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Influence of land use and nutrient flux on metabolic activity of *E. coli* O157 in river water

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Abstract

Infections caused by waterborne *Escherichia coli* O157 continue to pose a public health risk. An increase in faecal coliform loading of watercourses due to expanding populations, intensification of agriculture and climate change are predicted to amplify these risks. Understanding the effect of land use on the ecology of *E. coli* O157 in environmental waters is therefore important for implementing effective mitigation measures. In order to test the hypothesis that activity of waterborne *E. coli* O157 is affected by both land use type and the respective autochthonous microbial communities, we inoculated replicate microcosms of water collected from areas of contrasting land uses within a catchment with a chromosomally *lux*-marked *E. coli* O157. Pathogen metabolic activity, and its ability to reactivate following addition of nutrients, were quantified over time in both filter-sterilised and non-sterile microcosms. Metabolic activity differed significantly according to the land use type, the degree of competition from background microbes and the availability of nutrients. These results indicate that land use types associated with particular areas of a watercourse should be considered a central factor in models that aim to predict pathogen risk in environmental waters.

Keywords: animal waste; microbial pollution; Quantitative Microbial Risk Assessment (QMRA); sewage; verocytotoxigenic *E. coli* (VTEC); Water Framework Directive

1. Introduction

Enteric pathogens such as *Escherichia coli* O157 present a threat to human health and are a significant economic and emotional burden to society. Ruminant livestock are recognised as the major reservoir of *E. coli* O157 and the organism may be introduced into the water environment through direct deposition of their faeces or from overland flow (Jones 1999; Williams et al. 2008). Consequently, many cases of human *E. coli* O157 infection result from direct contact with the pathogen in contaminated water (Strachan et al. 2006). Contaminated environmental waters are also likely to perpetuate the cycling and re-infection of livestock (Avery et al. 2008).

Once in aquatic systems, *E. coli* O157 can persist for varying periods of time, depending on a number of biotic and abiotic factors, e.g. pH, available nutrients, competition and antagonism (Avery et al. 2008; Williams et al. 2007). However, although many of these variables are affected by land type and land use, little is known about how these factors affect pathogen survival and activity (Quilliam et al. 2011a; Pickup et al. 2003). Land use within a catchment can significantly contribute to microbial loading of waterways and can determine the presence of both point and non-point sources. For example, livestock farming is often associated with faecal contamination of water supplies via run-off from fields or drainage from areas where livestock are handled; in contrast upland forestry is rarely associated with faecal inputs to water systems (Kay et al. 2008). Furthermore, both land use and type have an impact upon water chemistry, which in turn will influence the composition of allochthonous microbial communities (Allan 2004). Therefore, land use can significantly influence both the ecology and survival of enteric microbes entering aquatic ecosystems.

Climate change is projected to lead to an increase in extreme rainfall events (Jones and Reid 2001). Following high precipitation and flooding events, excessive levels of both nutrients and microbial pathogens can be introduced into otherwise oligotrophic aquatic environments due to overland flow and sub-surface carriage through soil (Kistemann et al. 2002). Ultimately, this alters the dynamics of survival, predation and competition between introduced and autochthonous bacteria.

These impacts indicate the importance of considering land use and land type in the establishment of sampling site locations for water quality monitoring, development of land management programmes for improving water quality and for the generation of models for water quality predictions (He and He 2008; Kay et al. 2008; Quilliam et al. 2011b). Such models aim to predict faecal coliform (FC) numbers and therefore the public health risk in unmonitored water systems by correlation with environmental variables such as rainfall, temperature, slope gradient, and populations of grazing ruminants (Shanahan et al. 2001). Recently, a number of catchment studies have aimed to develop such models (Crowther et al. 2003, Kay et al. 2008, 2009; Oliver et al. 2009). Although land use has been included as a factor, there is limited applied research in this area, and models based on faecal indicator organisms (FIO) often make assumptions about pathogenic organisms that are too simplistic, e.g. predicting that grazing intensity or the percentage area of improved pasture within a catchment is correlated with the risk of waterborne pathogens (Hampson et al. 2010). However, recently Thorn et al. (2011) demonstrated that the activity of *E. coli* O157 was actually reduced in waters from areas of high grazing intensity relative to waters from areas of less intensive grazing.

In this study we aim to quantify the activity of *E. coli* O157 in water collected from areas of different land use types in a well-characterised catchment. In order to

test the hypothesis that both land use type and the respective autochthonous microbial communities affects the ecology of this pathogenic organism, we generated microcosms artificially inoculated with a strain of *E. coli* O157 carrying a stable chromosomal *lux* reporter (Ritchie et al. 2003).

2. Materials and methods

2.1. Study area and sample collection

The Conwy River system, situated in North Wales (UK), is 43 km long and has a catchment area of *ca*. 300 km^2 (Fig. 1). The catchment is typical of many in the UK, with land types ranging from relatively unproductive gley and peat soil moorland in upland areas, which predominantly support relatively low-intensity sheep farming and commercial forestry, to fertile lowland areas that support a variety of livestock farming. The river itself is used for recreational sports, while the estuary area is important for the commercial harvesting of shellfish and has several public beaches with designated EC bathing waters. During base flow and based on similar rivers, water is estimated to take 10–12 hours to flow from the source of the river in the upland, to the estuary (Ledger 1981). The catchment has a mean annual temperature of 10 °C.

Water samples were taken from sites representing six different land use types within the Conwy catchment, namely, where the river is tidal and saline (n = 5), agricultural (n = 12), forest (n = 9), upland grassland (n = 6), peat moorland (n = 5), and mountain (n = 6) (total n = 43; Fig. 1). Triplicate grab samples were collected at each site using sterile, 50 ml sterile polypropylene conical tubes (Falcon, Becton Dickinson Labware, USA). Just prior to collection, each sample tube was rinsed three

times in the water to be sampled. All samples were stored at 4 °C and processed within six hours of collection.

2.2. Characterisation of water samples

The Conwy catchment has been developed by the Centre for Ecology and Hydrology (UK) as a long-term focal point for landscape scale research from source to sea (CEH, 2011). As a result, water chemistry within the catchment has been subject to a continuing monitoring programme. Sub-samples from each location were subjected to the following analyses in triplicate: pH with a Fisherbrand Hydrus 300 meter (Fisherbrand, Leicestershire, UK); electrical conductivity with a Jenway 4010 EC meter (Jenway Ltd., Dunmow, UK); dissolved organic carbon using a TOC-V analyser (Shimadzu Corp., Kyoto, Japan); nitrate, ammonium and phosphate colorimetrically using a SEAL AQ2 analyser (SEAL Analytical Ltd., Hampshire, UK).

Indigenous waterborne heterotrophic bacteria were quantified using the SimPlate® system (IDEXX Laboratories Ltd., Buckinghamshire, UK) according to the manufacturer's instructions, and expressed as most probable number (MPN) ml^{-1}. Enrichment in modified Tryptone Soya broth and plating on CT-SMAC plates was used to test for the presence of background *E. coli* O157 in all environmental water samples.

2.3. Inoculation of microcosms and monitoring of activity

Paired microcosms for each land use type contained either 25 ml unfiltered environmental water or 25 ml of environmental water filtered through a 0.2 µm Millex® syringe-driven filter unit (Millipore, Co. Cork, Ireland). *Lux*-marked *E. coli*

O157 (Tn5 *lux*CDABE) cells were harvested from an overnight LB broth culture (18 h, 37 °C, 150 rev min^{-1}), washed and re-suspended in sterile water. Microcosms were inoculated with 25 µl of *E. coli* O157 cells to a final concentration of 10^5 CFU ml^{-1} and incubated at 10 °C with a 12 h photoperiod (irradiance 30 µmol m^{-2} s^{-1}). Bioluminescence (metabolic activity) was measured in plastic luminometer cuvettes with a SystemSURE 18172 luminometer (Hygiena Int., Watford, UK) and expressed as relative light units (RLU). Measurements were continued until bioluminescence was below the level of detection. At this stage, a nutrient solution containing glucose (10 mM) and glutamate (1 mM) was added to each microcosm, and bioluminescence measured for a further 72 h.

2.5. Statistical analyses

Measurements of bioluminescence and water physio-chemical characteristics were analysed by analysis of variance (ANOVA) and Tukey multiple comparison tests (Minitab 12.0 software, Minitab Inc., PA, USA).

3. Results

3.1. Characterisation of water samples

Electrical conductivity was significantly greater in tidal saline waters (Table 1), and pH ranged from mildly acidic (peat moorland), to slightly alkaline (tidal saline water). Differences in nutrient levels reflected differences in land use, with dissolved organic carbon significantly greater in waters draining from upland grassland and peat moorland than those found downstream, and a significantly higher concentration of phosphate and nitrate in water that was associated with agricultural land. Land use also had a significant effect on the numbers of heterotrophic bacteria with far greater

numbers in the tidal saline water. There were no background *E. coli* O157 cells in any of the water types.

3.2. Activity of *E. coli* O157

Generally, bioluminescence values increased in the first 24 h post-inoculation, then subsequently decreased in all but unfiltered forest and mountain waters in which bioluminescence values were significantly greater ($P < 0.001$) for approximately 4 d post-inoculation (Fig. 2A). In general, the rate of decline followed a relatively similar pattern in all microcosms from all land use types. Trends in activity were notably altered by filter sterilisation, with removal of the indigenous microbial community altering the initial level, and subsequent degree, of *E. coli* O157 activity in microcosms from each land use type (Fig. 2B). Whilst only negligible bioluminescence could be detected after 10 d in all unfiltered microcosms, bioluminescence was still detectable after 16 d in all filtered microcosms other than tidal saline (Fig. 2B). Indeed, activity was significantly lower ($P < 0.001$) in tidal saline water samples than all other waters in both filtered and unfiltered microcosms.

3.3. Reactivation of *E. coli* O157

Following the addition of the glucose and glutamate solution, the metabolic activity of the *E. coli* O157 cells was transiently stimulated (Fig. 3). The rate and degree of reactivity was clearly affected by land use type. This increase in bioluminescence associated with the addition of the nutrient solution was most evident in filtered water collected from agricultural areas, with bioluminescence being two orders of magnitude higher than at the beginning of the experiment ($P < 0.001$; Fig. 3B). Further, the reactivation response was more transient in the unfiltered

microcosms, with bioluminescence decreasing to background levels by 72 h post nutrient addition but was still detectable after 72 h in the majority of filtered waters. Reactivation was lowest in tidal saline waters in both unfiltered and filtered microcosms.

4. Discussion

Model forecasts for predicting the concentrations of FIO in surface waters are becoming an increasingly important management decision tool. However, predictive models tend to assume uniform patterns of waterborne pathogen survival, and although the effect of land use on water microbial quality is well known, the effect of associated land use on waterborne pathogen survival and activity is less clear. This study quantified the metabolic activity of *E. coli* O157 in a range of contrasting waters from areas of different land use in a large catchment, typical of many in the UK. The strain used accurately reflects survival patterns of toxigenic *E. coli* O157 strains (Ritchie et al. 2003); and although the behaviour of a single strain of *E. coli* O157 under laboratory conditions should not be extrapolated to predict the behaviour of all pathogenic strains under all environmental conditions, this work has demonstrated that metabolic activity can be significantly influenced by water associated with contrasting land use areas. The measurement of luminescence from a *lux*-reporter construct has previously been used as an estimation of pathogen infectivity (Hale and Bonventre 1979), and highlights the differential potential for *E. coli* O157 infections following exposure to river water.

Climate change is projected to result in an increase in the frequency of heavy rainfall events, which together with a higher proportion of soil becoming compacted due to extended dry periods, is likely to contribute to higher levels of run-off into

watercourses. This may culminate in greater inputs of faecal matter into water systems through increased diffuse pollution from agricultural lands and more frequent sewage discharges from water treatment plants, together with a higher base flow in rivers and higher rates of re-suspension and mixing of sediments. As a consequence, this is likely to result in an influx of nutrient 'pulses' to otherwise oligotrophic environments. *E. coli* has been found to lower its metabolic rate in different environments in order to utilise scarce nutrient supplies (Klein and Casida 1967), which ultimately improves its survival. However, this study has shown that *E. coli* O157 persisting in such a state can quickly (although transiently) increase its activity in response to a pulse of nutrients in a range of environmental waters.

Bioluminescence was measured over a number of days; however, the time taken for water to flow from the top to the bottom of the catchment is relatively short (10–12 h during base flow); illustrating how land use quickly and notably alters water properties. The waters used in this study contrasted in terms of their physico-chemical and microbiological characteristics and reflect how land use affects water quality. Biotic and abiotic factors are important variables in determining the survival and activity of *E. coli* O157, e.g., osmotic stress may have been responsible for the decreased activity in the tidal saline microcosm, and the high numbers of heterotrophic bacteria in the agricultural water microcosms may result from the elevated levels of nutrients (Williams et al. 2007; Avery et al. 2008; Thorn et al. 2011).

Recently, Thorn et al. (2011) showed that whilst there is greater risk of faecal coliforms and pathogens being present in waters from areas of high livestock intensity, their survival may be reduced due to correspondingly high levels of other microbes. In the current study, detectable levels of bioluminescence were found in

microcosms for significantly longer periods of time when background microbes had been removed. Bioluminescence was also greatest in forest and mountain waters, which had the lowest concentration of autochthonous microbes. Furthermore, the degree of reactivation upon nutrient addition was much more pronounced in many of the sterilised microcosms. This indicates that whilst its activity can increase in response to nutrient addition, *E. coli* O157 is a poor competitor for nutrients in the aquatic environment.

This study provides further insight into the effects of land use on waterborne pathogen ecology. The findings highlight the importance of catchment-scale evaluations on the persistence and infectivity of waterborne *E. coli* O157 and that models that aim to predict the level of risk posed by environmental waters should not assume uniform decay patterns after pathogens have entered the water environment.

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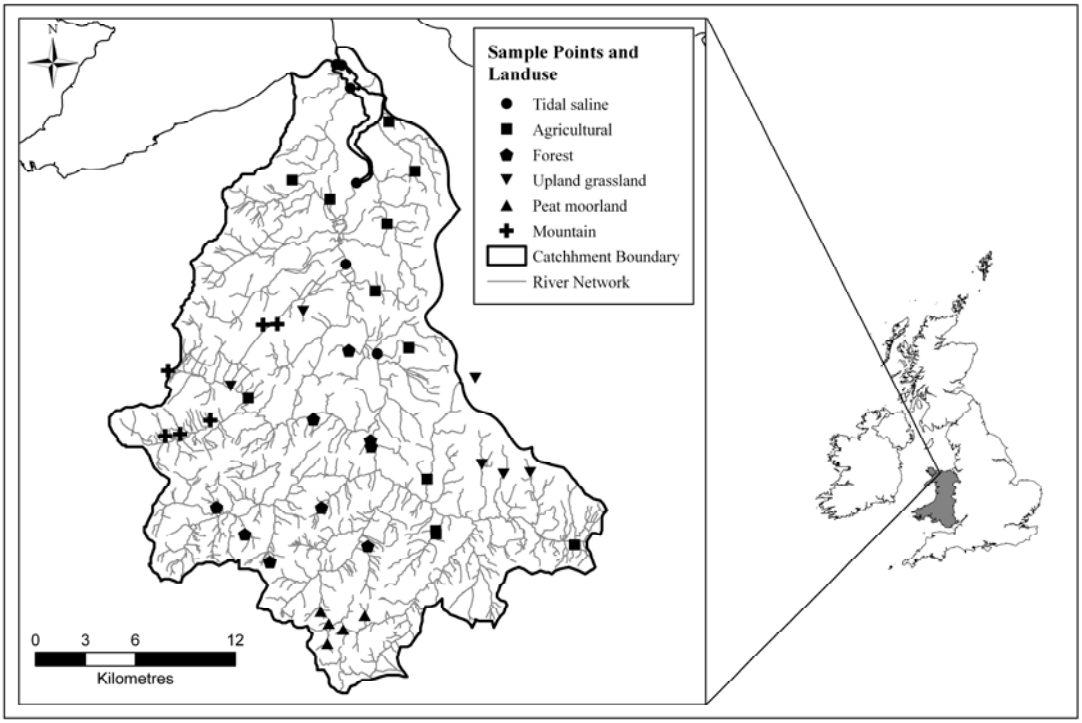

Figure 1: Location of the sampling points in the Conwy catchment, grouped according to the surrounding land class.

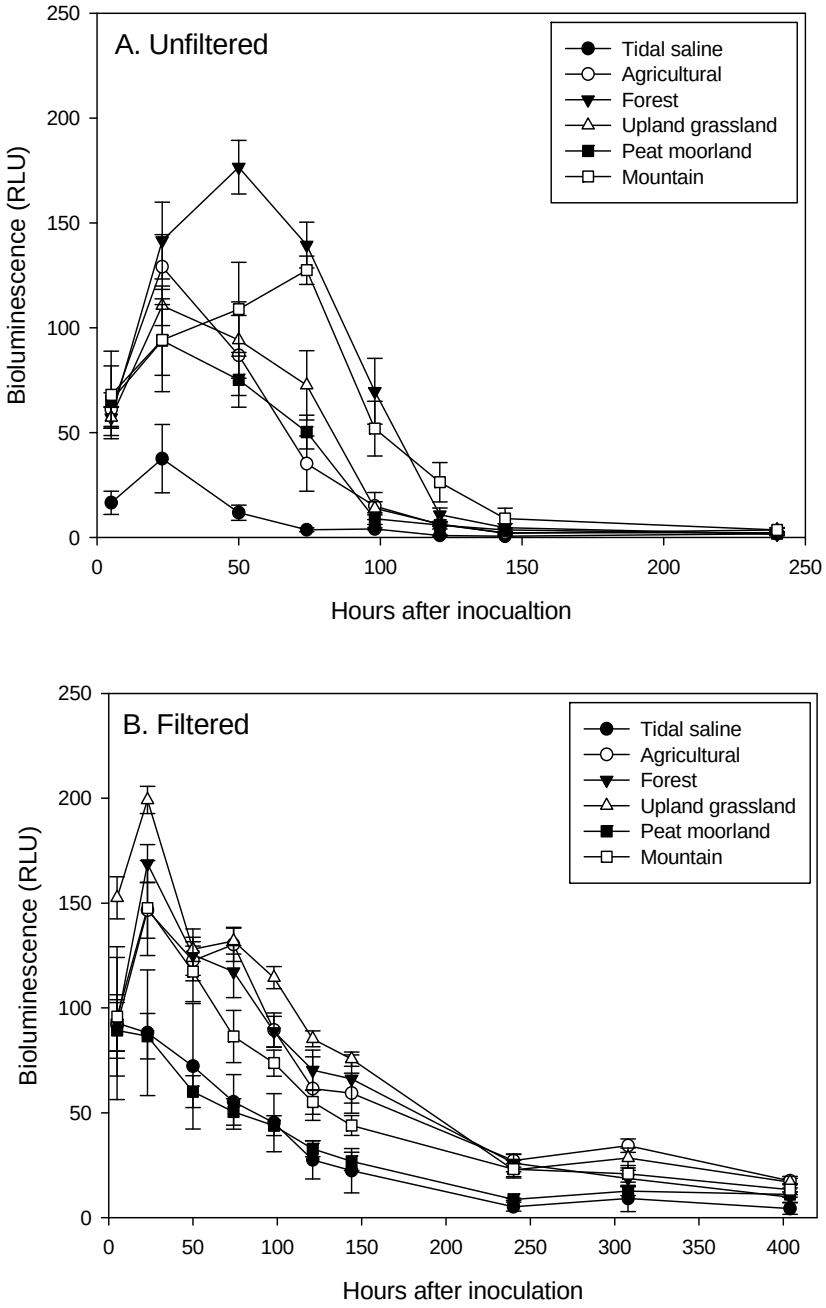

Figure 2: *E. coli* O157 activity in microcosms of unfiltered (A) and filtered (B) environmental water. Measurements were continued until bioluminescence was below the level of detection. Values represent the mean ± SEM ($n \geq 5$ for each water type). Note differences in time on the x-axes.

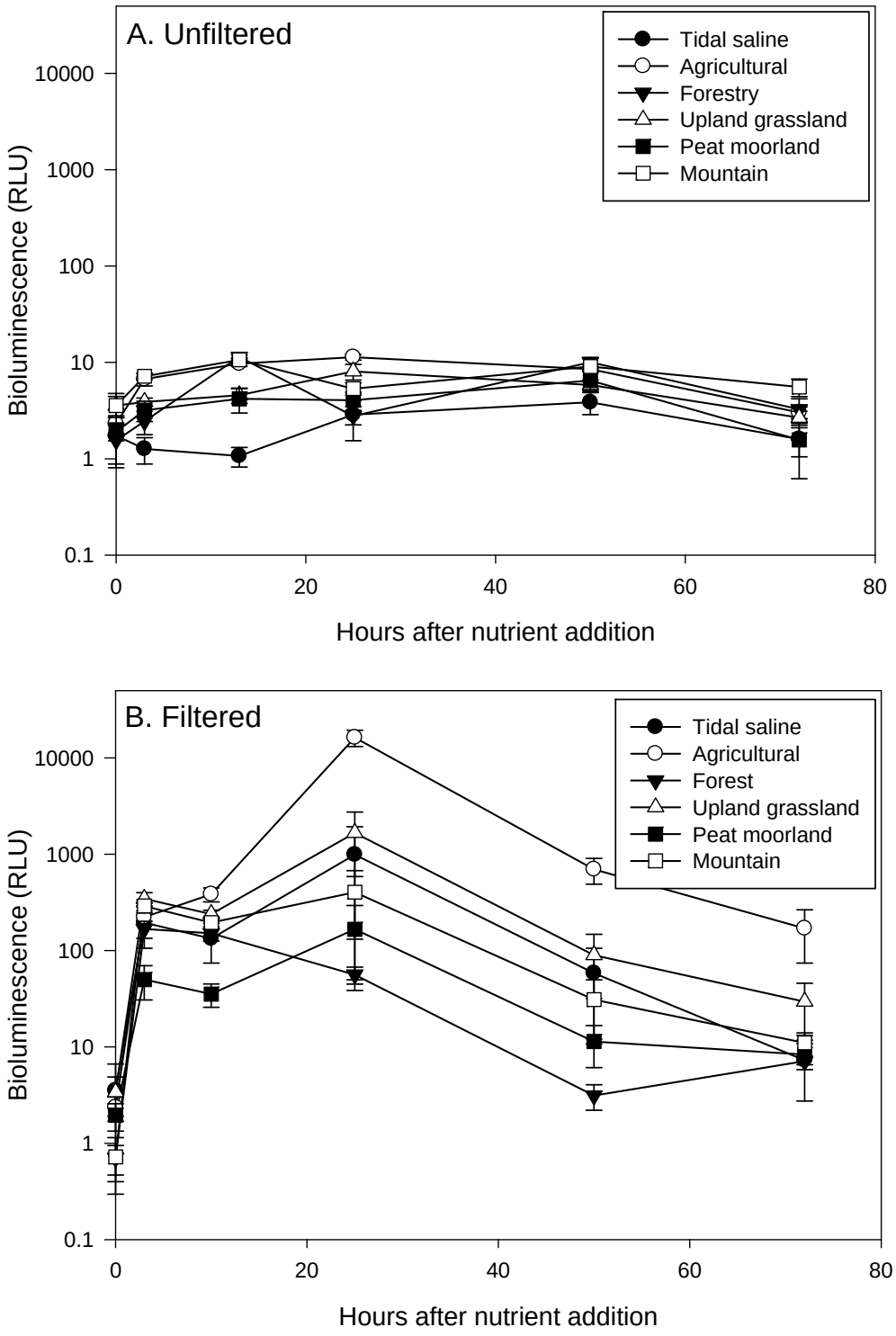

Figure 3: Reactivation of *E. coli* O157 activity in microcosms of unfiltered (A) and filtered (B) environmental water following the addition of a nutrient solution. Values represent the mean ± SEM ($n \geq 5$ for each water type).

Table 1: Intrinsic physico-chemical and microbiological characteristics of waters from different land use types. Values represent the mean ± SEM ($n \geq 5$ for each water type). EC = electrical conductivity; DOC = dissolved organic carbon. Different superscript letters denote significant differences between land use types at the $P < 0.05$ level.

Parameter	Tidal saline	Agricultural	Forest	Upland grassland	Peat moorland	Mountain
pH	7.29 ± 0.12^{d}	7.17 ± 0.08^{d}	5.97 ± 0.19^{abc}	6.04 ± 0.08^{ac}	5.40 ± 0.11^{ab}	6.08 ± 0.14^{ac}
EC ($\mu S\ cm^{-1}$)	21390 ± 6920^{c}	163 ± 19.7^{b}	61.2 ± 11.2^{a}	53.5 ± 7.2^{a}	40.9 ± 6.7^{a}	30.6 ± 2.8^{a}
DOC ($mg\ l^{-1}$)	4.4 ± 0.8^{a}	4.9 ± 0.6^{a}	4.9 ± 1.0^{a}	12.3 ± 2.1^{bc}	9.2 ± 1.0^{bc}	1.9 ± 0.3^{d}
NO_3^- ($mg\ l^{-1}$)	10.0 ± 5.4^{a}	119 ± 27.9^{b}	11.6 ± 3.2^{a}	3.0 ± 0.3^{a}	2.5 ± 0.6^{a}	10.6 ± 0.8^{a}
NH_4^+ ($mg\ l^{-1}$)	22.8 ± 7.95^{b}	2.28 ± 0.52^{a}	1.33 ± 0.21^{a}	1.25 ± 0.13^{a}	1.23 ± 0.08^{a}	1.23 ± 0.21^{a}
PO_4^{3-} ($\mu g\ l^{-1}$)	12.4 ± 1.71^{a}	11.0 ± 3.50^{a}	3.61 ± 0.94^{b}	5.25 ± 1.03^{a}	8.50 ± 2.31^{a}	2.50 ± 0.01^{b}
Heterotrophic bacteria (MPN ml^{-1})	1240 ± 102^{a}	864 ± 158^{b}	321 ± 65^{c}	730 ± 93^{abc}	753 ± 59^{abc}	333 ± 87^{c}