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High fecal indicator bacteria in temperate headwater streams at baseflow: implications for management and public health

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ABSTRACT

Fecal indicator bacteria (FIB) in rivers and streams serve as key markers of public health risks, but factors influencing spatiotemporal FIB variation in headwater streams at baseflow have received insufficient attention. We analyzed a 12-year dataset of FIB concentrations collected from 118 watersheds in the Delaware River Basin of the eastern United States across seasons to establish baseline conditions and investigate how landscape (watershed size and land cover) and in-stream (e.g., temperature and particulates) environmental variables relate to spatiotemporal FIB variations. We found that most probable number (MPN)-derived FIB levels in baseflow at 86 % to 96 % of sites (for *Escherichia coli* and *Enterococcus*, respectively) were above human health regulatory limits for primary contact during the recreational season. Variables affecting FIB dynamics included watershed size, seasonal variations in stream temperature, total particulate carbon and nitrogen in the water, and land cover types – specifically, the relative extent of forested, developed, and agricultural areas. Based on watershed size, smaller headwaters contained higher recreational period FIB concentrations than larger rivers. Headwater FIB concentrations were related to land cover, with lower concentrations observed in forested watersheds, and higher concentrations in developed and agricultural watersheds. Microbial source tracking suggested that FIB originated from human and bovine sources in headwaters with developed or agricultural land cover. FIB levels tended to be lower in non-recreational seasons (winter, spring, and fall) in small headwater streams. In addition to human, cow and other animals, we speculate that FIB survival and turnover in local environments might also cause FIB occurrence. These findings help guide choices of indicators to address fecal contamination of rivers and streams and prioritize restorative actions at the landscape scale.

1. Introduction

Microbial contaminants are a leading cause of water quality degradation in the United States, affecting 20 % of all rivers and streams (US EPA, 2017), and impairing water uses such as recreation and drinking water supply. Land and water uses, such as those associated with urbanized watersheds, significantly impact microbial contaminants (Zhang et al., 2020). As these risks are a concern for public health globally, extensive monitoring efforts are undertaken to assess the potential for pathogenic diseases spreading to humans (Abbas et al., 2021). However, such efforts are often time-consuming and resource-intensive, leaving many water bodies—especially smaller headwater streams—unmonitored. Currently fecal indicator bacteria (FIB) serve as valuable tools to for monitoring, enabling targeted restoration and management efforts to mitigate fecal pollution in watersheds (Verhougstraete et al., 2015).

Understanding and predicting relationships between watershed uses, in-stream processes, and FIB is inherently challenging due to many influential variables (Abbas et al., 2021; Petersen and Hubbart, 2020). In-stream variables such as water temperature, solar radiation, sediment transport, settling, and resuspension, runoff, pH, and nutrients can all influence spatiotemporal FIB dynamics (Pachepsky et al., 2024; Park

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et al., 2017; Petersen and Hubbart, 2020; Wang et al., 2018). At the landscape scale, watershed size or stream order, extent of agricultural and urban land covers, wastewater infrastructure, impervious surfaces, annual precipitation, and septic systems are variables shown to influence spatiotemporal FIB concentrations (Hsu et al., 2023; Lininger et al., 2022; Petersen and Hubbart, 2020; Price et al., 2018; Rafi et al., 2018; Sinclair et al., 2009; Verhougstraete et al., 2015). Thus, modeling and predicting FIB in streams typically involves high uncertainties (Abbas et al., 2021; Pandey et al., 2016). Further, the majority of investigations have focused on understanding and predicting stream FIB concentrations around storms, with little attention to FIB variability during baseflow throughout the year or among seasons (Park et al., 2017). Storm flows typically have predictably higher FIB concentrations than baseflow conditions due to the transport of sediment particles (Stumpf et al., 2010; Wang et al., 2018).

FIB in headwaters can originate from various human, agricultural, and ecosystem sources. Leaking septic systems can be a human FIB source during baseflow (Verhougstraete et al., 2015), as well as following precipitation events (McMinn et al., 2024). Human baseflow FIB sources can also include recreational activities such as swimming (Li et al., 2021). Agricultural FIB sources include pastureland adjacent to streams (Weller et al., 2022). Agricultural practices involving compost and natural fertilizers are potential origins of FIB in agricultural areas, including mushroom farms which involve manure and compost spreading, but the influence of mushroom farms on stream FIB concentrations is unknown (Noble and Gaze, 1994; Obire and Ogechi, 2013). There is also the potential for FIB from non-target wildlife (Badgley et al., 2019) and endemic FIB surviving in the environment (Devane et al., 2020). Particularly for headwater streams at baseflow conditions, when dilution of stream water is lowest and most human-water contact occurs, there is a poor understanding of FIB background conditions and environmental stressors causing high FIB levels with regards to watershed nonpoint-source pollution. Effectively identifying these stressors and addressing water quality impairments from fecal contamination requires understanding their seasonal influence on FIB concentrations including during baseflow conditions, as well as background stream FIB levels (Verhougstraete et al., 2015).

Although headwaters serve as critical sources of drinking and recreational water, FIB monitoring in these areas remains limited and insufficient. Relationships between small headwaters, watersheds, and land use patterns have not been fully analyzed or recognized, particularly as human, agricultural, and animal sources of FIB can be mixed in headwaters, creating complexities that lack sufficient information. We aimed to identify background FIB conditions, and factors influencing spatial and temporal FIB variability, for headwater streams across a series of watersheds in the mid-Atlantic region of the eastern United States during baseflow across seasons. Our study region covers portions of the Delaware River Basin from the Christina River in Newark, Delaware, across southeastern Pennsylvania, to the Paulins Kill River in Newton, New Jersey (Fig. 1). Mushroom farms are a common component of the agricultural landscape and, along with dairy farms, influence stream water quality such as nutrient concentrations in this region (Franks, 2024). We collected water samples monthly over a 12-year period, combined with microbial source tracking, to achieve this aim (Fig. 1). We hypothesized that intra-annual physicochemical variability, water temperature, and particulate concentrations would be related to the temporal variation in FIB dynamics within individual streams, while watershed size and land cover (e.g., developed and agricultural versus forest areas) would be related to the spatial variation among streams.

2. Results

2.1. Spatial patterns in fecal indicator bacteria concentration

Most of the study sites had *Escherichia coli* (*E. coli*) and *Enterococcus* levels that greatly exceed US Environmental Protection Agency (US EPA) fecal bacteria limits for *E. coli* (126 cells 100 mL^{-1}) and *Enterococcus* (35 cells 100 mL^{-1}) in recreational water, particularly during summer months (Fig. S1 a-d; US EPA, 2012). Including the auxiliary data sites for samples collected during the recreational period (to complement the more intensively sampled recreation period FIB monitoring group with additional sites; Fig. 1), 86 % of streams had *E. coli* most probable number (MPN) geometric means above the US EPA primary contact limit and 96 % of streams had mean *Enterococcus* above the limit (Fig. 2a-b).

Watershed characteristics, including size and agricultural plus developed land cover, were primary variables explaining differences in FIB concentrations across sites. Watershed size was negatively related to *Enterococcus* by logarithmic least squares regression, with smaller watersheds (e.g., <10 km^2) having greater concentrations of *Enterococcus* than larger ones (R^2=0.38, $p = 0.001$), and weakly (by variance explained) and marginally (by significance) related to *E. coli* concentration (R^2=0.14, $p = 0.055$; Fig. 2c-d). Using linear least squares regression for land cover analyses, *E. coli* (R^2=0.23, $p = 0.011$) and *Enterococcus* (R^2=0.20, $p = 0.021$) concentrations were negatively related to forest cover in our study region (Fig. 2e-f). Additionally, *E. coli* (R^2=0.42, $p < 0.001$) and *Enterococcus* (R^2=0.26, $p = 0.009$) concentrations significantly increased among watersheds with increasing agriculture plus developed area (Fig. 2g-h). However, we found no significant relationship between mushroom farm cover and baseflow FIB concentration (R^2=0.001 and $p > 0.9$ for both *E. coli* and *Enterococcus*; Fig. S2). The high variability of mean FIB concentrations in small watersheds (<10 km^2; Fig. 2c-d) can be explained by agricultural and developed land cover, which had a stronger relationship with FIB levels in these watersheds (R^2=0.61, $p = 0.002$ for *E. coli* and R^2=0.37, $p = 0.027$ for *Enterococcus*; Fig. S3). Further, *E. coli* and *Enterococcus* means were positively related among recreational period sites (R^2=0.54, $p < 0.001$; Fig. S4), but individual agricultural and developed classes (e.g., low intensity developed area on its own) did not have meaningful relationships with FIB (Figs. S5 and S6).

2.2. Temporal patterns in fecal indicator bacteria concentration

FIB concentrations exhibited strong seasonal patterns for the year-round sites. FIB levels peaked in the summer, when means were above the US EPA thresholds for *E. coli* (126 cells 100 mL^{-1}) and *Enterococcus* (35 cells 100 mL^{-1}; Fig. 3a-b). However, during the non-recreational season (fall, winter, and spring), mean FIB levels generally dropped below these limits. For *E. coli*, the highest mean concentration over all years was in June with 488 cells 100 mL^{-1}, while the lowest was in March with 30 cells 100 mL^{-1}. For *Enterococcus*, the highest mean concentration over all years was in July with 496 cells 100 mL^{-1}, while the lowest was in April with 13 cells 100 mL^{-1}. However, we observed several samples in January with high FIB concentrations that influenced a high monthly mean relative to other winter months.

Following these seasonal patterns, *E. coli* and *Enterococcus* concentrations were positively related to water temperature. Samples collected when stream water temperature was below 17 °C (typically from the non-recreational season) had mean FIB concentrations that were generally lower than or at the US EPA limit for primary recreation (Fig. 4a-b). However, in samples collected at water temperatures above 17 °C, primarily during the recreational period, mean FIB concentrations were consistently above US EPA recreational limits for *E. coli* and *Enterococcus*. Mean concentrations for *E. coli* were lowest at 10 °C (36 cells 100 mL^{-1}) and highest at 20 °C (411 cells 100 mL^{-1}). Meanwhile, mean concentrations for *Enterococcus* were lowest at 3 °C (13 cells 100 mL^{-1}) and highest at 21 °C (420 cells 100 mL^{-1}).

Total particulate carbon (C) and nitrogen (N) concentrations positively correlated with FIB concentrations among all samples collected at the study sites. Particulate C was strongly positively related with *E. coli* (linear least squares regression R^2=0.79, $p < 0.001$) and *Enterococcus* (R^2=0.73, $p < 0.001$) concentrations (Fig. 4c-d). Particulate N was even

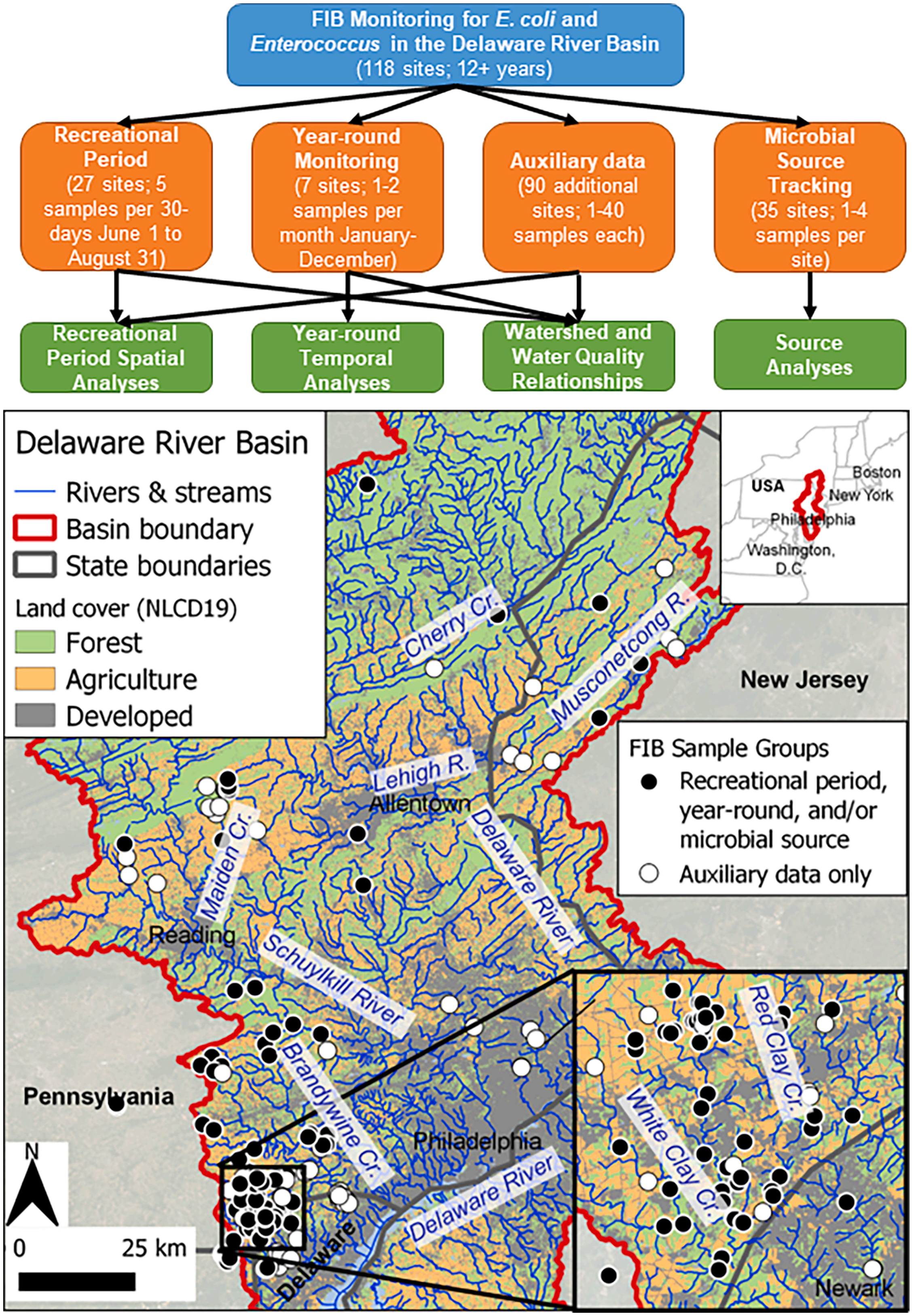

Fig. 1. Experimental design for the study, including the project-level framework (top), sampling frequency groups (middle row), and purposes for analyses (bottom row). Note there can be overlap between sampling frequency grouped sites. Map displays sampling sites and surrounding land cover, including an inset map of the White and Red Clay Creeks, over ESRI 2024 basemap. Reference Table S1 for more details of sampling.

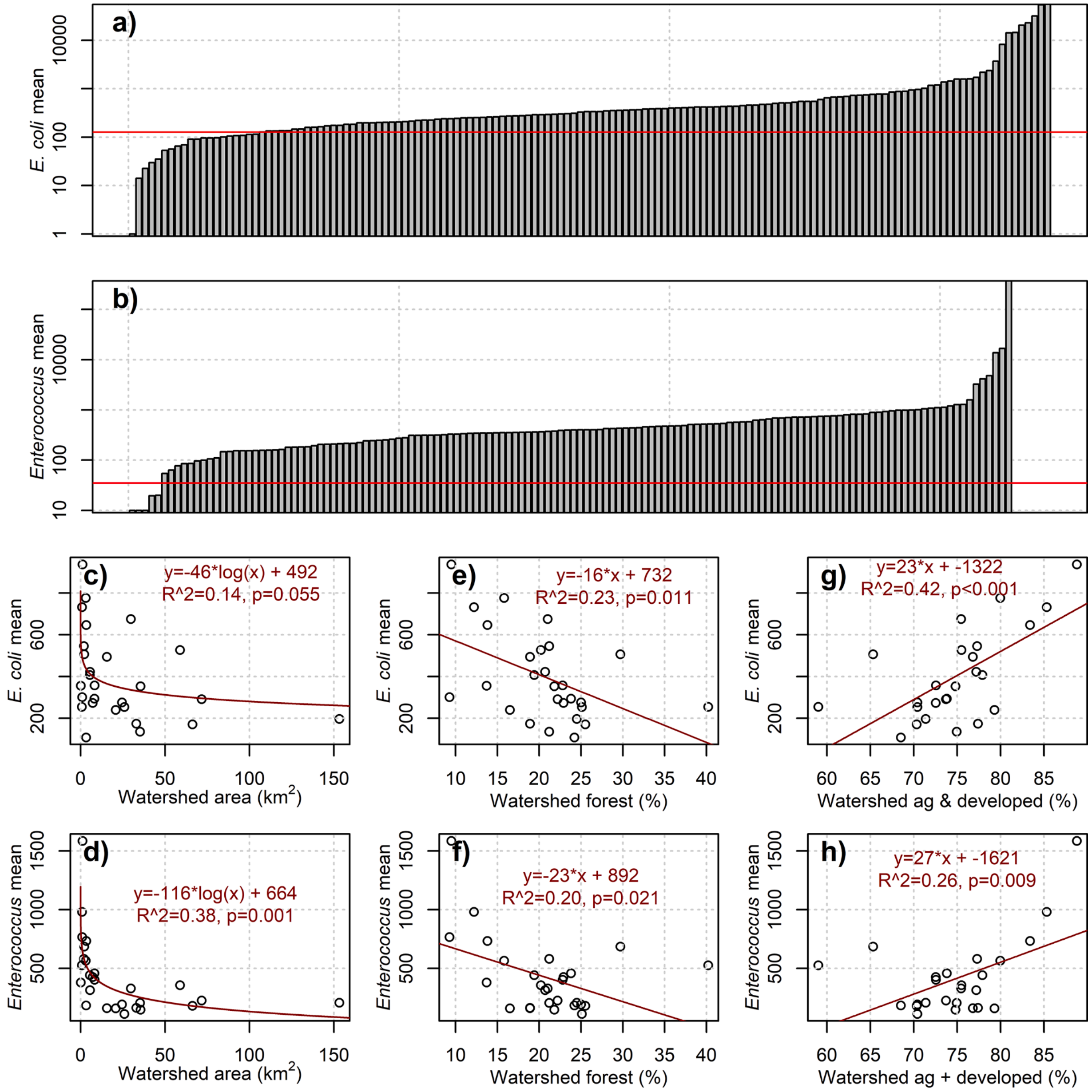

Fig. 2. Bar plots in log-scale of recreational period geometric mean MPN *E. coli* (a) and *Enterococcus* concentrations (b), compared with US EPA primary contact limits of 126 cells 100 mL^{-1} for *E. coli* and 35 cells 100 mL^{-1} for *Enterococcus*, respectively (red lines). Each bar (x-axis) represents one headwater site. Relationships between watershed size and geometric mean MPN (c) *E. coli* and (d) *Enterococcus*, with logarithmic least squares regressions. Geometric mean MPN (e) *E. coli* and watershed forest cover, (f) *Enterococcus* and watershed forest cover, (g) *E. coli* and watershed agriculture plus developed cover, and (h) *Enterococcus* and watershed agriculture plus developed cover, with linear least squares regressions.

more strongly positively related with *E. coli* and *Enterococcus* concentrations (R^2=0.89, p < 0.001 and R^2=0.81, p < 0.001, respectively) (Fig. 4e-f). The additional monitored water quality parameters, including pH and specific conductance, had weak relationships with FIB (Figs. S7 and S8).

2.3. Fecal indicator bacteria sources

Microbial source tracking (CowM2 for bovine-sourced DNA, and HF183 for human-sourced DNA; Griffith et al., 2013; US EPA, 2019) indicated that both human- and bovine-sourced FIB were present in our samples, supporting the influence of agricultural and developed land cover (and their related activities) on high FIB concentrations. Human-sourced FIB were found in 13 % of samples, including streams with as low as 59 % watershed agriculture plus developed cover (Fig. 5a, b). Bovine-sourced FIB were found in 78 % of samples, including streams with as low as 3 % agriculture plus developed cover (Fig. 5a, c; plots for agricultural and developed land cover separately can be found in Fig. S9). Notably, 18 % of headwater samples had FIB from neither human nor bovine sources (Fig. 5a). Watershed agriculture plus developed cover for samples with neither human nor bovine sources ranged from 70 % to 89 %.

3. Discussion

3.1. Headwaters contain higher FIB concentrations than larger rivers

We found that FIB levels in temperate headwater streams were often above regulatory thresholds and had a highly variable, non-linear relationship with watershed size, with small headwaters maintaining

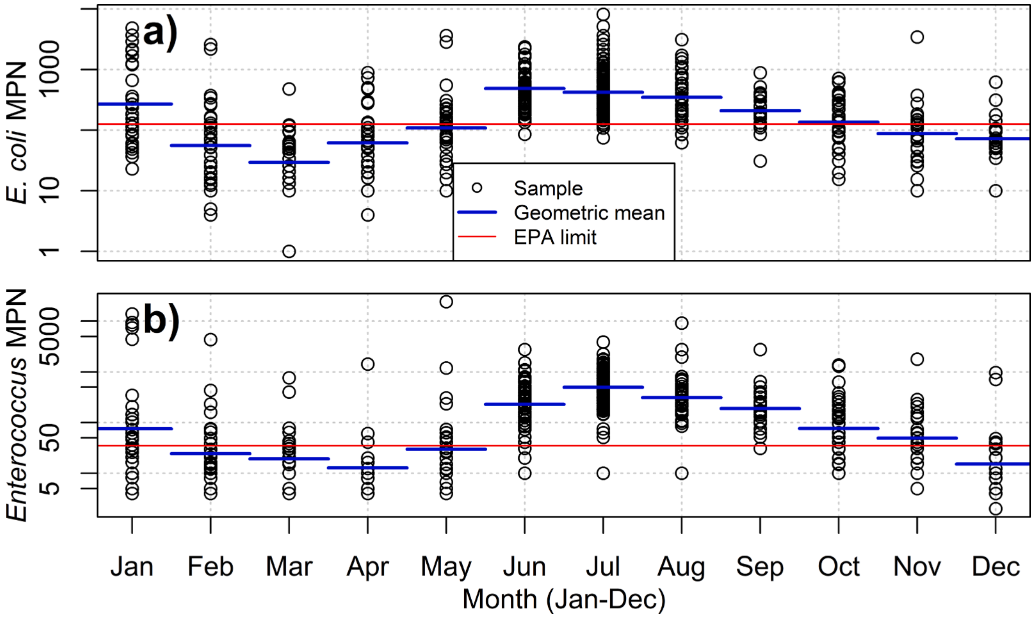

Fig. 3. Seasonal patterns in a) *E. coli* and b) *Enterococcus* MPN 100 mL^{-1} for the year-round study sites. The red lines mark the US EPA thresholds for *E. coli* (126 cells 100 mL^{-1}) and *Enterococcus* (35 cells 100 mL^{-1}) in recreational water. Y-axis values are displayed in log scale.

higher FIB concentrations than larger rivers. Prior research has also documented higher *E. coli* concentrations for low-order streams within anthropogenically influenced watersheds (Rafi et al., 2018), however, minimally-influenced and more natural land cover dominated watersheds in that study did not have higher *E. coli* concentrations in low-order streams (Rafi et al., 2018). Our work supports this finding because agricultural and developed land cover explained the high variations in mean FIB concentrations for small watersheds (Fig. S3).

Headwaters are strongly influenced by local landscape factors because of their close connectivity with the terrestrial ecosystem (e.g., less dilution and a more immediate connection with FIB sources; Klock et al., 2024), which may contribute to the high baseflow FIB concentrations we documented for smaller watersheds. Longitudinal flow connects headwater microbial communities with those downstream (Bier et al., 2023; Huang et al., 2022; Price et al., 2018), especially by transport on particles that reduce mortality rates compared to free floating microbes detached from particles during transport (Anderson et al., 2005; Nguyen et al., 2016). We found greater concentrations of baseflow FIB in waters with higher particulate C and N concentrations, supporting that sediment transport from upstream could be an FIB source.

Small headwaters contribute to larger swimmable rivers, drinking water sources, and activities such as recreation and shellfishing in receiving waters (Stumpf et al., 2010). Surface water from river and lake/reservoir systems contributes substantially to the drinking water supply for major cities around the globe (Blaine et al., 2006; McDonald et al., 2014; Turner et al., 2021). Headwater FIB data provides vital information about contamination sources and bacteria transport to areas with targeted monitoring, such as for beaches, large rivers for recreational use, or drinking water protection (Aufdenkampe et al., 2006; Kaplan et al., 2006). The higher FIB concentrations we documented for small developed watersheds support the protection of source water areas when considering management of recreation and drinking water, as well as planning watershed-scale land protection to incorporate headwaters (Aufdenkampe et al., 2006; Kaplan et al., 2006; Moravek et al., 2023).

3.2. Headwater FIB concentrations are related to land cover

We found that FIB concentrations increased with agricultural and developed land cover and that FIB sources included humans and cows. As our study watersheds were often rural with small population densities and without sewer connections to wastewater treatment plants, leaky or improperly maintained septic systems may be a source of human fecal pollution (McMinn et al., 2024). Study sites that had human-sourced FIB were often located in residential areas in the watershed and/or recreational areas upstream, such as parks and ponds with active canoeing and kayaking activities. Similarly, sites that had bovine-sourced FIB could be downstream of pastures (cattle for dairy or meat), cropland, and/or cows visibly in the stream. This highlights the value of combining watershed characteristics with source tracking information to guide watershed planning and address fecal contamination (Aufdenkampe et al., 2006; Hart et al., 2023; Kaplan et al., 2006; Tarek et al., 2023). However, we did not discern relationships between FIB concentrations and watershed mushroom farm cover, which could be due to insufficient temporal resolution of our samples, lack of data for manure and compost spreading application rates, or to the overwhelming influence of watershed total agricultural and developed cover on FIB. Previous research has found relationships between mushroom farm cover and nutrient water quality in the region (Franks, 2024). To better understand FIB and mushroom farm relationships, future research should consider a higher resolution, targeted study of FIB sources (e.g., poultry manure compost; Noble and Gaze, 1994) in small streams with numerous watershed mushroom farms and composting operations, such as Egypt Run of the White Clay Creek watershed (Homsey, 2020).

To the north of our study area in the state of New York, USA, land cover such as pasture adjacent to the stream was related to high FIB concentrations from human and agricultural sources (Weller et al., 2022). In watersheds south of our study area in Virginia, FIB concentrations can also be higher in more anthropogenically disturbed watersheds and during the recreational season (Badgley et al., 2019). However, there may have been ecosystem FIB sources beyond sources from agriculture and developed land such as non-target wildlife (Badgley et al., 2019). Further, in a Pennsylvania watershed west of our study area, developed land cover was associated with FIB concentrations that were higher than in nearby forested watersheds (Jeon et al., 2020). Climate and landscape changes as well as seasonal variability may affect future FIB (Robinson et al., 2022; Stallard et al., 2019).

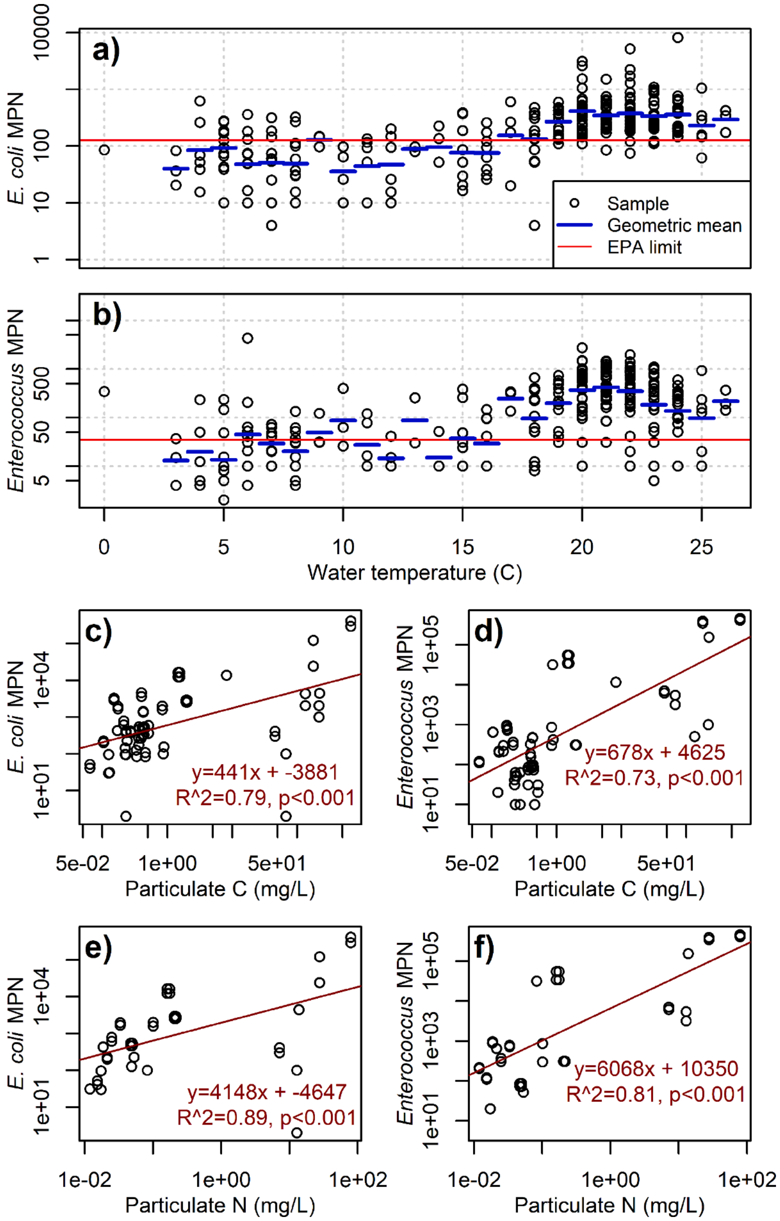

Fig. 4. Relationships between water temperature and mean a) *E. coli* and b) *Enterococcus* among all samples. The red lines mark the US EPA thresholds for *E. coli* (126 cells 100 mL^{-1}) and *Enterococcus* (35 cells 100 mL^{-1}) in recreational water. Relationships between particulate carbon (C) and *E. coli* 100 mL^{-1} (c), particulate C and *Enterococcus* 100 mL^{-1} (d), particulate nitrogen (N) and *E. coli* 100 mL^{-1} (e), and particulate N and *Enterococcus* 100 mL^{-1} (f) among all samples, with linear least squares regressions.

3.3. *Microbial survival and turnover in local environments might also cause FIB occurrence*

We found chronically high FIB concentrations even in small watersheds without evidence for human or bovine fecal contamination, supporting that there could be large FIB sources beyond the anthropogenically-driven inputs. Our pervasively high FIB levels in small, headwater streams at baseflow, along with the high percentage (18 %) of samples with neither human- nor bovine-sourced fecal bacteria, implies a potential endemic source. FIB can maintain viable populations in waters and sediments outside of their normal animal hosts (Devane et al., 2020; Lew et al., 2023; Weiskerger et al., 2019; Zhi et al., 2019). Also, our findings of positive relationships between water temperature and FIB concentrations, complemented by the seasonally higher FIB concentrations during the recreational period, may be due to greater survival of FIB in streams during the recreational period when there can be higher biological activity and more tolerable stream environments (Badgley et al., 2019; Petersen and Hubbart, 2020).

Although some FIB may ultimately be sourced from human or agricultural pollution, regulatory limits developed for fecal pathogens from contamination sources could lead to over-estimating public health risks in watersheds that also have these proximate-sourced, naturalized bacteria (Devane et al., 2020). On the other hand, fecally-sourced *E. coli* and *Enterococcus* in the environment can survive for days to weeks (Baker et al., 2021), meaning FIB may accumulate in small streams over time, and there can be unknown FIB sources (Bowen et al., 2024). There may also be the potential for high FIB concentrations due to legacy land uses (e.g., pasture), which have been shown to affect other modern-day components of stream ecosystems including fish and invertebrate communities (Harding et al., 1998). Monitoring microbial community structure using high throughput sequencing and machine learning could provide a wealth of information about microbial assemblies and

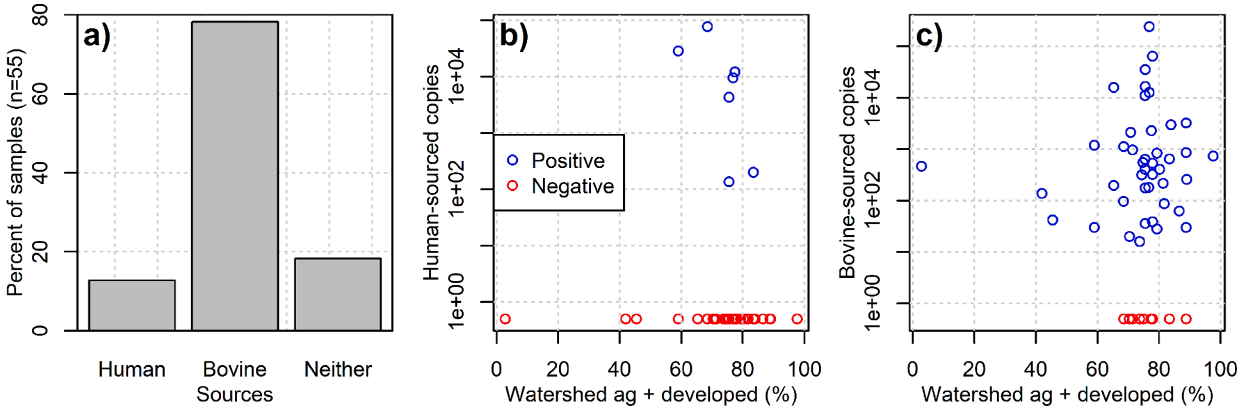

Fig. 5. Microbial source tracking results for a) human-positive, bovine-positive, or neither human- nor bovine-positive FIB in headwater streams. Then, relationship between watershed agricultural plus developed cover and b) human-sourced and c) bovine-sourced gene copies.

distributions, beyond specific fecal indicator bacteria concentrations, to help gauge these sources and human health risks (Arya et al., 2023; Bowen et al., 2024; Mo et al., 2024; Saleem et al., 2024; URycki et al., 2024).

3.4. Implications

A better understanding of FIB dynamics and sources can improve management of public waters (Hart et al., 2024; Stallard et al., 2019). Beyond the eastern US, swimming, bathing, and other human contact locations in the western and central US, Europe, Asia, and Africa can face chronic water quality problems when watershed fecal pollution is not adequately addressed. These regions could greatly benefit from improved knowledge about pollution sources (Aqel et al., 2024; Bisimwa et al., 2022; Lane et al., 2020; McNair et al., 2025, 2022; Ming et al., 2020; Reeves et al., 2004; Servais et al., 2007). Understanding the spatiotemporal variability and sources of baseflow FIB is particularly valuable for predictive models of fecal contamination. Such insights are critical for guiding management of water quality, particularly in the context of model development, validation, and uncertainty quantification (e.g., Myers et al., 2024; Xie et al., 2023). Improved prediction accuracy in FIB models could lead to reduce monitoring costs and more timely interventions (Lucius et al., 2019; Wheeler and Ledford, 2023).

4. Conclusions

Headwater streams offer significant benefits for downstream water quality and socio-ecological health but are often overlooked in FIB monitoring, which tends to prioritize more active recreational areas like large rivers, beaches, and lakes. From a regulatory perspective, headwater streams in our study region frequently contained FIB concentrations exceeding levels deemed safe for recreational human health. In some cases, elevated FIB levels were attributed to human and bovine fecal contamination. However, even small watersheds lacking apparent human or bovine FIB sources were found to have concerning FIB concentrations, raising questions about current approaches for identifying and addressing sources of FIB contamination. We found that seasonality was a major component in temporal variation of FIB during baseflow conditions, with concentrations peaking in the summer when stream temperature is high. Meanwhile, forested and developed plus agricultural land cover can in-part explain spatial variability in FIB concentrations at the watershed scale. Future work should consider using high throughput sequencing and machine learning to develop indicators that account for potentially endemic FIB population, which persist in soils and water independently of fecal contamination. Such efforts should particularly address the influence of seasonality and relationships with particulates to more effectively allocate resources and guide strategies for mitigating fecal contamination and addressing downstream water quality impairments in headwater streams.

5. Methods

5.1. Study area

The 34,965 km^2 Delaware River Basin is one of the most important in the eastern US for water supply, water quality, and environmental and cultural resources (Jackson et al., 2005; Moore, 2021). It provides drinking water for New York City and Philadelphia, two of the largest metropolitan economies in the nation, while also maintaining a multi-billion dollar agricultural industry for crops, livestock, and poultry (Kauffman, 2016). It also provides valuable ecosystem services from natural forests and wetlands including water filtration, flood control, and fish and wildlife habitat (Kauffman, 2016). The land cover of the Delaware River Basin consists of 21 % developed cover, 18 % agricultural, 50 % forests, and 11 % other (including open water, grasslands, and herbaceous wetlands; Dewitz and USGS, 2021). The study region also has a high density of mushroom farms and mushroom compost operations in the White Clay Creek and Red Clay Creek sub-watersheds, which are not common in most agricultural settings (Franks, 2024; Homsey, 2020).

5.2. Sampling design and purposes

Sampling groups for this study included sites for recreational period monitoring (June-August), year-round monitoring, auxiliary data (to complement and validate findings from more intensively sampled groups), and microbial source tracking (Fig. 1, Table S1). Sites of the recreational period sampling group were chosen to investigate possible human, agriculture, or wildlife sources of fecal pollution including water treatment plants, residential areas, golf courses, mushroom farms, and preserved forest and meadow land. The recreation period is when the most human contact with the water occurs during recreational activities such as paddling, fishing, and wading. Twenty-seven sites with the most intensive recreational period sampling (5 samples per 30-day period in June, July, and August, from 2016–2024) occurred in the White Clay Creek and Red Clay Creek (Fig. 1, inset). The majority of sites from White Clay Creek in southeast Pennsylvania and northwest Delaware were chosen by the White Clay Watershed Association (whiteclay.org) as part of the White Clay Creek Wild and Scenic Program (under the National Wild and Scenic River System of the US National Park Service) to monitor water quality within the watershed. Sites included headwater tributaries of the East Branch of White Clay as well as sites along the Middle and West Branches. The project also sampled two branches and one tributary of Red Clay Creek, which along with White Clay, serves as a major drinking water source for Newark, Delaware.

Sites of the year-round monitoring group were chosen to better our understanding of temporal and seasonal trends in FIB concentrations in the Delaware River Basin (Fig. 1). For year-round samples, four sites of particular interest were sampled bi-weekly throughout the year over a three year period, while an additional three sites were sampled monthly throughout the year (Table S1). Overlapping with some recreational period sites, year-round sample sites were focused on the White Clay Creek and Red Clay Creek watersheds (Fig. 1, inset).

Overlapping the recreational period and year-round sample sites and extending north to the Pocono-Kittatinny mountain range, as well as east to Brandywine Creek, 90 auxiliary sample sites were chosen as part of the Delaware River Watershed Initiative (4states1source.org; Fig. 1). Sites included a full range of land covers (forested, agricultural, suburban, and urban) and were generally centered around areas of interest for potential restoration projects. The purpose for the auxiliary sample sites was to validate and complement findings of recreational period and year-round FIB concentrations in the Delaware River Basin, and to provide a larger sample size for analyses with total particulate C and N (Fig. 1). Several sites were located within the Brandywine Valley unit of the First State National Historic Park and neighboring Brandywine Creek State Park in Delaware. Auxiliary sites in the Brandywine Creek watershed included a number of headwater tributaries ranging from pristine forested land to a heavily developed commercial district as well as the main stem of Brandywine Creek, which experiences extensive recreational impact from canoe/kayaking, river tubing, fishing, and wading. Samples at auxiliary sites were collected 2012–2024 although not all sites were sampled in all years, depending on the focus of related research activities of the larger project, with a range from 1 to 40 samples per site, median of 9 samples per site, and 91 % of sites having greater or equal to three samples (Fig. 1). This might include sampling a site before, during, and after a restoration effort as well as comparing that site to nearby baseline impaired or unimpaired sites. Auxiliary sites were primarily sampled during the recreational period with a target of three sample days per site. For instance, the Brandywine Creek watershed sites were sampled from 2017–2019 during the recreational period with three sample days per site (Table S1).

Finally, to better understand sources of fecal bacteria at priority sites, we used microbial source tracking to distinguish probable causes of the contamination (Fig. 1). Samples for microbial source tracking occurred primarily in the White Clay and Red Clay Creek watersheds, and also extended into the Brandywine Creek and Schuylkill River watersheds (Fig. 1, Table S1).

5.3. *Sample collection and processing*

Samples were collected during baseflow conditions. Streams were considered at baseflow 48 hours after a rainfall event of 0.25 inches or more. When sampling multiple sites within a watershed on the same day, sites were sampled in order from downstream to upstream. While sampling methods occasionally varied based on the overarching project, samples were most often collected in sterile one liter Nasco Whirl-Pak bags. Samples were transported on ice and processed on the same day as they were collected.

During the visits for FIB sample, physical and chemical water quality parameters were collected as part of the monitoring. Here, we describe relationships with stream temperature data, which were collected using hand-held Thermo Orion Star A329, Thermo Orion 5-Star, or YSI Pro Plus meters, and stream total particulate C and N, which were collected using grab samples for a subset of the samples ($n = 76$ and 40, respectively) and analyzed using US EPA method 440.0 elemental analysis. Temperature and total particulate C and N were selected based on the described literature for their strong influence on FIB. Additional physical and chemical water quality parameters (dissolved organic carbon (DOC), acid neutralizing capacity (ANC), chloride, nitrate-nitrogen (NO3-N), calcium, copper, iron, sodium, sulfur, ammonium-nitrogen (NH4-N), orthophosphate (PO4-P), total phosphorus, total nitrogen, total particulate nitrogen, total particulate carbon, peak T, Humification Index, Slope Ratio, pH, dissolved oxygen (DO), specific conductance, and stream temperature) are presented for reference in Table S2.

Fecal bacteria were quantified as most probable number (MPN) per 100 mL using Quanti-Tray 2000 kits from IDEXX Laboratories (Westbrook, Maine, USA). *E. coli* were measured using Colilert and *Enterococcus* were measured using Enterolert following instructions from the manufacturer (https://www.idexx.com). Samples were diluted to bring bacteria concentrations within the readable range of the assays (1 - 2419.6 cells 100 mL^{-1}). Dilutions were typically 10 × but could vary based on turbidity of the sample or land cover of the sample site (for instance, a very turbid sample or a sample from an agricultural or developed site with higher FIB concentrations may need to be diluted beyond 10 × to bring the bacteria concentration within the readable range of the assays). MPN results have been found to be essentially the same as colony forming units (CFU) analysis (Cowburn et al., 1994) but have the potential to differ because of the MPN's probabilistic sampling approach (Gronewold and Wolpert, 2008); regardless, MPN has the benefit of being much more rapid, and our methods are approved for monitoring purposes by the US EPA (US EPA, 2024).

Field replicates (duplicate samples collected from the sample site) or laboratory replicates (duplicate aliquots from a single field sample) were processed for approximately 10 % of a batch of samples. For example, if 20 samples were being processed, 2 replicates were included. Replicate samples were used for method validation, however, the replicate observations were removed from the dataset prior to analysis to prevent overleveraging or bias.

5.4. *Microbial source tracking*

For microbial source tracking analyses, we chose primers and probes targeting *Bacteroides* sp. 16S rRNA genes that are specific for each host organism. Monitoring of source-specific *Bacteroides* can provide a more precise indicator of fecal contamination than monitoring for generalist *E. coli* (Chavarria et al., 2024). *Bacteroides* have been previously used to identify sources of water quality impairments (Li et al., 2019; Verhougstraete et al., 2015). We followed established analytical procedures using two pairs of primers and probes: CowM2 for bovine-sourced DNA (Raith et al., 2013; Shanks et al., 2008), and HF183 for human-sourced DNA (Griffith et al., 2013; US EPA, 2019). For each sample, a 20 μL reaction was performed using 1 μL DNA for bovine-sourced copies, and 2 μL DNA for human-sourced copies, along with negative and positive controls (standards). TaqMan Environmental PCR Master Mix 2.0 (Applied Biosystems, Waltham, Massachusetts, USA) was used for qPCR reactions on a QuantStudio 3 thermocycler (Applied Biosystems). All samples were run in triplicates, with the result taking the average of the number of copies and only when two or more replicates were positive. We defined positive qPCR results as those that exhibited amplification (measured by fluorescence at each cycle) greater than any observed in the negative controls. 0.2 μL of bovine serum albumen (BSA) was added to samples to increase the effectiveness of PCR in the potential presence of inhibitors. The TaqMan PCR program was as follows: Initial denaturation of samples was at 50 °C for 2 min and 95 °C for 10 min, followed by 95 °C for 15 s and 60 °C for 1 min in each of 45 amplification cycles. Results past 45 cycles were removed to reduce the potential for false positives. Gene copy numbers were quantified by standard curves created using a 1:10 dilution series from commercially purchased standards (Integrated DNA Technologies, Coralville, Iowa USA) of known copy numbers (Griffith et al., 2013; US EPA, 2019).

5.5. *Data processing and statistical analysis*

Watershed boundaries were topographically delimited for each sampling site using ArcGIS Spatial Analyst tools (ESRI; Redlands, California, USA). Land cover was determined by National Land Cover

Database (NLCD) 2019 (Dewitz and USGS, 2021), aggregated by class (agriculture includes cropland and pasture/hay; developed includes open space, low, medium, and high intensity; and forest includes deciduous, evergreen, and mixed). We also included the proportion of watershed area as mushroom farms using a spatial mushroom farm dataset for White Clay and Red Clay watersheds (Homsey, 2020).

The US EPA provides recreational water quality criteria for *E. coli* and *Enterococcus*, the fecal bacteria which are the best indicators of health risks from recreational water contact (US EPA, 2012). The recommendations state that for fresh water the geometric mean of CFU or equivalent MPN numbers per 100 mL of water determined via a culture-based method should not exceed 126 for *E. coli* or 35 for *Enterococcus*. These guidelines were developed for use in primary contact recreation which includes swimming, bathing, surfing, or similar activities. However, the majority of sites in this study are not subject to this type of recreation. Instead, exposure is more likely to derive from wading, use for pets or livestock, and fishing. Canoeing and kayaking are also possible in the few larger tributaries.

Spatial relationships among sites and environmental variables were examined by taking the *E. coli* and *Enterococcus* concentration (expressed as geometric mean) for each monitoring site over the study period during the recreation season, and comparing with watershed-average land cover and in-stream water quality attributes, using linear or logarithmic least squares regression in R Statistical Software (v4.3.1; R Core Team, 2024). We based this approach on regressions of land cover against various water quality parameters including FIB, aggregated by a summary of the water quality data (mean, median, etc.), as a way to gauge environmental stressors to water quality across many sites and over time (Badgley et al., 2019; Myers et al., 2024). Linear regression was used for all analyses involving land cover and FIB relationships, as well as water quality (including particulate C and N) and FIB relationships. Logarithmic regression was used only for the relationships between watershed size and forest land cover with FIB geometric means because it better modeled the gentler slope for larger watersheds. Watershed size was used in spatial relationships rather than stream order because at our high resolution, streams of the same order could have meaningfully different watershed sizes. Agricultural and developed land were combined for analyses to represent whole-watershed human land cover impacts, because of additive relationships with FIB among land cover classes. Temporal patterns related to stream temperature were modeled by aggregating data to monthly geometric means as well as presenting individual sample results, and examining intra-annual variation due to seasonality. Note that seasonality due to the timing and extent of manure applications to fields was not included in temporal analyses due to a lack of data. For additional water quality parameters (e.g., total particulate C and N), all FIB samples with a corresponding laboratory water quality record were used in the analyses. Results were compared with the US EPA thresholds for *E. coli* and *Enterococcus* in recreational water to assess public health risks. Statistical relationships were considered significant if $p < 0.05$ and marginally significant if $p < 0.10$. Relationships were considered fit if $R^2 > 0.20$ and weak if $R^2 < 0.20$.

Results above or below the readable range were assigned the value of those limits so that calculations would not be biased against censored data exclusions (Corsi et al., 2021). We made this choice because censored data below the readable range (1 cell 100 mL^{-1}) accounted for 4.0 % of *E. coli* and 7.4 % of *Enterococcus* samples, which were both below the 15 % maximum percentage recommended for substitutions (US EPA, 2000). Censored data above the readable range (>2419.6 cells 100 mL^{-1}) accounted for only 0.45 % of *E. coli* and 0.30 % of *Enterococcus* samples.

Supplementary information

Supplementary information is available online including Table S1 and S2 and Figs. S1 to S8.

CRediT authorship contribution statement

Daniel T. Myers: Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis. **Laura Zgleszewski:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Raven Bier:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Jacob R. Price:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Shane Morgan:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Conceptualization. **John K. Jackson:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Diana Oviedo-Vargas:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Melinda Daniels:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **David B. Arscott:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Jinjun Kan:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2025.100347.

Data availability

All data and scripts used in this study are publicly available on figshare at https://doi.org/10.6084/m9.figshare.28216154.

References

Abbas, A., Baek, S., Silvera, N., Soulileuth, B., Pachepsky, Y., Ribolzi, O., Boithias, L., Cho, K.H., 2021. In-stream *E. coli* modeling using high-temporal-resolution data with deep learning and process-based models. Hydrol. Earth Syst. Sci. 25. https://doi.org/10.5194/hess-25-6185-2021.

Anderson, K.L., Whitlock, J.E., Harwood, V.J., 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Appl. Environ. Microbiol. 71. https://doi.org/10.1128/AEM.71.6.3041-3048.2005.

Aqel, H., Sannan, N., Al-Hunaiti, A., Fodah, R., 2024. Integrated water quality dynamics in Wadi Hanifah: physical, chemical, and biological perspectives. PLoS One 19. https://doi.org/10.1371/journal.pone.0298200.

Arya, S., George, A.B., O'Dwyer, J.P., 2023. Sparsity of higher-order landscape interactions enables learning and prediction for microbiomes. Proc. Natl. Acad. Sci. U. S. A 120. https://doi.org/10.1073/pnas.2307313120.

Aufdenkampe, A.K., Arscott, D.B., Dow, C.L., Standley, L.J., 2006. Molecular tracers of soot and sewage contamination in streams supplying New York City drinking water. J. North Am. Benthol. Soc. 25. https://doi.org/10.1899/0887-3593(2006)025[0928:MTOSAS]2.0.CO;2.

Badgley, B.D., Steele, M.K., Cappellin, C., Burger, J., Jian, J., Neher, T.P., Orentas, M., Wagner, R., 2019. Fecal indicator dynamics at the watershed scale: variable relationships with land use, season, and water chemistry. Sci. Total Environ. 697. https://doi.org/10.1016/j.scitotenv.2019.134113.

Baker, C.A., Almeida, G., Lee, J.A., Gibson, K.E., 2021. Pathogen and Surrogate Survival in Relation to Fecal Indicator Bacteria in Freshwater Mesocosms. Appl. Environ. Microbiol. 87. https://doi.org/10.1128/AEM.00558-21.

Bier, R.L., Mosher, J.J., Kaplan, L.A., Kan, J., 2023. Spatial scale impacts microbial community composition and distribution within and across stream ecosystems in North and Central America. Environ. Microbiol. 25. https://doi.org/10.1111/1462-2920.16396.

Bisimwa, A.M., Kisuya, B., Kazadi, Z.M., Muhaya, B.B., Kankonda, A.B., 2022. Monitoring faecal contamination and relationship of physicochemical variables with faecal indicator bacteria numbers in Bukavu surface waters, tributaries of Lake Kivu in Democratic Republic of Congo. Hygiene Environ. Health Adv. 3. https://doi.org/10.1016/j.heha.2022.100012.

Blaine, J.G., Sweeney, B.W., Arscott, D.B., 2006. Enhanced source-water monitoring for New York City: historical framework, political context, and project design. J. North Am. Benthol. Soc. https://doi.org/10.1899/0887-3593(2006)025[0851:ESMFNY]2.0.CO;2.

Bowen, M., Main, C.R., Farag, I.F., Biddle, J.F., 2024. Identifying potential introduced and natural sources of pollution in Delaware watersheds. Appl. Environ. Microbiol., e01958. -24.

Chavarria, K., Batista, J., Saltonstall, K., 2024. Widespread occurrence of fecal indicator bacteria in oligotrophic tropical streams. Are common culture-based coliform tests appropriate? PeerJ 12, e18007.

Corsi, S.R., De Cicco, L.A., Hansen, A.M., Lenaker, P.L., Bergamaschi, B.A., Pellerin, B.A., Dila, D.K., Bootsma, M.J., Spencer, S.K., Borchardt, M.A., McLellan, S.L., 2021. Optical Properties of Water for Prediction of Wastewater Contamination, Human-Associated Bacteria, and Fecal Indicator Bacteria in Surface Water at Three Watershed Scales. Environ. Sci. Technol. 55. https://doi.org/10.1021/acs.est.1c02644.

Cowburn, J.K., Goodall, T., Fricker, E.J., Walter, K.S., Fricker, C.R., 1994. A preliminary study of the use of Colilert for water quality monitoring. Lett. Appl. Microbiol. 19. https://doi.org/10.1111/j.1472-765X.1994.tb00902.x.

Devane, M.L., Moriarty, E., Weaver, L., Cookson, A., Gilpin, B., 2020. Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring. Water. Res. https://doi.org/10.1016/j.watres.2020.116204.

Dewitz, J., USGS, 2021. National Land Cover Database (NLCD) 2019 Products (ver. 2.0, June 2021). U.S. Geological Survey data release.

Franks, L., 2024. Surface Water Quality Patterns of White Clay Creek Subwatersheds in Delaware and Pennsylvania. University of Delaware Master's thesis.

Griffith, J.F., Layton, B.A., Boehm, A.B., Holden, P.A., Jay, J.A., Hagedorn, C., McGee, C. D., Weisberg, S.B., 2013. The California Microbial Source Identification Manual: a Tiered Approach to Identifying Fecal Pollution Sources to Beaches. Southern California Coastal Water Research Project, Costa Mesa, CA.

Gronewold, A.D., Wolpert, R.L., 2008. Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. Water. Res. 42. https://doi.org/10.1016/j.watres.2008.04.011.

Harding, J.S., Benfield, E.F., Bolstad, P.V., Helfman, G.S., Jones, E.B.D., 1998. Stream biodiversity: the ghost of land use past. Proc. Natl. Acad. Sci. U S A 95. https://doi.org/10.1073/pnas.95.25.14843.

Hart, J.J., Jamison, M.N., McNair, J.N., Woznicki, S.A., Jordan, B., Rediske, R.R., 2023. Using watershed characteristics to enhance fecal source identification. J. Environ. Manage. 336, 117642.

Hart, J.J., Jamison, M.N., Porter, A.M., McNair, J.N., Szlag, D.C., Rediske, R.R., 2024. Fecal Impairment Framework, A New Conceptual Framework for Assessing Fecal Contamination in Recreational Waters. Environ. Manage. 73. https://doi.org/10.1007/s00267-023-01878-x.

Homsey, A., 2020. MushroomFarms Layer. White Clay Basemap White Clay Creek Natl. Wild Scenic River. https://udel.maps.arcgis.com/apps/webappviewer/index.html?id=4bd6252384a44beca0105e5ed4cd64d0.

Hsu, T.T.D., Yu, D., Wu, M., 2023. Predicting Fecal Indicator Bacteria Using Spatial Stream Network Models in A Mixed-Land-Use Suburban Watershed in New Jersey, USA. Int. J. Environ. Res. Public Health 20. https://doi.org/10.3390/ijerph20064743.

Huang, T., Wollheim, W.M., Jones, S.H., 2022. Removal of Fecal Indicator Bacteria by River Networks. Water (Switzerland) 14. https://doi.org/10.3390/w14040617.

Jackson, J.K., Huryn, A.D., Strayer, D.L., Courtemanch, D.L., Sweeney, B.W., 2005. Atlantic coast rivers of the Northeastern United States. Rivers North Am. https://doi.org/10.1016/B978-012088253-3/50005-5.

Jeon, D.J., Pachepsky, Y., Coppock, C., Harriger, M.D., Zhu, R., Wells, E., 2020. Temporal stability of *E. coli* and Enterococci concentrations in a Pennsylvania creek. Environ. Sci. Pollut. Res. 27. https://doi.org/10.1007/s11356-019-07030-9.

Kaplan, L.A., Newbold, J.D., Van Horn, D.J., Dow, C.L., Aufdenkampe, A.K., Jackson, J. K., 2006. Organic matter transport in New York City drinking-water-supply watersheds. J. North Am. Benthol. Soc. 25. https://doi.org/10.1899/0887-3593(2006)025[0912:OMTINY]2.0.CO;2.

Kauffman, G.J., 2016. Economic Value of Nature and Ecosystems in the Delaware River Basin. J. Contemp. Water Res. Educ. 158. https://doi.org/10.1111/j.1936-704x.2016.03222.x.

Klock, A.M., Torgersen, C.E., Roberts, M.C., Vogt, D.J., Vogt, K.A., 2024. Environmental drivers and spatial patterns of antibiotic-resistant, enteric coliforms across a forest–urban riverscape. Fresh Water Sci. 43, 231–249.

Lane, M.J., Rediske, R.R., McNair, J.N., Briggs, S., Rhodes, G., Dreelin, E., Sivy, T., Flood, M., Scull, B., Szlag, D., Southwell, B., Isaacs, N.M., Pike, S., 2020. A comparison of *E. coli* concentration estimates quantified by the EPA and a Michigan laboratory network using EPA Draft Method C. J. Microbiol. Methods 179. https://doi.org/10.1016/j.mimet.2020.106086.

Lew, S., Glińska-Lewczuk, K., Burandt, P., Grzybowski, M., Obolewski, K., 2023. Fecal bacteria in coastal lakes: an anthropogenic contamination or natural element of microbial diversity? Ecol. Indic. 152. https://doi.org/10.1016/j.ecolind.2023.110370.

Li, D., Van De Werfhorst, L.C., Steets, B., Ervin, J., Murray, J.L.S., Blackwell, A., Devarajan, N., Holden, P.A., 2021. Sources of low level human fecal markers in recreational waters of two Santa Barbara, CA beaches: roles of WWTP outfalls and swimmers. Water Res. 202. https://doi.org/10.1016/j.watres.2021.117378.

Li, X., Sivaganesan, M., Kelty, C.A., Zimmer-Faust, A., Clinton, P., Reichman, J.R., Johnson, Y., Matthews, W., Bailey, S., Shanks, O.C., 2019. Large-scale implementation of standardized quantitative real-time PCR fecal source identification procedures in the Tillamook Bay Watershed. PLoS One 14. https://doi.org/10.1371/journal.pone.0216827.

Lininger, K.J., Ormanoski, M., Rodak, C.M., 2022. Observations and Correlations from a 3-Year Study of Fecal Indicator Bacteria in the Mohawk River in Upstate NY. Water (Switzerland) 14. https://doi.org/10.3390/w14132137.

Lucius, N., Rose, K., Osborn, C., Sweeney, M.E., Chesak, R., Beslow, S., Schenk, T., 2019. Predicting *E. coli* concentrations using limited qPCR deployments at Chicago beaches. Water. Res. X 2. https://doi.org/10.1016/j.wroa.2018.100016.

McDonald, R.I., Weber, K., Padowski, J., Flörke, M., Schneider, C., Green, P.A., Gleeson, T., Eckman, S., Lehner, B., Balk, D., Boucher, T., Grill, G., Montgomery, M., 2014. Water on an urban planet: urbanization and the reach of urban water infrastructure. Glob. Environ. Change 27. https://doi.org/10.1016/j.gloenvcha.2014.04.022.

McMinn, B.R., Korajkic, A., Kelleher, J., Diedrich, A., Pemberton, A., Willis, J.R., Sivaganesan, M., Shireman, B., Doyle, A., Shanks, O.C., 2024. Quantitative fecal pollution assessment with bacterial, viral, and molecular methods in small stream tributaries. Sci. Total Environ. 951, 175740.

McNair, J.N., Lane, M.J., Hart, J.J., Porter, A.M., Briggs, S., Southwell, B., Sivy, T., Szlag, D.C., Scull, B.T., Pike, S., Dreelin, E., Vernier, C., Carter, B., Sharp, J., Nowlin, P., Rediske, R.R., 2022. Validity assessment of Michigan's proposed qPCR threshold value for rapid water-quality monitoring of *E. coli* contamination. Water Res. 226. https://doi.org/10.1016/j.watres.2022.119235.

McNair, J.N., Rediske, R.R., Hart, J.J., Jamison, M.N., Briggs, S., 2025. Performance of Colilert-18 and qPCR for Monitoring *E. coli* Contamination at Freshwater Beaches in Michigan. Environments 12, 21. https://doi.org/10.3390/environments12010021.

Ming, H., Ma, Y., Gu, Y., Su, J., Guo, J., Li, J., Li, X., Jin, Y., Fan, J., 2020. Enterococci may not present the pollution of most enteric pathogenic bacteria in recreational seawaters of Xinghai bathing Beach, China. Ecol. Indic. 110. https://doi.org/10.1016/j.ecolind.2019.105938.

Mo, Y., Bier, R., Li, X., Daniels, M., Smith, A., Yu, L., Kan, J., 2024. Agricultural practices influence soil microbiome assembly and interactions at different depths identified by machine learning. Commun. Biol. 7, 1349.

Moore, S., 2021. Toward effective river basin management (RBM): the politics of cooperation, sustainability, and collaboration in the Delaware River basin. J. Environ. Manage. 298. https://doi.org/10.1016/j.jenvman.2021.113421.

Moravek, J.A., Andrews, L.R., Serota, M.W., Dorcy, J.A., Chapman, M., Wilkinson, C.E., Parker-Shames, P., Van Scoyoc, A., Verta, G., Brashares, J.S., 2023. Centering 30 × 30 conservation initiatives on freshwater ecosystems. Front. Ecol. Environ. 21. https://doi.org/10.1002/fee.2573.

Myers, D.T., Jones, D., Oviedo-Vargas, D., Schmit, J.P., Ficklin, D.L., Zhang, X., 2024. Seasonal variation in land cover estimates reveals sensitivities and opportunities for environmental models. Hydrol. Earth Syst. Sci. 28, 5295–5310. https://doi.org/10.5194/hess-28-5295-2024.

Nguyen, H.T.M., Le, Q.T.P., Garnier, J., Janeau, J.L., Rochelle-Newall, E., 2016. Seasonal variability of faecal indicator bacteria numbers and die-off rates in the Red River basin. North Vietnam Sci. Rep. 6. https://doi.org/10.1038/srep21644.

Noble, R., Gaze, R.H., 1994. Controlled Environment Composting for Mushroom Cultivation Substrates Based on Wheat and Barley Straw and Deep Litter Poultry Manure. J. Agric. Sci. 123. https://doi.org/10.1017/S0021859600067794.

Obire, O., Ogechi, A.A., 2013. Cultivation of mushroom (Pleurotus ostreatus) and the microorganisms associated with the substrate used. e- J. Sci. Technol. (e- JST) 8 (4), 49–59.

Pachepsky, Y., Harriger, M.D., Panko Graff, C., Wyatt-Brown, L., Stocker, M.D., Smith, J. E., 2024. Multiscale spatiotemporal variability of fecal indicator bacteria and associated particle size distributions in the sandy bottom sediments of a Pennsylvania creek. J. Environ. Qual. 53. https://doi.org/10.1002/jeq2.20531.

Pandey, P.K., Soupir, M.L., Ikenberry, C.D., Rehmann, C.R., 2016. Predicting streambed sediment and water column *E. coli* Levels at Watershed Scale. J. Am. Water Resour. Assoc. 52. https://doi.org/10.1111/1752-1688.12373.

Park, Y., Pachepsky, Y., Hong, E., Shelton, D., Coppock, C., 2017. *E. coli* Release from Streambed to Water Column during Baseflow Periods: a Modeling Study. J. Environ. Qual. 46. https://doi.org/10.2134/jeq2016.03.0114.

Petersen, F., Hubbart, J.A., 2020. Physical factors impacting the survival and occurrence of *E. coli* in secondary habitats. Water (Switzerland). https://doi.org/10.3390/w12061796.

Price, J.R., Ledford, S.H., Ryan, M.O., Toran, L., Sales, C.M., 2018. Wastewater treatment plant effluent introduces recoverable shifts in microbial community composition in receiving streams. Sci. Total Environ. 613–614. https://doi.org/10.1016/j.scitotenv.2017.09.162.

R Core Team, 2024. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org.

Rafi, K., Wagner, K.L., Gentry, T., Karthikeyan, R., Dube, A., 2018. *E. coli* Concentration as a function of stream order and watershed size. J. Environ. Qual. 47. https://doi.org/10.2134/jeq2017.12.0488.

Raith, M.R., Kelty, C.A., Griffith, J.F., Schriewer, A., Wuertz, S., Mieszkin, S., Gourmelon, M., Reischer, G.H., Farnleitner, A.H., Ervin, J.S., Holden, P.A., Ebentier, D.L., Jay, J.A., Wang, D., Boehm, A.B., Aw, T.G., Rose, J.B., Balleste, E., Meijer, W.G., Sivaganesan, M., Shanks, O.C., 2013. Comparison of PCR and quantitative real-time PCR methods for the characterization of ruminant and cattle fecal pollution sources. Water Res. 47. https://doi.org/10.1016/j.watres.2013.03.061.

Reeves, R.L., Grant, S.B., Mrse, R.D., Oancea, C.M.C., Sanders, B.F., Boehm, A.B., 2004. Scaling and Management of Fecal Indicator Bacteria in Runoff from a Coastal Urban Watershed in Southern California. Environ. Sci. Technol. 38. https://doi.org/10.1021/es034797g.

Robinson, J.M., Redvers, N., Camargo, A., Bosch, C.A., Breed, M.F., Brenner, L.A., Carney, M.A., Chauhan, A., Dasari, M., Dietz, L.G., Friedman, M., Grieneisen, L., Hoisington, A.J., Horve, P.F., Hunter, A., Jech, S., Jorgensen, A., Lowry, C.A., Man, I., Mhuireach, G., Navarro-Pérez, E., Ritchie, E.G., Stewart, J.D., Watkins, H., Weinstein, P., Ishaq, S.L., 2022. Twenty Important Research Questions in Microbial Exposure and Social Equity. mSystems 7. https://doi.org/10.1128/msystems.01240-21.

Saleem, F., Li, E., Edge, T.A., Tran, K.L., Schellhorn, H.E., 2024. Identification of potential microbial risk factors associated with fecal indicator exceedances at recreational beaches. Environ. Microbiome 19, 4.

Servais, P., Garcia-Armisen, T., George, I., Billen, G., 2007. Fecal bacteria in the rivers of the Seine drainage network (France): sources, fate and modelling. Sci. Total Environ. 375. https://doi.org/10.1016/j.scitotenv.2006.12.010.

Shanks, O.C., Atikovic, E., Blackwood, A.D., Lu, J., Noble, R.T., Domingo, J.S., Seifring, S., Sivaganesan, M., Haugland, R.A., 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Appl. Environ. Microbiol. 74. https://doi.org/10.1128/AEM.01843-07.

Sinclair, A., Hebb, D., Jamieson, R., Gordon, R., Benedict, K., Fuller, K., Stratton, G.W., Madani, A., 2009. Growing season surface water loading of fecal indicator organisms within a rural watershed. Water Res. 43. https://doi.org/10.1016/j.watres.2008.12.006.

Stallard, M.A., Winesett, S., Scopel, M., Bruce, M., Bailey, F.C., 2019. Seasonal Loading and Concentration Patterns for Fecal Bacteroidales qPCR Markers and Relationships to Water Quality Parameters at Baseflow. Water Air. Soil Pollut. 230. https://doi.org/10.1007/s11270-019-4083-3.

Stumpf, C.H., Piehler, M.F., Thompson, S., Noble, R.T., 2010. Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: hydrographic patterns and terrestrial runoff relationships. Water Res. 44. https://doi.org/10.1016/j.watres.2010.07.004.

Tarek, M.H., Hubbart, J., Garner, E., 2023. Microbial source tracking to elucidate the impact of land-use and physiochemical water quality on fecal contamination in a mixed land-use watershed. Sci. Total Environ. 872. https://doi.org/10.1016/j.scitotenv.2023.162181.

Turner, S.W.D., Rice, J.S., Nelson, K.D., Vernon, C.R., McManamay, R., Dickson, K., Marston, L., 2021. Comparison of potential drinking water source contamination across one hundred U.S. cities. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-27509-9.

URycki, D.R., Good, S.P., Crump, B.C., Ceperley, N.C., Brooks, J.R., 2024. Microbial community storm dynamics signal sources of "old" stream water. PLoS One 19, e0306896.

US EPA, 2024. Identification of test procedures (40 CFR 136.3). United States Environmental Protection Agency .

US EPA, 2019. Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay. 2019. US EPA, Washington, DC.

US EPA, 2017. National Rivers and Streams Assessment 2018/19: Quality Assurance Project Plan. EPA. - 841-B-17-001.

US EPA, 2012. Recreational Water Quality Criteria. U. S. Environmental Protection Agency Office of Water, 820-F-12-058.

US EPA, 2000. Guidance for data quality assessment. practical methods for data analysis. EPA QA/G9. QA00 UPDATE. Office of Environmental Information.

Verhougstraete, M.P., Martin, S.L., Kendall, A.D., Hyndman, D.W., Rose, J.B., 2015. Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. Proc. Natl. Acad. Sci. U S A 112. https://doi.org/10.1073/pnas.1415836112.

Wang, C., Schneider, R.L., Parlange, J.Y., Dahlke, H.E., Walter, M.T., 2018. Explaining and modeling the concentration and loading of *Escherichia coli* in a stream—A case study. Science of the Total Environment 635. https://doi.org/10.1016/j.scitotenv.2018.04.036.

Weiskerger, C.J., Brandão, J., Ahmed, W., Aslan, A., Avolio, L., Badgley, B.D., Boehm, A. B., Edge, T.A., Fleisher, J.M., Heaney, C.D., Jordao, L., Kinzelman, J.L., Klaus, J.S., Kleinheinz, G.T., Meriläinen, P., Nshimyimana, J.P., Phanikumar, M.S., Piggot, A.M., Pitkänen, T., Robinson, C., Sadowsky, M.J., Staley, C., Staley, Z.R., Symonds, E.M., Vogel, L.J., Yamahara, K.M., Whitman, R.L., Solo-Gabriele, H.M., Harwood, V.J., 2019. Impacts of a changing earth on microbial dynamics and human health risks in the continuum between beach water and sand. Water Res. https://doi.org/10.1016/j.watres.2019.07.006.

Weller, D.L., Murphy, C.M., Johnson, S., Green, H., Michalenko, E.M., Love, T.M.T., Strawn, L.K., 2022. Land Use, Weather, and Water Quality Factors Associated With Fecal Contamination of Northeastern Streams That Span an Urban-Rural Gradient. Front. Water 3. https://doi.org/10.3389/frwa.2021.741676.

Wheeler, C.T., Ledford, S.H., 2023. Stability in headwater chemical signatures across a dynamic flow regime in a highly urbanized Piedmont catchment. Hydrol. Process. 37. https://doi.org/10.1002/hyp.14825.

Xie, Z., Chen, S., Huang, J., Li, D., Lu, X., 2023. Patterns and drivers of fecal coliform exports in a typhoon-affected watershed: insights from 10-year observations and SWAT model. J. Clean. Prod. 406. https://doi.org/10.1016/j.jclepro.2023.137044.

Zhang, X., Zhi, X., Chen, L., Shen, Z., 2020. Spatiotemporal variability and key influencing factors of river fecal coliform within a typical complex watershed. Water Res. 178. https://doi.org/10.1016/j.watres.2020.115835.

Zhi, S., Banting, G., Stothard, P., Ashbolt, N.J., Checkley, S., Meyer, K., Otto, S., Neumann, N.F., 2019. Evidence for the evolution, clonal expansion and global dissemination of water treatment-resistant naturalized strains of *E. coli* in wastewater. Water Res. 156. https://doi.org/10.1016/j.watres.2019.03.024.