Detecting human faecal pollution in mixed use rural-residential catchments using a Markov Chain Monte Carlo source apportionment model

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Highlights

- A source apportionment model (SourceTracker) was used to determine the relative contribution of different sources faecal pollution in rural residential catchments.
- Human faecal pollution was detected in both rural residential and forested catchments, though at lower levels than other faecal sources such as waterbirds.
- Human faecal contribution was greatest under low flow conditions indicating possible groundwater pathways from septic systems to streams.

Introduction

Infectious pathogens associated with human faecal pollution pose a threat to human health when they enter waterways. Consequently, identifying sources and transport pathways of human faecal pollution in waterways is an important task for waterway managers. Distinguishing human faeces from other faecal sources in mixed-use rural residential catchments is complicated by the presence of other faecal sources including domestic, wild and agricultural animals. The Markov Chain Monte Carlo source apportionment model SourceTracker (Knights et al. 2011) can be used to identify the proportional contribution of different sources of faecal pollution by determining the proportional contribution of different faeces to the bacterial metagenome in aquatic environments (Henry et al. 2016, McCarthy et al. 2017). This study aimed to test the capacity of SourceTracker to detect human faecal pollution in small rural-residential catchments hosting multiple sources of faecal pollution, and better understand the conditions under which human faecal pollution is exported.

Methodology

Samples and processing

Water samples were collected from nine small (11 – 280 ha) rural-residential catchments, and one forested catchment in South East Australia. The rural residential catchments host dwellings with on-site septic (wastewater treatment) systems, as well as agricultural land-use, while the forested catchment contains no known sources of human faecal pollution. Samples of potential faecal sources were collected from within the catchments ('source samples'), complimented by samples previously collected by the Environment and Public Health Microbial Laboratory at Monash University. Human faecal pollution was represented by samples from wastewater treatment plants. The faeces of cattle, waterbirds, domestic cats and dogs, and several species of wild mammal were also included as sources in the model. Genomic DNA from faecal and water samples was extracted, and 16S rRNA amplicon sequencing undertaken to provide the SourceTracker model inputs.

Analysis

SourceTracker was used to determine the proportion of the microbial community present in stream water ('sink') samples that originated from each faecal 'source'. First, SourceTracker's capacity to differentiate between different sources of faecal pollution was tested by randomly splitting the faecal samples from each category (e.g. wastewater samples), treating half as sources of faecal pollution, and half as sinks, following the method of Staley (2018). This provides the proportion of each type of faecal pollution correctly apportioned, and how much is mis-apportioned to other faecal types. SourceTracker was then run to determine both the proportional contribution of all faeces sources to the total microbial community, and of human faeces to the faecal microbial community. Wilcoxon rank sum tests were used to test for significant differences in total and human faecal contribution between the two catchment types.

Results and discussion

Testing of SourceTracker showed that human faeces could be readily distinguished from other faecal sources, with high proportions (average 89 ± 6%) of human faecal samples being correctly identified, and low proportions (0.0 – 0.9%, average of 0.2%) of other faecal sources being incorrectly identified as wastewater.

Human faecal pollution was predicted to contribute to the microbial community in both rural residential and forested catchments (Figure 1). However other faecal sources contributed proportionally more, including wild waterbirds with an average contribution of 41% and 57% to the faecal community of rural residential and forested catchments, respectively. The contribution of faecal sources to the total microbial community was significantly higher (Wilcoxon W = 452, p = 9.5e-5) in rural-residential catchments (median = 0.59%) than in the nearby forested catchment (median = 0.21%). Rural residential catchments displayed a similar median contribution of human faeces to the total faecal community (19%) as forested catchments (18%) respectively, though some samples from rural residential catchments displayed comparatively high contributions from human faeces (Figure 1). The identification of human faecal pollution in the forested watershed may indicate a previously unknown source, such as informal toileting by recreational forest users. It may also indicate an uncharacterised environmental microbial source similar to human faeces.
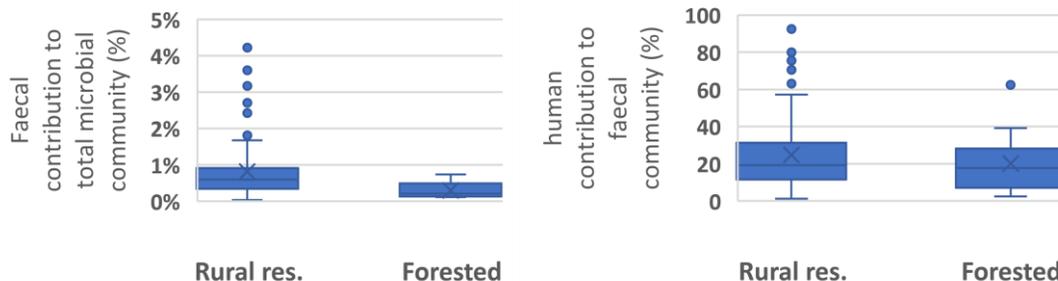

Figure 1. Left: proportional contribution of all faecal sources to the total microbial community in the two catchment types (rural residential (Rural res.) and Forested). Right: Proportional contribution of human faecal pollution to the total faecal community in the two catchment types.

High frequency monitoring following a wet weather event indicated that the proportional contribution of human wastewater to the total microbial community (Figure 2) increased as the stream returned to baseflow conditions following a rainfall event. This suggests increased transport of other sources of

faecal pollution during wet weather events, diluting human faecal pollution transported by discharging groundwater.
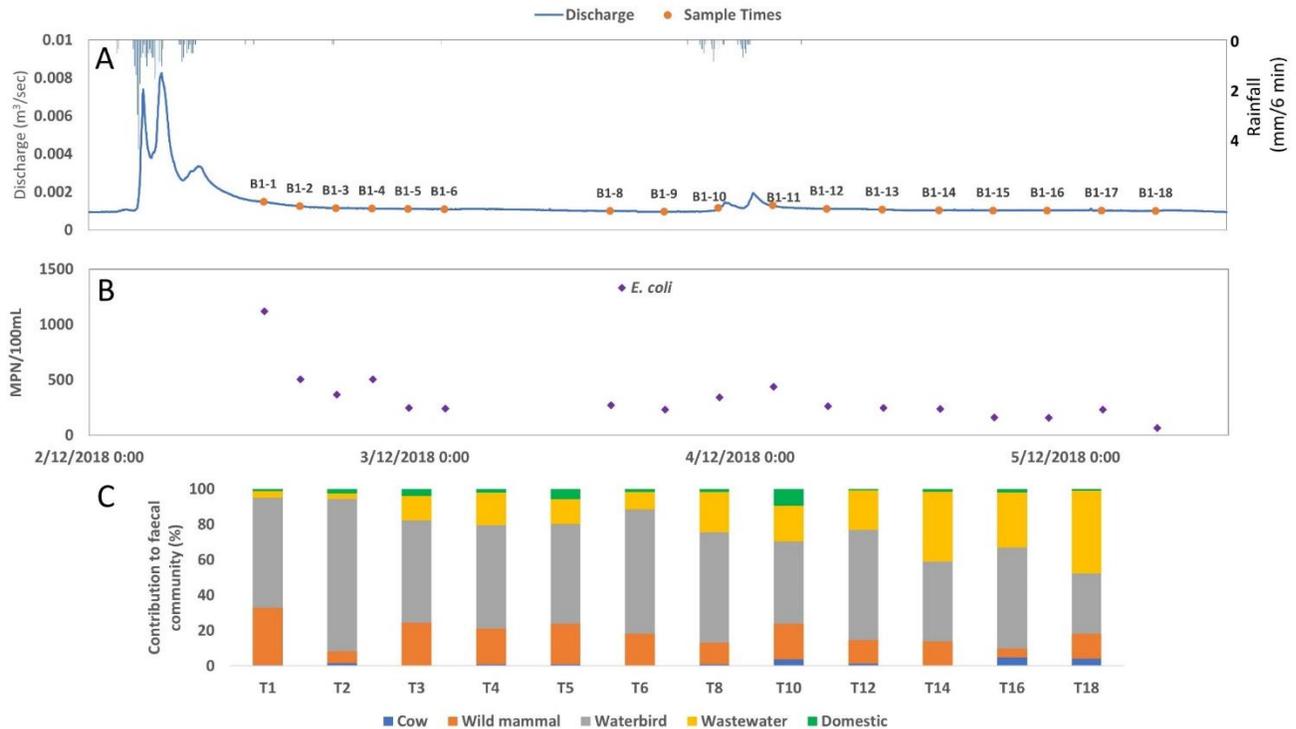

Figure 2 - Results of high-frequency sampling from a small rural residential catchment during December 2018. Panel A displays discharge, and the position of samples collected, panel B displays corresponding *E. coli* counts, and panel C displays the proportional contribution from different sources to the total faecal community across the sampling period.

Conclusions and future work

The Markov chain Monte Carlo apportionment model SourceTracker has substantial potential to assist in identifying the presence, and understanding the behaviour of human faecal pollution in complex mixed-use catchments. In this study, SourceTracker was used to detect human faecal pollution in rural-residential catchments and a nearby forested catchment, and identify groundwater discharge as a potentially important transport pathway. Higher concentrations of human faecal pollution detected under low flow conditions require further investigation, as this may indicate discharging groundwater carrying effluent from failing septic systems.

References

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