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Examination of the Watershed-Wide Distribution of *Escherichia coli* along Southern Lake Michigan: an Integrated Approach† ▽

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Recent research has highlighted the occurrence of *Escherichia coli* in natural habitats not directly influenced by sewage inputs. Most studies on *E. coli* in recreational water typically focus on discernible sources (e.g., effluent discharge and runoff) and fall short of integrating riparian, nearshore, onshore, and outfall sources. An integrated "beachshed" approach that links *E. coli* inputs and interactions would be helpful to understand the difference between background loading and sewage pollution; to develop more accurate predictive models; and to understand the differences between potential, net, and apparent culturable *E. coli*. The objective of this study was to examine the interrelatedness of *E. coli* occurrence from various coastal watershed components along southern Lake Michigan. The study shows that once established in forest soil, *E. coli* can persist throughout the year, potentially acting as a continuous non-point source of *E. coli* to nearby streams. Year-round background stream loading of *E. coli* can influence beach water quality. *E. coli* is present in highly variable counts in beach sand to depths just below the water table and to distances at least 5 m inland from the shore, providing a large potential area of input to beach water. In summary, *E. coli* in the fluvial-lacustrine system may be stored in forest soils, sediments surrounding springs, bank seeps, stream margins and pools, foreshore sand, and surface groundwater. While rainfall events may increase *E. coli* counts in the foreshore sand and lake water, concentrations quickly decline to prerain concentrations. Onshore winds cause an increase in *E. coli* in shallow nearshore water, likely resulting from resuspension of *E. coli*-laden beach sand. When examining indicator bacteria source, flux, and context, the entire "beachshed" as a dynamic interacting system should be considered.

The use of *Escherichia coli* as an indicator of sewage contamination has in recent years become increasingly controversial. It has been argued that the reliability of this bacterium as an indicator of recent sewage contamination is compromised by its persistence and common occurrence in nature (14, 42); its capacity to resuscitate or grow under selected conditions (31, 33); the length of analysis time required, leading to inadequate predictability (38); and differential survival rates with variations in light, substrate, salinity, and predatory community (4, 15, 24, 25). Lack of host or geographic specificity (17) may explain problems in identifying an *E. coli* contamination source. While many scientists agree that its presence in high densities suggests recent human or animal pollution, moderate to low densities of *E. coli* are more problematic for ascertaining pollution, and substantial effort and capital are spent to search for causes (of beach closures) when solutions are typically unclear.

For indicator bacteria, point source contributions are generally more straightforward than non-point sources, natural inputs, or seasonal effects. Beach closures are a familiar example of undesirable effects of elevated indicator bacteria; thousands of potential swimming days are lost every year due to excessive *E. coli* (26). In many cases, especially those where bacterial densities are near the regulatory limit (<235 CFU/100 ml) for safe swimming (35), the sources of the indicator bacteria are unknown. Even though the solution to these problems should ideally integrate both source and flux of *E. coli*, few studies include both aspects. Source studies are generally concentrated on animal or human origins, i.e., source tracking (32, 34); however, ambient contributions (soil and sediments) and environmental influences (bacterial regrowth, inactivation, and die off) are not adequately included in such exercises. Process, transport, and flux inquiries tend to be largely mathematical and grouped into mechanistic and empirical modeling approaches.

The ability to generalize from findings has been further limited by the focus of many investigations. Studies have typically been devoted to a particular type of environment, geographic location, or contamination source and have largely concentrated on effluent discharges or stream or beach water. Synoptic studies across shores and water are limited but suggest an interaction between land and surface water *E. coli* densities (12, 39). An integrated approach that links habitats within watersheds (e.g., riparian soils with creek and sediments, creeks/streams with receiving waters, and beach sand with swimming water) is important for understanding the distribution, proximate sources and sinks, transient storage, and transportation of microbial pollutants and the effects on apparent water quality. Eventually, an *E. coli* "budget" would then be possible, given a better understanding of local bacteria partitioning and flux. Further, by examining fluvial and lacustrine systems, perhaps a more integrated model of how the watershed influences *E. coli* concentrations in beaches will emerge. A better understanding of the groundwater, physico-

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FIG. 1. Sampling areas included in the study.

chemical, and hydrometeorological influences is needed, and knowledge of bacterial spatial distribution along and within the shore may provide clues to land-water interactions.

In this paper, we present data from a series of studies conducted over 10 years in the temperate coastal lacustrine and fluvial watersheds of Lake Michigan to show that *E. coli* from seemingly different local habitats can be related. Using the results of these studies and related published studies, we suggest that a more integrated model of land-water interaction emerges that may further our understanding of the environmental occurrence of this enteric bacterium. By studying numerous interrelated habitats, the continuum of potential contamination, from riparian forest soils to beach shoreline, is described along with the implications for recreational water quality. The derived concepts may influence how we approach contaminant tracking and have implications for beach monitoring and assessment, regulatory and public health, and remediation approaches.

MATERIALS AND METHODS

Site descriptions. The described studies occurred between 1991 and 2002 mostly within the coastal watersheds of southern Lake Michigan, focusing on three habitats: (i) riparian forest soils, (ii) creek basins, and (iii) beach shore and water (Fig. 1). Stream and forest studies were done in Derby Ditch and the Dunes Creek watershed, small perennial streams of Lake Michigan.

Lake studies were conducted in southern and northwest Lake Michigan in Illinois (63rd Street Beach), Indiana (Lakeview, West, Central Avenue, and Mount Baldy beaches), and Michigan (Warren Dunes, Good Harbor Bay, and Little Glen Lake). Most were wide, sandy beaches bounded by foredunes and forested uplands within public parks; 63rd Street Beach is an urban, partially embayed beach within the City of Chicago without foredunes, as described elsewhere (39). Little Glen Lake is a small inland lake in Sleeping Bear Dunes National Lakeshore.

Microbiological analysis. Substrate samples (beach sand and soil) were analyzed for *E. coli* by the Colilert-18 system; water was analyzed by the mTEC method (2) with positive (*E. coli* ATCC 25922) and negative blanks. Substrate collection and extraction are described elsewhere (5). At least 10% of the presumptive *E. coli* was confirmed by appropriate methods (3).

Field sampling: stream and watershed. (i) Stream reach. Water was sampled for *E. coli* from outfall to upper reaches of Dunes Creek and Derby Ditch at sites previously identified (37). Twelve previously established fixed locations were sampled weekly from 2 August to 20 September 1991 at Dunes Creek, and 42 randomly selected sites were sampled over a 1-week period at Derby Ditch. On 6 August 2001, creek water and sediment from 15 randomly chosen sites along 800 m of the main branch of Dunes Creek were sampled (5). The stream was only a few centimeters deep. Sediment was collected by hand to a depth of 2 to 3 cm and placed in a polyethylene bag; a bag was dipped below the stream's surface for water collection.

(ii) Population recovery. A 0.09-m^2 plot 5 m above the bank of Dunes Creek was treated with 8 liters of 88°C water on 7 June 2001. Leaf litter was removed prior to treatment. Monitored temperature showed that it was less than 25°C below 6 cm deep. Composite soil samples were collected with a sterile, 15-ml liquid medicine dispenser pre- and posttreatment. The plot was then covered with sterilized leaf litter. Soil samples were collected at various time intervals from June 2001 to November 2002; disturbance or waste in the area was documented. Limited animal disturbance (digging) was observed only on the day following treatment. An intensive sampling for *E. coli* distribution in the affected area was made 88 days posttreatment. Thirteen samples were taken within the plot along primary cardinal directions at the center (within 10 cm) and at intervals of 20, 40, and 60 cm from the center. Resultant densities were estimated and mapped using Surfer 8.0 (Golden Software).

(iii) In vitro growth. Fresh surface soil (0 to 6 cm depth) was collected from the Dunes Creek riparian forest. After thoroughly homogenizing the soil, aliquots (100 g) were weighed into five plastic containers. The soil in the containers was unamended (control) or amended with one of the following treatments: (i) bile salts, 0.15% (wt/wt) (Difco Laboratories, Sparks, MD); (ii) glucose, 1.0 g; (iii) liquid fertilizer, 1 ml (Expert Gardner Rose Food, Schultz Company, Bridgeton, MO), containing nitrogen (0.1 g), phosphorus (0.12 g), and potash (0.12 g); or (iv) bile salts, 0.15%, plus glucose, 1.0 g. The chemicals were dissolved separately in 5 ml of water and added to the corresponding containers. For the control, 5 ml of sterile water was added. The soil in each container was thoroughly mixed, and aliquots (20 g) were weighed into five Whirl-Pak bags. The bags were then incubated at 35°C. At 0, 24, 48, and 72 h of incubation, a bag from each treatment was randomly selected and analyzed for *E. coli*.

(iv) Riparian distribution. Dunes Creek water, adjacent bank seep, and artesian spring water and surrounding sediment were sampled along with intervening soils on 16 June 2001. Creek, seep, and artesian well waters were collected directly with polyethylene bags, and soil, bank, and creek sediment samples were taken by compositing 5-ml subsamples.

Field sampling: lake and shore. (i) Foreshore pore water. Weekly pore water samples were taken in triplicate at Lakeview and West Beach at 1-m intervals from the shoreline to 5 m inland during June to September 1993. In 1994, the sampling was repeated; additionally, monthly pore water samples were taken from Good Harbor Bay. Pore water samples were occasionally taken during 1993 and 1994 from Central Avenue ($n = 58$), Mount Baldy ($n = 12$), and Warren Dunes ($n = 15$) for reference. A post hole digger (12-cm diameter) that had been cleaned with ethanol and allowed to air dry was used to reach the groundwater; exposed water was sampled with a sterile pipette.

(ii) Pore water chemistry. Water chemistry was monitored weekly during July to August 1994 at Lakeview Beach. Three wells were buried 5 m inland from shore, 1 m apart, and up to 10 cm below the water table. Sample tubing was flushed 1 week prior to sampling. Using a 50-ml syringe, one full volume was discarded and another 100 ml of pore water was retrieved for analysis. Samples were analyzed for ammonia nitrogen, nitrate nitrogen, total phosphorus, total hardness, in situ-dissolved oxygen, pH, specific conductance, and temperature (2).

(iii) Foreshore hydrology. At West Beach in 1994, five water table wells were positioned at 1-m intervals from the shoreline to 5 m inland. Deep piezometers of the same construction were installed at the 1- and 2-m locations. Water level data were collected continuously using pressure transducers and data loggers. Seepage meters, to measure the amount of water being discharged or recharged below the lake, were used at 63rd Street Beach in 2000; five such setups were established in about 10 to 20 cm of water on two occasions. On one occasion, three seepage meters were used at West Beach in about 30 cm of water. Water was retrieved from shallow wells (piezometers) at various distances inland along the foreshore at both locations for *E. coli* analysis.

(iv) Vertical distribution. At Lakeview in 1999, sand was excavated to the water table using the method described above. Three holes 1 m apart and parallel to the shoreline were excavated at 1, 2, and 3 m from the shore. In each hole, 10 g of sand was retrieved from the exposed vertical wall surface at 5-cm intervals from the water table. The water table was reached at 10, 25, and 30 cm from the sand surface for distances 1, 2, and 3 m inland from the shore, respectively.

Deep sand vibracoring was used at 63rd Street Beach in 2000 at two foreshore locations that were 10 m inland and 10 m apart. Ten-centimeter pipes were forced into the sand using a vibrating device (10), with resulting core lengths of 1.26 and 1.92 m, including sand up to 1.76 m below the water table. Subsamples from the center of each core were taken at 5-cm intervals above the water table;

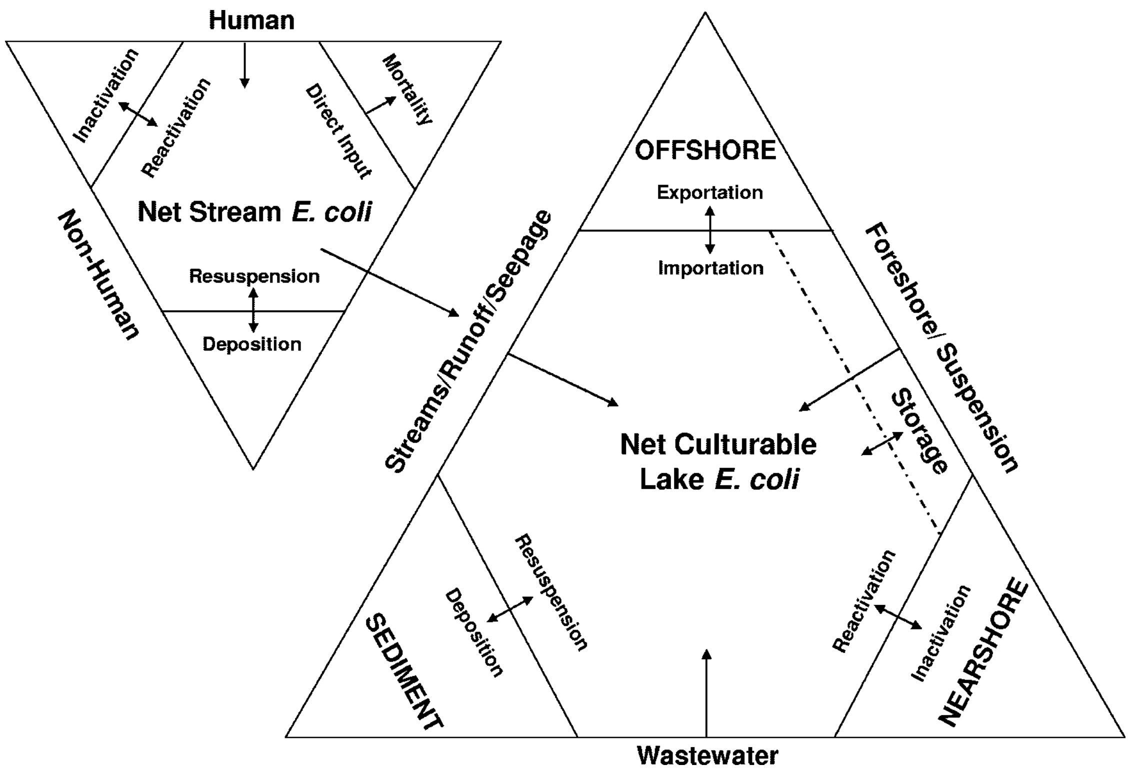

FIG. 2. Conceptual diagram of *E. coli* within and between stream and beach watersheds (left and right triangles, respectively). For streams, the model partitions stream inputs as human or nonhuman; the latter might include bacterial inputs originating from growth within riparian soils or long-term storage. Within the stream, major processes include (i) inactivation and reactivation, (ii) deposition (temporary or permanent loss) and resuspension, and (iii) death and direct input (e.g., animal defecation and multiplication). Culturable bacteria are delivered from the stream to the beachshed as surface water or groundwater. Internal inputs of *E. coli* into the lake include foreshore bacteria resuspension (from storage, defecates, and growth) and wastewater releases. Within the lake, there are dynamic interactions between inactivation/reactivation, deposition/resuspension, and offshore importation/exportation of bacteria. The whole beachshed system eventually yields the net culturable *E. coli* counts (enumerated) monitored by managers at a targeted beach location.

at 2-cm intervals to 20 cm below the water table (−20 cm); at 10-cm intervals to −60 cm; and then at −80, −100, −150, and −176 cm. Intervals for the shorter core ended at −110 cm.

(v) Sand-water interaction. *E. coli* from sand, pore water, and lake water was monitored intensively from 23 September to 2 October 1999 at Lakeview Beach. Sand was excavated using methods described above. Three holes were dug in the foreshore sand 2 m from the shoreline. A 10-ml water sample was collected from pore water using a sterile pipette. Twenty-five milliliters of sand was collected from the wall of the hole at 5-cm intervals. A lake water sample was collected simultaneously with a polyethylene bag. Rainfall began the third day of sampling, occurring periodically for 30 h and totaling 2.5 cm.

The coincidence of wind direction shifts on *E. coli* counts was examined at Little Glen Lake on 16 to 17 July 1997, where a nearly rainless front passed through the area during a 4-h period. Water samples were collected hourly at distances of 7.5, 15, 75, 150, and 225 m from the shore.

E. coli from sand and water were monitored at 63rd Street Beach from April through September 2000. Sampling and enumeration are detailed elsewhere (39). In short, five transects were sampled three times a week at the foreshore and submerged sands and at 45-, 90-, and 240-cm water depths.

Statistical path analysis was done using the AMOS version 5 software package (Smallwater Corp., Chicago, IL). Path analysis, also called structural equation modeling, is a diagrammatic use of the general linear model and is a powerful tool for testing potential relationships between complex and interacting factors. It considers both additive and multiplicative relations and can be used to test time series relationships. Its major feature is that it allows researchers to structure hypothetical factors in a diagrammatic format that can then be tested with routine parametric statistics. In this case the overall model was tested by chi-square, and relationships (using maximum likelihood) were assessed by correlation moments and regression weights. Statistical significance was set at $P = 0.05$.

All data were $\log_{10}$ transformed prior to quantitative assays.

RESULTS AND DISCUSSION

Numerous internal and external sources and sinks of *E. coli* in swimming water complicate the accuracy of water samples collected for beach monitoring. With inputs from point and non-point sources and the internal processes of deposition, inactivation, and exportation, *E. coli* counts can change within moments of collecting a water sample. In order to assess the factors influencing *E. coli* levels in the water, an integrated approach incorporating the upland as well as the nearshore processes needs to be used. In this series of studies, we examined upland areas, beach areas, and interactions between sand and water to evaluate the interconnectedness of the system; based on the results and on previous studies, we then developed an illustrative diagram to highlight the complexity of the numerous interacting processes (Fig. 2). While this figure is itself oversimplified, it provides a conceptual framework that helps structure the general research questions put forth in this paper.

Stream and watershed. The relationship between a stream and its basin has been long emphasized by both physical scientists and biologists (19, 22, 23). Studies have demonstrated the relationships of watersheds to contaminant, nutrient, and sediment loadings and consequential relationships to eukaryotic ecology, especially fish and invertebrates. Far less is known

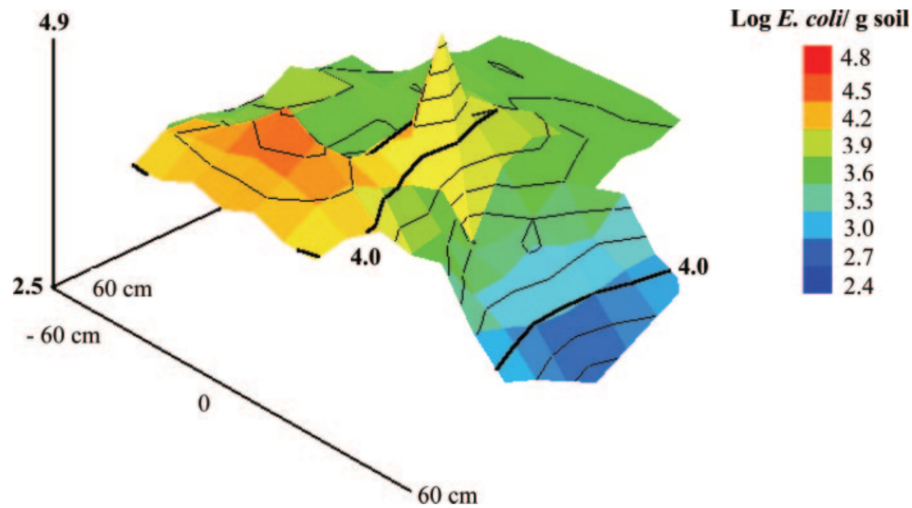

FIG. 3. Spatial distribution of *E. coli* 5 months after treatment in a plot treated with hot (88°C) water. The plot was located in Dunes Creek forest soil, approximately 5 m upland from the creek.

about interactions of watersheds, non-point pollution, and microbial response. Most of the research that has been done has focused on wet weather events during warm seasons or anthropogenic disturbance (e.g., deforestation, ditching, erosion, farm animals, and sludge applications). We posit that *E. coli* levels within Dunes Creek are strongly influenced by background levels of *E. coli* within soils, springs, and submerged sediments and that these sources help explain background bacterial levels even in the absence of human contamination.

***E. coli* recovery.** Previous research has revealed the high and variable counts of *E. coli* in Dunes Creek forest soils (5), but original sources remain unknown. After hot water treatment, surface, 4-, and 6-cm-deep soil temperature was 72, 48, and 41°C, respectively. Thus, only the top few centimeters was hot enough to kill the bacteria. Pretreatment *E. coli* count in the experimental plot was 150 most probable numbers (MPN) g^{-1}, and no culturable *E. coli* was found in samples taken 10 min after treatment to a depth of 5 cm. After this, surface populations quickly recovered to maintain pretreatment densities or higher for over 8 months: 1,112, 95, 134, >2,400, 166, 171, and 140 MPN g^{-1} at respective days 5, 20, 28, 81, 160, 216, and 250 (mean, >603 MPN g^{-1}).

The rapid recovery of the surface *E. coli* was likely due to introduction of organisms from adjacent or underlying soil. Regrowth remains a possibility (20); see below for additional evidence. Regardless of the recolonization source, the experiment shows that once established, *E. coli* can persist for months, through winter and into the next year. The hot water may have also reduced the densities of potential competitors and predators, allowing the *E. coli* to flourish. *E. coli* population pulses have been witnessed in soil when existing predators and/or competitors are removed by desiccation (33) or inhibitory substances are added (6). Also, hot water undoubtedly released a surplus of organic material that otherwise would have been trapped in the soil and litter. Neither mechanism explains why *E. coli* was able to persist through the following year.

Mapping the posttreatment *E. coli* population (Fig. 3) shows the variable density in a given area (<0.1 m^2) and emphasizes the patchiness of microbial distribution in soil (28): *E. coli* populations on June 15 varied from 3×10^2 to 8×10^4 MPN g^{-1}. This highlights the need for multiple samples to approximate *E. coli* concentration and also shows that high *E. coli* levels in the soil can be independent of stream concentrations and present even without apparent contamination (7). The experimental plot was within a few meters of Dunes Creek but was located well above the creek's normal flood plain. We surmise that soilborne *E. coli*, like that described here, could easily enter the creek by runoff or erosion events, resulting in elevated *E. coli* levels in the creek. In the collective watershed, the overall background load in the stream could be substantial.

In vitro growth. With the establishment of naturally occurring *E. coli* in soil, creeks, and beach sand, the question of the potential for growth must be considered. In a laboratory experiment, *E. coli* counts increased by 2 logs after 72 h in unamended (control) soil; its increase in the amended soils was much more rapid: 2 to 5 logs in 24 h (Fig. 4). *E. coli* growth patterns in glucose and fertilizer-amended soils were generally similar, as were the patterns in soils amended with bile salts and bile salts plus glucose, although the growth rate and relative counts were higher in the soil amended with bile salts plus glucose. When the experiment was terminated after 72 h, *E. coli* counts in soil amended with bile salts as well as that amended with bile salts plus glucose were at least 10 times higher those in the control, fertilizer-, or glucose-amended soils.

As *E. coli* growth requirements in soil are relatively simple (8), we hypothesized that *E. coli* can grow in the organically rich Dunes Creek forest soils at least during summer months. The delayed *E. coli* growth in the unamended soil is an indication that zymogenous (opportunistic) bacteria (e.g., culturable heterotrophic bacteria), which are more abundant (for every *E. coli* bacterium, there were 1.46×10^5 to 2.58×10^6 heterotrophic bacterial counts g^{-1}) and perhaps better adapted to soil conditions, may have been competing for limited nutrients. On the other hand, by reducing competition through the addition of bile salts (0.15%), a known inhibitor of nonfecal bacteria, *E. coli* counts increased by approximately 4.0

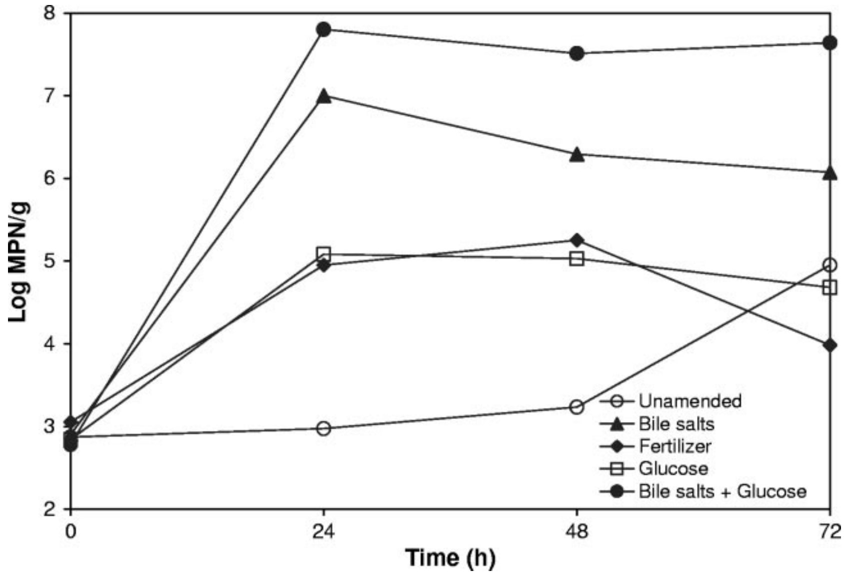

FIG. 4. In vitro growth potential of *E. coli* in unamended (control) and amended (bile salts, fertilizer, glucose, or bile salts plus glucose) forest soils at 35°C.

logs in 24 h. These results suggest that in natural soils, *E. coli* may grow under certain conditions: in the presence of metabolizable substrates and reduced or minimal competition from other microflora (6). Two potential factors support this hypothesis: (i) warm soil conditions during summer months (June to September) and (ii) nutrient availability—it is believed that the hot-water application experiment generated a nutrient pool (simple carbohydrates and other essential nutrients) from dead microbiota and macrobiota; these nutrients were perhaps sufficient to promote *E. coli* recolonization and growth in the soil. The sudden increase in nutrients can explain short-term responses; it does not, however, explain the long-term persistence of *E. coli* in the treated plot.

Springs. Soil moisture limits *E. coli* in riparian areas (5), which is influenced by precipitation and groundwater in the form of seeps along the banks and forest springs. While there was no *E. coli* in the emanating spring some 20 m from the stream, surrounding spring sediments contained 6, 2, and 1 MPN g^{-1} *E. coli* at the pool center, pool margin, and in the forest soil between pool and creek (Fig. 5). This pattern of decreasing counts along the margins of spring pools where coarser textured sediments grade to soils and litter has been seen elsewhere (5). *E. coli* may do well in substrates with moderately low levels of organic contents but not as well in finer organically rich soils due to habitat restrictions and negative interspecific relationships. *E. coli* counts were lower in the creek water than in submerged sands; surrounding bank seeps had high counts similar to those of submerged sands. It appears that *E. coli* accumulates in a downstream direction, largely due to bacterial accrual from sources, such as soils (18, 33), seeps, and bank sediments, and not necessarily from apparent pollution.

Stream water. Earlier studies have shown that *E. coli*, as well as nutrients, typically increase over stream order, with the highest concentrations often found at the outfall and the lowest concentrations near the stream origin or below wetlands (5, 37). *E. coli* densities in the Dunes Creek main branch ranged from 10^2 to 10^3 CFU/100 ml, but just below wetlands or large pools *E. coli* was nearly absent. The negative correlation with distance from the outfall ($P = 0.04$; $R = -0.535$) indicates the

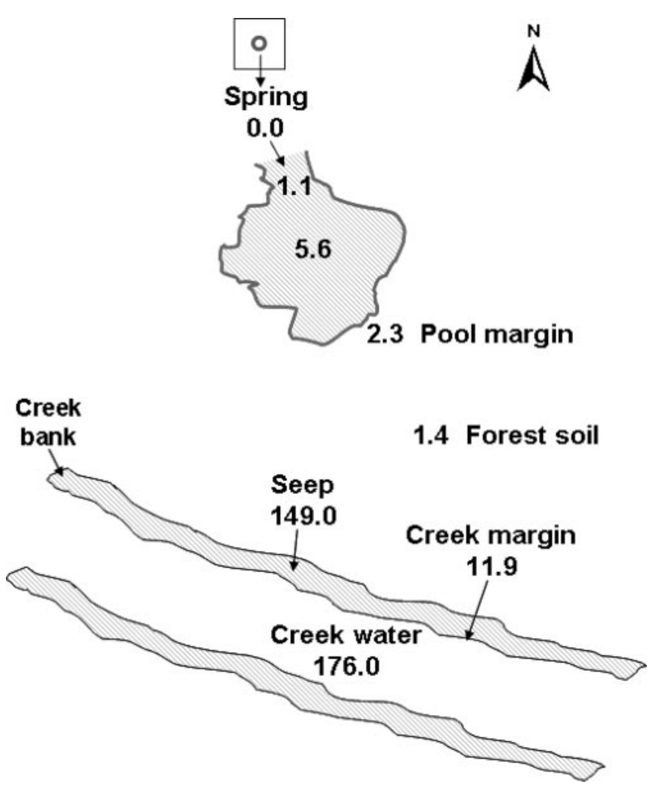

FIG. 5. Diagram of sampling locations and *E. coli* counts in creek, bank, soil, and seep in the Dunes Creek watershed. *E. coli* counts are in most probable number/gram of dry weight for soils and most probable number/100 ml for water.

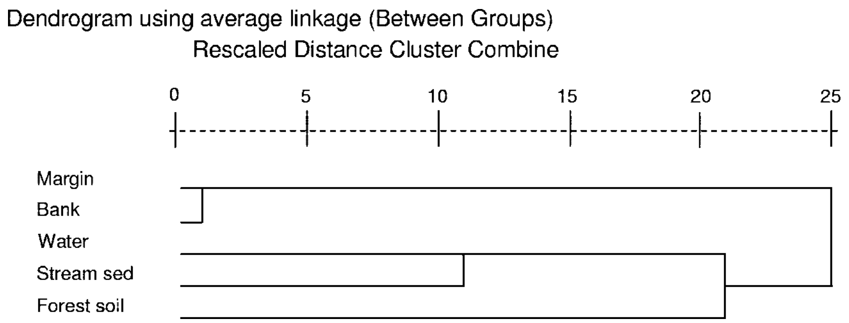

FIG. 6. Hierarchical cluster for *E. coli* counts in Dunes Creek water and upland sites. Media sampled included the creek water (at the center), creek sediment (at the center), creek margin (0.25 m from the creek center), creek bank (1 m from the creek center), and forest (4 m from the creek center).

trend of *E. coli* increasing from headwaters to outfall. *E. coli* levels were higher in stream sediments than in overlying water, usually by 2 logs, and they were significantly correlated ($P < 0.05$; $R = 0.516$). Levels of *E. coli* in the secondary branches of Derby Ditch and Dunes Creek were significantly lower than those in the main branch of either, but the creeks were not significantly different from one another ($P = 0.05$).

In an experiment conducted previously in Dunes Creek, the potential for watershed and stream interaction was explored by comparing *E. coli* counts in creek water, bottom sediment, margin sediment, and sediment at 1 and 4 m from the creek (5). Hierarchical clustering by squared Euclidean distance of those data reveals similarities between stream margins and the bank 1 m from the stream edge and also stream bottom sediment and overlying water (Fig. 6). The lack of similarity between upland and basin *E. coli* concentration does not necessarily negate a relationship. Perhaps more extensive sampling along a longer stretch of stream would yield stronger basin-riparian interaction. This approach shows more clearly the similarity and potential interaction between the water-stream bottom and margin-bank components of the watershed.

Past studies show that Dunes Creek no longer has any apparent major inputs of point source contamination (37, 40), but it has been impacted by heavy historical ditching and loss of wetlands. *E. coli* isolates ($n = 188$) from Dunes Creek water, soil, and sediments were tested for multiple antibiotic resistance, and over 95% of the isolates tested were susceptible to the antibiotics, suggesting that the source of *E. coli* within the watershed was less likely from sewage contamination, as sewage (point source)-associated strains exhibit greater resistance to many commonly used antibiotics in humans and animals (30). *E. coli* densities increased as these streams neared the outfalls and recreational beaches and remained relatively high throughout the warm season. The transport of bacteria from creek sediment to the water can be high even during base flow (21), which highlights a large potential source of indicator bacteria for downstream areas. During weekly monitoring in 2001, there was a significant correlation between stream and beach water *E. coli* concentrations at the Dunes Creek outfall ($R = 0.67$; $P < 0.005$; 16 df).

Lake and shore. The literature on *E. coli* in large lakes, such as Lake Michigan, is heavily weighted toward point source effects on recreational water quality, with special emphasis on water quality management and health effects. Studies on non-point source contamination are more sparse, and these tend to lack the integrative synthesis of observations necessary to develop a broad perspective of the beach-water system and the influences of environmental factors on water quality.

Foreshore. Foreshore sands studied were generally similar in characteristics, being more than 95% sand and less than 1% organics, and the organic component was mostly detritus. Of the physical parameters monitored, only dissolved oxygen (positively) and ammonia (negatively) were significantly correlated with *E. coli* (<0.05). These findings suggest that *E. coli* does better in pore water that has moderate amounts of organic matter and aerobic conditions. *E. coli* may lack a competitive advantage in reducing, organically rich environments, or possibly the abundant fine material that clogs interstitial spaces excludes microbial productivity and inhibits pore water circulation (36).

Groundwater influences. At 63rd Street Beach and West Beach, *E. coli* was not present in the groundwater discharging to the lake, nor was it normally retrieved in well water, similar to findings reported elsewhere (11). *E. coli* already present is likely held within the sand and cannot readily move horizontally or vertically. These results help explain why most well tests in beach sands rarely show substantial *E. coli* content unless sediments are saturated with contaminants or testing is in karst environments (15). Vertical fluctuations in the foreshore water table tracked lake level fluctuations, although they were much attenuated. Mean amplitude was 6 cm and 15 min apart 2 m inland, and flow reversals occurred on short time scales. The reoccurring displacement of water suggests there is adequate water exchange for the nutrient and waste fluxes required to promote microflora in the shallow groundwater.

Horizontal distribution. There was no significant difference in log mean *E. coli* concentration in pore water taken from West Beach (2.45 ± 0.336 standard deviations) and Lakeview Beach (2.37 ± 0.679) over the sampling period. *E. coli* counts in pore water were higher than in lake water, but they did not vary with distance from shore. While both beaches have streams nearby, their respective watersheds are quite different, and *E. coli* concentrations in lake water at West Beach are lower in mean and variance. West Beach, however, is more subject to direct sewage contamination, being near the outfall of the Little Calumet River (27). Regardless of beach location or type, *E. coli* in sand and pore water is persistent, similar to previous findings (1, 39).

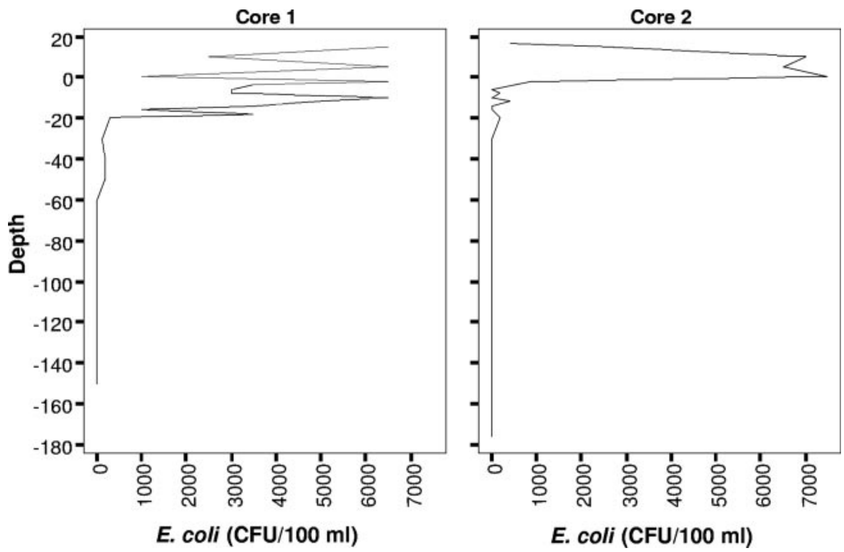

FIG. 7. Depth distribution of *E. coli* in two deep sand cores collected at 63rd Street Beach.

Vertical distribution. Vertical patterns of distribution in the sand above the water table showed no significant difference ($P = 0.536$) with distance from shore (1 to 3 m). While sand *E. coli* concentrations at Lakeview were lower than those at 63rd Street, results from both locations again showed that *E. coli* on a volume comparison was higher in sand than in lake water.

Results from deep sand coring at 63rd Street Beach showed that *E. coli* in sand was somewhat variable in depth distribution but not relative pattern (Fig. 7). The depth range for the first core sample was 6 cm above (+6 cm) to 100 cm below (−100 cm) the water table. Maximum *E. coli* counts from this core were at −8 cm (6,500 MPN/100 ml), although concentrations of 1,000 or more MPN/100 ml were consistently found to depths of −18 cm. Below this depth *E. coli* concentrations exponentially decreased; no *E. coli* was detected by −60 cm. In the second core, maximum *E. coli* concentrations occurred at the water table, but bacterial concentrations were very similar from +15 to −2 cm. *E. coli* levels fell precipitously below −4 cm, with only occasional occurrence of cells at a depth of −20 cm and none thereafter.

The high bacterial concentration within the first 30 cm of surface sand means that swash and wave action can resuspend these bacteria, affecting beach water *E. coli* content, especially in shallow water. Pore water was, on average, about 25% by volume of the sand content. Assuming that the entire 30-cm depth of sand can be resuspended by wave action and wind setup or seiches, it follows that there is more than an adequate quantity of *E. coli* stored within the foreshore sand (~5 to 6 log/100 ml) to exceed the current U.S. Environmental Protection Agency criterion (2.38 log *E. coli*/100 ml) for closing beaches. This estimation assumes no lakeward dilution and a 22% bottom gradient.

Interaction of water and sand. Fig. 8 shows the results of structural equation modeling of morning *E. coli* in sand and water at 63rd Street Beach during the swimming season of 2000. The overall model, which uses maximum likelihood to optimize potential regression relationships, failed to be rejected ($n = 66$; chi-square = 6.6; 5 df; $P = 0.251$). It should be noted that the model represents the "average" conditions and that relationships between components would likely change during varying ambient conditions (e.g., wind direction, rainfall, and wave heights). The strongest relationships were between waters at 240-, 90-, and 45-cm depths. This is understandable, since waters are contiguous and exchange readily. The relationship between shallow water and shore sand is less but is still highly significant. We would have guessed that the net flow would be from the sand to the beach water, but the model suggests a depositional trend; likewise, there was a relationship between foreshore and submerged sand. Perhaps this is because under summer conditions winds are predominately offshore, which encourages deposition as opposed to onshore winds, which tend to resuspend and export *E. coli*-laden foreshore sands (37). Overall, the model accounts for a cumulative correlation coefficient of 0.66 in knee-deep water, where routine sampling normally occurs. The model emphasizes and reinforces findings that foreshore sands are an important component of sediment-water interactions and both deeper water and submerged and exposed sediments can act as

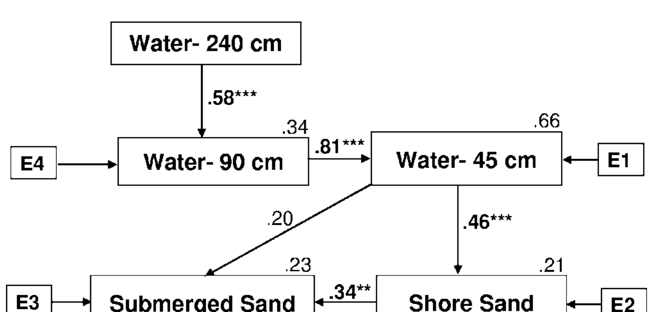

FIG. 8. Structural equation model of *E. coli* in water and beach sediments at 63rd Street Beach from May through September 2000. Cumulative R^2 values are above the box, and regression weights are above the arrows. Regression weight significance is as follows: *, 0.05; **, <0.01; ***, <0.001. E1 to E4 represent residual error.

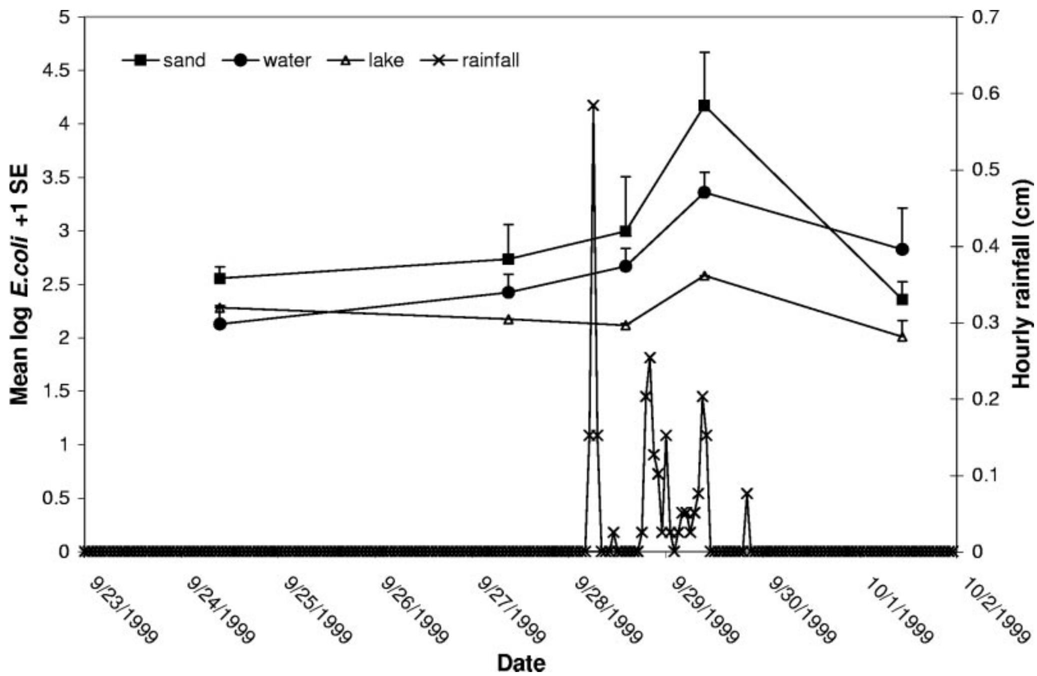

FIG. 9. Response of *E. coli* in beach sand, pore water, and lake water to a rain event. SE, standard errors.

potential sources of *E. coli* under certain conditions (39). These sources should be identified as proximate sources, since they may not be the original source but only sinks and avenues of contamination.

Seasonal distribution. *E. coli* counts generally show a seasonal pattern in recreational waters, with higher counts associated with late summer, a pattern not examined for pore water. In pore water (West and Lakeview) and sand (63rd Street) studies, *E. coli* counts were significantly different between sampling years, which may be due to a combination of evolving sampling techniques and real differences between the Chicago, Indiana, and Michigan beaches. These data were standardized for differences in sampling techniques for years and locations by converting yearly concentrations to *z* scores, which indicates the number of standard deviations a value differs from the mean of the population. While *E. coli* levels in sand during April and early May were lower than during summer months, there were no particular trends during summer. *E. coli* levels in lake water, in contrast, increased during the summer. Yearly monitoring of sand *E. coli* strongly suggests that these populations are in equilibrium (9), but it is not known if this is due to importation (gulls and lake deposition) or from the bacteria itself (multiplication and persistence). The founding population in the latter case remains unknown, but past research demonstrates that origins are varied (39).

Factor interaction. Background *E. coli* counts at Lakeview Beach prior to a rainstorm were log mean 2.55 ($\pm$0.11 standard errors) for sand, 2.13 ($\pm$0.18) for pore water, and 2.28 CFU/100 ml for lake water (Fig. 9). Counts in all three media responded to the 1-cm, 8-h rain event, and the counts increased more with an additional 0.6 cm of rainfall. By the third day after all rainfall had subsided, *E. coli* counts had fallen to levels below those prerainfall for sand (2.36 $\pm$ 0.17) and lake water (2.00 $\pm$ 0.15). Counts in pore water were still slightly elevated over initial counts (2.83 $\pm$ 0.39). The immediate increase in *E. coli* counts in the lake water indicates that it was not a runoff-driven event. Rather, the resuspension of *E. coli* in the sand and possibly the physical turbulence caused an increase in *E. coli* in sand, pore water, and lake water. This phenomenon indicates the potential for *E. coli* to move lakeward from sand, a process that may be greatly elevated during rain events.

At Little Glen Lake, background *E. coli* counts in the lake were higher closest to shore (163 CFU at 7.5 m from shore) and decreased with increasing depth and distance from the shore: the *E. coli* count was 0 at 150 m offshore, a phenomenon seen in larger lakes (39). After the wind shifted, counts fell to zero at all locations except 7.5 m from shore. Two hours later, the wind was calm and *E. coli* was detected in one sample (75 m) at very low counts (21 CFU).

The movement of *E. coli* between beach sand and swimming water is the direct link between background *E. coli* and recreational water quality. Because the flux is influenced by numerous factors, including rainfall, wind direction, concentration of *E. coli*, and beach orientation, *E. coli* concentration in the water can be directly impacted by naturally occurring *E. coli* populations in the beach sand (9, 39). Once *E. coli* cells are adsorbed onto particulate matter (sand and silt) in moist, nearshore areas, they are protected from environmental stresses, such as desiccation and solar radiation (41). Bacteria that are introduced can establish and perhaps grow in the warm, moist sand and in the presence of nutrients, e.g., decomposing vegetation or other available sources, and suitable temperature (5). *E. coli* cells suspended in the open water are not only stressed by biotic and abiotic factors but soon settle or become diluted such that concentration decreases exponentially with depth or distance from the shore.

The ubiquity of *E. coli* in beach sand from the shoreline 5 m inland and to a depth of up to 70 cm below the surface in the backshore indicates this is a persistent or even a naturally occurring population. There are obvious potential onsite sources (birds, visitors, etc.) of *E. coli*, but the expanse over which the *E. coli* population extends indicates sources must either be widespread or autochthonous in nature. Also, the common occurrence of *E. coli* in beach sand indicates it may be a recurring source of *E. coli* for the nearshore water, accounting for some of the beach closures during increased wave conditions.

The watershed-wide occurrence and documented persistence of *E. coli* throughout a coastal watershed has significant consequences for downstream recreational waters. Regardless of original source, *E. coli* is abundant in forest soil, natural streams, deep backshore sand, pore water, and foreshore sand, all of which may eventually travel into receiving waters (Fig. 2). The direct input of stream outfalls has already been established as a cause of higher counts of *E. coli* delivered to the monitored swimming water (5), and beach sand has also been implicated as a potential source (39).

While the contribution of sewage and other sources (e.g., runoff and streams) to increased *E. coli* levels in water is periodically a problem for many recreational beaches (26), the continuous presence of endogenous sources within watershed soils and sands and throughout the year is likely to have an effect on beach water quality. Several studies have shown the common occurrence of indicator bacteria in beach sand (1, 16, 29, 39) and soil (13), but few have connected indicator bacteria densities throughout the watershed with adjacent beach water quality. Original sources of these bacteria are likely wide ranging and dependent on location, but persistence throughout the watershed indicates a certain interconnectedness that would affect downstream water quality.

The description of the distribution of *E. coli* from forest floor to creek bank, creek, outfall, beach water, and foreshore and backshore sand should not necessarily imply a unidirectional movement from upland to beach sinks. There are several transitory pathways in this regard, most notably cell inactivation, death, burial, dilution, and deep-water deposition. Likewise, while riparian areas may be an important source, sewage, streams, wetlands, and coastal wildlife may significantly contribute to *E. coli* loadings. All of the contaminants represent the collective net *E. coli* concentrations cultured from beach water during sampling (i.e., apparent concentration), which makes the partitioning of these various sources difficult even with the aid of modern molecular techniques (34) or sanitary surveys. Determining the components, fluxes, and interactions of a dynamic conceptual model or simply an *E. coli* "budget" will help define the anticipated pathogen concentration at a given beach and the real health consequences (Fig. 2). Understanding the relationships between the factors that ultimately lead to the net *E. coli* concentrations at bathing beaches will help optimize monitoring approaches and define remediation strategies for using indicators that reflect actual, not apparent, fecal pollution.

Conclusions. The interaction of *E. coli* between the watershed and beach shows there is a complex system with many interacting factors. Once established in forest soil, *E. coli* can persist throughout the year and for many months, potentially acting as a continuous non-point source of *E. coli* for nearby streams. Storage of *E. coli* in the fluvial-lacustrine system may include forest soils, sediments surrounding springs, bank seeps, stream margins and pools, foreshore sand, and surface groundwater, and year-round background loading from these components can influence beach water quality. Further, along the shore *E. coli* is present in highly variable counts in beach sand to depths just below the water table and distances of at least 5 m inland from the shore, which provides a large potential area of input to beach water. All of these potential *E. coli* inputs may be released into the lake when there are interacting factors, such as wind and rainfall. In these studies, rainfall events increased *E. coli* counts in the foreshore sand and lake water, but concentrations quickly declined to prerain concentrations. Onshore winds caused an increase in *E. coli* in shallow nearshore water, likely resulting from resuspension of *E. coli*-laden beach sand. Overall, when examining indicator bacteria source, flux, and context, the entire "beachshed" as a dynamic, interacting system should be considered.

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