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Using microbiological tracers to assess the impact of winter land use restrictions on the quality of stream headwaters in a small catchment

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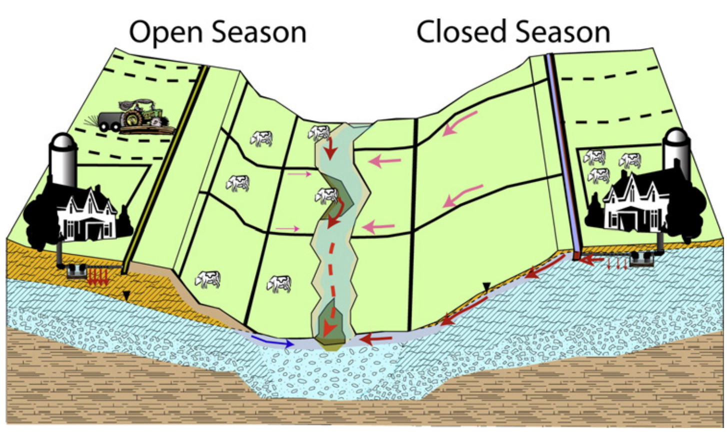
HIGHLIGHTS

- Investigation of impact of winter land use restrictions on surface water quality
- Relative concentration of common FIOs indicates consistently fresh pathogen sources.
- Microbial source tracking demonstrates different fecal material sources during year.
- Summer surface water contamination associated with direct livestock access
- Contamination from sewage delivered during periods of higher hydraulic connectivity

GRAPHICAL ABSTRACT



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ABSTRACT

Diverse land use activities can elevate risk of microbiological contamination entering stream headwaters. Spatially distributed water quality monitoring carried out across a 17 km^2 agricultural catchment aimed to characterize microbiological contamination reaching surface water and investigate whether winter agricultural land use restrictions proved effective in addressing water quality degradation. Combined flow and concentration data revealed no significant difference in fecal indicator organism (FIO) fluxes in base flow samples collected during the open and prohibited periods for spreading organic fertilizer, while relative concentrations of *Escherichia coli*, fecal streptococci and sulfite reducing bacteria indicated consistently fresh fecal pollution reached aquatic receptors during both periods. Microbial source tracking, employing *Bacteroides* 16S rRNA gene markers, demonstrated a dominance of bovine fecal waste in river water samples upstream of a wastewater treatment plant discharge during open periods. This contrasted with responses during prohibited periods where human-derived signatures dominated. Differences in microbiological signature, when viewed with hydrological data, suggested that increasing groundwater levels restricted vertical

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infiltration of effluent from on-site wastewater treatment systems and diverted it to drains and surface water. Study results reflect seasonality of contaminant inputs, while suggesting winter land use restrictions can be effective in limiting impacts of agricultural wastes to base flow water quality.

1. Introduction

That hydrological processes in stream headwaters influence the water quality and discharge regimes further downstream has been recognized for some time (Alexander et al., 2007). Although initial studies focused on processes operating in upland catchments where pollution pressures are lower, a large number of rivers and streams across Ireland have headwaters in more intensively cultivated lowland areas with greater water pollution pressures. These pressures have the potential to impact water quality, which in turn can pose a threat to aquatic ecological health and/or drinking water supplies (Archbold et al., 2010).

Pathogenic microorganisms (pathogens), such as pathogenic bacteria, viruses and parasitic protozoans, display a particularly high capacity to impact drinking water. Their widespread occurrence and high source concentrations, coupled with their ability to cause illness at low doses, means small quantities of pathogen-bearing wastes can render large volumes of water unfit for human consumption (Mara and Horan, 2003). As a consequence the raw quality of surface waters in many Irish catchments displays potential to affect human health, either through consumption of water and/or contaminated foodstuffs, e.g. shellfish in receiving coastal waters (Whyte et al., 2004).

Surface water acts as the principal source of drinking water across much of the European Union (EU), as well as for over 80% of the population of Ireland (Environmental Protection Agency, 2015). Although most potable water in Ireland is provided through large public water supplies, a significant proportion of the population is served by smaller public networks, or private supplies, where water may receive more limited treatment prior to provision to end users, thus elevating risk of exposure. Routine water quality monitoring provides a basis for assessing contamination risks. However, analyzing for pathogenic microorganisms can prove expensive, time consuming and inconclusive (Pepper et al., 2011). As a consequence, microbiological water quality monitoring focuses on more robust fecal indicator organisms (FIOs), which are easier to analyze (Mara and Horan, 2003). More recently, microbial source tracking (MST) has further assisted in determining the origin of FIOs detected in water supplies (Seurinck et al., 2005). In Ireland a library-independent method, developed by Kildare et al. (2007), has permitted its application to typical environmental settings and has designed host-specific qPCR assays based on the order *Bacteroidales*. Since this bacterial group are more prevalent in the gastrointestinal tract than the FIOs, and make up approximately one-third of the human fecal microflora (Noble et al., 2006), qPCR assays targeting variable regions of the *Bacteroidales* 16S rRNA gene allow for the discrimination between human and ruminant sources of fecal pollution.

Despite their widespread occurrence and potential to impact health, natural attenuation processes such as inactivation (loss of infective capacity), reduce intestinal bacteria concentrations when they exit their hosts (Mara and Horan, 2003). Attenuation rates vary depending on microorganism type and ambient environmental conditions (Pepper et al., 2011). Although these processes serve to reduce risks to human health, recent Irish Environmental Protection Agency (Environmental Protection Agency, 2015) drinking water reports have noted that microbiological contaminants, as reflected by the presence/absence of *Escherichia coli* (*E. coli*), remain the principal threat to water quality across Ireland (Environmental Protection Agency, 2015).

The EU Water Framework Directive (WFD) requires member states to protect water resources, and includes for the provision of microbiologically safe drinking water (Directive 2000/60/EC) (European Commission, 2000). The WFD's requirement to achieve goals in an integrated manner at the catchment scale entails developing an understanding of the nature and distribution contaminant sources and the risks they pose to receptors. The risks posed to catchment water quality by agricultural activities have been recognized by the EU and transposed into law through the Nitrates Directive (Directive 91/676/EEC) (European Commission, 1991) (subsequently subsumed into the WFD). To address the risk of agricultural pollution of water supplies the WFD requires implementation of land use measures to reduce the risk of contaminant delivery. In the Republic of Ireland this has been addressed through the application of winter farming measures (Government of Ireland, 2014). These measures aim to restrict nutrient application (including organic fertilizer) to farmland during wetter, colder periods of the year when there is little plant growth, and when hydrological processes are more likely to deliver diffuse agricultural pollution to water courses. More specifically the Irish Department of Agriculture, Food and the Marine (2014) has implemented prohibited spreading periods (, or a closed season,) when no application of fertilizer is permitted. These periods vary by region across Ireland, but correspond to times when livestock are housed indoors and where organic wastes must be stored (and accumulate) in concentrated areas, most notably farmyards. Poor storage practices can result in farmyards acting as point sources for agricultural contaminants, particularly during prolonged wet periods (Edwards et al., 2008). Conversely, appropriate storage practices can prevent wastes from reaching water courses, thus reducing pollutant loads that may potentially impact drinking water supplies.

In Irish rural settings, characterizing the impacts of agricultural practices on water quality can be complicated by the widespread occurrence of additional pollutant sources, including on-site wastewater treatment systems (OSWWTS), mainly septic tanks (Arnscheidt et al., 2007). Although these systems have been demonstrated as effective technologies for wastewater treatment in free draining Irish subsoils ((Keegan et al., 2014), their efficiency in lower permeability deposits where high water tables limit treatment capacity, including the removal of FIOs, have proved more questionable (Donohue et al., 2015). Characterizing attenuation processes associated with OSWWTSs has proved particularly challenging in geologically heterogeneous catchments, where highly variable subsoil cover complicates characterization of the capacity of the ground to accept percolating effluent for part or all of the year. Where ranges of groundwater fluctuation prove substantial, this may result in intermittent direct discharge to the surface, potentially resulting in significantly reduced levels of effluent treatment before reaching aquatic receptors (Donohue et al., 2015).

Assessing the impact of land use control measures and distinguishing the effects of agriculturally-derived microbiological contaminants from other sources at the catchment scale proves challenging, particularly in those catchments underlain by aquifers classed as poorly productive (based on their limited aquifer transmissivity and storativity, along with the low well yields obtained from them), which underlie over 65% of Ireland (Moe et al., 2010). High spatial and temporal variations in transmissivity (due to significant water table fluctuations in low storage media) can potentially influence those hydrological pathways delivering pollutants to aquatic receptors across a catchment at different times of the year. Focused water quality monitoring assists considerably in characterizing these impacts at the catchment (drainage basin) scale (Archbold et al., 2010). However, failure to characterize hydrological conditions during sampling can result in ambiguity or incorrect interpretation. Conversely, significant synergies can be achieved by combining water quality data with hydrological