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Long-Term Monitoring of Waterborne Pathogens and Microbial Source Tracking Markers in Paired Agricultural Watersheds under Controlled and Conventional Tile Drainage Management

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Surface waters from paired agricultural watersheds under controlled tile drainage (CTD) and uncontrolled tile drainage (UCTD) were monitored over 7 years in order to determine if there was an effect of CTD (imposed during the growing season) on occurrences and loadings of bacterial and viral pathogens, coliphages, and microbial source tracking markers. There were significantly lower occurrences of human, ruminant, and livestock (ruminant plus pig) *Bacteroidales* markers in the CTD watershed in relation to the UCTD watershed. As for pathogens, there were significantly lower occurrences of *Salmonella* spp. and *Arcobacter* spp. in the CTD watershed. There were no instances where there were significantly higher quantitative loadings of any microbial target in the CTD watershed, except for F-specific DNA (F-DNA) and F-RNA coliphages, perhaps as a result of fecal inputs from a hobby farm independent of the drainage practice treatments. There was lower loading of the ruminant marker in the CTD watershed in relation to the UCTD system, and results were significant at the level $P = 0.06$. The odds of *Salmonella* spp. occurring increased when a ruminant marker was present relative to when the ruminant marker was absent, yet for *Arcobacter* spp., the odds of this pathogen occurring significantly decreased when a ruminant marker was present relative to when the ruminant marker was absent (but increased when a wildlife marker was present relative to when the wildlife marker was absent). Interestingly, the odds of norovirus GII (associated with human and swine) occurring in water increased significantly when a ruminant marker was present relative to when a ruminant marker was absent. Overall, this study suggests that fecal pollution from tile-drained fields to stream could be reduced by CTD utilization.

Tile drains or artificial subsurface drainage is commonly used to drain fields in agricultural regions throughout the world to help facilitate crop production. However, it is well documented that conventional tile drainage can serve as an efficient means by which agricultural pollutants from field systems can enter the broader surface water environment (1–4). Fecal pollution in tile drainage as derived from land application of manure or municipal biosolids is well documented (5–11).

Controlled tile drainage (CTD) is a beneficial management practice (BMP) that physically regulates tile discharge from tile-drained fields through the use of water flow control structures (4, 12). Documented benefits of the practice include reduced export of agricultural contaminants from fields to surface water systems (4, 13–15) as well as improved crop yields as a result of the conservation of nutrients and water (16). Controlled tile drainage, which is part of a family of drainage water management practices (17), is a practice that is increasing in use worldwide. Its potential impact on water quality targets can be nontrivial since in many tile-drained landscapes a significant amount of water input to streams comes from tile drainage networks. For instance, Sunohara et al. found that watershed-scale adoption of CTD employed just during the growing season can significantly reduce mass fluxes of water and nutrients (M. D. Sunohara, N. Gottschall, G. Wilkes, E. Craiovan, E. Topp, Z. Que, O. Seidou, S. Frey, and D. R. Lapen, submitted for publication). Notwithstanding these benefits, controlled tile drainage is currently not a practice that is ubiquitous in tile-drained regions throughout the world, and little is known about how this practice, when imposed *en masse* at a watershed scale, impacts the sources and degree of fecal pollution in surface water. A majority of experimental research on CTD is set at the field/plot scale and has focused primarily on other pollution targets (18–20). However, recently Schmidt et al. (21) found that CTD could potentially increase instantaneous loads and concentrations of fecal indicator bacteria and *Campylobacter* spp. in watersheds, but at plot scales, Frey et al. (9) found that regulating tile drainage has the potential for significantly reducing bacterial movement to surface water relative to conventional tile drainage following land applications of liquid swine manure. Other studies at field scale that completely shut down tile flow following manure application found a marked reduction in fecal indicator bacteria loads in comparison to free drainage (22). However, fully controlling tile drainage in this way may not be practical to carry out or necessarily beneficial with respect to field trafficking or water ponding potential at the soil surface.

In watersheds, which are open systems, the efficacy of a beneficial management practice (BMP) on microbial water quality will

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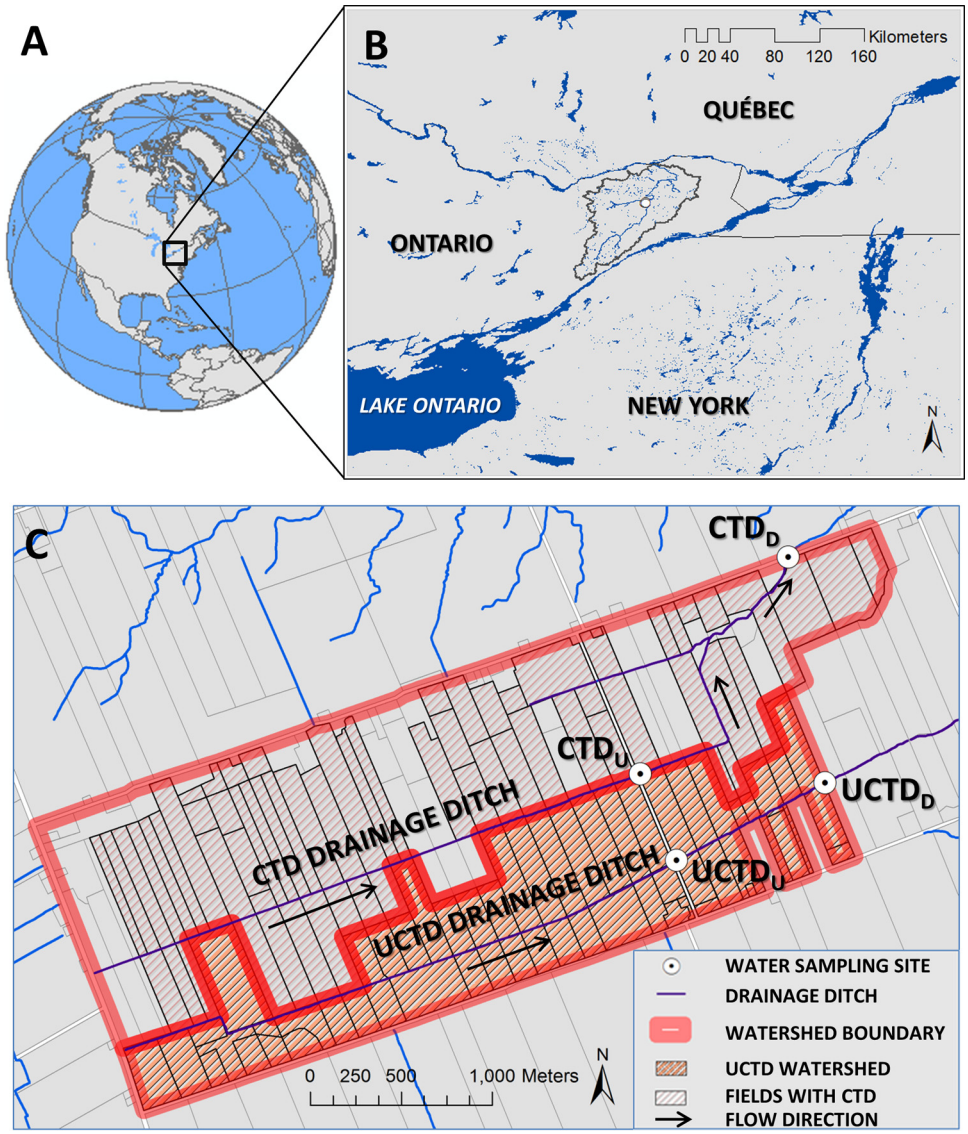

FIG 1 (A) Study location in North America. (B) Study location in Eastern Ontario, Canada, and outline of the South Nation River basin, as well as sample locations (small white dots). (C) Map of the CTD and UCTD watersheds and water sample locations.

potentially be masked by multiple sources of fecal pollution (23–28). This was underscored in a study by Wilkes et al. (24), whereby occurrences of source-specific *Bacteroidales* microbial source tracking markers shifted as a result of restricting livestock access to streams. In many tile-drained landscapes, tile drainage can contribute a significant proportion of flow to surface water drainage systems (Sunohara et al., submitted), and it is hypothesized here that CTD imposed *en masse* on a field-to-field basis in a watershed will impact the sources of fecal contamination and pathogen occurrence in streams by virtue of CTD's considerable control of drainage water from farm field to stream.

A 7-year study was undertaken to examine the effects of CTD, employed during the growing season only, on the loading and/or occurrences of selected *Bacteroidales* microbial source tracking markers, viruses (pathogens and coliphages), and bacterial pathogens in paired agricultural watersheds in eastern Ontario Canada. The "test" watershed was under CTD intervention (CTD watershed), and the "reference" watershed was under conventional, freely draining conditions (uncontrolled tile drainage [UCTD] watershed).

MATERIALS AND METHODS

Study site and controlled tile drainage. Schmidt et al. (21) and Sunohara et al. (submitted) provide background on the CTD and UCTD watersheds. The paired watersheds situated in eastern Ontario, Canada (Fig. 1A) are located in the South Nation River basin (Fig. 1B). The total surface catchment areas of the CTD and UCTD watersheds are 467 and 250 ha, respectively. The tile drainage-contributing areas (tile shed) for the CTD and UCTD watersheds are 415 and 225 ha, respectively. Due to the very flat topography of the watersheds, and the fact that the drainage ditches are manmade, the tile shed was used in the "load" calculations discussed later in the text. The two watersheds have similar flat topographies, soils, and land uses. Soils are dominated by Bainsville silt loams of the Gleysolic

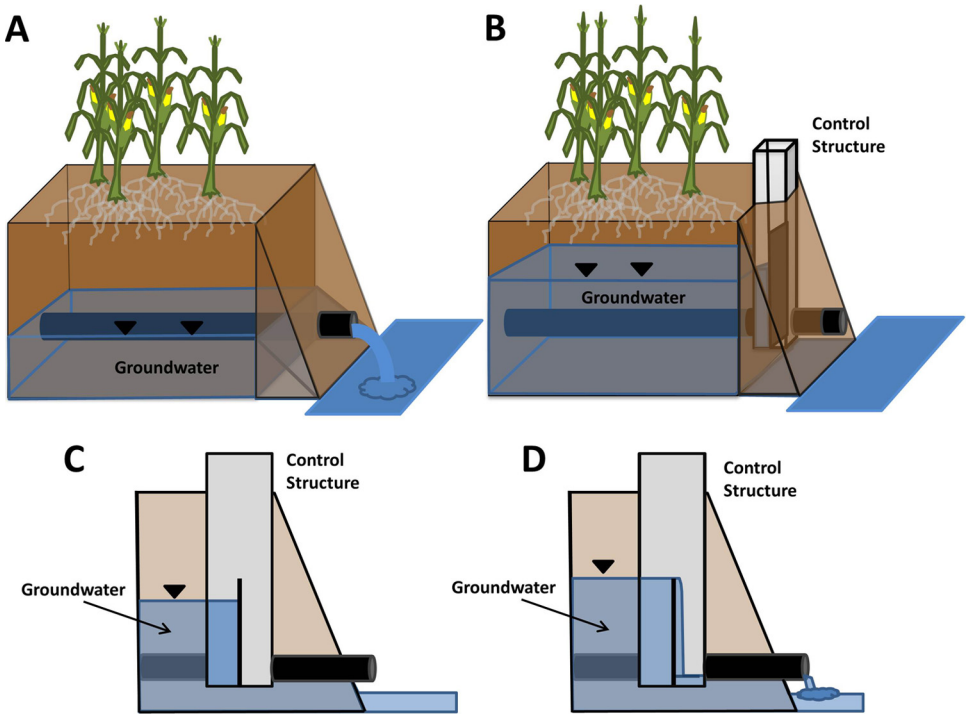

FIG 2 (A) Tile in field under conventional or free drainage. (B and C) Tile with water flow control structure fully restricting tile flow to adjacent stream. (D) Tile with water flow control structure when water table depth exceeds height of control structure stop logs. Flow to adjacent stream is only partially restricted. Black triangles indicate the groundwater level.

order (29). Mean daily temperatures peaked in July (~22°C) and were lowest in January to February (~5°C to −10°C) for the period from 2005 to 2011, whereas total yearly precipitation for 2005 to 2011 ranged between 600 and 1,100 mm (historical climate data for Russell, Ontario, from Environment Canada [http://climate.weather.gc.ca/] [climate ID 6107247], accessed 4 October 2013). The surface water systems are ice covered in winter. Tile drainage from fields is the dominant water input to the streams/ditches described in this study. Sunohara et al. (submitted) estimates that over 73% of stream flow is derived from tile drainage for these watersheds.

There are multiple farms on this watershed that operate independent cash/livestock cropping operations. Based on yearly field surveys of the watersheds made from 2005 to 2012, the CTD watershed was under ~29 to 50% corn, ~8 to 37% soybean, and ~27 to 38% pasture/forage. Similar land cover percentages were found for corn (~28 to 45%), soybean (~5 to 22%), and pasture/forage (33 to 50%) on the UCTD watershed. Livestock fecal inputs to streams would be derived exclusively from land applications of dairy cow manure above sites CTD_U and $UCTD_D$ (Fig. 1C). Land applications of manure (usually surface spread plus incorporation) occur in spring prior to planting (roughly an order of days prior) and in fall after crops have been harvested. Usually liquid bovine manure is applied to fields at application rates of around 47,000 to 75,000 liters ha^{-1}. Within 200 m upstream of site 18, there exists a small hobby farm that consists of penned animals of low and variable seasonal intensity (observed were up to 10 to 20 goats, 1 donkey, and several horses) (31). Wildlife in these watersheds consisted of, at least, raccoons, skunks, muskrats, voles, mice, frogs, fish, turtles, white-tailed deer, and birds (including, but not limited to songbirds, geese, wild turkeys, crows, gulls, and ruffed grouse).

Tile-drained fields occupy roughly 89% and 90% of total catchment areas for the CTD and UCTD watersheds, respectively. The years 2005 and 2006 represented years where CTD intervention in the CTD watershed was insignificant, occupying only 4 to 9% of tile-drained fields in the watershed (pre-CTD intervention). From 2007 on, CTD was installed more intensely, representing ~79% of the tile-drained fields in the watershed (CTD intervention period). Therefore, for 2007 to 2011, the CTD watershed was under CTD intervention and within the paired-watershed context was considered the "test" watershed. The UCTD watershed is considered a nontreated watershed, or within the paired-watershed context the "reference" watershed (where conventional or free tile drainage is employed exclusively) (Fig. 2A).

Tile drainage on fields in the CTD watershed was controlled by means of inline water-level control structures (Agri Drain Corporation, Adair, IA) fitted with stop logs (or weir boards) (Fig. 2B to D). Flow control on fields in the CTD watershed was practiced between roughly planting/fertilizing to harvest of each year (i.e., the growing season). Flow control structures in the CTD watershed between roughly harvest and planting (winter season) were set for free drainage. Hence, during this harvest-to-planting time period (winter), the CTD watershed behaved effectively like the UCTD watershed regarding tile drainage. However, often manure applications conducted on the fields were done so during freely draining conditions in order to facilitate field trafficking. During spring, drainage control was nominally imposed on the CTD watershed soon after spring manure applications in timing with planting operations. Manure applied postharvest in the fall would typically be done so on fields that were freely draining, but not always, depending on producer practices and antecedent soil water conditions. During flow control times (approximately from planting/fertilizer application to harvest), the stop logs were set at a depth of ~0.6 m below the soil surface so that when the water table was less (or shallower) than this depth below the surface, field tile water would overflow the stop logs and drain into the drainage ditch. This CTD attribute helped reduce waterlogging potential during wetter periods and facilitated root-water table interaction for crops. When water levels were below this depth (>0.6 m below surface), tile flow from field to adjacent stream was fully abated.

Stream monitoring and microbiological analyses. Water sampling/monitoring sites CTD_D and $UCTD_D$ represent the downstream monitoring sites for the CTD and UCTD watersheds, respectively (Fig. 1C). Water monitoring sites CTD_U and $UCTD_U$ represent, respectively, upper reach monitoring sites in the CTD and UCTD watershed systems. Site CTD_D is the furthest site downstream relative to all other sites and is thereby impacted by a larger area of land uses, including a small hobby farm immediately upstream of the site. For the other monitoring sites, there was no upstream influence from livestock fecal pollution outside bovine manures applied to fields potentially contaminating tile drainage.

From 2005 to 2011, water samples were collected on a biweekly basis (capturing base flow and storm flow events over many seasonal conditions and years) at the water sampling sites (total of 145 sampling occasions) targeting the following: (i) the detection and quantification of total *Bacteroidales* and host-specific *Bacteroidales* markers, including human, pig, ruminant, muskrat, and Canada goose markers (from 2005 to 2010); and (ii) detection of bacterial pathogens, including *Listeria* spp. and *Listeria monocytogenes* from 2005 to 2006 and *Salmonella* spp., *Campylobacter* spp. (Public Health Agency of Canada [PHAC]), and *Escherichia coli* O157:H7 from 2005 to 2011. Water samples were shipped overnight and processed the next day following the methodologies outlined by Marti et al. (32) for *Bacteroidales* detection, Lyautey et al. (33, 34) for *Listeria* and *Listeria monocytogenes* detection, and Jokinen et al. and Wilkes et al. (26, 35–37) for *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 detection.

Briefly, to detect *Bacteroidales* source markers, 25 to 300 ml of water was filtered through 0.45-μm-pore-size Nuclepore membrane filters (Whatman, Thermo Fisher Scientific, Ottawa, Ontario, Canada). Filters were placed in a 15-ml Falcon tube containing 0.5 ml of guanidine isothiocyanate (GITC) buffer (5 mol $liter^{-1}$ guanidine isothiocyanate, 100 mmol $liter^{-1}$ ethylenediamine tetraacetic acid, 0.5% Sarkosyl) and frozen at −80°C until extraction. DNA was extracted using the DNeasy tissue kit (Qiagen, Mississauga, Ontario, Canada) following the manufacturer's instructions, except that the proteinase K step was omitted (elution volume, 100 μl). The PCR settings, amplification programs, and markers used have been described by Marti et al. (32). Two microliters of template DNA was used for each replicate. Negative controls (no template DNA) were performed in triplicate for each run. The presence/absence of PCR inhibitors was verified on a 10× template DNA dilution by using a TaqMan exogenous internal-positive-control kit (Applied Biosystems, Toronto, Ontario, Canada) following the manufacturer's instructions (46). If inhibitors were present, a 100× dilution of the DNA template proceeded. The number of marker copies per 100 ml was converted to copies day^{-1} ha^{-1} using total daily stream discharge (total volume of water discharged per day at the monitoring site) and the catchment area tile-drained fields upstream of the monitoring site. After sample processing for *Bacteroidales* source markers, ruminant detections were combined with pig detections into a new "livestock" category, and Canada goose plus muskrat markers were combined into a new "wildlife" category. It should be noted that the "total *Bacteroidales*" parameter is not discussed in depth in this paper because it is nonspecific in regards to source.

In 2008 to 2010, additional biweekly samples were collected targeting the detection of the following viruses: hepatitis A virus, astrovirus, norovirus genogroup I (GI), norovirus GIV, sapovirus, human Torque teno virus, adenovirus 40/41, and general adenovirus. All of these viruses are primarily associated with (a/w) humans. Additionally, norovirus GIII (a/w bovines) and Torque teno sus virus (a/w swine), hepatitis E virus, norovirus GII (a/w swine and human), and rotavirus were analyzed for detection. Samples were processed as described by Wilkes et al. (24, 31). In synchrony with the virus samples, samples were also collected for detection and quantification of F-specific RNA (F-RNA) and F-DNA coliphages as described by Wilkes et al. (24), resulting in F-RNA coliphages of human origin and animal origin.

Campylobacter spp. (Eastern Cereal and Oilseed Research Centre [ECORC]) were quantified by new most probable number (MPN) methods for samples from 2010 to 2011 as described by Schmidt et al. (21). Here, isolation and quantification of *Campylobacter* cells were performed using an 11 tube (1 of 500 ml, 5 of 100 ml, and 5 of 10 ml) MPN protocol. Each water sample was filtered through a 0.22-μm-pore filter, incubated in enrichment broth, streaked on agar, and reincubated under a microaerophilic condition. The putative *Campylobacter* colonies selected based on morphology and Gram staining reaction were confirmed by genus- and species-specific PCR assays. The density of *Campylobacter* cells (MPN, 100 ml^{-1}) was calculated according to the method described by Oblinger and Koburger (38) and subsequently converted to a load identical to the method described previously for the *Bacteroidales* source markers.

Arcobacter quantification was conducted on samples for 2008 to 2011 according to Whiteduck-Leveillee (39). Water sample volumes of 10, 50, and 100 ml were analyzed by membrane filtration using 0.45-μm-pore filters; enumeration was expressed as CFU 100 ml^{-1} and subsequently converted to a daily load, as described previously for the quantified water quality endpoints. The filters were aseptically transferred to ASIA (*Arcobacter* selective isolation agar) medium and incubated under a microaerophilic condition. Putative colonies were further purified on m-AAM agar (modified agarized *Arcobacter* medium) and then confirmed by PCR assay (39).

Statistical and CART analyses. Microbial source tracking (MST) marker and pathogen loads were calculated using tile drainage catchment area normalized daily flows (termed "normalized daily loads" for both markers and pathogens). Site and treatment (CTD watershed versus UCTD watershed) comparisons for these calculated loads were made using Mann-Whitney U tests. Occurrence data were also analyzed by treatment, using 2-by-2 contingency tables and Fisher's exact tests for consideration of significance. These analyses were split into preintervention (2005 to 2006) and CTD intervention (2007 to 2011) period data groupings regarding CTD on the CTD test watershed, as previously described. For the Mann-Whitney U and Fisher's exact tests, significance was deemed at P values of <0.05.

Interactions among CTD and UCTD treatments and season (with both drainage treatment and season considered independent variables in analysis) in terms of MST marker copies day^{-1} ha^{-1} (dependent variables) were examined by means of classification and regression tree analysis (CART) (Salford Systems, San Diego CA) on CTD intervention period data, following methods described by Wilkes et al. (31, 36) for regression trees. Succinctly, CART is a "machine-driven," nonparametric and recursive partitioning method that splits dependent data (for, e.g., loads of *Bacteroidales* source marker copies day^{-1} ha^{-1}) into homogenous (low-variance) groupings using independent data splitting conditions (for, e.g., season and/or treatment factors, considered potential predictors). CART systematically iterates through a data set and tests all possible split conditions of the dependent data (for, e.g., the aforementioned loads) using all possible independent variable data-dependent variable splitting possibilities. CART then selects the best independent variable split criteria that group (by minimizing variance) the dependent data into what are termed nodes and then repeats the process on these nodes. This process is terminated by the method and/or by user-defined constraints (thereby creating terminal nodes). The result of the process is a tree structure of independent variable splitting rules that identify potentially meaningful groupings of dependent data. The analysis is purely exploratory in the context of this article.

Odds ratio (OR) estimates and 95% confidence intervals (95% CIs) for the estimates were calculated between pathogens and ruminant and wildlife markers (markers deemed to reflect CTD effect most pertinently). For this study, OR values with 95% confidence values that did not bracket the value of 1 were considered for discussion purposes "proxies" for significance. The Mann-Whitney U test, Fisher's exact tests, CART analyses, and OR estimates between pathogens and markers were applied to all available data in pre-CTD intervention (2005 to 2006) and CTD intervention (2007 to 2011).

TABLE 1 The occurrence of microbial source tracking markers and pathogens prior to CTD intervention during years 2005 to 2006 of the study

Microbial target[a]	% of samples positive for microbial target[b]								*n*			
	CTD_D	CTD_U	$UCTD_D$	$UCTD_U$	$CTD_D - UCTD_D$	$CTD_D - UCTD_U$	$CTD_U - UCTD_D$	$CTD_U - UCTD_U$	CTD_D	CTD_U	$UCTD_D$	$UCTD_U$
Total *Bacteroidales*	100	100	LD	88	LD[c]	12	LD	12	29	21	LD	25
Bacteroidales markers												
Human	7	0	LD	4	LD	3	LD	−4	29	21	LD	25
Pig	3	0	LD	0	LD	3	LD	0	29	21	LD	25
Ruminant	10	19	LD	12	LD	−2	LD	7	29	21	LD	25
Muskrat	3	14	LD	16	LD	−13	LD	−2	29	21	LD	25
Canada goose	7	0	LD	0	LD	7	LD	0	29	21	LD	25
Livestock	14	19	LD	12	LD	2	LD	7	29	21	LD	25
Wildlife	10	14	LD	16	LD	−6	LD	−2	29	21	LD	25
Other bacteria												
Listeria spp.	89	100	94	100	−5	−11	6	0	18	16	17	15
Listeria monocytogenes	33	19	12	13	21	20	7	6	18	16	17	15
Salmonella spp.	6	9	9	13	−3	−7	0	−4	35	32	33	31
Campylobacter spp.	31	31	24	35	7	−4	7	−4	35	32	33	31
E. coli O157:H7	0	0	0	0	0	0	0	0	35	32	33	31

[a] Viral pathogens and coliphages were not collected in this period. "Livestock" and "Wildlife" are summary classes: "Livestock" represents pigs and/or ruminants, and "Wildlife" represents muskrats and/or Canada geese.
[b] No significant differences between site pairwise comparisons were observed using Fisher's exact test (significant at $P < 0.05$). Note that there were percentage point differences for most CTD site − UCTD site percentage point values shown. Negative values indicate the occurrence of a greater percentage in UCTD versus pre-CTD by the indicated percentage point value and site comparison.
[c] LD, limited data.

RESULTS

Occurrences of pathogens and *Bacteroidales* markers. By comparing monitoring site data that had roughly similar temporally collocated support, or in other words, similar total sample numbers during the 2005 to 2006 pre-CTD intervention period, it was found that there were no significant differences among the CTD and UCTD watersheds in terms of the occurrence of any source specific MST marker (with CTD_D, CTD_U, and $UCTD_U$ as the sites considered) (Table 1). This finding was expected given the lack of significant CTD intervention during these years on the CTD watershed (Table 1); albeit only 2 years of pre-CTD intervention monitoring was achieved. For the same sites, and also including $UCTD_D$, there were likewise no significant differences in the occurrences of selected bacterial pathogens among the CTD and UCTD watersheds during this preintervention period (Table 1).

During the CTD intervention years of 2007 to 2011, for sites CTD_U and $UCTD_U$, which had relatively equal numbers of total samples for the aforementioned pairwise comparisons, there were significantly lower occurrences of livestock (pig plus ruminant)-, ruminant-, and human-specific MST markers in the CTD treatment watershed in relation to the UCTD watershed (Table 2). There were also significantly lower occurrences of *Salmonella* spp. and *Arcobacter* spp. at the CTD_D site in relation to both UCTD sites. Otherwise, outside a significantly higher occurrence of F-DNA coliphage in the CTD watershed, there were no other significant differences in occurrences of other microbial targets among watershed treatments (Table 2).

Odds ratio analysis, using all available data, showed that *Salmonella* spp. had higher odds of occurring when the ruminant marker was present relative to when it was absent (OR, 6.37; 95% CI, 1.00 to 40.67) (Table 3), and the association had a *P* value of 0.050. Yet for *Arcobacter* spp., the odds of this pathogen occurring was lower if a ruminant marker was present, relative to when it was absent (OR, 0.28; 95% CI, 0.08 to 0.95). Norovirus GII (a/w human or swine) was found to have a greater odds of occurring in the presence of the ruminant marker, in relation to when it was absent (OR, 4.71; 95% CI, 1.07 to 20.85).

Stream loads of pathogens and *Bacteroidales* markers. There were no significant differences among the CTD and UCTD watershed monitoring sites for the pre-CTD intervention period (2005 to 2006) regarding loads of copies day^{-1} $ha^{-1}$of source-specific markers (Table 4). For the CTD intervention period (2007 to 2011), the distributions of F-DNA and F-RNA coliphage normalized loads were significantly higher in the CTD watershed relative to the UCTD watershed (Table 5). Of note, the ruminant marker was only marginally insignificant at the 0.06 level for upstream monitoring sites (CTD_U and $UCTD_U$), where loads of mean copies day^{-1} ha^{-1} were higher in the UCTD watershed in relation to the CTD watershed. Other loads of microbial endpoints, including some bacterial pathogens, were not significantly different among watershed treatments during the CTD intervention period.

Interactions among season and tile drainage practice for pathogens and *Bacteroidales* markers. CART analyses of copies day^{-1} ha^{-1} for *Bacteroidales* MST markers and loads of coliphages and a subset of enumerated pathogens indicated that optimal data split structures were dominated by drainage practice and seasonal interactions (Table 6 and Table 7). Only *Arcobacter* spp. exhibited a sole seasonal effect (summer > spring and fall) for regression tree growth under the CART frameworks defined previously (Table 6). Drainage treatment was found to be a sole data split variable for only F-RNA coliphage GII (a/w human) (UCTD > CTD) and the muskrat marker (UCTD > CTD). The CART analysis regression trees for other microbial endpoints demonstrated interactions among season and drainage treatment. For specific tree

TABLE 2 Occurrence of microbial source tracking markers and pathogens within the CTD intervention period 2007 to 2011 on the CTD watershed

Microbial target[a]	% of samples positive for microbial target[b]								*n*			
	CTD_D	CTD_U	$UCTD_D$	$UCTD_U$	$CTD_D - UCTD_D$	$CTD_D - UCTD_U$	$CTD_U - UCTD_D$	$CTD_U - UCTD_U$	CTD_D	CTD_U	$UCTD_D$	$UCTD_U$
Total *Bacteroidales*	91	96	LD[c]	74	LD	17*	LD	22*	57	57	LD	46
Bacteroidales markers												
Human	2	0	LD	9	LD	−7	LD	−9*	57	57	LD	46
Pig	9	0	LD	0	LD	9	LD	0	57	57	LD	46
Ruminant	16	0	LD	9	LD	7	LD	−9*	57	57	LD	46
Muskrat	5	23	LD	11	LD	−6	LD	12	57	57	LD	46
Canada goose	4	0	LD	0	LD	4	LD	0	57	57	LD	46
Livestock	24	0	LD	9	LD	15	LD	−9*	54	57	LD	46
Wildlife	7	23	LD	11	LD	−4	LD	12	54	57	LD	46
Coliphages												
F-DNA	34	20	10	23	24*	11	10	−3	44	46	39	39
F-RNA a/w humans	5	15	13	15	−8	−10	2	0	44	46	39	39
F-RNA a/w animals	34	15	18	18	16	16	−3	−3	44	46	39	39
Viruses												
Hepatitis A virus	2	0	0	0	2	2	0	0	46	47	39	40
Astrovirus a/w humans	9	9	11	4	−2	5	−2	5	32	33	27	28
Norovirus GI a/w humans	9	9	5	5	4	4	4	4	46	47	39	40
Norovirus GIII a/w bovines	13	15	11	7	2	6	4	8	32	33	27	28
Norovirus GIV a/w humans	6	12	7	4	−1	2	5	8	32	33	27	28
Sapovirus a/w humans	0	0	0	0	0	0	0	0	32	32	27	28
Torque teno virus	24	17	23	23	1	1	−6	−6	45	47	39	40
Torque teno sus virus	27	15	15	15	12	12	0	0	44	47	39	40
Adenovirus 40/41 a/w humans	3	6	0	3	3	0	6	3	32	47	39	40
General adenovirus a/w humans	3	0	3	0	0	3	−3	0	32	47	39	40
Pathogenic bacteria												
Campylobacter spp. (ECORC)	70	100	68	73	2	UDS[d]	UDS	27	27	12	22	11
Arcobacter spp.	69	79	78	94	−9	−25*	1	−15	58	43	51	34
Salmonella spp.	0	2	8	5	−8*	−5	−6	−3	58	45	50	37
Campylobacter spp. (PHAC)	57	73	62	76	−5	−19	11	−3	58	45	50	37
E. coli O157:H7	0	0	0	0	0	0	0	0	58	45	50	37
Other viruses												
Hepatitis E virus GIII (swine)	0	4	5	8	−5	−8	−1	−4	46	47	39	40
Norovirus GII a/w human and swine	9	6	3	8	6	1	3	−2	46	47	39	40
Rotavirus	6	0	0	7	6	−1	0	−7	32	33	27	28

[a] *Listeria* was not monitored in this period. "Livestock" and "Wildlife" are summary classes: "Livestock" represents pigs and/or ruminants, and "Wildlife" represents muskrats and/or Canada geese.

[b] Note that there were percentage point differences for most of the CTD − UCTD site percentage point values shown. Negative values indicate the occurrence of a percentage greater in UCTD versus CTD by the indicated percentage point value and site comparison. *, significant result by Fisher's exact test ($P < 0.05$) for pairwise comparison of sites.

[c] LD, limited data.

[d] UDS, data support among sites not considered equal enough to support comparative statistical analyses.

models generated, the groups with the highest mean copies day^{-1} ha^{-1} among the source tracking markers associated with the UCTD system, under seasonal interaction, were ruminants (UCTD in spring) and humans (UCTD in fall) (Table 7). For specific CART analysis regression trees produced, the highest groupings in mean copies day^{-1} ha^{-1} for the CTD system were found for the pig *Bacteroidales* marker (CTD in summer [site CTD_D versus $UCTD_U$]) and the muskrat *Bacteroidales* marker (CTD in spring [site CTD_U versus $UCTD_U$]). For *Campylobacter* spp. (ECORC), F-DNA coliphage, F-RNA coliphage, and F-RNA

TABLE 3 Odds ratios and 95% confidence intervals for selected pathogens and ruminant and wildlife MST markers for all years of the study (2005 to 2011)[a]

Pathogen	*Bacteroidales* marker	Lower 95% CI of OR	OR	Upper 95% CI of OR	*P* value[b]
Salmonella spp.	Ruminant	1.00	6.37	40.67	0.050
	Wildlife	0.45	2.82	17.47	0.267
Arcobacter spp.	Ruminant	0.08	0.28	0.95	0.041*
	Wildlife	0.76	5.94	46.61	0.090
Norovirus GII	Ruminant	1.07	4.71	20.85	0.041*
Adjusted norovirus GII[c]	Wildlife	0.01	0.17	3.01	0.228

[a] Note that other pathogens did not have enough data support and/or had confidence intervals that straddled the value of 1 and therefore are not presented here. Only ruminant and wildlife markers were used here since they were deemed more directly influenced by CTD practices in these experimental watersheds.
[b] *, result considered significant due to the confidence interval not bracketing unity and $P < 0.05$.
[c] Here we added 0.5 within cells of the 2-by-2 matrix to perform OR and OR CI calculation due to a "zero" cell (i.e., there were no positive samples for both norovirus GII and *Bacteroidales* wildlife markers at the same site and time of sample).

GI coliphage (a/w animal), the highest mean load groups were associated with the CTD watershed, under different seasonal dispositions (coliphages higher in summer and *Campylobacter* spp. [ECORC] higher in fall).

DISCUSSION

Significant differences in frequencies of occurrence of host-specific DNA markers and selected pathogens were not found for the pre-CTD intervention years of 2005 to 2006. This is consistent with initial expectations because, first, there was no significant CTD intervention to control water discharge from fields to the water courses during this time, and second, the environmental and land use affinities among the two watersheds were effectively similar. For the CTD intervention period of 2007 to 2011, when on average during these years ~79% of the tile-drained fields were tile drain managed in the CTD watershed, significant differences associated with CTD and UCTD treatment effects for microbial source tracking host-specific DNA marker and pathogen occurrences were found. Overall, there were significantly lower occurrences in human-, ruminant-, and livestock (pig and ruminant)-specific *Bacteroidales* markers in the CTD watershed, by approximately 9 percentage points each. The finding of modestly lower ruminant marker occurrences associated with the upstream monitoring sites (upstream of the influence of the small hobby farm in the CTD watershed and therefore more purely influenced by tile drainage management practice) makes biophysical sense since CTD can physically control the degree of fecal pollution being transported from fields, where manure is applied and wildlife interact, to adjacent surface water. In these watersheds, a prevailing driver of input of livestock fecal material into streams is tile drainage. Sunohara et al. (submitted) estimated that ~73% of stream discharge is derived from tile drainage in these watersheds. The predominant ruminants in the watershed are dairy cattle (Holstein), and dairy cow manure is always applied to various fields in this watershed in both spring and fall. Fecal pollution by other ruminants in this agricultural watershed is considerably rescinded to livestock sources. Frequent surveillance of livestock and wildlife activity in these watersheds supports such contentions. Nevertheless, since dairy operations dominate the livestock activities in these experimental watersheds, the ruminant findings are promising in terms of the potential for CTD to reduce fecal contamination of surface waters from fields that receive manure applications. The relatively higher occurrences of the human *Bacteroidales* marker in the upstream UCTD monitoring site cannot be easily explained on the basis of land use activities, since there was no known location upstream of CTD_U and $UCTD_U$ where human fecal inputs were known to have systematically occurred.

TABLE 4 Mann-Whitney U test results and descriptive statistical summaries for specific *Bacteroidales* markers for the pre-CTD intervention period (2005 to 2006)[a]

Site comparison[b]	*Bacteroidales* microbial marker[c]	Total copies day^{-1} ha^{-1}: Mean		Total copies day^{-1} ha^{-1}: Median		Mean rank sum for:		*n* for:		Mann-Whitney U test *P* value[d]
		CTD site	UCTD site	CTD site	UCTD site	CTD site	UCTD site	CTD site	UCTD site	
CTD_D vs $UCTD_U$	Total	4.04×10^9	2.02×10^{10}	5.17×10^8	6.65×10^9	16.3	25.3	23	16	0.017*
	Human	8.04×10^6	0	0	0	20.3	19.5	23	16	0.434
	Pig	0	0	0	0	20.0	20.0	23	16	0.989
	Ruminant	5.25×10^6	3.76×10^5	0	0	20.6	19.2	23	16	0.498
	Muskrat	1.36×10^4	4.42×10^7	0	0	18.8	21.8	23	16	0.136
	Canada goose	6.12×10^4	0	0	0	20.3	19.5	23	16	0.434
CTD_U vs $UCTD_U$	Total	4.49×10^9	2.02×10^{10}	1.47×10^9	6.65×10^9	14.0	17.9	15	16	0.244
	Human	0	0	0	0	16.0	16.0	15	16	0.984
	Pig	0	0	0	0	16.0	16.0	15	16	0.984
	Ruminant	1.86×10^6	3.76×10^5	0	0	16.1	15.9	15	16	0.963
	Muskrat	2.43×10^6	4.42×10^7	0	0	15.4	16.6	15	16	0.579
	Canada goose	0	0	0	0	16.0	16.0	15	16	0.984

[a] Arithmetic means are used here for purely descriptive purposes.
[b] $UCTD_D$ had limited data, and therefore data are not presented.
[c] Densities of pathogens, viruses, and coliphages were not monitored in this period.
[d] *, significant ($P < 0.05$).

TABLE 5 Mann-Whitney U test results and descriptive statistical summaries for specific *Bacteroidales* markers, coliphages, and pathogens in the CTD intervention period (2007 to 2011)[a]

Site comparison	Microbial target	Result for: Mean		Result for: Median		Avg rank sum for:		*n* for:		Mann-Whitney U test *P* value[b]
		CTD site	UCTD site	CTD site	UCTD site	CTD site	UCTD site	CTD site	UCTD site	
CTD_D vs $UCTD_U$	Coliphages (total PFU day^{-1} ha^{-1})									
	F-DNA	4.32×10^6	4.26×10^5	0	0	37.8	32.0	36	33	0.142
	F-RNA	5.89×10^6	9.62×10^5	0	0	39.1	30.6	36	33	0.040*
	F-RNA GI (a/w animal)	5.88×10^6	1.02×10^5	0	0	38.1	31.6	36	33	0.065
	F-RNA GII (a/w human)	2.19×10^3	8.60×10^5	0	0	33.4	36.7	36	33	0.195
	F-RNA GIII (a/w human)	0	0	0	0	35.0	35.0	36	33	0.995
	F-RNA GIV (a/w animal)	0	0	0	0	35.0	35.0	36	33	0.995
	Bacteroidales (total copies day^{-1} ha^{-1})									
	Total	8.95×10^9	7.49×10^{10}	2.35×10^9	4.34×10^8	46.3	37.9	46	38	0.116
	Human marker	3.58×10^6	1.13×10^7	0	0	41.4	43.8	46	38	0.233
	Pig marker	1.12×10^8	0	0	0	44.2	40.5	46	38	0.066
	Ruminant marker	5.77×10^7	1.90×10^9	0	0	44.6	39.9	46	38	0.152
	Muskrat marker	2.78×10^5	3.15×10^6	0	0	41.8	43.3	46	38	0.490
	Canada goose marker	5.08×10^6	0	0	0	42.9	42.0	46	38	0.376
CTD_D vs $UCTD_D$	*Campylobacter* spp. (ECORC) (total MPN day^{-1} ha^{-1})	3.02×10^5	1.19×10^5	8.40×10^3	6.21×10^3	22.0	21.9	24	19	0.990
	Arcobacter spp. (total CFU day^{-1} ha^{-1})	4.93×10^6	2.91×10^7	4.43×10^5	1.57×10^6	40.5	47.0	46	40	0.222
	Coliphage (total PFU day^{-1} ha^{-1})									
	F-DNA	4.32×10^6	7.87×10^5	0	0	38.3	29.1	36	31	0.012*
	F-RNA	5.89×10^6	3.87×10^5	0	0	37.3	30.2	36	31	0.088
	F-RNA GI (a/w animal)	5.88×10^6	3.12×10^5	0	0	36.2	31.4	36	31	0.181
	F-RNA GII (a/w human)	2.19×10^3	7.75×10^4	0	0	32.8	35.4	36	31	0.292
	F-RNA GIII (a/w human)	0	0	0	0	34.0	34.0	36	31	0.995
	F-RNA GIV (a/w animal)	0	0	0	0	34.0	34.0	36	31	0.995
CTD_U vs $UCTD_U$	*Campylobacter* spp. (ECORC) (total MPN day^{-1} ha^{-1})	2.39×10^5	1.85×10^5	2.21×10^4	0	11.2	8.9	9	10	0.373
	Arcobacter spp. (total CFU day^{-1} ha^{-1})	3.40×10^7	5.82×10^6	4.74×10^5	3.29×10^5	30.9	29.0	32	27	0.673
	Coliphages (total PFU day^{-1} ha^{-1})									
	F-DNA	4.23×10^4	4.26×10^5	0	0	34.4	36.8	37	33	0.466
	F-RNA	4.74×10^4	9.62×10^5	0	0	35.1	35.9	37	33	0.814
	F-RNA GI (a/w animal)	2.29×10^4	1.02×10^5	0	0	35.3	35.7	37	33	0.890
	F-RNA GII (a/w human)	2.38×10^4	8.60×10^5	0	0	34.8	36.3	37	33	0.599
	F-RNA GIII (a/w human)	2.31×10^3	0	0	0	35.9	35.0	37	33	0.360
	F-RNA GIV (a/w animal)	0	0	0	0	35.5	35.5	37	33	0.995
	Bacteroidales (total copies day^{-1} ha^{-1})									
	Total	1.35×10^{10}	7.49×10^{10}	1.95×10^9	4.34×10^8	45.4	37.0	44	38	0.107
	Human marker	0	1.13×10^7	0	0	40.0	43.2	44	38	0.061
	Pig marker	0	0	0	0	41.5	41.5	44	38	0.996
	Ruminant marker	0	1.90×10^9	0	0	40.0	43.2	44	38	0.061
	Muskrat marker	1.54×10^7	3.15×10^6	0	0	44.0	38.6	44	38	0.102
	Canada goose marker	0	0	0	0	41.5	41.5	44	38	0.996
CTD_U vs $UCTD_D$	*Arcobacter* spp. (total CFU day^{-1} ha^{-1})	3.40×10^7	2.91×10^7	4.74×10^5	1.57×10^6	34.5	38.1	32	40	0.462
	Coliphage (total PFU day^{-1} ha^{-1})									
	F-DNA	4.23×10^4	7.87×10^5	0	0	35.3	33.6	37	31	0.558
	F-RNA	4.74×10^4	3.87×10^5	0	0	33.8	35.3	37	31	0.663
	F-RNA GI (a/w animal)	2.29×10^4	3.12×10^5	0	0	33.6	35.5	37	31	0.503
	F-RNA GII (a/w human)	2.38×10^4	7.75×10^4	0	0	34.2	34.9	37	31	0.800
	F-RNA GIII (a/w human)	2.31×10^3	0	0	0	34.9	34.0	37	31	0.376
	F-RNA GIV (a/w animal)	0	0	0	0	34.5	34.5	37	31	0.995

[a] Arithmetic means are presented here for purely descriptive purposes.
[b] *, significant ($P < 0.05$).

TABLE 6 Daily normalized loads of pathogens and coliphages associated with final CART least-square regression tree analysis[a]

Microbial target	CART data split criterion/criteria	*n*	Mean ± SD of terminal node microbial target data
Bacteria			
Campylobacter spp. (total MPN day^{-1} ha^{-1})	Spring, summer	44	$2.08 \times 10^4 \pm 4.12 \times 10^4$
	Fall and UCTD	10	$3.90 \times 10^5 \pm 3.52 \times 10^5$
	Fall and CTD	8	$1.09 \times 10^6 \pm 1.05 \times 10^6$
Arcobacter spp. (total CFU day^{-1} ha^{-1})	Fall, spring	71	$2.92 \times 10^6 \pm 5.93 \times 10^6$
	Summer	74	$3.28 \times 10^7 \pm 1.38 \times 10^8$
Coliphages (total PFU day^{-1} ha^{-1})			
F-DNA	Fall, spring	66	$8.13 \times 10^3 \pm 3.59 \times 10^4$
	Summer and UCTD	33	$1.16 \times 10^6 \pm 3.02 \times 10^6$
	Summer and CTD	38	$4.12 \times 10^6 \pm 1.61 \times 10^7$
F-RNA	Fall, spring	66	$3.62 \times 10^5 \pm 2.30 \times 10^6$
	Summer and UCTD	33	$6.71 \times 10^5 \pm 2.62 \times 10^6$
	Summer and CTD	38	$5.56 \times 10^6 \pm 3.23 \times 10^7$
F-RNA GI (a/w animal)	Fall, spring	66	$8.94 \times 10^4 \pm 4.20 \times 10^5$
	Summer and UCTD	33	$2.81 \times 10^5 \pm 1.53 \times 10^6$
	Summer and CTD	38	$5.54 \times 10^6 \pm 3.23 \times 10^7$
F-RNA GII (a/w human)	CTD	73	$1.31 \times 10^4 \pm 7.22 \times 10^4$
	UCTD	64	$4.81 \times 10^5 \pm 2.49 \times 10^6$
F-RNA coliphage GIII (a/w human)	No tree created[b]		
F-RNA coliphage GIV (a/w animal)	No tree created[c]		

[a] Shown are the mean (arithmetic) daily normalized loads (and standard deviation [SD]) of pathogens and coliphages associated with final CART least-square regression tree analysis using as input drainage practice (CTD or UCTD) and season (spring, summer, or fall) as independent criteria. The results presented here are limited to the CTD intervention period (2007 to 2011) but include all available site data.

[b] There were only 3 values above zero for this endpoint in this case, limiting the possibility of split combinations available for the program to test for group differences in microbial targets. After running CART on these data, CART lists the following as the classic output for this condition: "No useful split was found. No tree created."

[c] There were no nonzero data to apply splitting rules to in this case. (All data here were 0.) CART lists the following as the classic output for this condition after running the routine: "No learn sample variance for target."

In terms of loading processes associated with fecal markers, the lack of significant differences among the host-specific *Bacteroidales* markers for the watershed treatments during 2005 to 2006 (pre-CTD intervention) is consistent with the explanations already provided. During the CTD intervention years, however, no significant differences in source-specific *Bacteroidales* marker copies day^{-1} ha^{-1} were observed. However, it is important to note though that the differences in ruminant marker copies day^{-1} ha^{-1} between site CTD_U in relation to site $UCTD_U$ were almost significant ($P = 0.06$ versus the significance threshold of 0.05) with lower copies day^{-1} ha^{-1} associated with site CTD_U (Table 5). These marginally insignificant findings complement the significant ruminant marker occurrence results and support contentions of CTD being a beneficial management practice. It is speculated that the inline water-level control structures limit the transport of fecal pollution from fields where manure is applied and wildlife interact to adjacent surface water; as the upstream differences in marker endpoints observed here were not confounded by downstream land uses. Such downstream-confounding land uses include the hobby farm located just upstream of CTD_D. The significantly higher loading of F-RNA and F-DNA coliphages associated with site CTD_D in relation to the UCTD sample sites suggests some impact from the small hobby farm since comparisons among sites upstream of the farm on the CTD watershed (CTD_u) and UCTD sites revealed no significant differences. Why these differences would have manifested themselves for coliphages and not the other microbial endpoints is unclear. However, it may have something to do with persistence of these organisms in environmental matrices, and moreover, they can be significantly shed by both animals and humans alike (40).

It should be noted again that controlled tile drainage was imposed roughly between planting and harvest (growing season), since farmers most often require free tile drainage outside this time to some degree in order to traffic their fields. Manure applications on fields typically occur just prior to planting and after harvest, when tile drainage control in the CTD watershed is not imposed. Therefore, the abatement potential of CTD regarding ruminant fecal pollution would manifest itself most strongly following spring manure applications at a minimum, since the period between manure applications on fields and conversion of free drainage on the CTD watershed to CTD was on the order of days. Thus, given the use of CTD during the growing season only, these results are especially promising as a basis to support CTD-based mitigation strategies of fecal pollution of surface waters. Such beneficial impacts of CTD on MST endpoints were further demonstrated in the CART analysis employed in this study.

In this study, CART analyses uncovered seasonal interactions with watershed tile drainage treatment for a majority of the mi-

TABLE 7 Daily normalized loads of microbial source tracking endpoints associated with final CART least-square regression tree analysis[a]

Site comparison	*Bacteroidales* microbial target	CART data split criterion/criteria	*n*	Mean ± SD of terminal node microbial target data (total copies day^{-1} ha^{-1})
CTD_D vs $UCTD_U$	Total	Fall, summer	54	$6.29 \times 10^9 \pm 1.38 \times 10^{10}$
		Spring and CTD	16	$1.17 \times 10^{10} \pm 2.24 \times 10^{10}$
		Spring and UCTD	14	$1.95 \times 10^{11} \pm 6.69 \times 10^{11}$
	Human marker	CTD	46	$3.58 \times 10^6 \pm 2.40 \times 10^7$
		UCTD and spring, summer	32	$6.84 \times 10^6 \pm 2.68 \times 10^7$
		UCTD and fall	6	$3.53 \times 10^7 \pm 7.89 \times 10^7$
	Pig marker	Fall, spring	44	$7.12 \times 10^6 \pm 4.67 \times 10^7$
		Summer and UCTD	18	0 ± 0
		Summer and CTD	22	$2.21 \times 10^8 \pm 7.04 \times 10^8$
	Ruminant marker	Fall, summer	54	$2.16 \times 10^7 \pm 1.15 \times 10^8$
		Spring and CTD	16	$1.42 \times 10^8 \pm 5.16 \times 10^8$
		Spring and UCTD	14	$5.11 \times 10^9 \pm 1.84 \times 10^{10}$
	Muskrat marker	CTD	46	$2.78 \times 10^5 \pm 1.31 \times 10^6$
		UCTD	38	$3.15 \times 10^6 \pm 1.37 \times 10^7$
	Canada goose marker	No tree created[b]		
CTD_U vs $UCTD_U$	Total	Fall, summer	52	$9.98 \times 10^9 \pm 2.79 \times 10^{10}$
		Spring and CTD	16	$1.18 \times 10^{10} \pm 1.51 \times 10^{10}$
		Spring and UCTD	14	$1.95 \times 10^{11} \pm 6.69 \times 10^{11}$
	Human marker	CTD	44	0 ± 0
		UCTD and spring, summer	32	$6.84 \times 10^6 \pm 2.68 \times 10^7$
		UCTD and fall	6	$3.53 \times 10^7 \pm 7.89 \times 10^7$
	Pig marker	No tree created[c]		
	Ruminant marker	Fall, summer	52	$1.52 \times 10^7 \pm 1.09 \times 10^8$
		Spring and CTD	16	0 ± 0
		Spring and UCTD	14	$5.11 \times 10^9 \pm 1.84 \times 10^{10}$
	Muskrat marker	Fall, summer	52	$3.98 \times 10^6 \pm 1.69 \times 10^7$
		Spring and UCTD	14	$3.25 \times 10^6 \pm 1.17 \times 10^7$
		Spring and CTD	16	$3.41 \times 10^7 \pm 7.56 \times 10^7$
	Canada goose marker	No tree created[c]		

[a] Shown are the arithmetic mean and standard deviation (SD) of microbial source tracking endpoints associated with final CART least-square regression tree analysis using as input drainage practice (CTD or UCTD) and season (spring, summer, or fall) as independent criteria. The results presented here are limited to the CTD intervention period (2007 to 2011).

[b] There is only 1 positive value for this case, limiting the possibility of split combinations available for the program to test for group differences in microbial targets. CART lists the following as the classic output for this particular CART analysis routine: "No useful split was found. No tree created."

[c] There were no nonzero data to apply splitting rules to in this case. (All data here were 0.) CART lists the following as the classic output for this condition after running the CART routine: "No learn sample variance for target."

crobial water quality targets quantified as a "load." The groups with the greatest mean ruminant marker copies day^{-1} ha^{-1} were delineated for the UCTD watershed in spring when manure is applied to fields. Moreover, in middle to late spring, tile flow is controlled in the CTD watershed, accounting for lower flows in the CTD watershed in relation to the UCTD watershed (21; Sunohara et al, submitted). Also in spring, tile flows are usually higher in relation to summer tile flow (Sunohara et al., submitted). Controlled tile drainage would indeed have a maximal effect on ruminant loading during these times given that dominant sources of ruminant fecal material would be derived from livestock manure applications and that CTD controls contaminated tile water discharge from field to stream on a field-to-field basis in this experimental watershed setting. This maximal effect was observable and evidenced at site CTD_U in spring, which had no observable ruminant detection during the treatment period. However, there were also seasonal-drainage treatment interactions that identified higher relative loads in the CTD watershed during the CTD intervention period for *Campylobacter* spp. (ECORC), F-RNA coliphage, F-DNA coliphage, and F-RNA coliphage GI (a/w animal). This was the only time coliphage level was determined, as it was not done in 2005 to 2006 prior to CTD intervention. Reasons for these differences are not entirely clear, but since there were at least animal affinities with the coliphage data and affinities among

Campylobacter spp. and avian source-classed *Cryptosporidium* identified in other studies (26), it is plausible that reduction of field-based water inputs to a stream via CTD reduces flushing and dilution of fecal material in the stream system, thereby increasing concentrations (see reference 21 for a similar discussion). This consideration is supported by the fact that (i) summer is a time that farmers who grow crops do not apply manure to their fields, (ii) tile flow control is strongest during the growing season (15; Sunohara et al., submitted), (iii) stream flow is lowest during this period to begin with, which may have been especially critical in terms of exposing streambed sediments to rainfall erosion (more so in the flow-controlled CTD watershed), (iv) wildlife frequently interact and produce fecal droppings in the stream or near the stream corridor in the summer months (31), (v) the hobby farm animals are present in open pens and pasture in the summer, and (vi) summer had a higher coliphage load in the stream in relation to the other seasons.

It was found that occurrences of *Salmonella* spp. and *Arcobacter* spp. were significantly lower at CTD monitoring sites in relation to the UCTD monitoring sites during the CTD intervention period (by 8 and 25 percentage points, respectively). *Salmonella* spp. was found to be associated with livestock-source-classed *Cryptosporidium* (26) and ruminant *Bacteroidales* markers (32) in surface waters in the study region. Wilkes et al. (36) indicated that relatively significant discharge-generating events may need to transpire in order for the detection of *Salmonella* spp. to occur in surface waters in the area (i.e., runoff, erosion, and drainage events). These results are supporting evidence of *Salmonella* spp. originating directly from on-farm sources or sediment/soil receptors that are influenced by agricultural activities (S. K. Frey, N. Gottschall, G. Wilkes, D. Gregoire, E. Topp, K. D. M. Pintar, M. Sunohara, and D. R. Lapen, submitted for publication). The link between *Salmonella* spp. and livestock sources is underscored by the odds ratio results where the odds of *Salmonella* spp. occurring is higher (~6.4) when the ruminant marker occurs in water than when the marker does not occur in water. Thus, controlling tile water flow from fields where manures are applied could be a means to control at least some movement of *Salmonella* spp. to surface water bodies. Frey et al. (9) also found that controlling tile drainage in fall after manure application significantly reduced fecal indicator bacterial loads in surface water, supporting the above contention. In addition, Frey et al. (41) found strong links among *Salmonella* detection and ruminant markers in a nearby watershed in the study area. *Arcobacter* spp. can exist in the gastrointestinal tracts of healthy cattle (42) and therefore could enter surface water in this watershed via cattle manure leaching into tile drains, as would be the case for any other manure-derived pathogen. However, unlike *Salmonella* spp., odds ratios among *Arcobacter* spp. and the ruminant marker indicated that the odds of this pathogen occurring in water decreases when the ruminant marker occurs, relative to when the marker is absent (OR, ~0.3). However, the odds ratios among *Arcobacter* spp. occurrence and wildlife markers were nearly significant in the context of 95% confidence intervals bracketing 1, with a lower 95% confidence value of 0.76 and an OR value of ~5.9. These findings are worth noting on their own since they are opposite from the trends that occur among the *Salmonella* spp. and the ruminant marker. Similarly, *Campylobacter* spp. versus ruminant ORs, although having confidence intervals bracketing 1, were lower than the ORs for *Campylobacter* spp. versus wildlife (data not shown). Thus, there may be an association between wildlife fecal pollution and *Arcobacter* spp. and the closely related *Campylobacter* spp. in these watersheds. The finding of statistically lower occurrences of *Arcobacter* spp. in the CTD watershed is in support of potential wildlife pollution sources (for, e.g., avian [43]) on fields where crops are grown. The resulting fecal pollution from these sources appears, at least statistically speaking, to be partially mitigated by tile drainage control.

An interesting and unexpected finding is the odds ratio associated with norovirus GII (a/w human and swine) and the ruminant marker (Table 3). The OR indicates the odds of this virus occurring increases when a ruminant marker occurs, relative to when the marker is not present. For now, the presence of norovirus GII (a/w human and swine) in bovine fecal material has not been reported. However, Mattison (44) identified partial GII.4 norovirus genomic sequences for the first time in cattle feces from a Canadian farm. Recombination, which is common in noroviruses, can occur when a host is infected with 2 different strains of viruses and has been recognized as partially responsible for the genetic diversity and continuing emergence of new noroviruses (45). Sequencing as well as other confirmatory evaluations regarding norovirus GII (a/w human and swine) links with bovine sources was not conducted in this study. It should be noted that there are reports in many rural areas in North America of human septage being put into manure storage systems. If that kind of material is applied to land as a manure amendment on farms in this study, it could account for the ruminant marker-norovirus GII (a/w human and swine) relationships seen in this study. Although the data were not presented, we did not find odds ratios among norovirus GII (a/w human and swine) and the human *Bacteroidales* marker that had confidence intervals that did not bracket the value of 1.

Some important conclusions can be drawn from this work. They include the following. (i) There were no differences in occurrences of pathogens and microbial source tracking markers and stream loading of microbial endpoints among the tile drain managed (CTD) and uncontrolled or conventional/free tile drained (UCTD) watersheds during the 2 years (2005 to 2006) before CTD was implemented broadly in the CTD watershed ($P \geq 0.05$).

(ii) During the CTD intervention period (2007 to 2011) when the CTD watershed had widespread tile drainage control (with 79% of tile-drained fields controlled during the growing season during these years), lower occurrences of the human, ruminant, and livestock (ruminant plus pig) markers were found for the CTD watershed in relation to the UCTD watershed ($P < 0.05$). As for pathogens and other microbial water quality parameters, there were only lower occurrences of *Salmonella* spp. and *Arcobacter* spp. in the CTD watershed ($P < 0.05$). There were no instances, other than for F-DNA coliphage, where there were significantly higher (at $P < 0.05$) occurrences of any marker or microbial target in the CTD watershed. This supports the general contention that water flow control was generally effective at reducing inputs of fecal material from fields to streams during the growing season.

(iii) It was found that the odds of *Salmonella* spp. occurring increased (OR, 6.37) when a ruminant marker was present (relative to marker absence), yet for *Arcobacter* spp., the opposite was true (OR, 0.28). Moreover, the odds of *Arcobacter* presence increased when a wildlife marker was present (OR, 5.94), relative to when the wildlife marker(s) was absent. Additionally the odds of norovirus GII (a/w human and swine) occurring in water in-

creased when a ruminant marker was present relative to when a ruminant marker was absent (OR, 4.71). The mechanisms for this particular association are not entirely clear. From these findings we conclude, in combination with factors identified in conclusion ii above, that CTD reduces transport of some pathogens from tile-drained fields to streams.

(iv) Regarding loads of microbial source tracking markers, pathogens, and viral indicators, there were only significantly higher loads of F-RNA and F-DNA coliphages from the CTD watershed during the CTD intervention period ($P < 0.05$). Coliphage loading was higher in the CTD watershed associated with a downstream monitoring site, possibly as a result of fecal material derived from a small hobby farm located upstream of the site. Yet it is also worthy of note that there was lower loading of the ruminant marker in the CTD watershed, but results were only marginally insignificant at the $P = 0.06$ level (in relation to the significance threshold of 0.05).

(v) Data mining by means of classification and regression tree (CART) (Salford Systems, San Diego, CA) analyses delineated a vast array of seasonal and drainage control interactions among microbial load targets of importance. For example, it was found for the ruminant marker that highest loads existed during spring in the UCTD watershed, which is consistent with reduced manure inputs into the stream system via tile drainage control in the CTD watershed. F-RNA coliphage loads associated with animals were highest in summer in the CTD watershed which may have been primarily of wildlife origin and the hobby farm as noted above. Irrespective, during this period, stream flushing was likely constrained and dilution inhibited in the CTD watershed.

(vi) While overall, this study suggests that CTD imposed at the watershed scale has a beneficial impact on occurrences of some pathogens and markers of ruminant origin, it should be noted that in this region, free tile drainage (to reduce soil water contents) is usually used around harvest time and immediately prior to planting to allow for field traffic activities such as tillage, planting, and manure/fertilizer application to land. Unfortunately, during these earlier spring and fall periods, manure is applied to fields, and thus abatement of fecal pollution potential by CTD would be reduced during these periods, and the CTD watershed would be expected to behave more in line of a freely draining watershed (UCTD watershed). Controlled tile drainage could be employed as a temporary manure management practice as described by Frey et al. (9). However, to reduce manure borne fecal contamination to streams in more temperate regions of the world where tile drainage is utilized, CTD is often imposed more readily during winter or "fallow" periods of the year, and therefore, we would expect greater potential for CTD to mitigate field-based fecal pollution of streams on a yearly basis. Nevertheless, as documented in this study, there are significant environmental benefits that can be garnered via the use of CTD during just the growing season.

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