**Supplementary Information 1.**

**Microbial Source Tracking Pilot Study Methods**

Samples from the ISCO 6712 portable sampler were filtered through 0.2µm cellulose nitrate membrane filters to concentrate bacterial cells, which then underwent chemical extraction of total nucleic acids. The following nucleic acid extraction method was adapted from Carrigg *et al*. (2007): the filter was cut into six segments; each segment was added to a 1.5 ml micro-centrifuge tube. To each tube, 500 μl of CTAB buffer, 500 μl of lysis buffer (50 μM Tris–HCl [pH 8]; 40 μM ethylene diamine tetraacetic acid [EDTA; pH 8]; 750 μM filter sterilised sucrose) and 20 μl of lysozyme (10 mg ml−1; Sigma-Aldrich, Germany) were added; mixtures were briefly vortexed (30 seconds) and incubated at 37°C for 15 min and then vortexed again before a further incubation at 37°C for 15 min. Sodium dodecyl sulphate was added to a final concentration of 2% (w/v); the samples were again vortexed and then incubated at 70°C for 1 h. Following this, 6 μl of proteinase K (Sigma-Aldrich) were added. Samples were then vortexed and incubated at 50°C for a further 30 min, followed by centrifugation for 15 min (10,000×g). The supernatants were transferred to fresh 2 ml micro-centrifuge tubes and the aqueous phase was extracted by mixing with an equal volume of chloroform–isoamyl alcohol (24:1), followed by centrifugation (10,000×g) for 15 min. Nucleic acids were then precipitated from the extracted aqueous layer with addition of 0.6 vol of isopropanol in fresh 1.5 ml micro-centrifuge tubes, incubated overnight at room temperature, followed by centrifugation (10,000×g) for 60 min and then a further centrifugation step (12,000×g) for 15 min. The pelleted nucleic acids were washed with 70% (v/v) ice-cold ethanol by centrifugation (12,000×g) for 15 min and air dried before re-suspension in 30 μl DEPC-treated water. Extracts from the six filter segments for each sample were recombined after re-suspension.

DNA was extracted from various animal faecal sources from within the catchment to build a DNA archive of potential faecal pollution sources and also to evaluate qPCR assay specificity. Animal faecal samples from the catchment included cow (n = 7), sheep (n = 10), horse (n = 4), pig (n = 6) and goat (n = 4). Extracted DNA from human faecal samples (n = 10) was donated by University College Hospital Galway. The animal faecal samples (25 mg) underwent nucleic acid extraction using the Powersoil DNATM Isolation Kit (MoBio, Carlsbad, CA) with some modifications to the method: after solution C1was added a 10 min incubation at 70oC was introduced, and there were two washes with solution C5. Each faecal extract was mixed into species-specific genomic pools.

For all TaqMan assays, 10 µl of nucleic acid extract were assayed in a final reaction volume of 25 µl. Each 25 µl reaction contained a final concentration of 1x TaqMan® Environmental Master Mix 2.0 (Applied Biosystems) with 400 nM each of forward and reverse primer, and 80 nM probe. The samples were placed in 96-well plates and amplified in an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems). Standard amplification conditions were used: 2 min at 50oC and 10 min at 95oC, followed by 40 cycles of 15 s at 95oC and 1 min at 60oC. Cycle threshold (Ct) values were evaluated with a threshold that was specific to each assay (Kildare *et al.*, 2007); 0.018 for universal Bacteroidales marker (BacUni- UCD) detection, 0.026 for human-specific Bacteroidales marker (BacHum-UCD) detection, and 0.022 for ruminant specific Bacteroidales marker (BacCow-UCD) detection. Tables S2 and S3 summarise the assays used in this study.

The qPCR data for the MST *Bacteroidales* assays were converted into loads by multiplying the data, which is given as gene copy (gc)/ml, by mean hourly discharge (m3/h) and correcting to gc/m3 and then gc/h.