagraj (Figure 3B), as well as input from the Gyanpur irrigation canal. At breakpoint 2A, ~ 250 km downstream of the Yamuna and Tamas Rivers' confluences, nano-chlorophytes, diatoms, pico-chlorophytes, and two groups of cryptophytes (crypto

2 and 3) markedly increase, as well as TP, NO_2-N, NO_3-N and Cl at Mirzapur (G20). There were also high concentrations of TDP, Si, Cl, NO_3-N and DOC in the Yamuna River (T18) where discharge increased significantly (Figure 2T). Prayagraj is a large city (population ~ 1.5 million) (Population Census, n.d.), with localised inputs from a range of sources including industrial, agricultural, and wastewater, which can increase organic and inorganic concentrations, contributing to the increase in phytoplankton observed (Cotner and Wetzel, 1992; Reinl et al., 2022). Within Zone 2A and upstream of breakpoint 2B, the Yamuna and Tamas Rivers (T18 & T19) join the Ganga River with elevated Si, Cl, NO_3-N, SO_4, and DOC (Figure 2). The convergence of these tributaries, particularly the Yamuna (3951 m^3/s), substantially increases the discharge of the Ganga from 404 m^3/s (G17) to 4737 m^3/s at Mirzapur (Figure 2T). The Yamuna River runs through the cities of New Delhi, Ghaziabad and Agra, and has been reported to be highly contaminated (Trisal et al., 2008). The individual impacts of these rivers were not identified within this sampling survey due to there being no Ganga sampling point between the convergence of the Yamuna and Tamas rivers, although systematic tributary contributions should be considered in the future. The dilution effects observed may relate to the post-monsoon timing of sampling in comparison to other studies in the lower flow pre-monsoon season (Bowes et al., 2020). The microbial composition changing with downstream mixing of the Yamuna and Tamas rivers indicates a eutrophic input (Trisal et al., 2008), further supported by the higher SRP:TP proportion (~ 10 %) in the Yamuna River, likely related to bioaccumulation of SRP (Bowes et al., 2020; Jarvie et al., 2006).

The breakpoints observed here are largely consistent with Richards *et al*. (2022) transitions in Ca:Na and Mg:Na previously reported at 1400 ± 70 km downstream, and shifts in oxidation reduction potential (ORP), Sr:Ca, Cl:Na, Mg:Ca, pH, electrical conductivity (EC), Mg, and Na reported ~1570 ± 70 km (Richards et al., 2022), although the zone definitions are slightly different. At breakpoint 2B (1573 ± 42 km) an increase in Cl, SO_4 and NO_3-N, and a decrease in TP and TDP, was observed. The increase in EC observed by Richards *et al* (2022) can be indicative, in part, of increased nutrient concentrations (Ma et al., 2015), and is corroborated by increases in Cl, NO_3-N and SO_4 (Figures 2F, H & I).

In Zone 2B the peak in population density and percentage urban cover occurred at Varanasi (G23). In addition to tributaries (Varuna, Gomti, Karamnasa) joining in the Ganga River near Varanasi, many industries (*e.g.* tanneries and paper mills) and anthropogenic activities likely contribute to the increase in nutrients (Soni et al., 2022). Varanasi is a major Hindu spiritual city where humans are cremated and released into the river for spiritual rebirth (Singh et al., 2004). However, ash produced from cremations contribute towards the increase in SO_4 and DOC of the Ganga River (Verma and Shrivastav, 2018). The Varuna River has been shown to introduce effluent from agriculture and textile, metal, food, and paint industries (Trombadore et al., 2020) into the Ganga River which can affect photosynthetic rates, increasing the pH due to increased consumption of CO_2 by microorganisms (Bai et al., 2022).

3.4.4 Zone 3

Zone 3 (1453 – 2378 km) covers the Ganga segment between Ghazipur (G25) and Berhampore (G61), where the river name changes to the Hooghly. Throughout this ~ 1000 km, Patna is the only major city with a population of greater than 2.5 million people, with increased urban cover and population density. Many of the nutrient and physicochemical parameters (*e.g.* SRP, TDP, TP, Cl; see Figure 2) are relatively stable in Zone 3, see Figure 2, despite the convergence of three major tributaries (Ghaghara: T29 & T30, Sone; T33 & T34 and Ghandak; T38 & T39) within 100 km upstream and near Patna. This stable water quality is likely due to a dilution effect from each of the tributaries, see Figure 2T. Changes in irrigated crop cover (Figure 4C), are likely associated with increased population density (Figure 2Q).

Approximately 200 km downstream of Patna (1903 ± 50 km) between Bahr (G43) and Mipur (G44) there were minor parameter breakpoints with reductions in NO_3-N and Cl^-, and increased cryptophytes 3 and nano-chlorophytes. Another 300 km downstream between Manihari (G51) and Kishanpur (G52) at 2269 ± 36 km there were minor parameter breakpoints with increases in Si, SO_4 and discharge (Figure 2E, 2I & 2T), consistent with the Koshti and Fulahar Rivers joining the Ganga.

3.4.5 Zone 4

The transition between Zone 3 and Zone 4 occurs around Berhampore (G61; 2532 ± 54 km), approximately 100 km downstream of the Farakka Barrage. Population density and urban cover increase around Nabadwip (G69) and Kal (G71), before peaking at Kolkata (G76 - 78) with ~ 13,500 people/km and 66.84%, respectively. There is also an increase in NH_4-N at G74 which then decreases over the next few sampling locations (Figure 2 D), indicating an increased sewage input, whilst there is a gradual increase in NO_2-N between G75 and G79 (Figure 2 G) and NO_3-N between G75 – G81 (Figure 2 H). The relative oxic conditions of the Ganga River (Figure 2 K) suggests NH_4-N is unstable and therefore a transient store of N as it is likely that both ammonia-oxidising and nitrite-oxidising bacteria will act as microbial intermediaries in the formation of NO_3-N, further demonstrating the dynamic nature of a large river system. Zone 4 starts with a minor breakpoint for SRP, DOC, tryptophan-like fluorescence, cyanobacteria 2 and modelled discharge. This is the only breakpoint that was affected by a major change (i.e. reduction) in discharge, which decreases substantially from ~ 1450 m^3/s (G55) to ~ 2 m^3/s at G56 (post-Farakka barrage; Figure 2T). SRP also decreases post-Farakka Barrage, with localised reductions observed in cyanobacteria 2 and DOC (only observed at G61). Interestingly, tryptophan-like fluorescence decreases leading towards the Farakka Barrage (G58 – G61) but increases after the Ajay River joins the Hooghly (T65 and G66, respectively). The Ajay River has been shown to be heavily contaminated with heavy metals associated with agricultural (e.g. manure use for agriculture) and industrial discharge (Singh and Kumar, 2017), and the high organic load may explain the increased tryptophan-like fluorescence at this location. These water quality shifts are most likely related to the considerably reduced discharge. There was also an observed transition in ORP, pH and DO by Richards et al (2022) at a similar 2380 ± 80 km distance downstream. A substantial increase of Cl much further downstream ~ 2700 km is indicative of an estuarine shift, and transitions in Na, Sr:Ca and Mg:Ca were also noted in the lower reaches by Richards et al (2022).

3.5 Implications and Future Directions

This study provides insight into the variety of potential impacts that can affect nutrient availability and biological water quality, in particular, how changes in water quality are influenced by anthropogenic activities or tributary confluences. This information is valuable as the Ganga River is an example of a large river system of global importance and can help to build an understanding of the relative importance of localised versus cumulative impacts on spatially dependent water quality parameters and overall water quality. Notwithstanding the unique opportunity afforded by this ~ 2700 km longitudinal survey providing important baseline water quality across the Ganga River in India, a limitation is the spatio-temporal resolution. Further sampling would be recommended to capture seasonal and diurnal variations, to improve spatial resolution in strategic locations, and to better understand any variability in pollution patterns and key areas, such as around Kanpur, Varanasi and post-Farakka Barrage. Improving the understanding of distinct water quality zones and associated approximate locations of localized shifts in water quality, can help enable water management approaches and/or remediation interventions to be appropriately considered and/or developed for each zone while taking the entire system into consideration. This will help to identify locations that may require additional management to increase or maintain good water quality. For example, the understanding gained from this large-scale baseline study help prioritise locations, such as Kanpur and Varanasi, for the installation of remediation interventions and high-resolution *in-situ* monitoring approaches (Chowdury et al., 2019) to aid river and pollution management in the future. In addition, such data could help identify locations that potentially encourage the formation of algal blooms that in turn impact water quality through reducing nutrient availability, that is then released upon algal/diatom breakdown.

4 Conclusions

This study presents the first ~ 2700 km water quality sampling survey of the Ganga River focussing on key nutrient and microbial parameters, as well as key physicochemical parameters. Strategic locations were identified where systematic water quality shifted through changing nutrient availability and/or microbial activity/abundance were likely impacted by tributary convergences, barrages and anthropogenic activities (population density, urban cover and irrigated crop cover). This study demonstrated the effects of fluctuations in population density and urban areas, such as Kanpur, Prayagraj and Varanasi, and tributaries impacted by pollution, such as the Yamuna and Varuna rivers, have on large river systems. Segmented regression demonstrated the locations where systematic water quality shifts occur, resulting in distinct changes in water quality, with additional NMDS analysis further supporting compositional distinctions including a major breakpoint at ~ 1020 km downstream from source around Kanpur. This study also demonstrated that anthropogenic influences (population density and urban cover) can have greater influences on water quality parameters including SRP, NO_3-N, DO (mg/L), tryptophan-like fluorescence and green diatoms at local catchment scale (e.g. ~ 10 km upstream), in comparison to total catchment area. The multipollutant focus illustrates the complex biogeochemical and hydrological processes and influences affecting downstream compositional patterns in nutrients and microbial water quality. The approach employed here could be applied more widely to improve understanding of biogeochemical processes in complex large river systems and to provide important baseline data to aid river and pollution management in such settings.

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Data availability

Relevant data can be made available upon request.

Author contributions

GEC: investigation, formal analysis, data visualization, writing – original and final draft. LAR: investigation, formal analysis, methodology, visualization, supervision, writing – original and final draft. BGF: investigation, conceptualization, formal analysis, methodology, data curation, writing – original draft. RMST: investigation, methodology, funding acquisition. MJB: investigation, conceptualization, methodology, funding acquisition, resources. DSR: data visualisation, methodology, resources. HJT: investigation, methodology, data curation. KK: investigation; software, methodology. TKD: funding acquisition. AK: investigation, methodology. MH: investigation BH: investigation, methodology. US: interpretation. LKA: Investigation. DJEN: analysis, resources. HD: Methodology, data visualisation. DMH: investigation, funding acquisition. HN: investigation. AG: conceptualization, funding acquisition, methodology, resources. HJ: Conceptualisation, funding acquisition DCG: conceptualization, funding acquisition. DAP: conceptualization, funding acquisition, resources. StK: conceptualization, funding acquisition. DMR: conceptualization, funding acquisition, resources. All authors have contributed to original or final manuscript reviewing and editing.

Conflict of interest statement

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.

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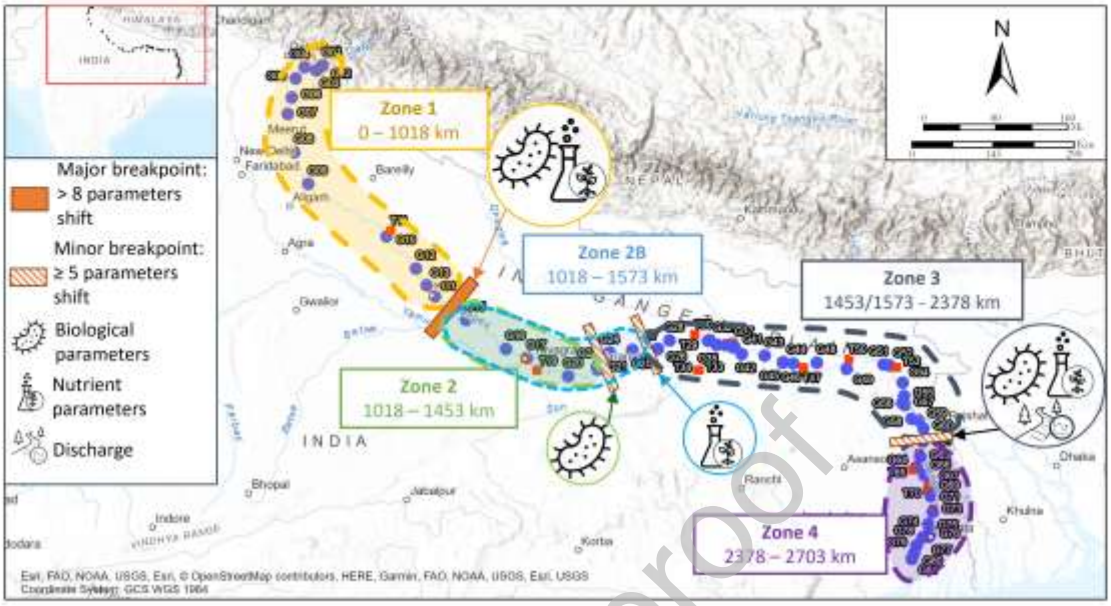
Graphical abstract



Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: