Groundwater and stream *E. coli* concentrations in coastal plain watersheds served by onsite wastewater and a municipal sewer treatment system

Charles Humphrey, Algernon Finley, Michael O'Driscoll, Alex Manda and Guy Iverson

ABSTRACT

The goal of this study was to determine if onsite wastewater treatment systems (OWS) were influencing groundwater and surface water *Escherichia coli* concentrations in a coastal plain watershed. Piezometers for groundwater monitoring were installed at four residences served by OWS and five residences served by a municipal wastewater treatment system (MWS). The residences were located in two different, but nearby (<3 km), watersheds. Effluent from the four septic tanks, groundwater from piezometers, and the streams draining the OWS and MWS watersheds were sampled on five dates between September 2011 and May 2012. Groundwater *E. coli* concentrations and specific conductivity were elevated within the flow path of the OWS and near the stream, relative to other groundwater sampling locations in the two watersheds. Groundwater discharge in the OWS watershed could be a contributor of *E. coli* to the stream because *E. coli* concentrations in groundwater at the stream bank and in the stream were similar. Stream *E. coli* concentrations were higher for the OWS in relation to MWS watersheds on each sampling date. Water quality could be improved by ensuring OWS are installed and operated to maintain adequate separation distances to water resources.

Key words | coastal watersheds, fecal bacteria, groundwater quality, onsite wastewater

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INTRODUCTION

Onsite wastewater treatment systems (OWS) are used by approximately 23% of US citizens, and each day an estimated 15 billion liters of OWS-treated effluent is discharged to the environment (United States Environmental Protection Agency 2002). OWS typically include a septic tank, drainfield trenches, vadose zone (aerated soil) beneath the trenches, and setback distances from the trenches to features of potential concern such as a well or stream. Effluent discharged from septic tanks to drainfield trenches contains elevated concentrations of pathogenic microorganisms including *Cryptosporidium*, *Escherichia coli* (*E. coli*), *Salmonella*, and *Shigella* (United States Environmental Protection Agency 2002; Lowe *et al.* 2007). If pathogen concentrations in septic tank effluent are not reduced in the vadose zone beneath the OWS trenches, then groundwater and possibly surface waters near the OWS may become contaminated and thus threaten public and environmental health. For example, studies by Scandura & Sobsey (1997), Conn *et al.* (2011), and Humphrey *et al.* (2011) have shown that concentrations of fecal indicator bacteria including *E. coli* and/or enterococci concentrations in groundwater beneath OWS can exceed water quality standards, especially when the separation distances from drainfield trenches to groundwater are less than 60 cm and the OWS are installed in sandy soils. Linkages have been established between fecal contamination of water resources and public health. For example, Borchardt *et al.* (2003) discovered that, in central Wisconsin, high densities of OWS were associated with endemic diarrhea illness in children that drank groundwater from wells (near OWS). OWS have also been identified as a major contributing source of fecal bacteria to economically and ecologically

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important coastal waters of North Carolina (Cahoon *et al.* 2006), Florida (Bhat & Danek 2012), and Australia (Ahmed *et al.* 2005). The United States Environmental Protection Agency (2003) has reported statistically significant correlations between elevated concentrations of fecal indicator bacteria (*E. coli* and enterococci) in recreational waters and waterborne disease incidences. Therefore, it is important for public and environmental health that we have a good understanding of the factors which influence the effectiveness of OWS in reducing fecal indicator bacteria concentrations.

Some studies have shown associations between OWS and elevated concentrations of fecal indicator bacteria in groundwater using septic tank effluent and groundwater well data (Scandura & Sobsey 1997; Conn *et al.* 2011; Humphrey *et al.* 2011). Other studies have used surface water quality data, land-use characteristics and differences or changes in wastewater treatment mechanisms to infer links between OWS and surface water fecal indicator bacteria concentrations (Cahoon *et al.* 2006; Meeroff *et al.* 2008; Bhat & Danek 2012). However, none of the aforementioned studies included groundwater monitoring in watersheds served by OWS and municipal sewers to fully quantify the groundwater transport of fecal indicator bacteria from residential properties to surface waters. This study incorporated groundwater and surface water monitoring for both OWS and municipal wastewater treatment system (MWS) watersheds, thus providing a more holistic analysis of the contributions of fecal indicator bacteria from OWS to water resources.

The goal of this project was to determine if *E. coli* concentrations in groundwater and surface water were significantly elevated in a watershed served by OWS in comparison to an adjacent watershed served by an MWS. Site-specific characteristics such as separation distances from OWS to groundwater, soil type, and wastewater strength were evaluated in the context of concentrations of *E. coli* in groundwater and surface waters from the OWS and MWS watersheds. This information may be useful for developing policies with regards to OWS design criteria and watershed management of non-point sources of pollution.

METHODS

Site selection

Two watersheds within the central coastal plain of Pitt County, North Carolina (USA) were selected for this study (Figure 1). These watersheds were chosen because the predominant land-use for both was residential development, they shared many of the same soil series (Table 1), the watersheds were close to each other (<3 km), and both were relatively small (201 to 440 ha), thus reducing the likelihood for potential differences in water quality related to land-use, geological setting, and climate. There were no livestock or other animal containment operations in either watershed. One watershed drained to Fork Swamp and was served by an MWS, and the other drained to Hardee Creek and was served by OWS. The watershed draining to Fork Swamp will be referred to as the MWS watershed, and the watershed draining to Hardee Creek will be referred to as the OWS watershed. Automated rain gauges were installed in both watersheds to determine precipitation totals and for comparison with long-term precipitation averages for the study area.

Four residential properties with OWS were instrumented with piezometers ($n = 24$) for monitoring the groundwater transport of *E. coli* from OWS. Piezometers were installed up-gradient and near the OWS drainfields for all sites (site numbers 100–400) and also down-gradient from OWS and near the creek for sites 100 (15 m from the OWS) and 200 (22 m from the OWS) (Figure 1). Information about the age of the OWS, septic tank capacities, and design wastewater loading rates were collected from permits for the systems at sites 100–400 (Table 2). Water-use records for the sites were obtained from the public water provider, and were used in conjunction with the OWS disposal field design to determine observed wastewater loading rates (Table 2). Piezometers were also installed at five residences (sites 600–1000) that were served by the MWS. The MWS sites were not instrumented as intensively, with two or three piezometers at each location (Figure 1). A total of 11 different piezometers were sampled at the five MWS sites. Soil profiles were described to determine the soil series at each site during piezometer installations. The specific soil series for the groundwater monitoring sites included Lynchburg, Goldsboro and Bibb for sites 100, 200, and 1000; Ocilla for sites 300, 400, and 800; Portsmouth for sites 600 and 700; and Pantego for site 900. Characteristics of these soils can be found in Table 1.

Piezometer networks

Boreholes for the piezometers were created using soil augers. Subsoil samples were collected from the boreholes near the OWS drainfields. The soil samples were analyzed

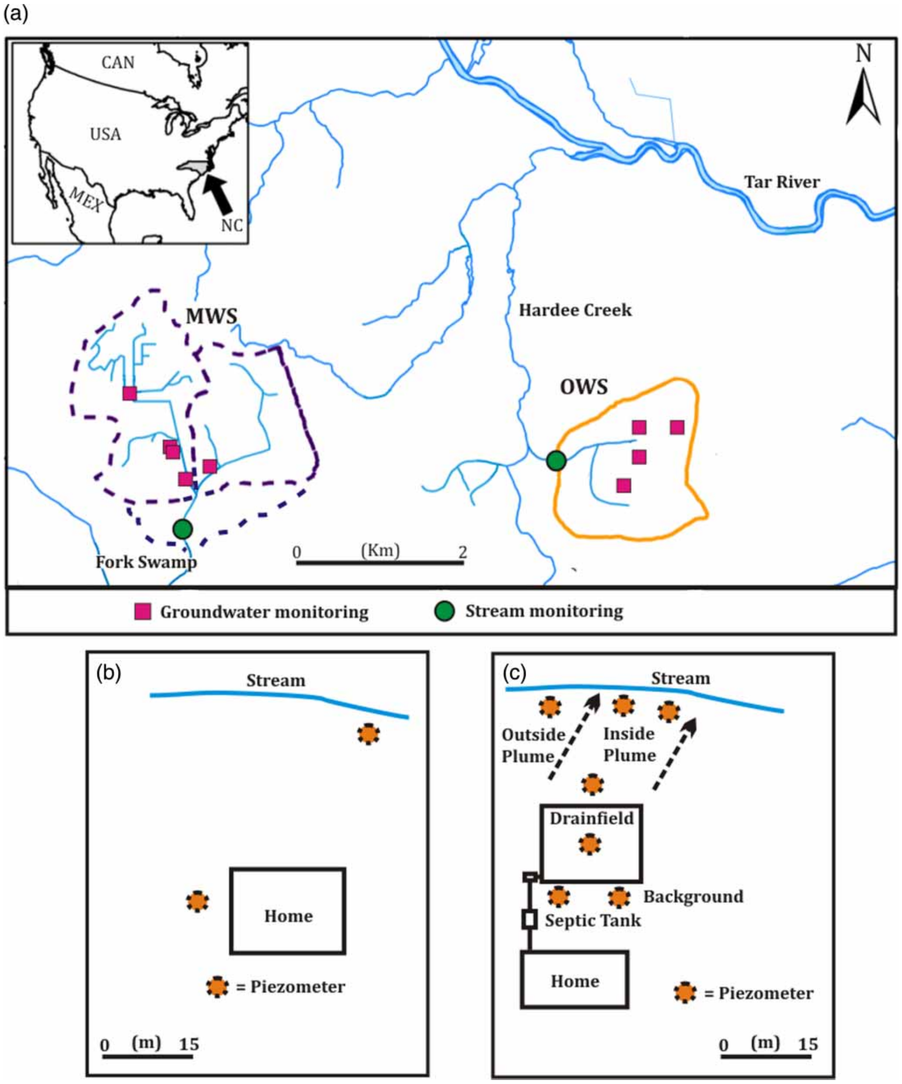

Figure 1 | (a) Groundwater and stream monitoring sites in two watersheds within the coastal plain of North Carolina. One watershed was served by onsite wastewater systems (OWS) and the other by a municipal wastewater treatment sewer system (MWS). (b) Two or three piezometers were installed at each of the five MWS sites. (c) Networks of piezometers were installed up-gradient and down-gradient of onsite wastewater systems at four sites.

for particle size distribution using the hydrometer method (Day 1965). The piezometers were constructed using either 3.18 cm or 5.08 cm diameter solid PVC pipe coupled to a 0.9 m well screen with a cap cemented to the bottom of the screen section. Piezometers were driven into the boreholes using sledge hammers, and sand was used to fill the annular space surrounding the piezometer screen. A mixture of sand, bentonite, and borehole soil was used to fill the annular space above the well screen. The top of the borehole was sealed with bentonite. The relative elevations of the top of the piezometer casings were determined using a laser level. A Trimble global positioning system was used to determine the coordinates of each piezometer. Depths to groundwater levels were measured in each piezometer and subtracted from the relative elevation of the top of the piezometer casing to determine elevations of groundwater levels. Groundwater flow direction was calculated using the relative elevation of groundwater, the coordinates of the piezometers, and the three-point contouring method (Domenico & Schwartz 1998). Based on the groundwater flow direction and OWS drainfield dimensions, piezometers were then installed down-gradient from the OWS and within the 'plume' area. Piezometers were also installed up-gradient from the drainfield and down-gradient and outside the 'plume' area for groundwater quality comparison (Figure 1). Vertical separation from OWS drainfield

Table 1 | Soil characteristics including soil series, Natural Resources Conservation Service (NRCS) hydrologic groups (A–D, with A indicating low runoff potential and D indicating high runoff potential), drainage classification, surface horizon permeability, depth to seasonal high water table, flooding frequency, and percent of the watersheds with the specific soils

Soil series	NRCS soil groups	Drainage classification	Surface horizon permeability (cm/hr)	Seasonal high water table depth (cm)	Flooding frequency	% MWS	% OWS
Alaga	A	Excessively	16–50.8	>150	None	0.1	0
Bibb	D/B	Poorly	5–16	0	Very frequent	0	9.8
Byars	D	Very poorly	5–16	0	Infrequent	1.7	0
Coxville	D/C	Poorly	16–50.8	0	Frequent	10.7	2.3
Craven	C	Moderately well	1.6–5	75	None	0	1
Exum	C/B	Moderately well	1.6–5	75	None	4.3	4.2
Goldsboro	C/B	Moderately well	5–16	75	None	4.4	4.6
Lynchburg	C/B	Somewhat poorly	5–16	45	None	19.1	1.9
Norfolk	B	Well drained	5–16	>150	None	0.9	2.7
Ocilla	C/B	Somewhat poorly	5–16	75	None	4.5	37.7
Osier	D	Poorly	16–50.8	0	Frequent	1.1	5.2
Pantego	D/C	Very poorly	5–16	0	Frequent	14.7	0
Portsmouth	D/C	Very poorly	5–16	0	Frequent	2.4	0
Rains	D/B	Poorly drained	5–16	0	Frequent	35.1	13.2
Wagram	A	Somewhat excessively	16–50.8	>150	None	1.1	17.4

Table 2 | Characteristics of onsite wastewater treatment systems in the study region. Repaired systems are indicated by (*)

Site	Tank capacity (L)	System age (yrs)	Observed wastewater loading rate ($L/(d{\cdot}m^2)$)	Design wastewater loading rate ($L/(d{\cdot}m^2)$)	Subsoil percentage clay/silt/sand	Mean vertical separation from drainfield to groundwater (cm)
100	3,785	13/8*	4.2	8.73	25/6/69	0
200	3,785	34/7*	6.16	16.3	25/10/65	21
300	3,785	25	3.44	12.22	35/15/50	48
400	3,785	12	8.12	12.22	26/8/66	222

trenches to groundwater was based on the difference in measured depths to groundwater and trench installation depths (based on measurements using tile-probes in the field).

Groundwater, septic tank, and stream sampling

Groundwater readings were recorded and samples were collected from the piezometers seasonally for 1 year on September 2011, November 2011, January 2012, March 2012, and May 2012. Depth to groundwater was measured first in each piezometer using a handheld Solinst water level meter. Piezometers were then purged using a new disposable bailer. Each piezometer had a dedicated bailer that was unwrapped immediately prior to bailing/sampling and new neoprene gloves were used for each sample location. Groundwater was collected from each piezometer using a dedicated bailer and transferred to a calibration cup for field determination of pH, temperature, specific conductance (μS/cm), and dissolved oxygen (DO; mg/L) using a YSI-556 MultiProbe Meter. Groundwater was also transferred from the bailer to a sterile sample bottle that had an identification label for the specific sampling location.

Septic tank effluent from sites 100–400 was sampled on each date (five events) by removing the manhole covers from the outlet compartments of the tanks and lowering a bailer into the liquid layer. Effluent samples in the bailer were transferred to the field meter calibration cups for environmental readings, and also into pre-labeled sample bottles for microbial analysis in the laboratory.

Surface water samples were collected downstream from the groundwater monitoring sites for environmental readings and microbial analysis on the same days that the piezometers and septic tanks were sampled, plus 3 additional months (August 2011, December 2011, and June 2012). Stream discharges were determined on each sampling date by measuring the stream cross-sectional areas and velocities as described by the United States Environmental Protection Agency (1998). Stream samples were collected by dipping the open sterile bottles into the streams, filling the bottles, and securing the lids. Streams and groundwater were sampled during baseflow conditions (no rain 3 days prior to sampling) to reduce the potential influence of stormwater runoff on the concentration of *E. coli* in the streams. Stormflow samples were also collected for microbial analysis during January 2012 and May 2012. Sample bottles were placed in ice-filled coolers after collection and transported to the East Carolina University Environmental Health Sciences Water Laboratory for analysis. Samples were prepared and incubated within 6 hours of collection. Water samples were analyzed for *E. coli* using the IDEXX Colilert substrate with Quantitray 2000 for most probable number (MPN) determination. The samples were incubated at 35 °C for 24 hours and wells which illuminated under a black light were recorded as positives for *E. coli*. Septic tank effluent and some groundwater samples were diluted (dilution factors of 10–10,000) because of elevated *E. coli* concentrations. For quality control and assurance, field blanks were inserted for approximately 10% of samples.

Comparison groups

Concentrations of *E. coli* in effluent from the septic tanks were compared to concentrations of *E. coli* in groundwater down-gradient and within the groundwater flow path of the OWS (plume) to assess the treatment effectiveness of the OWS. The *E. coli* concentrations in groundwater down-gradient of the OWS were compared to concentrations up-gradient of the OWS (background) and to groundwater at MWS sites to determine if OWS were influencing groundwater quality. Groundwater *E. coli* concentrations were compared to *E. coli* concentrations in streams at the OWS and MWS sites to determine if significant differences were observed. If groundwater *E. coli* concentrations were greater than or not statistically different from stream *E. coli* concentrations, then groundwater may be considered a contributor of *E. coli* to the streams. Data from each of the OWS sites was pooled based on sampling location to get a broader perspective on influence that OWS have on groundwater quality in the watershed. The pooled data from the OWS sites were compared to pooled data from the MWS sites. The groundwater data from piezometers installed within 1.5 m of the stream banks and down-gradient and within the flow path of the OWS were pooled (NS-I) and compared to septic tank effluent data, groundwater near the stream but outside the flow path of the system (NS-O), and to stream data to determine if OWS were likely influencing stream water quality. Mann–Whitney nonparametric tests were used to determine if differences between the comparison groups were statistically significant at $P \leq 0.05$ when comparison group data did not show a normal distribution, even after $\log_{10}$ transformation. Otherwise, paired *t*-tests were used to determine if statistical significance at $P < 0.05$ was observed between comparison groups. The statistical software MINITAB 16 was used to perform the analysis.

Environmental readings including pH, specific conductance, temperature, and DO were summarized for the main comparison groups. Pearson correlation analysis was performed to determine if there were significant relationships between *E. coli* concentrations and the specific conductivity (SC) of water samples. Prior studies have shown that groundwater influenced by wastewater typically has elevated salt concentrations and SC, and thus SC may be used an indicator of wastewater-impacted groundwater plumes (Geary 2005; Del Rosario *et al.* 2014; Humphrey *et al.* 2014; O'Driscoll *et al.* 2014).

RESULTS AND DISCUSSION

Treatment efficiency

The geometric mean of *E. coli* concentrations of septic tank effluent from the four sites was variable and ranged from 32,454 MPN/100 mL at site 200 to 1,243,629 MPN/100 mL at site 400. Comparisons are more easily visualized by converting these values to a logarithmic scale: $\log_{10}$ of $32{,}454 = 4.51$ and $\log_{10}$ of $1{,}243{,}629 = 6.09$, respectively (Figure 2). Therefore, the geometric mean and the $\log_{10}$ conversion of the geometric mean of *E. coli* concentrations will be reported where appropriate. The *E. coli* concentrations observed at the sites in this study are similar to values reported in other studies conducted in eastern North Carolina (Conn *et al.* 2011; Habteselassie *et al.* 2011; Humphrey *et al.* 2011, 2014). All field blanks tested negative for *E. coli*. Groundwater down-gradient from the OWS (plume) contained lower *E. coli* concentrations ($P < 0.05$) relative to the OWS effluent at each of the four sites (Figure 2). More specifically, on the $\log_{10}$ scale there was a reduction of 3.1

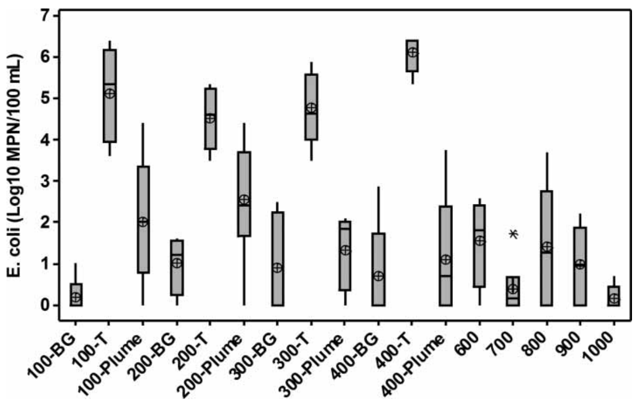

Figure 2 | *E. coli* concentrations for sites served by onsite wastewater treatment systems (100–400) and municipal wastewater treatment systems (600–1000). Groundwater up-gradient from the onsite wastewater systems (BG), septic tanks (T), groundwater down-gradient from the onsite wastewater systems (Plume) are displayed.

in the geometric mean of the *E. coli* concentration at site 100, a 1.96 reduction at site 200, a 3.44 reduction at site 300, and a 5.00 reduction at site 400. The geometric mean of *E. coli* concentrations down-gradient from sites 300 and 400 were also lower relative to sites 100 and 200; therefore sites 300 and 400 were more efficient at reducing *E. coli* concentrations in comparison to sites 100 and 200 (Figure 2). Sites 300 and 400 had larger mean vertical separation distances from trench bottom to groundwater, 48 and 222 cm respectively, relative to site 100 (0 cm) and site 200 (21 cm) (Table 2). Subsoil beneath the trenches at site 300 also had the highest mean percentage of clay (35%) of the sites (Table 2).

Prior research has shown that OWS with larger separation distances from trench bottom to groundwater and/or higher clay contents are typically more effective at reducing fecal indicator bacteria concentrations than OWS with relatively small separation distances and lower clay content because of increased filtration and die-off of *E. coli* in the thicker unsaturated zone and smaller void spaces (Karathanasis *et al.* 2006; Humphrey *et al.* 2011; Humphrey *et al.* 2014; Stall *et al.* 2014). Groundwater down-gradient from the OWS had *E. coli* concentrations greater than groundwater up-gradient from the OWS at site 100 ($P = 0.0117$) and site 200 ($P = 0.0191$), indicating that OWS were influencing groundwater quality at those sites. The geometric mean of *E. coli* values for groundwater down-gradient from the OWS at site 300 (21 MPN/100 mL, $\log_{10}$ of $21 = 1.32$) and site 400 (12 MPN/100 mL, $\log_{10}$ of $12 = 1.08$) was larger than values up-gradient from the OWS (site 300: 8 MPN/100 mL, $\log_{10}$ of $8 = 0.90$; site 400: 5 MPN/100 mL, $\log_{10}$ of $5 = 0.69$), but the differences were not statistically significant (site 300 $P = 0.7465$ and site 400 $P = 0.5038$). This indicates that the OWS at these sites (300 and 400) were not having a significant influence on groundwater quality with regards to *E. coli*. The OWS at sites 300 and 400 were in compliance with the North Carolina minimum vertical separation distances requirement (30 cm) from trench bottom to groundwater for OWS installed in sandy clay loam and sandy clay soils, but sites 100 and 200 were not in compliance with the regulations (Table 2) (North Carolina Division of Environmental Health 1998).

Site-specific groundwater comparisons

Groundwater *E. coli* concentrations down-gradient from the OWS at site 200 (geometric mean: 354 MPN/100 mL, $\log_{10}$ of $354 = 2.55$) were significantly greater ($P \leq 0.05$) than groundwater *E. coli* concentrations at each of the MWS sites (geometric mean range: 1 to 35 MPN/100 mL, $\log_{10}$ of $1 = 0$ to $\log_{10}$ of $35 = 1.54$) and were elevated relative to United States Environmental Protection Agency (2003) standards for surface waters (235 MPN/100 mL, $\log_{10}$ of $235 = 2.371$ for a single sample) (Figure 2). Groundwater down-gradient from site 100 (geometric mean: 101 MPN/100 mL, $\log_{10}$ of $101 = 2.00$) had greater *E. coli* concentrations than groundwater at MWS sites 600–700 and 900–1000 at $P < 0.05$ and greater than site 800 at $P = 0.059$. There were significant correlations between SC and *E. coli* concentrations at sites 100 ($r = 0.949$ and $P = 0.05$) and site 200 ($r = 0.981$ and $P = 0.019$). These comparisons also show that the OWS at sites 100 and 200 were influencing the *E. coli* concentrations and SC of groundwater.

Groundwater *E. coli* concentrations down-gradient from site 400 (geometric mean: 12 MPN/100 mL, $\log_{10}$ of $12 = 1.079$) were not statistically different than *E. coli* concentrations in groundwater at any of the MWS sites. The groundwater *E. coli* concentrations down-gradient from site 300 (geometric mean: 21 MPN/100 mL, $\log_{10}$ of $21 = 1.322$) were significantly different ($P = 0.0147$) than groundwater *E. coli* concentrations at site 1000 (geometric mean: 1 MPN/100 mL, $\log_{10}$ of $1 = 0$), but other MWS site groundwater comparisons were not significantly different (all $P > 0.05$). Therefore, groundwater quality adjacent to the OWS at sites 300 and 400 was typically similar to groundwater quality at the MWS sites. The groundwater *E. coli* concentrations up-gradient from the OWS at sites 100–400 were also similar to groundwater *E. coli* concentrations at the MWS sites. There were significant correlations between *E. coli* concentrations and SC at sites 300 and 400 combined ($r = 0.856$ and $P = 0.007$).

Pooled comparisons

When combining the data from all sites for each sampling location, it is evident that OWS were influencing groundwater quality. For example, the sampling location with the highest geometric mean of *E. coli* concentrations was the septic tank (131,219 MPN/100 mL, $\log_{10}$ of 131,219 = 5.12), followed by groundwater near the stream and within the flow path of the OWS, respectively (845 MPN/100 mL, $\log_{10}$ of 845 = 2.93) (Figure 3). The other groundwater sampling locations had significantly ($P \leq 0.05$) different *E. coli* concentrations than observed in the septic tanks and in groundwater down-gradient near the stream and within the flow path of the OWS. More specifically, groundwater down-gradient near the stream and outside the flow path of the OWS (geometric mean: 16 MPN/100 mL, $\log_{10}$ of 16 = 1.20), groundwater at the MWS sites (8 MPN/100 mL, $\log_{10}$ of 8 = 0.90) and groundwater up-gradient from OWS (5 MPN/100 mL, $\log_{10}$ of 5 = 0.69) had geometric mean *E. coli* concentrations at least one order of magnitude lower than septic tank effluent and groundwater within the flow path of the OWS (Figure 3). Similar trends were observed when comparing the mean SC values of water samples at the various sampling locations (Table 3). The average depth to groundwater measured from the piezometers for the OWS sites (1.4 ± 0.9 m) was lower than the MWS sites (2.2 ± 0.5 m), but within one standard deviation.

Wastewater contains elevated concentrations of salts and other ions which influence the conductive properties of water, and thus lead to relatively high SC values in comparison to most non-saline groundwater (Del Rosario et al. 2014; Humphrey et al. 2014; O'Driscoll et al. 2014). In this study, septic tank effluent had the highest mean SC of all sampling locations (781 ± 233 μS/cm) followed by groundwater near the stream and within the flow path of the OWS (238 ± 163 μS/cm) (Table 3). These values were elevated relative to groundwater SC up-gradient from the OWS (203 ± 137 μS/cm), near the stream and outside of the plume area (122 ± 85 μS/cm), and in groundwater at the MWS sites (121 ± 58 μS/cm) (Table 3). Pearson correlation analysis revealed a significant positive correlation ($r = 0.856$ and $P = 0.000$) between the pooled mean SC values and $\log_{10}$-transformed geometric mean of the *E. coli* concentrations for the sampling groups at the OWS sites. Therefore, sampling locations with elevated SC values typically had elevated *E. coli* concentrations within the OWS watershed. However, there was not a statistically significant correlation between SC and *E. coli* concentrations at the MWS sites ($r = -0.039$ and $P = 0.950$). Septic effluent typically had lower DO concentrations (2.0 ± 1.1 mg/L), and warmer temperatures (20.4 ± 4.6 °C) than groundwater sampled from all piezometers. The mean water temperature, pH, and DO concentrations were similar for groundwater sampled from piezometers at the different locations (Table 3).

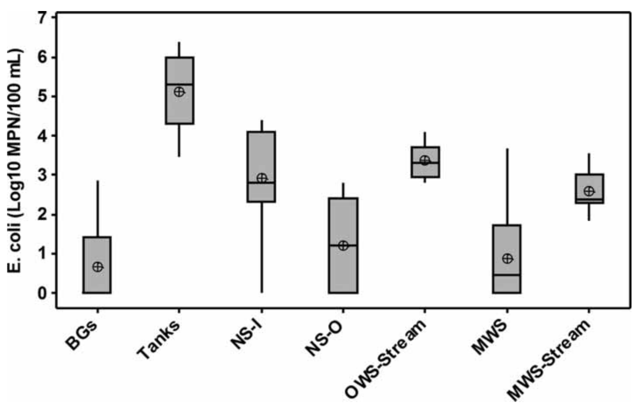

Figure 3 | The combined $\log_{10}$ of *E. coli* concentration data for all sampling locations including groundwater up-gradient from the onsite wastewater systems (BGs), septic tanks (Tanks), groundwater within the flow path of the systems and near the stream (NS-I) and groundwater outside the flow path of the systems and near the stream (NS-O), and the streams draining the onsite system watershed (OWS-Stream) and municipal wastewater system watershed (MWS-Stream).

Stream comparisons

Stream *E. coli* concentrations were variable and elevated for both the OWS (geometric mean: 2,296 MPN/100 mL, $\log_{10}$ of 2,296 = 3.36) and MWS watersheds (389 MPN/100 mL, $\log_{10}$ of 389 = 2.59) during baseflow conditions (Figure 4). The *E. coli* concentrations were higher for the OWS watershed relative to the MWS watershed on each of the baseflow sampling dates and differences were statistically different ($P = 0.003$) between the watersheds. Stream SC values were also significantly ($P = 0.034$) elevated for the OWS stream (138 ± 26 μS/cm) relative to the MWS stream (103 ± 22 μS/cm) (Table 3). The MWS stream had higher mean pH (6.3) and DO (6.7 mg/L) than the OWS stream (pH: 6.0; DO: 5.6 mg/L), but the differences were not statistically significant. Mean water temperatures were 16.2 and 16.3 °C, respectively, for the OWS and MWS streams (Table 3). There was less than a 3% difference in the total precipitation received for the MWS watershed (118 cm) relative to the OWS watershed (115 cm). On each sampling date, stream baseflow measured at the OWS watershed (1,484 ± 968 m^3/d) was lower than the MWS (1,919 ± 1,467 m^3/d), most likely because the MWS watershed had a larger drainage

Table 3 | Mean and standard deviation values of physical and chemical parameters of samples collected from locations served by onsite wastewater systems (OWS) and municipal wastewater systems (MWS)

Sample location	Specific conductivity (μS/cm)	pH	Dissolved oxygen (mg/L)	Temperature (°C)	Flow (m^3/d)
OWS background	203 ± 137	6.3 ± 1.1	2.6 ± 1.0	19.5 ± 3.9	–
Septic tanks	781 ± 233	6.2 ± 0.5	2.0 ± 1.1	20.4 ± 4.6	–
OWS near stream (in plume)	238 ± 163	5.8 ± 0.6	2.5 ± 1.3	18.3 ± 4.1	–
OWS near stream (outside plume)	122 ± 85	5.9 ± 0.4	2.2 ± 0.8	19.1 ± 3.6	–
OWS stream (baseflow)	138 ± 26	6.0 ± 0.2	5.6 ± 1.6	16.2 ± 5.5	1,484 ± 968
OWS stream (stormflow)	125 ± 5	6.2 ± 0.4		16.8 ± 3.4	9,964 ± 8,778
MWS groundwater	121 ± 58	5.2 ± 1.2	2.7 ± 1.1	19.0 ± 3.5	–
MWS stream (baseflow)	103 ± 22	6.3 ± 1.0	6.7 ± 1.5	16.3 ± 6.4	1,919 ± 1,467
MWS stream (stormflow)	76 ± 15	6.8 ± 0.1		17.6 ± 5.4	18,055 ± 9,895

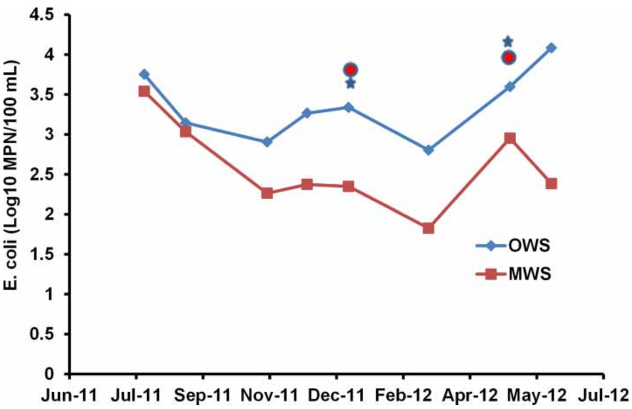

Figure 4 | Baseflow stream *E. coli* concentrations observed during the study period. Storm sample *E. coli* concentrations are indicated by circles for MWS and by stars for OWS streams.

area and received more rainfall. However, normalizing the stream discharge by drainage area shows that the OWS watershed yielded more discharge (7.38 m^3/(d·ha)) relative to the MWS watershed (4.36 m^3/(d·ha)), potentially because of increased recharge from the 328 OWS. During the two storm events, stream pH, temperature, and discharge were also higher for the MWS stream, relative to the OWS stream (Table 3). Stream SC was higher for the OWS stream relative to the MWS stream during the two storms (Table 3). Rainfall for the study period was slightly (3–6 cm) above the long-term average of 112 cm for Greenville, North Carolina (State Climate Office of North Carolina 2015).

Stream *E. coli* concentrations during storms were elevated relative to baseflow conditions for the OWS and MWS watersheds, and during May relative to January sampling periods for both watersheds (Figure 4). During the January storm event, the MWS and OWS stream *E. coli* concentrations were 7,027 MPN/100 mL or $\log_{10}$ of 7,027 = 3.85 and 6,499 MPN/100 mL or $\log_{10}$ of 6,499 = 3.81, respectively. During the May storm event, the *E. coli* concentrations of the streams were 8,555 MPN/100 mL or $\log_{10}$ of 8,555 = 3.93 and 12,098 MPN/100 mL or $\log_{10}$ of 12,098 = 4.08, for the MWS and OWS watersheds, respectively. The difference in baseflow and stormflow *E. coli* concentrations was more pronounced in the MWS stream, indicating a stronger influence of urban runoff on stream *E. coli* concentrations.

The *E. coli* concentrations in septic tank effluent were significantly different ($P = 0.0003$) than in the OWS stream during baseflow conditions. Stream *E. coli* concentrations were higher than *E. coli* concentrations in groundwater up-gradient from the OWS ($P = 0.0001$) and groundwater outside the plume area ($P = 0.0043$) of the OWS. However, statistically significant differences were not observed when comparing *E. coli* concentrations in groundwater within the flow path of the OWS to *E. coli* concentrations in the OWS stream ($P = 0.3875$). Therefore, it is possible that the effluent from OWS that was discharged to the subsurface and transported by groundwater to the stream was a contributing source of *E. coli*. One each sampling date, there were some piezometers within the flow path of the OWS and near the creek that had *E. coli* concentrations elevated relative to stream concentrations. Groundwater at the MWS sites had significantly ($P = 0.0029$) different *E. coli* concentrations (lower concentrations) than the MWS stream, indicating that groundwater transport was an unlikely source of surface water *E. coli* in that watershed.

Sources of *E. coli*

Data in this study suggest that OWS installed with minimal vertical separation to groundwater (less than 48 cm) and in close proximity to surface waters (15–22 m) may be a significant contributor to elevated concentrations of *E. coli* and elevated SC values of groundwater down-gradient from the OWS and to surface waters. Because groundwater at the MWS sites contained significantly lower concentrations of *E. coli* than the stream draining the MWS watershed, groundwater was an unlikely source of bacteria in that watershed. Stream *E. coli* concentrations in both watersheds increased during storms relative to baseflow conditions, possibly due to urban runoff and transport of *E. coli* from pet and animal waste into the streams. Prior studies have indicated that wildlife, pets, and livestock can be significant sources of surface water fecal bacteria in some settings (Whitlock *et al.* 2002; Kon *et al.* 2009; Wright *et al.* 2009). There were no livestock farms operating in either the OWS or MWS watersheds, but pets and some wildlife were observed during the study, and thus are likely sources of bacteria in the MWS and OWS watersheds.

CONCLUSIONS

This study suggests that effluent discharged to the subsurface via some OWS can increase the concentration of *E. coli* and SC of groundwater adjacent to and down-gradient (15–22 m) from the systems relative to groundwater up-gradient from the OWS, and relative to groundwater in watersheds served by MWS. Groundwater with elevated *E. coli* concentrations can contribute to elevated concentrations of *E. coli* in streams. The OWS that were installed with the largest vertical separation distances to groundwater (both more than 45 cm) had *E. coli* concentrations in groundwater similar to what was observed in groundwater at the MWS sites. Therefore, ensuring that OWS are installed and operated with 45 cm or more separation distance to the water table and more than 15 m from surface waters may help reduce the likelihood of elevated *E. coli* concentrations in groundwater and surface waters. Groundwater discharge in MWS watersheds served to dilute concentrations of *E. coli* in the MWS stream, while groundwater discharge was a potential contributing source of *E. coli* to the OWS stream during baseflow conditions. In both the OWS and MWS watersheds, wildlife and pets were a likely significant source of *E. coli* in streams. Urban runoff transported elevated concentrations of *E. coli* to the waterways as evidenced by elevated *E. coli* concentrations in both streams during stormflow in comparison to baseflow conditions. Homeowner education regarding managing pet waste, maintaining their OWS, and reducing runoff may help improve water quality in these watersheds if the residents adopted the various best management practices associated with these non-point sources of pollution.

This study was conducted entirely in coastal North Carolina where the soils have a high sand content, groundwater is shallow, and the region relies heavily on the use of conventional style OWS. However, there are other coastal states including Florida (Meeroff *et al.* 2008) and countries such as Australia (Ahmed *et al.* 2005) that have similar soil and site conditions, and use similar OWS technologies. Therefore the findings of the study may be useful for other states and countries that are implementing policies for OWS design and installation.

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