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Research article

Seasonal persistence of faecal indicator organisms in soil following dairy slurry application to land by surface broadcasting and shallow injection

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ABSTRACT

Dairy farming generates large volumes of liquid manure (slurry), which is ultimately recycled to agricultural land as a valuable source of plant nutrients. Different methods of slurry application to land exist; some spread the slurry to the sward surface whereas others deliver the slurry under the sward and into the soil, thus helping to reduce greenhouse gas (GHG) emissions from agriculture. The aim of this study was to investigate the impact of two slurry application methods (surface broadcast versus shallow injection) on the survival of faecal indicator organisms (FIOs) delivered via dairy slurry to replicated grassland plots across contrasting seasons. A significant increase in FIO persistence (measured by the half-life of *E. coli* and intestinal enterococci) was observed when slurry was applied to grassland via shallow injection, and FIO decay rates were significantly higher for FIOs applied to grassland in spring relative to summer and autumn. Significant differences in the behaviour of *E. coli* and intestinal enterococci over time were also observed, with *E. coli* half-lives influenced more strongly by season of application relative to the intestinal enterococci population. While shallow injection of slurry can reduce agricultural GHG emissions to air it can also prolong the persistence of FIOs in soil, potentially increasing the risk of their subsequent transfer to water. Awareness of (and evidence for) the potential for 'pollution-swapping' is critical in order to guard against unintended environmental impacts of agricultural management decisions.

1. Introduction

Water used for recreation, drinking or food production (including shell fisheries) is routinely screened for faecal indicator organisms (FIOs) by regulators to track compliance with health related standards and associated legislation (Pachepsky et al., 2016; Clements et al., 2015). The detection of FIOs in environmental matrices is indicative of faecal contamination and their presence in high numbers can suggest a risk to human health in addition to posing wider economic and environmental threats (Oliver et al., 2016a,b; Quilliam et al., 2015). The Water Framework Directive (WFD), a significant piece of EU water legislation, was designed to protect and improve the quality of water bodies throughout Europe. Microbial pollution of water is integral to the WFD, with bathing and shellfish harvesting waters designated as 'Protected Areas' within Article 6 of the WFD. In the USA, total maximum daily loads (TMDLs) are calculated under the Clean Water Act, and FIOs are the leading cause of TMDL exceedance, and thus impairment, of river and stream water quality (USEPA, 2015). In recent years significant international effort has focused on minimising diffuse pollution from agriculture in recognition of the complexity of

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challenges in mitigating its impact on the water environment (Collins et al., 2016; Brown and Froemke, 2012; McGongle et al., 2012).

In 2013 there were 9.84 million cattle and calves in the UK of which 1.78 million were dairy cattle (Defra, 2014). The majority of these cattle are concentrated in the west of the UK, where grassland agriculture dominates. Approximately 65% of the dairy cattle housing systems are slurry-based systems (Anon, 2006), where the slurry is a mixture of faeces, urine and water. These systems produce *ca*. 50 million tonnes of slurry annually in the UK (Williams et al., 2000). The livestock industry in the UK is intensifying with slurry-based systems being favoured over systems producing solid manures and subsequently the larger animal enterprises are producing greater volumes of slurry. Slurry remains liquid during storage and therefore does not compost, potentially allowing prolonged survival of FIOs and pathogens and opportunities for frequent re-inoculation from recurring inputs to slurry stores (Blaiotta et al., 2016; Hutchison et al., 2005). Animal slurries contain significant concentrations of nutrients, are a desirable farm resource, and are routinely applied to agricultural lands as a crop fertiliser and soil conditioner. However, manure applications can pose a risk for the transfer of pathogenic microorganisms to watercourses via overland flow from fields or from diffuse inputs via artificial drainage or other subsurface hydrological pathways (Cho et al., 2016). The FIO concentrations contained within animal manures are highly variable depending on shedding rates, manure type (liquid or solid manure) and storage conditions. Their rate of decline after manure application to land has been shown to be dependent on environmental factors, such as UV exposure, temperature, soil type and desiccation (Park et al., 2016; Stocker et al., 2015). Agricultural land that receives manure and demonstrates hydrological connectivity to surface waters has the potential to contribute to diffuse microbial pollution of water (Dymond et al., 2016). However, the risk of microbial loss from land to water will vary across contrasting seasons and according to different methods of manure application, and this warrants further investigation.

One hypothesis is that FIOs delivered to injection slots in soil will survive for longer than FIOs in slurry that has been surface broadcast, with potential for prolonging the risk of FIO contamination of the wider environment. This is because of increased cell protection from UV, desiccation and extremes in temperature afforded by the soil habitat. However, an effective method for reducing emissions of ammonia (NH_3) from the land application of organic manures is to inject slurries into the soil (Häni et al., 2016; Misselbrook et al., 2002). Thus, in many European countries it is the norm to inject slurries below the sward and into the soil, though in the UK slurry application to land via broadcast application, often using a splash plate applicator, remains standard practice. Consequently, there is a need to determine if reducing NH_3 emissions through shallow injection of slurry will simultaneously increase the potential survival of FIOs, and hence the subsequent risk of increased subsurface transfers of FIOs to water, i.e. the potential for so-called ‘pollution swapping’. The management and mitigation of such risk is becoming a priority for environmental guardians who seek practical tools to facilitate effective microbial risk assessments of agricultural systems (Muirhead, 2015; Oliver et al., 2010, 2009). The ability of emerging risk-based decision support tools to estimate FIO survival (or rather the accumulation of an FIO burden) in the landscape from a range of on-farm activities, for example the applications of animal slurries to crops and pasture, is important for helping to understand the contribution of diffuse agricultural sources to the impairment of microbial water quality.

Slurry applications to land are influenced by seasons, generally guided by crop requirement, but also by the farmers' need to relieve stress in the capacity of slurry stores prior to housing livestock over the winter. Relatively large volumes of slurries are often applied to agricultural land through the spring, summer and autumn (Smith et al., 2001) and in the UK the Nitrate Pollution Prevention Regulations 2008 (Anon, 2008) have resulted in ~68% of agricultural land in England being designated as Nitrate Vulnerable Zones (NVZs). NVZs stipulate closed periods during which organic manures with high available nitrogen contents, such as livestock slurries, cannot be applied to land. For grasslands, predominantly livestock farms in the ‘wetter west’ of England, these closed periods extend from 1 September to 31 December on sandy and shallow soils and from 15 October to 15 January on all other soils (Defra, 2013). While much research has focused on nitrate and ammonia emissions from slurry applications to grasslands in NVZs, less attention has been given to microbial pollutants such as FIOs and potential pathogens.

The overall aim of this study was to evaluate the persistence profiles of two key FIOs, *E. coli* and intestinal enterococci (IE), following their delivery to grassland soil through contrasting slurry application methods. The specific objectives were to: (i) determine decay rates and half-lives of *E. coli* and IE at the plot scale following shallow injection and surface broadcast (splash-plate) application of slurry to grassland; and (ii) evaluate whether the resulting FIO die-off patterns in soil were influenced by contrasting season of slurry application in the UK.

2. Materials and methods

2.1. Site description

Experiments were conducted at the North Wyke Research farm, Devon UK (50°45′N, 3°50′W), on an experimental grassland field. The average annual air temperature of the site is 9.6 °C, and the annual precipitation is 1055 mm (30-year mean, climate record of North Wyke, 1982–2012). The soil type was a poorly drained silty clay loam (Halstow Series; Findlay et al., 1984). Slurry was applied to replicated grassland plots, approximately 2 m × 2 m, using two simulated spreading techniques; surface broadcast (splash-plate) and shallow injection. Broadcast spreading was simulated using an adapted watering can with a spoon attachment to provide a suitable splash-plate spread pattern. In order to simulate the shallow injection, 5–6 cm deep slots were cut into the ground, 20 cm apart, and a watering can was used to pour the appropriate quantity of slurry into the slots to match the same application rate of the surface broadcast slurry. Dairy slurry was obtained from a slurry lagoon on a nearby dairy farm. One day prior to the date of application, slurry was collected from the farm in a 1 m^3 intermediate bulk container (IBC) and transported to the field. On the morning of application (day 0) the slurry was thoroughly mixed prior to decanting into clean 10 l galvanised steel watering cans for spreading. The slurry was stored in the IBC in the field for the duration of each experiment to enable FIO die-off rates in stored slurry to be determined in addition to the soil-associated FIO die-off rates.

Slurry was applied at the equivalent rate of 45 m^3 ha^{-1} for all treatments. The plot-scale experiment comprised fifteen 4 m^2 randomised plots accommodating three treatments (five replicates of; (i) broadcast applied; (ii) shallow injection; and (iii) control plots, no amendments applied) which were investigated during three distinct periods of the year: spring (day 0 = May), summer (day 0 = July) and autumn (day 0 = October). The plots had no history of livestock grazing, manure application or fertiliser addition during the previous 20 years. A different set of fifteen randomised plots was used for each seasonal experiment and a 2 m buffer surrounded each plot to minimise cross contamination of the plots. Meteorological data was collected in the field using a Skye Minimet 4 meteorological station (Skye Instruments Ltd., UK).