

Spatial and Temporal Dynamics of Chemical and Microbial Contamination in Nonurban Floodwaters

Minna Saaristo,* Christopher P. Johnstone, Phoebe Lewis, Simon Sharp, Timothy Chaston, Molly Hoak, Paul Leahy, Darren Cottam, Luke Noble, John Leeder, and Mark Patrick Taylor



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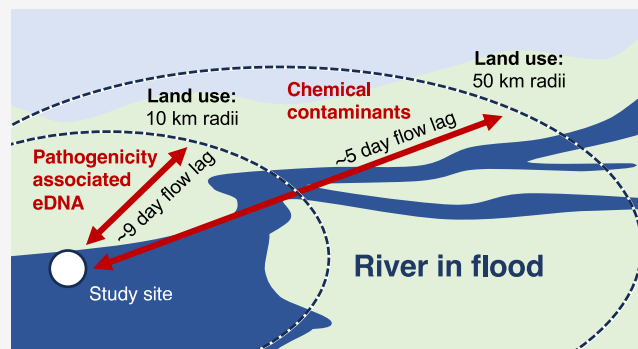
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ABSTRACT: During major flood events, waterborne contaminants are relatively poorly characterized. This is due to logistical difficulties associated with obtaining water samples in potentially dangerous flood conditions. Herein, we report analyses of water samples from a large, flooded landscape in Victoria, Australia, during a major flood event. We collected 83 samples from seven rivers and 18 river locations as far apart as 520 km. The sampling campaign covered a 26-day window, with 3 samples taken weekly from each site. Floodwater samples were analyzed for 778 contaminants and 544 microbial species were identified using eDNA. Our study shows that 85 contaminants were detected in floodwaters. Fungicides, phthalates, plant macronutrients, metal(loid)s and PPCPs were better explained by land uses, whereas herbicides and insecticides were explained by a mixture of land use and water flow data. Potentially pathogenic orders with the highest detection rates were Enterobacterales (82.4%), Mycobacteriales (70.6%) and Legionellales (58.8%). Contaminants and microbial signatures responded to rainfall, water flow and water level, demonstrating increased and varied human and environmental risks of exposure during the sampling window. Our work underlines the importance of rigorous and timely monitoring and provides an evidence-base for decision making during increasingly frequent and intense climate driven flood events.



KEYWORDS: climate change, contaminants of emerging concern, environmental DNA, mass flux, land use and surface water flow models

INTRODUCTION

Anthropogenic climate change is increasing the frequency, duration and severity of extreme weather events,^{1–4} prompting global concerns about flood-borne contaminant risks.^{5,6} During 13–14 October 2022, up to 300 mm of rain fell in parts of Victoria, Australia (Figure 1). Due to prevailing wet conditions, rivers and catchments had limited capacity to absorb additional rainfall. The result was one of the worst flooding events in Victoria’s history.⁷ The Environment Protection Authority Victoria (EPA), in partnership with State Emergency Service (VICSES) and Natural Hazards Research Australia sampled floodwaters across the impacted systems to assess biochemical contamination risks.

Although direct flooding effects (e.g., infrastructure damage, erosion, postflood cleanup^{8–19}) have been studied previously, relatively fewer studies characterize anthropogenic chemical and microbial contamination of flood waters^{19–21} Additionally, extreme flood events have been studied more extensively in relatively stable river systems^{6,9,22,23} than in those subject to regular extreme wetting and drying cycles. Some of the rivers in Victoria dry out entirely or form chains of “billabongs” connected by underground flows. Studies of such systems are important, because climate predictions suggest a future of

more energetic and unpredictable extremes of wetting and drying globally.^{1,2}

Previous studies of flood contaminants report physical and chemical characteristics of floodwaters (e.g., dissolved oxygen,²⁴ nitrate/nitrite,²⁵ cations,²⁶ trace elements²²), or a restricted suite of contaminants, such as pharmaceutical and personal care products (PPCPs) or per- and polyfluoroalkyl substances (PFAS).^{15,27} Microbial investigations have been largely restricted to culturable taxa, such as *Escherichia coli*, and eDNA studies tend to focus on faecal indicators.^{16,28} These narrow within-publication scopes have tended to limit comparisons across distinct chemical groups and/or taxa within a given flooded system.

Large scale studies of flooded systems indicate increasing complexity of hydrological, geological, anthropogenic and landscape interactions in larger catchment areas.²² Yet many

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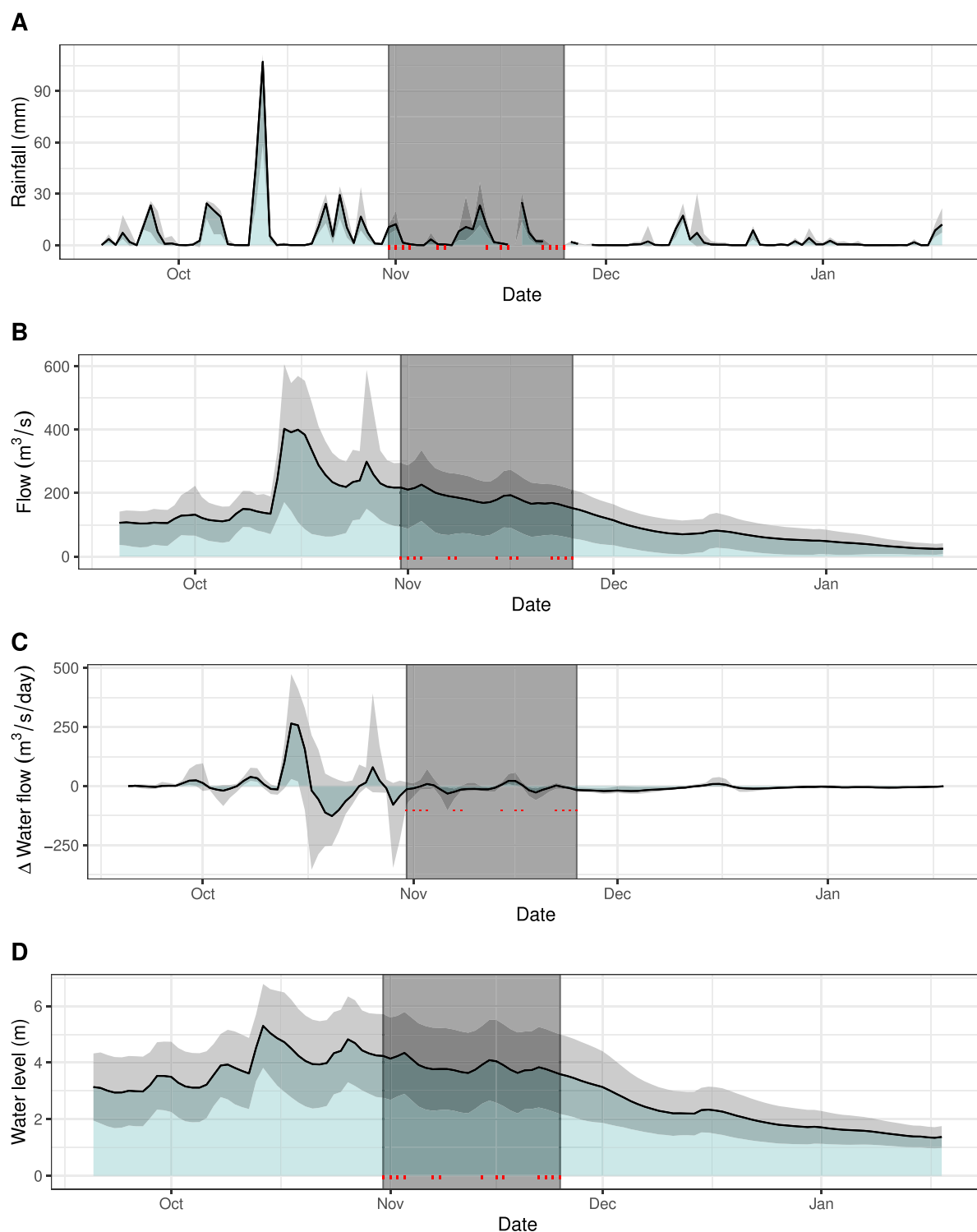


Figure 1. Measures of rainfall and flooding averaged over the study area during the major flooding in Victoria 2022: (A) rainfall (mm), (B) water flow ($\text{m}^3 \text{s}^{-1}$), (C) Δ Water flow ($\text{m}^3 \text{s}^{-1} \text{day}^{-1}$) (an index of flood acceleration/deceleration), and (D) water level (m). The gray shaded bar represents the window during which water samples ($n = 83$ samples) were obtained across 18 river locations. A longer span of flood-affected months (October 2022 through January 2023) (i.e., outside the chemical and eDNA sampling window) is shown for context. Red lines indicate sampling dates. Gray shaded areas above and below lines represent 95% CI of the line.

studies focus on small rivers in urban environments,²⁹ and the effect of spatial scale on contaminant-flood relationships is not well characterized. Further, where large scale land usage has been assessed, the categories outside of urban environments are predominantly coarse grained, rely on satellite imagery,^{26,30}

and/or exclude important land uses, such as sewage treatment and intensive animal production.

Herein, we analyze relative responses of chemical groups and eDNA signatures to major flooding. Over 26 days, we measured 778 chemical analytes at 18 sites (with 3 revisits) and identified 544 microbial operational taxonomic units

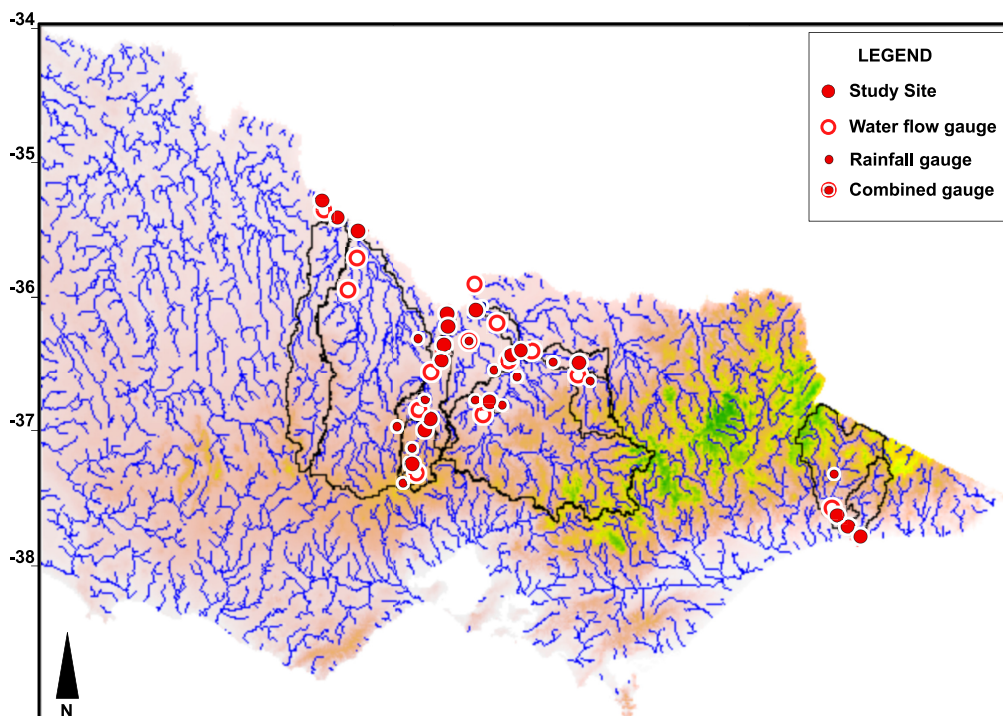


Figure 2. A map of the state of Victoria, Australia showing approximate study locations and major urban centers (underlying topographic map via Wikimedia commons). Black lines enclose catchments that empty into the study sites. Blue lines are natural water courses. Green indicates higher elevation and white indicates lower elevation.

(OTU) in floodwaters. Random forests analyses were used to identify relationships between land uses, hydrology, contaminant groups and waterborne microbes during a major flooding event across multiple rivers and catchments. Conclusions are drawn in the context of risk management and decision making during and postflooding.

METHODS AND MATERIALS

Sampling. All sites used for sampling were impacted by flooding and were chosen in discussion with EPA VIC and State Emergency Services (Figure 2). Sites were located on the Broken River ($n = 2$), Campaspe River ($n = 4$), Lake Eppalock ($n = 1$), Goulburn River ($n = 3$), Little Murray River ($n = 1$), Loddon River ($n = 1$), Murray River ($n = 2$) and Snowy River ($n = 3$). Sampling frequencies and exact locations were largely a function of logistical and safety considerations. Sites were resampled (83 visits across all sites) on Oct 31, and on Nov 1, 2, 3, 7, 8, 14, 16, 17, 22, 23, 24, and 25. The method for sampling followed EPA publication IWRG 701 (2009). In brief, grab samples were collected at each site using prerinsed bottles (amber glass or HDPE) supplied by the relevant analytical laboratory. Bottles were triple-rinsed in floodwater before collecting a sample by pole. Samples were immediately placed on ice and transported to the relevant laboratory. Supplementary methods and materials for expanded methodological descriptions and underlying study design approach) (see S1.1–S1.13 and Table S1.1).

Analysis. We tested for 778 analytes (here, defined as chemicals that are quantifiable as concentrations in water) (refer Table S1.2 and S3.1–S3.2). The methods for sample collection, handling, transport, storage, and quality assurance and control were consistent with EPA, national and international best practice and standards.^{31,32} Chemical contaminant concentrations were determined at Leeder Analytical (Mel-

bourne). Blanks and spiked control samples were used to address quality control requirements. No quality control reports indicated possible method inconsistencies or contamination. Quality assurance (QA) and Quality Control (QC) information is provided in Section S2 of the Supporting Information (Tables S2.1–S2.6).

Analyte Classification. For clarity, we classified analytes into 16 groups for data analysis (Table S1.2).

Sample Analysis (eDNA). The eDNA assessments were conducted by EnviroDNA (Melbourne). Full details are described in S1.3 of the supplementary methods.

Taxonomic Focus. Waterborne disease associated microbes were compiled from Chahal, Van Den Akker, Young, Franco, Blackbeard and Monis³³ and Fonti, Di Cesare, Sangulin, Del Negro and Celussi³⁴ (genera unless otherwise stated): *Acinetobacter*, *Aeromonas*, Family Arcobacteraceae (including *Aliacobacter*), *Campylobacter*, *Coxiella*, *Empedobacter*, *Enterococcus*, *Helicobacter*, *Klebsiella*, family Legionellaceae, *Leptospira*, Family Leptotrichiaceae, *Mycobacterium*, *Paracoccus*, *Prevotella* (in: Bacteroidales), *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus*, *Trichococcus*, *Vibrio*, and *Yersinia*. *Escherichia coli* and order Bacteroidales are also summarized and discussed, as these are associated with wastewater effluent²⁸ and have the potential to cause gastroenteritis if ingested.

Environmental Data: Hydrological. Rainfall (mm), water flow ($\text{m}^3 \text{s}^{-1}$) and water level (m) data were provided by the Australian Bureau of Meteorology (BOM). For each study site, the nearest upstream water flow/level monitor(s) ($n = 14$) and rainfall gauge ($n = 12$) were identified. Data were downloaded for all records from 20 Sep 2022 to 18 Jan 2023. Daily means of waterflow and level were obtained, and rainfall was summed. From flow data we calculated three-day Δ flow, capturing the acceleration/deceleration of flow for each site

over the previous 3 days. See S1.5 (Supplementary Methods and Materials) for further information on hydrological data.

Environmental Data: Land Use/Surface Water Flow Model. To identify and summarize all land uses from which water flows to each sample site, we developed a catchment boundary geographical information system using a 10×10 m digital elevation model (DEM) data set from Vicmap Elevation.³⁵ Land use boundaries and classifications were obtained from Victorian land use information system (VLUIS) (Agriculture Victoria)³⁶ and the Australian Bureau of Statistics.³⁷ Land uses were captured at 10 and 50 km radii of each site, such that land use parcels fell within the upstream catchment. See S1.8 for a detailed description of the land use model (Figures S1.1–S1.3). Summed land use categories and areas for the study are in Table S1.3.

Environmental Data: Upstream Range. A measure of distance from the farthest downstream sampling site (allocated 0 km) and sites upstream. Distances were measured in Google Earth along watercourses on a satellite image. See S1.9 (Supplementary Methods and Materials) for a detailed description.

Enrichment and Dilution. We define “enrichment” and “dilution” of contaminants as functions of their concentration correlated against water volume (i.e., a C–Q concentration–discharge relationship³⁸). A detailed description is in S1.10.

Multiscale Analysis. We tested two spatial scales for land uses: 10 and 50 km radii around study points. The land uses were parcels that fell inside the watershed for the study site at the study radii. For temporal scale, we used “lagged” rainfall, water flow, water level and Δ flow on the day of sample (day 1) and 2–10, 15, and 20 days prior to sample date (i.e., where the “2 day lag” is the environmental flow data for the previous day, etc.).

Mass Flux. Mass flux values were calculated for all chemical analytes in water using the R Package “calcLoad” in “rmodel” for each sample (kd/day). See S1.11 for a detailed description.

Method Limited of Reporting Imputations. All descriptive statistics (median, mean, standard error etc.) and inferential statistics (random forests, correlations) use zero imputation for Method Limit of Reporting (<MLR) values. This means that the values represent underestimates. However, because variance in MLR among analytes differs on orders of magnitude (even within the same category, such as ‘insecticides’), and “detection” assays (as versus quantifications) were not obtained, the use of half-MLR or MLR in this study was not viable.

Data Analysis. All data analysis was conducted in RStudio 2023.12.1 + 402³⁹ for macOS and R 4.3.2 (2023-10-31).⁴⁰ Data analysis was applied to chemical concentrations and eDNA using random forests (“cforest” in “party” and “partykit”: $n = 20,000$, replacement = false) to characterize the meaningfulness of 105 environmental variables including land use areas (km²) (at 10 and 50 km radii) and lagged environmental variables (i.e., rainfall on the day, and 1–10, 15, and 20 days prior to water grab sampling). All explanatory variables in the “expanded” analysis are in Supplementary Table S1.4. Models were checked for stability and +10,000 iterations were added until stability was achieved. No model needed >50,000 iterations. Two sites on the Murray River were excluded from Random Forests analysis due to insufficient land use data. Summary statistics (mean, median etc.) were calculated using ‘ddply’ in ‘plyr’. Plotting used ‘ggplot2’ and ‘ggparty’.

Alpha Level. Because of the large number of comparisons, a significance level of <0.005 is used instead of <0.05. Note that random forests are not discussed in terms of “significance”. Instead, “importance” is used when discussing the relative importance within the model, and “meaningful” denotes an explanatory variable that is both better than random and above the error of the model. Note that a variable can satisfy $P < 0.005$ in some iterations of a Random Forests model averaging method, but still not reach “meaningful” explanatory power. Determining “meaningfulness” is a two-step process. (1) Comparison of variables to explanatory power using randomized data, and (2) elimination of any variable that falls within the error of the model both add additional stringency beyond the <0.005 alpha level (see S1.12 and S1.13 for additional information).

RESULTS AND DISCUSSION

Detection of Chemical Analytes. A total of 85 analytes were detected (Tables S3.1, S3.2, and Figure 3A). Total detected chemicals per group were: biphenyls ($n = 1$), erosion prone salts ($n = 3$), fungicides ($n = 7$), herbicides ($n = 16$), insecticides ($n = 3$), metal(loid)s ($n = 19$), PAHs ($n = 5$), PFAS ($n = 11$), phthalates ($n = 8$), Plant macronutrients ($n = 6$), PPCPs ($n = 1$), TPH ($n = 4$ brackets; C6–C9, C10–C14, C15–C28, C29–C36) and VOCs ($n = 1$). No antiparasitics, mammalian hormones, plant growth regulators or sweeteners were detected, and these are not discussed further.

Mass Fluxes. Mass flux indicates the volume of contaminant moving through the landscape during floods. The largest mass fluxes were for the erosion prone salts, with the highest value being Na (897,744 kg/day at one site), followed by Ca (389,022 kg/day) and Mg (299,248 kg/day). Plant macronutrients were the next highest category, with site maximums of 287,000 kg/day (K) and 123,000 kg/day (N). The highest orthophosphate mass flux recorded was 3213 kg/day. Metal(loid)s also showed high mass fluxes with maximums of 3348 (Al), 1387 (Ba), 31,566 (Fe), and 8191 (Mn) kg/day. Among metals and metalloids, As showed the highest maximum mass flux (136.8 kg/day). In comparison, Pb mass flux was only 22.0 kg/day. Total petroleum hydrocarbon mass flux was high, particularly C15–C28 (8141 kg/day). The highest mass fluxes of pesticides were captan (fungicide; 4.02 kg/day), diuron (herbicides; 3.23 kg/day) and imidacloprid (0.94 kg/day). C2 and C3 alkyl-naphthalene gave equal highest PAH mass flux values (3.2 kg/day), and among PFAS, perfluorooctanoic acid was the most prevalent (0.43 kg/day). The phthalates di-isooctyl phthalate, di-isobutyl phthalate and di-*n*-butyl phthalate were quantified at 35.0, 6.57, and 6.10 kg/day, respectively (Table S3.3).

It is increasingly recognized that exposures to mixtures of contaminants can lead to synergistic endocrine effects in humans and animals,⁴¹ as shown in recent studies of phthalates.^{42–44} We observed an average daily passage of ~1.5 kg of phthalates in floodwaters daily during the study window, representing a substantial contaminant load.¹⁹ The most “meaningful” land use predictor of phthalate concentrations was sewage and wastewater treatment plants (WWTP) ($\tau + 0.088$; see relative importance below). Investigation of effluent from WWTPs in the area might be warranted. Note that “meaningful” is not equivalent to ‘significant.’ Meaningful predictors were both better than random and had greater predictive power than the error of the random forests models.

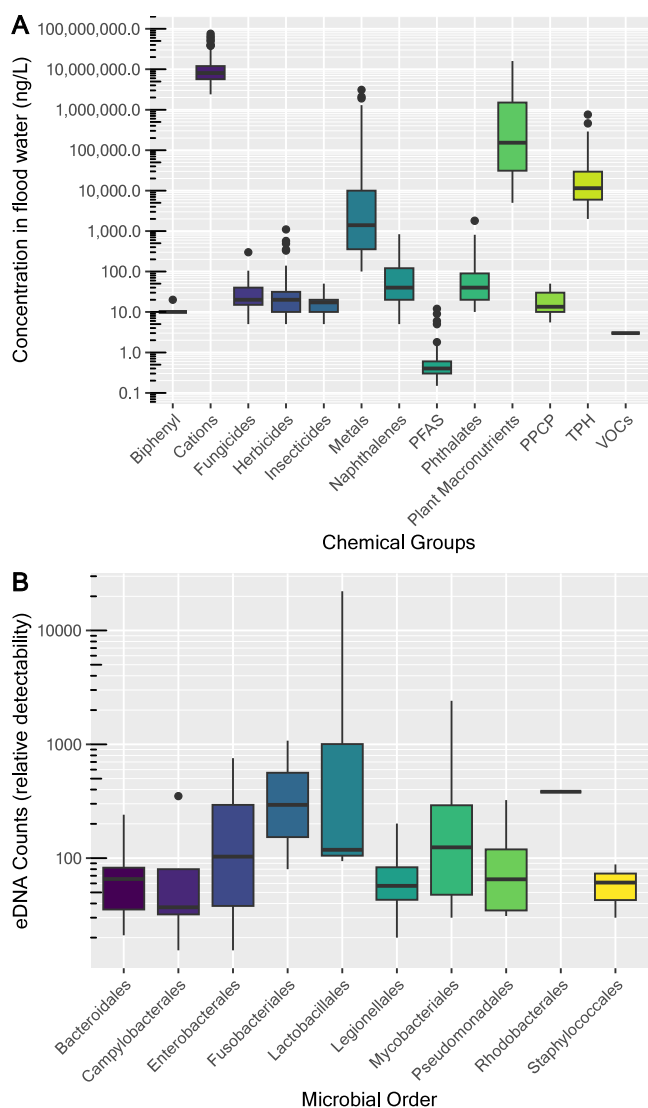


Figure 3. (A) Boxplots showing concentration (ng/L) medians, upper and lower quartiles for the detected chemical contaminant groups. The jittered red data points are individual detections. Note that the concentrations are on a log scale, and that there is a gap between the limit of reporting (LOR) and zero in each category. (B) Boxplots showing medians, upper and lower quartiles for eDNA counts each of the detected pathogenicity associated microbial taxa. Only pathogenicity associated species are plotted.

Although mass flux of PFAS was much less than that of PAHs, downstream deposition may represent considerable risk given the high persistence, bioaccumulation and toxicity of PFAS.^{45,46} Of the remaining chemicals that were assessed, lincomycin showed an average of ~42 g/day. This is a high value for an antibiotic that has activity at low concentrations and can lead to selection pressure favoring resistance genes in microbes.^{47,48}

Relative Importance of Land Use and Surface Water Flows. In analyses of land use and hydrology predictors, chemical contaminant concentrations were variably attributable to land uses and/or water flow and rainfall. Fungicides, phthalates, plant macronutrients, metal(loid)s and PPCPs (here, lincomycin) were better explained by land uses, whereas herbicide and insecticide contamination were explained by a mixture of land use and water flow data. At the other extreme,

erosion prone salts (cations), PAHs, PFAS and TPH were best explained by water flows, and to a lesser extent rain and water levels. Although we detected biphenyls and VOCs in some samples, no clear predictors were implied by our analyses. Figure 4A–F shows correlations for six environmental categories that were repeatedly predictive of contaminants: water flow (5 day lag), rain (5 day lag), lakes and reservoirs (50 km), upstream range, natural vegetation (50 km) and cropping (50 km) (refer Figures S3.1–3.13).

Within the limitations of the random forest models, the most frequently identified land use correlations were with water bodies, natural vegetation and to a lesser extent cropping, agricultural services and sawmills. Fungicides, insecticides, herbicides and lincomycin were all positively associated with water bodies. Lakes are known to act as reservoirs for pesticides⁴⁹ and PPCPs,⁵⁰ suggesting that flooding “flushed out” upstream waterbodies, either by resuspending contaminants from sediments and/or potentially mobilizing dissolved contaminants. Because sewage and WWTP (including retardation ponds) are distinct land uses, we suggest that the positive association with lakes and reservoirs is not due to WWTP overflow. Natural vegetation was negatively associated with metal(loid)s and phthalates, perhaps acting as a reverse index of anthropogenic activity or alternatively as sinks for chemical contaminants, as shown for forests.²⁶ Cropping, on the other hand, was positively related with fungicides, perhaps reflecting heavier use of agrichemicals in these areas.^{51–53} The cropping land use category also excluded grazing or tree horticulture, and soil runoff could be exacerbated by regular ploughing and machine harvesting.⁵³

The 50 km land use radii around study points outperformed the 10 km radii as a predictor of contaminants across all contaminant groups (Figures S3.1–3.13). In a contrasting study of anthropogenic land uses (patch density, contagion, interspersed and juxtaposition), landscape indices measured 200 m on either side of a flooded river explained cation and anion concentrations in flood waters better than at other scales (100–2000 m).²⁶ In another study of small catchment scales (<15 km across), higher zinc and TPH contents in artificial wetlands were related to the presence of industry covering >10% of the upstream study area.⁵⁴ Urban environments are highly heterogeneous,⁵⁵ and our analyses likely tend toward larger scale causal relationships because of this. Prior studies have argued that “typical” patterns in flooded rivers might be restricted to smaller rivers in urban environments.²⁵ Taken together, these studies warrant a multiscale approach to understanding risks and management options in flooded landscapes.

In temporal analyses of “lagged” water flow, level and rainfall (1–10 days, 15 and 20 days prior to sampling), contaminant concentrations at study sites were predominantly determined by discharge volumes 5–10 days prior. Higher rating correlations were negative for lagged flow and rainfall relationships, suggesting broadscale discharge-dilution effects and dominance of source limitations in the entire river system (i.e., negative concentration–discharge (C–Q) relationships). The lack of concentrating effects is consistent with some previous floods studies,¹⁹ but not with others.¹⁶ Both temporal and landscape scales may play a role here. Large-scale, nonurban watersheds might behave differently to smaller urban rivers, but a lack of research focus on larger systems in flood limits the certainty of this assertion.

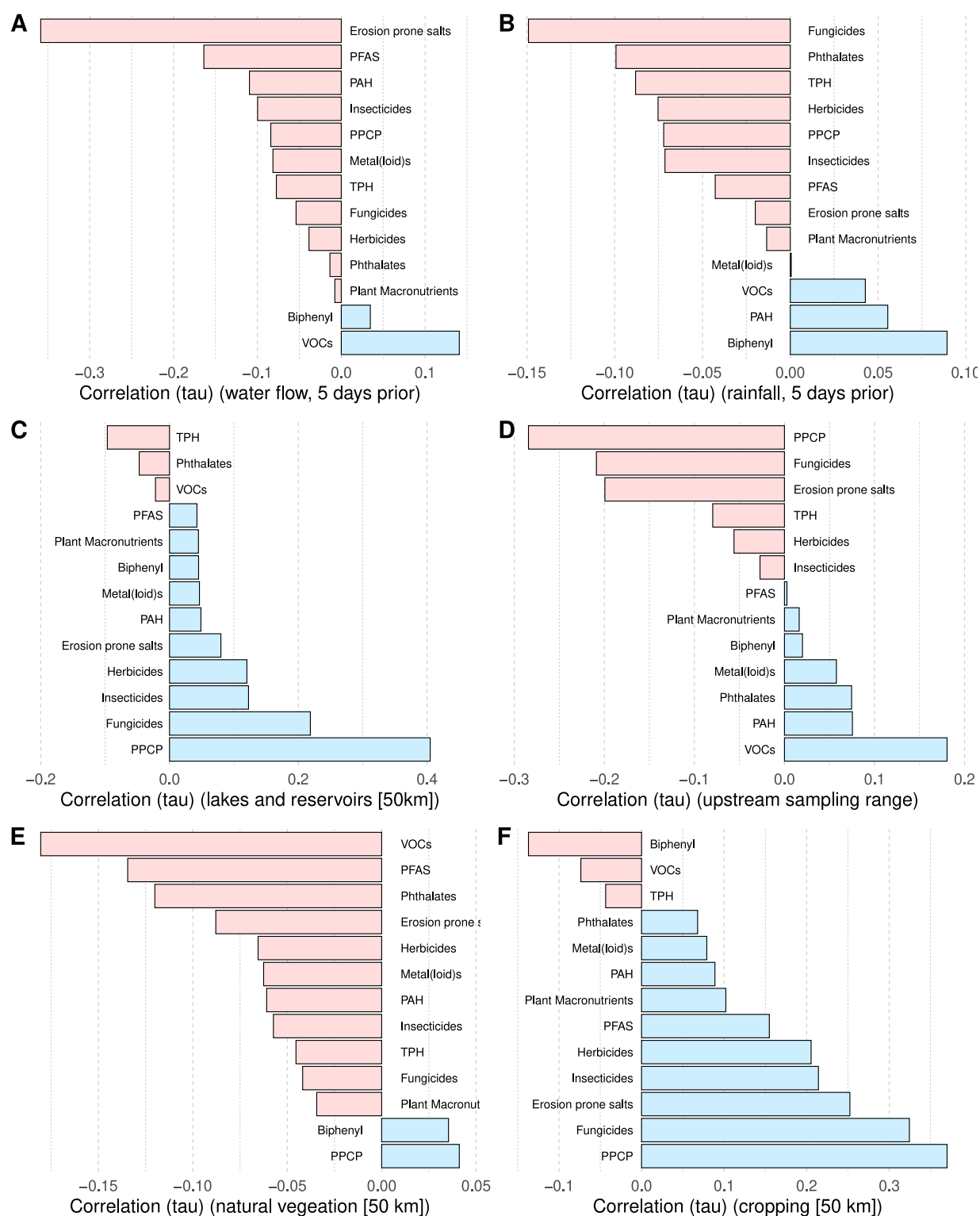


Figure 4. Bar graphs (red = negative, blue = positive) showing concentration ($\mu\text{g/L}$) tau correlations with (A) water flow (5 days prior), (B) rainfall (5 days prior), (C) lakes and reservoirs (50 km), (D) upstream sampling range, (E) natural vegetation (50 km), (F) cropping (50 km). Hydrological lags and land use categories were chosen based on relative importances of predictors in random forests models.

Comparisons to other reports of contaminants in flooded or postflooded waterways reveal seemingly few clearly generalizable trends across reported data (some examples^{8–17,56}). For example, He, Dong, Sun, Zhang, Zhang, Hua, and Guo¹⁵ reported 17 PPCPs in floodwater, whereas we detected just one, lincomycin. The same study reported potentially

concerning levels of natural estrogens in floodwaters, whereas no mammalian hormones or analogues were detected in this study. Triclosan is a widely used antimicrobial (e.g. sunscreen, and hand soaps) and frequently detected in surface waters,^{15,57,58} yet we saw no indication of its presence in the flooded landscape. Quite different patterns tend to be reported

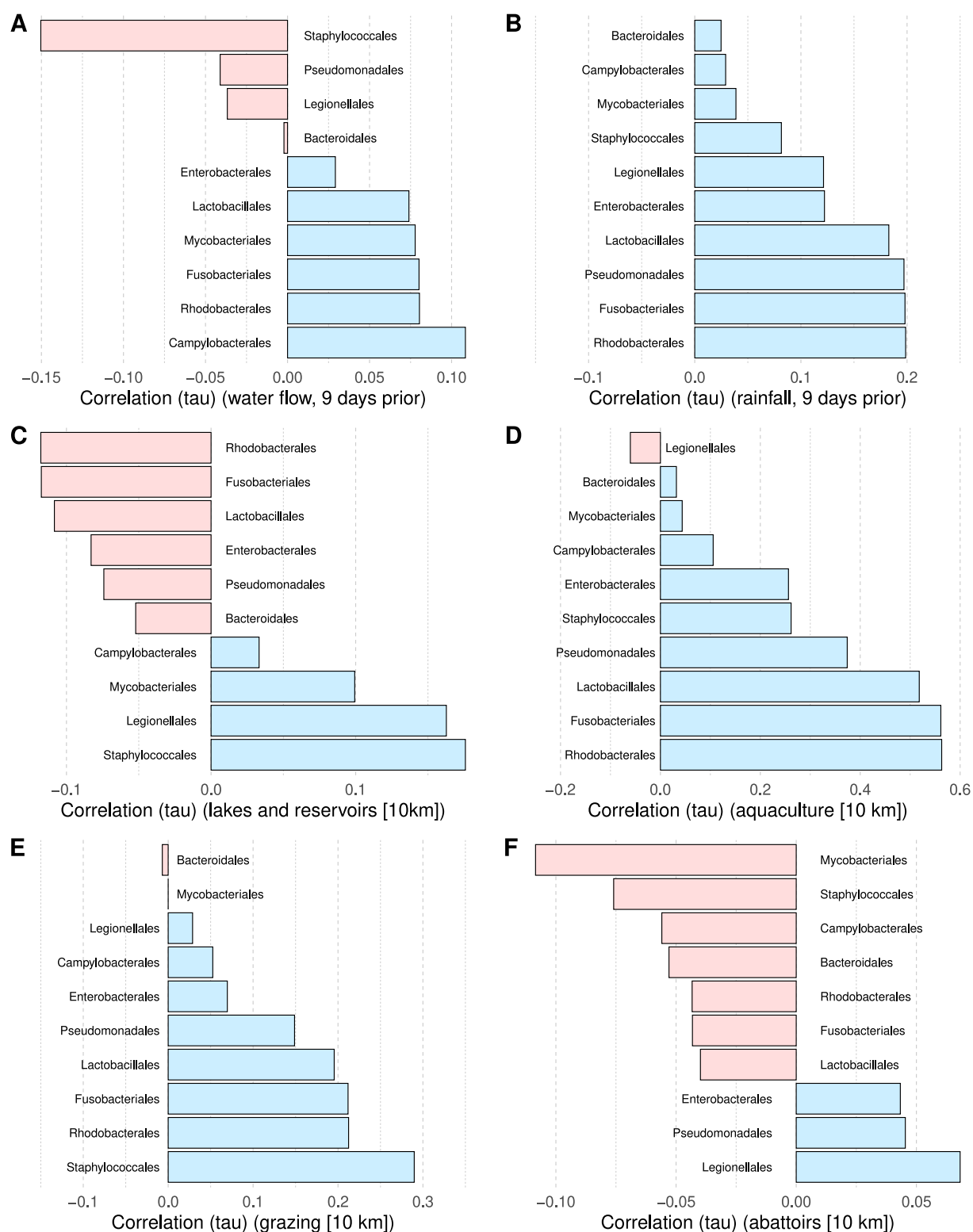


Figure 5. Bar graphs showing eDNA [potentially pathogenic Orders] “count” tau correlations (red = negative, blue = positive) with (A) water flow (9 days prior), (B) rainfall (9 days prior), (C) lakes and reservoirs (10 km), (D) aquaculture (10 km), (E) grazing (10 km), (F) abattoirs (10 km). Hydrological lags and land use categories were chosen based on relative importances of predictors in random forests models.

across different studies (some examples^{8–17,56}), and other authors have drawn attention to apparent trends in the published data that suggest the contaminant makeup of flooded or postflood waterways is highly context dependent.^{19–21} Certainly, this seems to be the case here. Different contaminant groups, and even chemicals within a group, show

quite varied and distinct responses to land use, water flow and rainfall. Based on the current results, it seems possible that no single overarching model of contaminants in floods is viable. Contextual relationships between the landscape, hydrology and specific chemical attributes are potentially too locally variable to be sensibly transposed from one environment to another.

We used total suspended solid concentrations, as a proxy for suspended sediments, and observed quite different responses to the dissolved concentrations of contaminants in water. Whereas meaningful flow relationships with the contaminants tended to be negative, indicating dilution, TSS had a strong positive (concentrating) association with flow at 6 days. Interestingly, TSS also tended to respond at the 10 km radius limit where land uses were important predictors, such as for stormwater and natural vegetation (both positive). Previous studies have argued that for chemicals (e.g., heavy metals) that bond to sediment, up to 90% of the contaminant load might be in the suspended sediment in a flood,⁵⁹ meaning the primary pathway of the contaminant might not be strongly different to the pathway of sediments. Future research on contaminant-sediment relationships in large-scale flooded landscapes are needed, especially for sediment-binding groups, such as phthalates⁶⁰ and heavy metals.⁵⁹

Fungicides, insecticides, herbicides, erosion prone salts and lincomycin were more concentrated at sites farther downstream than upstream (Figure 4D). These contaminants include a PPCP (lincomycin) and agrichemicals, which can cause harm to humans and to the environment at low concentrations. For example, a recent study reported that even at very low concentrations (pyrethroids; 96 h exposure range 0.0007–0.0010 $\mu\text{g L}^{-1}$), pesticide exposure can affect fish behavior and gene expression for generations.⁶¹ Our study is in agreement with previous work that recommends postflood monitoring to continue farther downstream of a flooded catchment to assess risks to the health of the water system.²⁰

Assessments of contaminants in alluvial sediments deposited by floodwaters should be standard practice. This has been undertaken more frequently in postflood urban environments²² than in agricultural landscapes. Some authors⁵ have argued that flood-borne chemical contaminants may enter human food chains after deposition on agricultural or horticultural land, potentially triggering the 'next big' food scandal.¹⁹ There is clearly a need to improve postflood assessment of farmed landscape soils, crops, and stock animals (or animal products, e.g., milk) after serious flooding. Some very high mass fluxes in the current study in combination with the observation that at least some contaminants were concentrated at downstream sites suggests this system would benefit from postflood sediment monitoring.

Detection of Disease Associated Microbes. In total, 554 OTU (species level) were identified across 26 microbial phyla in floodwater via eDNA metabarcoding. The phyla with the highest maximum eDNA counts (relative detectability) were Firmicutes (66,822), Asgardarchaeota (24,998) and Proteobacteria (12,543). The phyla with the highest mean count values were Asgardarchaeota (2,319.9), Firmicutes (586.4) and Armatimonadota (188.6). The phyla Actinobacteriota, Armatimonadota, Bacteroidota, Cyanobacteria, Desulfobacterota, Firmicutes, Proteobacteria and Verrucomicrobiota returned positive signals for presence in all study sites. Putative OTU belonging to potentially pathogenic and/or effluent associated taxa were detected (Table S3.4 and Figure 3B). The number (n) of OTUs per taxon were: *Acinetobacter* ($n = 3$), *Aeromonas* ($n = 3$), Arcobacteraceae ($n = 3$), Bacteroidales (*Prevotalla*) ($n = 1$), *Enterococcus* ($n = 2$), *Escherichia* ($n = 1$), *Klebsiella* ($n = 4$), Legionellaceae ($n = 2$), Leptotrichiaceae ($n = 2$), *Mycobacterium* ($n = 4$), *Paracoccus* ($n = 1$), *Pseudomonas* ($n = 1$), and *Streptococcus* ($n = 4$). Notably,

some of these taxa include potentially serious pathogens (e.g., *Mycobacterium* spp. and *Legionella* spp.).

Microbial Profile in Water. Microbial risks of exposure in floodwaters have been explored previously,^{13,62,63} however most prior work has focused on culturable taxa and/or bacterial toxins. Increasingly, there is a recognition that metagenomic surveillance of pathogenic microbes is required for thorough monitoring.⁶⁴ Of the taxa detected (Table S3.5), *Acinetobacter*, Legionellaceae, *Mycobacterium*, *Paracoccus*, and *Pseudomonas* are commonly found in environmental soil and/or water, but can cause infectious disease in humans or other animals upon entering the body. Arcobacteraceae and *Klebsiella* can persist in both the environment (water, soil) or living organisms, and are typically considered either opportunistic pathogens and/or pathogenic only in some infection-adapted lineages. Leptotrichiaceae and *Streptococcus* include some well-known pathogens, though also form part of the commensal microbiota in animals. *Aeromonas*, Order Bacteroidales (including *Prevotalla*), *Enterococcus*, and *Escherichia* are found in human and animal guts, and are either suspected or known to cause gastroenteritis. This last set of microbes is typically not considered likely to originate in the environment (i.e., they tend not to persist in soil or water), and are typically attributed to wastewater effluent, rural septic tank leakage, or agricultural sources (feeding pens, milking yards etc.). Overall, a diversity of potential pathogenic groups was detected, including taxa of environmental origin (naturally persisting in soil and water) and others that are more likely to originate from animal or human faecal waste entering the river system during a flooding event.

Microbe and Environmental Patterns. Overall, microbial eDNA counts tended to be better explained by land uses than water flow, rainfall or water level, although some Orders were highly taxonomically dominated in their explanatory variables. Figure 5A–F shows correlations for six environmental categories that were repeatedly important for predicting microbial eDNA signatures: water flow (9 day lag), rain (9 day lag), lakes and reservoirs (10 km), aquaculture (10 km), grazing (10 km) and abattoirs (10 km). As with chemical contaminants, responses to environmental variables were not consistent across taxa (see Figures S3.14–S3.25).

Aquaculture at either 10 or 50 km showed positive associations with Orders Campylobacterales, Enterobacteriales, Lactobacillales, Pseudomonadales and Rhodobacteriales. Aquaculture in marine environments has been associated with some contaminants.⁶⁵ Hence, our observations warrant targeted investigations to determine whether aquaculture in or near river systems promotes proliferation of pathogen associated microbes. The presence of Fusobacteriales, Legionellales and Staphylococcales was better explained by rural land uses, such as grazing (Fusobacteriales and Staphylococcales) and intensive animal production (Fusobacteriales) or abattoirs (Legionellales and Staphylococcales). Contrary to expectations, Bacteroidales and Enterobacteriales were not associated with Sewage and WWTP and Rural Residential (septic tanks) land use categories. Instead, Bacteroidales was predominantly explained by diluting and/or survivorship reducing effects of lakes and reservoirs, flow, and water level. Previous work has shown that Bacteroidales are good indicators of faecal discharge in flood waters because they do not survive in environmental water, whereas *E. coli* can persist for days or weeks.⁶⁶ Enterobacteriales, which includes *E. coli*, was better explained by taxon-specific predictors rather than any land use,

with the exception of aquaculture. Overall, our study indicates that microbes might respond to water flows on a slightly longer time scale, but over a smaller landscape scale compared to contaminants.

Environmental Management Implications. The detection of several PAHs might be of particular concern in an Australian context. PAHs, including naphthalene, are used in industrial processes, and are also produced by combustion of petrochemicals or wood.⁶⁷ Naphthalene and all derivative PAHs are considered probable carcinogens in humans.⁶⁷ These chemicals may also have endocrine disrupting effects.⁶⁸ Although roads and industrial facilities are present in the study area, wood combustion is an important source of PAHs in Australia, particularly in regional areas,⁶⁹ where bushfires can increase soil erosion, destroy vegetation root systems and transfer of ash substances.^{70,71} Extreme rainfall events and extreme fire events are likely to increase in frequency and intensity with climate change,¹ potentially elevating PAH contamination in waterways.

Another point of particular concern in a strongly wetting/drying environment are the high observed loads of plant macronutrients. Although eutrophication associated with plant macronutrients (agricultural contaminants) is a long-standing focus of environmental research and management,⁷² the issue requires further consideration in the Australian context. Australian water systems tend to pass through 'wetting' and 'drying' cycles, and plant macronutrients are the metabolic currency required to underwrite periodic algae blooms. In recent years, algal blooms have been observed in Australian rivers during drying cycles, leading to deoxygenation of the water and subsequent 'fish kill' or 'black water' events.^{73,74} Although all macronutrients are required for algal growth, in most freshwater systems, phosphorus is the limiting element. Carlson's trophic scale index classifies total phosphorus levels $>24 \mu\text{g L}^{-1}$ as satisfying the requirements for eutrophication.⁷⁵ In our study, 44% of floodwater samples had orthophosphate concentrations above this threshold, with a maximum of $142 \mu\text{g L}^{-1}$. We did not monitor the effects of drying after the flood subsided, but if 44% of samples were potentially eutrophic at the height of the flood, the receding waters might have concentrated orthophosphate to an even larger extent in postfloodwater bodies (see Table S3.6 for some baseline values).

Many of the chemical contaminants studied here undergo chemical, biological and physical transformations in the environment. These downstream processes are replete with complex interactions,²⁹ and the resulting metabolites can be more toxic and concentrated than the parent compounds.⁷⁶ Assessing metabolites, or hydrolytic, thermolytic and photolytic products, and their relations with parent molecules in the environment could form an avenue of future research in this system.²⁹ A recent metanalysis proposed that most glyphosate in European rivers might originate from aminopolyphosphonates converted to glyphosate in WWTPs, and not from agriculture.⁷⁷ Failing to consider possible chemical derivative pathways might lead to false conclusions. Relationships between chemical concentrations and mass fluxes, with biomagnification and bioaccumulation in the food web, is also a point of concern, especially for contaminants that are known to bioaccumulate (e.g., Hg, PFAS).

It remains unclear how metabarcoding eDNA counts should be interpreted in the context of human health risks. Yet, *Vibrio* infections and gastrointestinal illnesses have been reported

postflooding.²⁸ Mean probable colonies per 100 mL reached 1200 in some of the present samples, exceeding the EPA guideline of 260 Most Probable Number (MPN)/100 mL in recreational water. Hence, monitoring bodies could establish clear guidelines for safe levels of potential pathogens in environmental water based on eDNA metabarcoding counts.

In conclusion, if environmental values and human health are to be conserved, it is essential to know what contaminants have moved through a flooded landscape, and their respective concentrations. The present dataset is an evidence-base for decision making during increasingly frequent and high magnitude extreme weather events.^{24,25} Our explanatory models show that potential contaminant risks are highly context dependent and illustrate a pressing need to allocate resources to monitoring of contaminants during and after flooding.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.4c03875>, and includes:

28 figures and 16 tables, and further information about the study scope, sample extraction, and analysis, QA/QC, eDNA analysis, hydrological data, Δ flow calculations, human footprint index, land use, surface water flow models, mass flux calculations, and extended models for the data analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Minna Saaristo – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia; orcid.org/0000-0002-9632-8611; Email: minna.saaristo@epa.vic.gov.au

Authors

Christopher P. Johnstone – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Phoebe Lewis – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Simon Sharp – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Timothy Chaston – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Molly Hoak – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Paul Leahy – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Darren Cottam – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia
Luke Noble – EnviroDNA, Melbourne, Victoria 3056, Australia
John Leeder – Leeder Analytical, Melbourne, Victoria 3078, Australia
Mark Patrick Taylor – Environment Protection Authority, EPA Science, Macleod, Victoria 3085, Australia

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acs.est.4c03875>

Notes

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REFERENCES

- (1) Morán-Ordóñez, A.; Duane, A.; Gil-Tena, A.; De Cáceres, M.; Aquilué, N.; Guerra, C. A.; Geizendorffer, I. R.; Fortin, M. J.; Brotons, L. Future impact of climate extremes in the Mediterranean: Soil erosion projections when fire and extreme rainfall meet. *Land Degradation & Development* **2020**, *31* (18), 3040–3054.
- (2) Touma, D.; Stevenson, S.; Swain, D. L.; Singh, D.; Kalashnikov, D. A.; Huang, X. Climate change increases risk of extreme rainfall following wildfire in the western United States. *Sci. Adv.* **2022**, *8* (13), No. eabm0320.
- (3) Milly, P. C. D.; Wetherald, R. T.; Dunne, K.; Delworth, T. L. Increasing risk of great floods in a changing climate. *Nature* **2002**, *415* (6871), 514–517.
- (4) Rahmstorf, S.; Coumou, D. Increase of extreme events in a warming world. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (44), 17905–17909.
- (5) Foulds, S. A.; Brewer, P. A.; Macklin, M. G.; Haresign, W.; Betson, R. E.; Rassner, S. M. E. Flood-related contamination in catchments affected by historical metal mining: an unexpected and emerging hazard of climate change. *Sci. Total Environ.* **2014**, *476*, 165–180.
- (6) Coble, A. A.; Wymore, A. S.; Shattuck, M. D.; Potter, J. D.; McDowell, W. H. Multiyear trends in solute concentrations and fluxes from a suburban watershed: Evaluating effects of 100-year flood events. *Journal of Geophysical Research: Biogeosciences* **2018**, *123* (9), 3072–3087.
- (7) VICSES. *Victorian Floods 2022 (VICSES, Summer 2022) (VICSES)*; V. S. E. S., Ed.; VICSES: Melbourne, 2022; vol 21, pp 4–5.
- (8) Ashley, N. A.; Valsaraj, K. T.; Thibodeaux, L. J. Elevated in-home sediment contaminant concentrations—The consequence of a particle settling–winnowing process from Hurricane Katrina floodwaters. *Chemosphere* **2008**, *70* (5), 833–840.
- (9) Ciesielczuk, T.; Kusza, G.; Poluszyńska, J.; Kochanowska, K. Pollution of flooded arable soils with heavy metals and polycyclic aromatic hydrocarbons (PAHs). *Water, Air, Soil Pollut.* **2014**, *225*, 2145.
- (10) Crampon, M.; Soulier, C.; Sidoli, P.; Hellal, J.; Joulain, C.; Charron, M.; Guillemoto, Q.; Picot-Colbeaux, G.; Pettenati, M. Dynamics of soil microbial communities during diazepam and oxazepam biodegradation in soil flooded by water from a WWTP. *Front. Microbiol.* **2021**, *12*, 3451.
- (11) Donald, D. B.; Hunter, F. G.; Sverko, E.; Hill, B. D.; Syrgiannis, J. Mobilization of pesticides on an agricultural landscape flooded by a torrential storm. *Environmental Toxicology and Chemistry: An International Journal* **2005**, *24* (1), 2–10.
- (12) Dong, J.; Shang, M.; Feng, R.; Song, X.; Yan, D.; Xie, H. Export and risk from antibiotic remobilization from surrounding water to lake in the extreme 2020 Yangtze River basin flooding. *Science of The Total Environment* **2022**, *834*, No. 155176.
- (13) Fewtrell, L.; Kay, D.; Watkins, J.; Davies, C.; Francis, C. The microbiology of urban UK floodwaters and a quantitative microbial risk assessment of flooding and gastrointestinal illness. *Journal of flood risk management* **2011**, *4* (2), 77–87.
- (14) Gallen, C.; Baduel, C.; Lai, F. Y.; Thompson, K.; Thompson, J.; Warne, M.; Mueller, J. F. Spatio-temporal assessment of perfluorinated compounds in the Brisbane River system, Australia: impact of a major flood event. *Marine pollution bulletin* **2014**, *85* (2), 597–605.
- (15) He, S.; Dong, D.; Sun, C.; Zhang, X.; Zhang, L.; Hua, X.; Guo, Z. Contaminants of emerging concern in a freeze-thaw river during the spring flood. *Science of the total environment* **2019**, *670*, 576–584.
- (16) Kiaghadi, A.; Rifai, H. S. Physical, chemical, and microbial quality of floodwaters in Houston following Hurricane Harvey. *Environ. Sci. Technol.* **2019**, *53* (9), 4832–4840.
- (17) Liu, D.; Zhao, J.; Jeon, W. H.; Lee, J. Y. Solute dynamics across the stream-to-riparian continuum under different flood waves. *Hydrological Processes* **2019**, *33* (20), 2627–2641.
- (18) Merz, B.; Kreibich, H.; Schwarze, R.; Thieken, A. Review article “Assessment of economic flood damage”. *Natural Hazards and Earth System Sciences* **2010**, *10* (8), 1697–1724.
- (19) Crawford, S. E.; Brinkmann, M.; Ouellet, J. D.; Lehmkuhl, F.; Reicherter, K.; Schwarzbauer, J.; Bellanova, P.; Letmathe, P.; Blank, L. M.; Weber, R.; Brack, W.; van Dongen, J. T.; Menzel, L.; Hecker, M.; Schüttrumpf, H.; Hollert, H. Remobilization of pollutants during extreme flood events poses severe risks to human and environmental health. *J. Hazard. Mater.* **2022**, *421*, No. 126691.
- (20) Best, J.; Ashmore, P.; Darby, S. Beyond just floodwater. *Nature Sustainability* **2022**, *5*, 811–813.
- (21) Mansour, I.; Heppell, C. M.; Ryo, M.; Rillig, M. C. Application of the microbial community coalescence concept to riverine networks. *Biological Reviews* **2018**, *93* (4), 1832–1845.
- (22) Barber, L. B.; Paschke, S. S.; Battaglin, W. A.; Douville, C.; Fitzgerald, K. C.; Keefe, S. H.; Roth, D. A.; Vajda, A. M. Effects of an extreme flood on trace elements in river water—from urban stream to major river basin. *Environ. Sci. Technol.* **2017**, *51* (18), 10344–10356.
- (23) Mayes, W.; Perks, M.; Large, A.; Davis, J.; Gandy, C.; Orme, P.; Jarvis, A. Effect of an extreme flood event on solute transport and resilience of a mine water treatment system in a mineralised catchment. *Sci. Total Environ.* **2021**, *750*, No. 141693.
- (24) Meingast, K. M.; Kane, E. S.; Coble, A. A.; Marcarelli, A. M.; Toczydlowski, D. Climate, snowmelt dynamics and atmospheric deposition interact to control dissolved organic carbon export from a northern forest stream over 26 years. *Environmental Research Letters* **2020**, *15* (10), 104034.
- (25) Fazekas, H. M.; Wymore, A. S.; McDowell, W. H. Dissolved organic carbon and nitrate concentration-discharge behavior across scales: Land use, excursions, and misclassification. *Water Resour. Res.* **2020**, *56* (8), No. e2019WR027028.
- (26) Chang, C.-T.; Lee, L.-C.; Song, C.-E.; Chiang, J.-M.; Liao, C.-S.; Liou, Y.-S.; Wang, S.-F.; Huang, J.-C. Divergent effect of landscape patterns on stream water chemistry and seasonal variations across mountainous watersheds in a Northwest Pacific island. *Ecological Indicators* **2024**, *158*, No. 111581.
- (27) Mourier, B.; Labadie, P.; Desmet, M.; Grosbois, C.; Raux, J.; Debret, M.; Copard, Y.; Pardon, P.; Budzinski, H.; Babut, M. Combined spatial and retrospective analysis of fluoroalkyl chemicals in fluvial sediments reveal changes in levels and patterns over the last 40 years. *Environ. Pollut.* **2019**, *253*, 1117–1125.
- (28) Kapoor, V.; Gupta, I.; Pasha, A. T.; Phan, D. Real-time quantitative PCR measurements of fecal indicator bacteria and human-associated source tracking markers in a Texas river following Hurricane Harvey. *Environmental Science & Technology Letters* **2018**, *5* (6), 322–328.
- (29) Mofijur, M.; Hasan, M.; Ahmed, S. F.; Djavanroodi, F.; Fattah, I.; Silitonga, A.; Kalam, M.; Zhou, J. L.; Khan, T. Y. Advances in identifying and managing emerging contaminants in aquatic ecosystems: Analytical approaches, toxicity assessment, transformation pathways, environmental fate, and remediation strategies. *Environ. Pollut.* **2024**, *341*, No. 122889.
- (30) Coble, A. A.; Wymore, A. S.; Potter, J. D.; McDowell, W. H. Land use overrides stream order and season in driving dissolved organic matter dynamics throughout the year in a river network. *Environ. Sci. Technol.* **2022**, *56* (3), 2009–2020.
- (31) EPA, V. *IWRG 701*; Victoria, E., Ed.; EPA Publication, 2009.

- (32) EPA, V. *NEMP 3*; FAS National Environmental Management Plan, 2023.
- (33) Chahal, C.; Van Den Akker, B.; Young, F.; Franco, C.; Blackburn, J.; Monis, P. Pathogen and particle associations in wastewater: significance and implications for treatment and disinfection processes. *Advances in applied microbiology* **2016**, *97*, 63–119.
- (34) Fonti, V.; Di Cesare, A.; Šangulin, J.; Del Negro, P.; Celussi, M. Antibiotic resistance genes and potentially pathogenic bacteria in the central Adriatic Sea: are they connected to urban wastewater inputs? *Water* **2021**, *13* (23), 3335.
- (35) Vicmap. *Vicmap Elevation*; 2024. <https://www.land.vic.gov.au/maps-and-spatial/spatial-data/vicmap-catalogue/vicmap-elevation> (accessed 2024).
- (36) VLUIS. *Victorian land use information system (VLUIS)*; 2017. <https://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/vluis> (accessed 2024).
- (37) ABS. *Australian Bureau of Statistics Meshblock Areas*; 2024. <https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1270.0.55.001July%202016?OpenDocument> (accessed June 2024).
- (38) Chorover, J.; Derry, L. A.; McDowell, W. H. Concentration-discharge relations in the critical zone: Implications for resolving critical zone structure, function, and evolution. *Water Resour. Res.* **2017**, *53* (11), 8654–8659.
- (39) RStudio: Integrated Development Environment for R. *Posit Software*; PBC: Boston, MA, 2023. <http://www.posit.co/> (accessed June 2024).
- (40) R: A language and environment for statistical computing. *R Foundation for Statistical Computing*; Vienna, Austria, 2023. <https://www.R-project.org/> (accessed June 2024).
- (41) Kassotis, C. D.; Phillips, A. L. Complex Mixtures and Multiple Stressors: Evaluating Combined Chemical Exposures and Cumulative Toxicity. *Toxics* **2023**, *11*, 487.
- (42) Chen, H.; Chen, K.; Qiu, X.; Xu, H.; Mao, G.; Zhao, T.; Feng, W.; Okeke, E. S.; Wu, X.; Yang, L. The reproductive toxicity and potential mechanisms of combined exposure to dibutyl phthalate and diisobutyl phthalate in male zebrafish (*Danio rerio*). *Chemosphere* **2020**, *258*, No. 127238.
- (43) Hamid, N.; Junaid, M.; Manzoor, R.; Jia, P.-P.; Pei, D.-S. Prioritizing phthalate esters (PAEs) using experimental in vitro/vivo toxicity assays and computational in silico approaches. *Journal of Hazardous Materials* **2020**, *398*, No. 122851.
- (44) Wang, Z.; Ma, J.; Wang, T.; Qin, C.; Hu, X.; Mosa, A.; Ling, W. Environmental health risks induced by interaction between Phthalic Acid Esters (PAEs) and biological macromolecule: A review. *Chemosphere* **2023**, *328*, No. 138578.
- (45) Kurwadkar, S.; Dane, J.; Kanel, S. R.; Nadagouda, M. N.; Cawdrey, R. W.; Ambade, B.; Struckhoff, G. C.; Wilkin, R. Per- and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. *Science of The Total Environment* **2022**, *809*, No. 151003.
- (46) Ragnarsdóttir, O.; Abdallah, M. A.-E.; Harrad, S. Dermal uptake: An important pathway of human exposure to perfluoroalkyl substances? *Environ. Pollut.* **2022**, *307*, No. 119478.
- (47) Tello, A.; Austin, B.; Telfer, T. C. Selective pressure of antibiotic pollution on bacteria of importance to public health. *Environ. Health Perspect.* **2012**, *120* (8), 1100–1106.
- (48) Bengtsson-Palme, J.; Kristiansson, E.; Larsson, D. J. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS microbiology reviews* **2018**, *42* (1), fux053.
- (49) Imfeld, G.; Payraudeau, S.; Tournebise, J.; Sauvage, S.; Macary, F.; Chaumont, C.; Probst, A.; Sánchez-Pérez, J.-M.; Bahi, A.; Chaumet, B.; Gilevska, T.; Alexandre, H. The role of ponds in pesticide dissipation at the agricultural catchment scale: a critical review. *Water* **2021**, *13* (9), 1202.
- (50) Ebele, A. J.; Abdallah, M. A.-E.; Harrad, S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg. Contam.* **2017**, *3* (1), 1–16.
- (51) Wightwick, A. M.; Bui, A. D.; Zhang, P.; Rose, G.; Allinson, M.; Myers, J. H.; Reichman, S. M.; Menzies, N. W.; Pettigrove, V.; Allinson, G. Environmental fate of fungicides in surface waters of a horticultural-production catchment in southeastern Australia. *Arch. Environ. Contam. Toxicol.* **2012**, *62*, 380–390.
- (52) Allinson, G.; Allinson, M.; Bui, A.; Zhang, P.; Croatto, G.; Wightwick, A.; Rose, G.; Walters, R. Pesticide and trace metals in surface waters and sediments of rivers entering the Corner Inlet Marine National Park, Victoria, Australia. *Environmental Science and Pollution Research* **2016**, *23*, 5881–5891.
- (53) Allinson, G.; Bui, A.; Zhang, P.; Rose, G.; Wightwick, A. M.; Allinson, M.; Pettigrove, V. Investigation of 10 herbicides in surface waters of a horticultural production catchment in southeastern Australia. *Archives of environmental contamination and toxicology* **2014**, *67*, 358–373.
- (54) Sharley, D. J.; Sharp, S. M.; Marshall, S.; Jeppe, K.; Pettigrove, V. J. Linking urban land use to pollutants in constructed wetlands: Implications for stormwater and urban planning. *Landscape and Urban Planning* **2017**, *162*, 80–91.
- (55) Band, L. E.; Cadenasso, M. L.; Grimmond, C. S.; Grove, J. M.; Pickett, S. T. Heterogeneity in urban ecosystems: patterns and process. *Ecosystem function in heterogeneous landscapes* **2005**, 257–278.
- (56) Zhou, M.; Wu, S.; Zhang, Z.; Aihemaiti, Y.; Yang, L.; Shao, Y.; Chen, Z.; Jiang, Y.; Jin, C.; Zheng, G. Dilution or enrichment: the effects of flood on pollutants in urban rivers. *Environ. Sci. Eur.* **2022**, *34* (1), 61.
- (57) Roberts, J.; Kumar, A.; Du, J.; Hepplewhite, C.; Ellis, D. J.; Christy, A. G.; Beavis, S. G. Pharmaceuticals and personal care products (PPCPs) in Australia's largest inland sewage treatment plant, and its contribution to a major Australian river during high and low flow. *Science of the total environment* **2016**, *541*, 1625–1637.
- (58) Anim, A. K.; Thompson, K.; Duodu, G. O.; Tscharke, B.; Birch, G.; Goonetilleke, A.; Ayoko, G. A.; Mueller, J. F. Pharmaceuticals, personal care products, food additive and pesticides in surface waters from three Australian east coast estuaries (Sydney, Yarra and Brisbane). *Mar. Pollut. Bull.* **2020**, *153*, No. 111014.
- (59) Ciszewski, D.; Grygar, T. M. A review of flood-related storage and remobilization of heavy metal pollutants in river systems. *Water, Air, Soil Pollut.* **2016**, *227*, 239.
- (60) Ghosh, S.; Sahu, M. Phthalate pollution and remediation strategies: a review. *Journal of Hazardous Materials Advances* **2022**, *6*, No. 100065.
- (61) Hutton, S. J.; Siddiqui, S.; Pedersen, E. I.; Markgraf, C. Y.; Segarra, A.; Hladik, M. L.; Connon, R. E.; Brander, S. M. Multigenerational, Indirect Exposure to Pyrethroids Demonstrates Potential Compensatory Response and Reduced Toxicity at Higher Salinity in Estuarine Fish. *Environ. Sci. Technol.* **2024**, *58*, 2224–2235.
- (62) ten Veldhuis, J.; Clemens, F.; Sterk, G.; Berends, B. Microbial risks associated with exposure to pathogens in contaminated urban flood water. *Water research* **2010**, *44* (9), 2910–2918.
- (63) Taylor, J.; Lai, K. m.; Davies, M.; Clifton, D.; Ridley, I.; Biddulph, P. Flood management: prediction of microbial contamination in large-scale floods in urban environments. *Environ. Int.* **2011**, *37* (5), 1019–1029.
- (64) Ko, K. K.; Chng, K. R.; Nagarajan, N. Metagenomics-enabled microbial surveillance. *Nature Microbiology* **2022**, *7* (4), 486–496.
- (65) Holmer, M. Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. *Aquaculture Environment Interactions* **2010**, *1* (1), 57–70.
- (66) Staley, Z. R.; Chuong, J. D.; Hill, S. J.; Grabuski, J.; Shokralla, S.; Hajibabaei, M.; Edge, T. A. Fecal source tracking and eDNA profiling in an urban creek following an extreme rain event. *Sci. Rep.* **2018**, *8* (1), 14390.
- (67) Rengarajan, T.; Rajendran, P.; Nandakumar, N.; Lokeshkumar, B.; Rajendran, P.; Nishigaki, I. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine* **2015**, *5* (3), 182–189.

- (68) Zhang, Y.; Dong, S.; Wang, H.; Tao, S.; Kiyama, R. Biological impact of environmental polycyclic aromatic hydrocarbons (ePAHs) as endocrine disruptors. *Environmental pollution* **2016**, *213*, 809–824.
- (69) Steiber, R. S. Organic combustion fingerprints of three common home heating fuels. *Air & Waste* **1993**, *43* (6), 859–863.
- (70) Pérez-Cabello, F.; Echeverría, M. T.; Ibarra, P.; de la Riva, J. Effects of fire on vegetation, soil and hydrogeomorphological behavior in Mediterranean ecosystems. *Earth observation of wildland fires in Mediterranean ecosystems* **2009**, 111–128.
- (71) Yang, X.; Zhu, Q.; Tulau, M.; McInnes-Clarke, S.; Sun, L.; Zhang, X. Near real-time monitoring of post-fire erosion after storm events: a case study in Warrumbungle National Park. *Australia. International Journal of Wildland Fire* **2018**, *27* (6), 413–424.
- (72) Loewald, A.; Ryan, P.; Kim, J. *A Review of Phosphorous and Nitrogen in Groundwater and Lakes*; 2020.
- (73) Koehn, J. D. Key steps to improve the assessment, evaluation and management of fish kills: lessons from the Murray–Darling River system, Australia. *Marine and Freshwater Research* **2022**, *73* (2), 269–281.
- (74) Jackson, S.; Head, L. Australia's mass fish kills as a crisis of modern water: Understanding hydrosocial change in the Murray-Darling Basin. *Geoforum* **2020**, *109*, 44–56.
- (75) Opiyo, S.; Getabu, A. M.; Sitoki, L. M.; Shitandi, A.; Ogendi, G. M. Application of the Carlson's trophic state index for the assessment of trophic status of lake Simbi ecosystem, a deep alkaline-saline lake in Kenya. *Int. J. Fish. Aquat. Stud.* **2019**, *7* (4), 327–333.
- (76) Yin, L.; Wang, B.; Yuan, H.; Deng, S.; Huang, J.; Wang, Y.; Yu, G. Pay special attention to the transformation products of PPCPs in environment. *Emerging Contaminants* **2017**, *3* (2), 69–75.
- (77) Schwientek, M.; Rügner, H.; Haderlein, S.; Schulz, W.; Wimmer, B.; Engelbart, L.; Bieger, S.; Huhn, C. Glyphosate contamination in European rivers not from herbicide application? *Water Res.* **2024**, *263*, No. 122140.