An investigation of the impacts of unrestricted cattle access to watercourses on freshwater physicochemical and microbial parameters

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List of Publications and Presentations

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O'Callaghan, P., Kelly-Quinn, M., Jennings, E., Antunes, P., O'Sullivan, M., Fenton, O., and Ó hUallacháin, D., 2018. The environmental impact of cattle access to watercourses: a review. J. Environ. Qual. 48(2), 340 - 351

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List of abbreviations

AEOS	Agri-Environment Options Scheme
AES	Agri-Environment Scheme
AIC	Akaike Information Criterion
Al	Aluminium
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BK	Brackan River catchment
BMP	Best Management Practice
BW	Munster Blackwater catchment
BWD	Bathing Water Directive
Ca	Calcium
$CaCO_3$	Calcium carbonate
CAP	Common Agricultural Policy
CAS	Cattle Access Site
CFU	Colony forming units
Cl	Chloride
CM	Commons River catchment
COSAINT	Cattle exclusion from watercourses: Environmental and Socio-economic Implications
CSA	Critical source area
CSO	Central Statistics Office
D	Defecation
DAFM	Department of Agriculture, Food and the Marine
DED	District Electoral Division
DF	Degrees of freedom
DG	Douglas River catchment
DO	Dissolved Oxygen
DoHPLG	Department of Housing, Planning and Local Government
DWD	Drinking Water Directive
DS	Downstream
E. coli	*Escherichia coli*
EC	European Commission
ECD	Estimated Cattle Density
ECDC	European Centre for Disease Prevention
edf	Estimated degrees of freedom
EEA	European Environment Agency
EEA	European Economic Area
EFSA	European Food Safety Authority
EG	Early grazing season
EPA	Environmental Protection Agency
EU	European Union
Fe	Iron
FIB	Faecal Indicator Bacteria

FST	Faecal Source Trackers
GAM	General Additive Models
GF/C	Glass Fibre Filters Grade C
GLAS	Green Low carbon Agri-environment Scheme
GLS	General Least Squares
GO	Gentle Owen
HCl	Hydrochloric acid
IMS	Industrial Methylated Spirits
INT	Interface
ISO	International Organisation for Standardisation
KH_2PO_4,	Potassium phosphate
KS	Kolmogorov-Smirnov
LG	Late grazing season
LOI	Loss on Ignition
LSU	Livestock Units
MARS	Managing Aquatic ecosystems and water Resources under multiple Stress
MG	Mid-grazing season
Mn	Manganese
MPN	Most Probable Number
MS	Member State
MT	Milltown Lake catchment
N	Nitrogen
N2	Dinitrogen
Na_3PO_4	Sodium phosphate
$NaNO_3$	Sodium nitrate
NAP	Nitrates Action Plan
ND	Nitrates Directive
NE	North-East
NH4	Ammonium nitrogen
NH_4Cl	Ammonium chloride
NO_3	Nitrate
NO_x-N	Oxidised Nitrogen
NSPPP	National Source Protection Pilot Project
NVZ	Nitrates Vulnerable Zone
OC	Organic Carbon
OPW	Office of Public Works
P	Phosphorus
PCR	Polymerase Chain Reaction
PG	Post-grazing season
RBMP	River Basin Management Plan
REPS	Rural Environmental Protection Scheme
RHI	Riparian Habitat Index
SAC	Special Area of Conservation
SD	Standard deviation
SE	Standard error

SRP	Soluble reactive phosphorus
TH	Tullycaghney
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TON	Total Oxidised Nitrogen
TP	Total Phosphorus
TRP	Total Reactive Phosphorus
TSS	Total suspended solids
TV	Tievnamara
U	Urination
UK	United Kingdom
US	Upstream
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VBNC	Viable but non-culturable
VTEC	Verocytotoxigenic *E. coli*
WFD	Water Framework Directive
WHO	World Health Organisation

Abstract

Where pastoral agriculture dominates, the practice of allowing livestock access to farmland watercourses as a cheap and low maintenance source of drinking water has been shown to have an adverse impact on water quality. In Ireland, agriculture, which is predominantly cattle-based, has been linked to the downward trend in water quality observed in recent decades, which conflicts with the goals of the Water Framework Directive. However, the research investigating the potential impacts of cattle access to watercourses on freshwater systems has predominantly been conducted in the USA and Australasia. In these regions, climate and farming practices typically contrast with those observed in Ireland and in Europe, thus making comparison difficult. This study aimed at contributing to an understanding of the impacts of cattle access to watercourses on freshwater systems in the Irish setting. Specifically, the current study investigated the impacts of cattle access to streams on freshwater abiotic parameters (i.e. nutrients, sediment) and on freshwater faecal contamination. It also addressed the efficacy of streamside fencing as a mitigation measure for such impacts.

The findings of this study show that faecal contamination in watercourses draining agricultural areas in Ireland is widespread. *Escherichia coli* (*E. coli*) concentrations in the streambed sediment compartment were in the order of 10^3 to 10^4 CFU g dry wt^{-1} at stream reaches with no cattle access, during grazing season. However, at stream reaches with unrestricted cattle access, *E. coli* sediment concentrations were significantly higher, with maximum average concentrations of 1.6 x 10^7 CFU g dry wt^{-1} in mid-grazing season. Sediment *E.* coli concentrations were found to decrease but persist in post-grazing season, with concentrations at upstream reaches of up to 10^3 CFU g and up to 10^5 CFU g dry wt^{-1} at cattle access sites. Furthermore, the study found a significant negative correlation between the Riparian Habitat Index scores of the sites, which reflect the intensity of cattle access impact (and whereby a lower score indicates higher site degradation) and *E. coli* sediment concentrations in mid-grazing season.

Nutrient (TP, TN and OC) reservoirs in sediments at cattle access sites were assessed. Results here indicate that cattle access to watercourses does not generally result in localised nutrient accumulation in streambed sediments. However, this study found a significant positive relationship between cattle density at the access sites and all three nutrient concentrations in the silt and clay fraction of the sediments. The results suggest that while cattle access may contribute to sediment nutrient reservoirs, rapid flushing downstream of nutrients at access sites may occur. Additionally, results suggest that sediment nutrient concentrations in agricultural streams are mainly driven by diffuse pollution at the catchment scale.

Potential changes in water quality parameters during cattle in-stream activity were assessed in a near-real time experiment which showed that cattle access led to significant increases in water loads of *E. coli* bacteria, TSS and NH_4-N. While increases were observed in TP loads, these were not significant in the context of the natural variation at the experimental site. NO_3-N and SRP did not show significant variation in response to cattle in-stream activity.

A short study on the effects of streamside fencing showed a positive effect in streambed sediment concentrations of *E. coli* and nutrients. However, this study also highlighted the need of adopting a site-specific, holistic approach that combines cattle exclusion measures with other measures to control diffuse pollution if cattle-based agriculture pressures are to be successfully managed.

The current research has thus demonstrated that cattle access to watercourses contributes to both faecal and nutrient contamination of freshwaters, and that while microbial pollution is mainly governed by field-scale management, nutrient pollution is driven by catchment-scale practices. Results presented here support the implementation of fencing to exclude cattle from watercourses; however, this needs to be considered as part of wider, integrated catchment management plans. The study contributes to the literature describing agricultural pressures in headwater streams and provides relevant information for stakeholders and policy makers.

1. Introduction

Chapter 1. Introduction

Freshwater accounts for only 0.01% of the water volume in the world and 0.8% of the land surface cover (Pham et al., 2019), but supports almost 6% of all species described to date (Dudgeon et al., 2006). Additionally, freshwaters provide a wide range of ecosystem services, such as provisional services (e.g. drinking water, irrigation water for agriculture, food), regulating services (e.g. water purification, flood buffering), supporting services (e.g. nutrient cycling) and recreational services (e.g. spiritual, leisure and aesthetic value) (Pham et al., 2019). However, largely due to their vital role in supporting human populations, freshwater systems are one of the most impacted systems in the world (Dudgeon et al., 2006), threatened by urbanisation and industry activities, agriculture intensification, water abstraction, flow diversion and damning, introduction of invasive species and diseases, and climate change (Malmqvist and Rundle, 2002).

Agriculture has been long recognised as a major pressure on freshwater systems globally (Vörösmarty et al., 2010). In the 1990s, agriculture was the main cause of pollution of freshwater resources in the USA (Cooper, 1993), and has been identified as the main source of contaminants to freshwater systems in New Zealand (Howard-Williams et al., 2010) and throughout Europe (Ulén et al., 2007). Much of this pollution, which includes excess nitrogen (N) and phosphorus (P) from fertiliser and slurry application, excess fine sediment loadings and faecal contaminants, reaches surface waters through diffuse pathways of contamination (Deakin et al, 2016; Heathwaite, 2010; Muirhead and Monaghan, 2012), which, by definition, do not have a single origin, but are rather scattered in the landscape.

Excess nutrients can result in eutrophication of freshwater resources (Dodds and Oakes, 2008; Smith et al., 1999), thereby impacting aquatic biota and affecting drinking water supplies (Smith et al., 1999). Excess fine sediments can reduce water clarity and affect primary producers and aquatic foodwebs (Hickey et al., 1994; Davies-Colley et al., 2008, Izagirre et al. 2009), and also smother bed substrates with resulting implications for benthic fauna (Braccia and Voshell, 2006), as well as potentially interfering with biogeochemical cycles in the hyporheic zone (Jones et al., 2015). Faecal material can be associated with pathogens and with nutrient enrichment and can therefore affect drinking water supplies and preventing recreational use of surface waters (Howard-Williams et al., 2010), and may also contribute to eutrophication (James et al., 2007).

Recognising the need to reduce the pressures on freshwater systems, several pieces of legislation have been developed to protect freshwaters and ensure their sustainable use. In Europe, water protection legislation began in 1975, with the introduction of standards for rivers and lakes used for drinking water abstraction, and saw a major development with the introduction of the Water Framework Directive (2000/60/EC) (WFD) in 2000 (European Commission [EC], 2020a). The WFD aggregated previous legislation aimed at water quality protection, expanded their scope to all waterbodies, introduced water management based at river basin scale, and defined a concrete goal: to achieve at least good ecological status of all European waterbodies. Such developments at the policy and management level were fruitful in improving water quality, having, for instance, led to a reduction of N surpluses by 18% between 2000 and 2015 (European Environmental Agency [EEA], 2018a). Nevertheless, the recent State of Environment Report (SOER) 2020 of the EEA stated that, despite such recent improvements, environmental pressures on European freshwaters remain substantial, with diffuse pollution from agriculture and hydromorphological degradation being by far the major issues (EEA, 2019). Currently, approximately 50% of the European surface waterbodies are classified as moderate or bad status, thereby failing to meet the WFD goals (Hering and Birk, 2018). The MARS – *Managing Aquatic ecosystems and water Resources under multiple Stress* Project (2014 – 2018), launched by the EU with the aim of understanding how multiple stressors affect surface waters and informing management and policy, acknowledged the complexity the environmental pressures currently faced by European freshwaters. In its recommendations for mitigation, the MARS report indicated that, in particular for rivers, a consistent and broad scale implementation of riparian buffer strips was necessary to address both diffuse pollution and hydromorphological deterioration (Hering and Birk, 2018).

In regions where pastoral agriculture dominates, as is the case with Ireland, additional sources of pollution can include discrete areas in streams or rivers that are used by livestock to gain access to drinking water and/or and to cross between parcels of land. Such practice has been reported as having negative effects on riparian vegetation (Platts and Nelson, 1985), streambank stability and resistance to erosion and stream channel morphology (Kauffman et al. 1983; Trimble 1994; Trimble and Mendel 1995; Sovell et al. 2000), leading to increased sedimentation, loss of in-stream habitat and alterations in benthic fauna communities (Braccia and Voshell, 2007; Zaimes and Schultz, 2011). Moreover, where livestock have unrestricted access to watercourses, they often void faeces and urine within the channel or in the proximity to waters. This has been linked to increases in water and sediment faecal contamination (Davies-Colley et al., 2004), with potential implications for both human and animal health.

Despite the evidence that unrestricted cattle access to watercourses can have detrimental impacts on water quality (O'Callaghan et al., 2018), the body of literature addressing the topic is relatively small, and the extent of the impacts as well as the mechanisms involved remain unclear (O'Callaghan et al., 2018). Factors such as stocking density and hydroclimatic conditions are likely to influence the extent to which unrestricted cattle access can impact the aquatic system, making it challenging to quantify such impacts and implement appropriate mitigation measures (Madden et al., 2019). To date, the majority of the studies addressing the topic has been conducted in the USA, Canada and New Zealand, where management, hydrological and climate conditions differ from Europe. There is, therefore, a lack of detailed understanding of the contribution of cattle access to watercourses on freshwater systems in a European context, and of the effectiveness of mitigation measures that address this issue in protecting water quality. Despite this, in Ireland, mitigation measures that exclude cattle from watercourses have been incorporated in agri-environmental policy since the implementation in 1994, almost three decades ago, of the first Irish agri-environmental scheme (AES), the Rural Environment Protection Scheme (REPS).

1.1. Aims and objectives of this research

The work presented in this thesis aimed at contributing to the limited body of literature on the topic by investigating how cattle access to watercourses affects freshwater physicochemical parameters and potentially contributes to excess nutrients and faecal contamination of waters. The levels of these contaminants were assessed in the stream sediment compartment given its widely recognised ability to act as both a reservoir to nutrients and bacteria and a source of these pollutants to overlying waters. Despite this important function, little attention has been given specifically to the sediment compartment in studies focusing on the impacts of livestock in-stream activity on freshwater quality. Therefore, the specific objectives of this study were:

1. To investigate the impact of unrestricted cattle access to watercourses on stream sediment concentrations of *Escherichia coli*
2. To assess the contribution of unrestricted cattle access to stream sediment levels of nitrogen (N), phosphorus (P) and organic carbon (OC)
3. To quantify the near real time (i.e. through 3 minutes interval continuous sampling) changes in water physicochemical parameters (i.e. dissolved phosphorus and nitrogen, total phosphorus, total suspended solids) and faecal contamination (*E. coli* bacteria) before, during and after cattle in-stream activity

4. To assess the effectiveness of cattle exclusion fencing as a mitigation measure in terms of key sediment and freshwater parameters

1.2. Thesis structure

This thesis aims to address the objectives identified above through four integrated data chapters, followed by an overall discussion chapter. Chapter 1 provides a background for the study and outlines its specific objectives. A detailed literature review is presented in Chapter 2, focusing on the impacts of agriculture on freshwater systems globally and in Ireland, and how cattle-based agriculture and unrestricted cattle access to watercourses can further contribute to water quality degradation. Chapter 3 provides a description of the selected sites sampled in this study. Sites were clustered at the catchment scale incorporating both intensively and extensively managed catchments. Inclusion of a variety of sites facilitated the evaluation of local/reach scale effects in relation to catchment-scale effects. Chapter 4 examines the impacts of cattle access to watercourses on bed sediment concentrations of *E. coli* bacteria, commonly used as indicators of faecal contamination and consequent health risk to human populations. The results provide estimates of the level of *E. coli* contamination of stream bed sediment from cattle access. Similar to Chapter 4, Chapter 5 investigates how cattle access to watercourses contributed to enriched nutrient concentrations in stream sediments. Chapter 6 describes a high temporal resolution monitoring experiment to assess the impacts of cattle in-stream activity on a number of water quality parameters, such as nutrients, total suspended solids and *E. coli* bacteria. Intensive sampling of stream water was undertaken at a selected cattle access point capturing both periods of cattle in-stream activity and periods of cattle absence. Chapter 7 describes a short study with the aim of assessing the efficacy of fencing watercourses (to exclude cattle) as a mitigation measure. The study investigated the potential benefits of fencing in the short-term (after ~ one year), in terms of the parameters analysed in Chapters 4 and 5, and also presents an assessment of the effectiveness of fencing on water quality by analysing freshwater parameters (nutrients, *E. coli* bacteria) in two streams (one fenced ~ nine years and one unfenced for that time) over a one year study. Each of these four chapters contains a more targeted literature review on their specific topic. Finally, Chapter 8 reviews the findings of the previous chapters, presents conclusions and discusses the implications of this research in agricultural management and policy.

2. Literature Review

Sections of this literature review contributed to the following review paper:

O'Callaghan, P., Kelly-Quinn, M., Jennings, E., Antunes, P., O'Sullivan, M., Fenton, O., Ó hUallacháin, D., 2018. The environmental impact of cattle access to watercourses: a review. J. Environ. Qual. 48(2), 340 - 351

Chapter 2. Literature Review

2.1. Agriculture is one of the major threats to freshwater systems globally

Deterioration of freshwater resources, as a result of physical alteration and degradation, habitat loss, nutrient enrichment, introduction of alien species, overexploitation, pollution and climate change is a global primary concern (Malmqvist and Rundle, 2002;Dudgeon et al., 2006; Heathwaite, 2010; Vörösmarty et al., 2010). Degradation of freshwater systems has been documented in many parts of the world (Malmqvist and Rundle, 2002). In Europe, freshwater ecosystems and their associated ecosystem services have been deteriorating since the 1950s, mainly due to a combination of physical modification of rivers and streams, water abstraction, drainage and eutrophication caused by diffuse pollution sources such as agriculture (Follett and Hatfield, 2001; Malmqvist and Rundle, 2002; Harrison et al., 2010; Ulén et al., 2007; Vidon et al., 2008; Heathwaite, 2010; Smolders et al., 2015).

One of the most concerning threats to freshwater systems is eutrophication, which has been defined by Nixon (1995) as ‘an increase in the supply of organic matter to an ecosystem’. High concentrations of nutrients in freshwater systems can result in an increase in primary producer biomass, particularly algae or cyanobacteria in the system, with subsequent high rates of decomposition and decreases in dissolved oxygen (Smith et al., 1999). This in turn impacts aquatic biota, whereas hypoxic conditions can cause sediment-bound contaminants to be released into waters (Correll, 1998). The addition of high loads of organic matter from increased primary production or other sources, for example as animal or human faeces, and its subsequent decomposition, can also lead to reductions in dissolved oxygen in waters and therefore affect oxygen-sensitive macroinvertebrate communities (Braccia and Voshell, 2006). Separately, high ammonium inputs from animal or human sources can pose a direct toxicity threat to aquatic communities (Camargo et al., 2005). Excess nutrients, particularly phosphorus, generally the main limiting nutrient in freshwater systems, can also accumulate in the stream sediments, potentially causing internal chronic pollution effects (Sharpley et al., 2013; Fox et al., 2016).

2.2 Pastoral agriculture in Europe and in Ireland

In 2016, 173 million ha in the European Union (EU-28) were used for agricultural production, representing about 47.1% of the total land area (Eurostat, 2019). In Ireland, agriculture accounts for approximately 67.4% of the land area (Central Statistics Office [CSO], 2020). Livestock production accounts for approximately 65% of the EU agricultural land (Leip et al., 2015), with 92% of the agricultural area of Ireland dedicated to grassland and rough grazing (CSO, 2020). There were 87 million bovine animals in EU in 2018 (Eurostat, 2019), with the majority of these animals kept in seven Member States (MS): France (21.2 %), Germany (13.7 %), the United Kingdom (11.0 %), Ireland (7.5 %), Spain (7.4 %), Italy (7.2 %) and Poland (7.1 %) (Eurostat, 2019). Cattle constitute the majority of livestock in two MS: Luxembourg (84%) and Ireland (82%) (Eurostat, 2020).

Leip et al. (2015) estimated that livestock agriculture in Europe was responsible for 73% of water pollution (both N and P) caused by agriculture. Diffuse sources of agricultural pollution affected 38% of the waterbodies surveyed by the European Environment Agency (EEA) (EEA, 2018b). The EEA reported in 2018 that nutrient enrichment from agriculture and loss of habitat due to hydromorphological changes were amongst the main pressures on European surface waters, with less than half (40%) of the surface waters were in good or high ecological status or potential (EEA, 2018b).

In Ireland, water quality has been declining in recent decades. In its most recent water quality monitoring programme (2013 – 2018), the Environmental Protection Agency (EPA), reported that one-third of river and lakes were failing to meet nutrient environmental quality standards and a quarter of rivers and lakes showing increasing nutrient concentrations (EPA, 2019). Indeed, this assessment showed an overall net decline in water quality in comparison to the previous full assessment (2010-2015), with 47.2% of surface water bodies currently in less than good ecological status (EPA, 2019). This change was nearly entirely driven by a decrease in river water quality, with 4.4% of the monitored river water bodies declining in status (EPA, 2019). Additionally, the EPA highlighted a concerning steady deterioration of the highest quality Irish river bodies over the past decades, with watercourses considered pristine falling from 13.4% (575 sites) in 1987 – 1990 to only 0.7% (20 sites) in 2016 – 2018. This downward trend in quality conflicts with the goals of the EU Water Framework Directive (WFD; Directive 2000/60/EC, 2000), whereby all EU Member States are required to achieve at least good chemical and ecological water quality in all surface water bodies, as well as maintaining high water quality sites, preventing deterioration of these systems. Moreover, surface waters constitute the main source of drinking water in Ireland (81.5%) (EPA, 2016).

2.3. Key contaminants from agriculture

2.3.1. <u>Phosphorus</u>

Phosphorus is generally the limiting nutrient for primary production in freshwater systems (Smith, 2003; Carpenter, 2008), and, as such, plays a major role in eutrophication of surface waters. Agriculture is considered the major source of P to surface waters in agricultural catchments (Neidhart et al., 2019). Phosphorus is applied to agricultural soils in the form of slurries and animal manures, which contain predominantly organic P forms, or chemical fertilisers, which contain mainly inorganic soluble or bound forms of P. Soluble inorganic P is readily available to plants; however P is also a highly particle-reactive element (McGechan et al., 2005), particularly in the presence of clays and calcium, aluminium and iron ions (Reddy et al., 1999; Withers and Jarvie, 2008). Soil inorganic P is in equilibrium with P that is loosely bound to soil particles, so that bound inorganic P is converted to soluble P in soil pore water when plant uptake reduces the concentrations of soluble P. However, continuous P applications can result in P-saturated soils, with increased total P pools and higher soluble P levels (Fox et al., 2016).

Delivery of phosphorus to surface waters typically occurs in particulate forms in surface runoff (Deakin et al., 2016), particularly in poached or eroded soils. The greatest agricultural losses of P typically occur during storm events (Palmer-Felgate et al., 2009; Sharpley et al., 2013). In P-saturated soils, significant quantities of dissolved phosphorus can also be transported to surface waters through subsurface pathways (Jennings et al., 2003; Deakin et al., 2016). Both dissolved and particulate forms of P can be transported through subsurface preferential flowpaths (Records et al., 2016). At times of low flows, when the proportion of P contributed to freshwaters by diffuse catchment sources is lower, point sources of P can be relatively important (Heathwaite, 2010).

In aquatic systems, P exists as a dissolved inorganic molecule, adsorbed onto particulate material, incorporated in biomass or incorporated within organic molecules of varying complexity (Jennings et al., 2003). Bioavailable P, defined as phosphorus in those fractions 'that can be that are readily assimilated by organisms, or can be made assimilable through the activities of organisms, and that portion which has already been assimilated' (Reynolds and Davies, 2001), is in the orthophosphate form (PO_4^{3-}). The biogeochemical cycling in freshwater systems is complex and depends on several factors including climate, hydrology, and reactivity of soils and sediments (House, 2003). Processes of P retention within lotic systems include biological uptake (by macrophytes, periphyton and microorganisms), P adsorption and precipitation onto sediments (e.g., precipitation with Fe or Mn hydroxides, co-precipitation of phosphate with Ca), and deposition of particulate forms of P during stable or falling stream discharge (House, 20039). Conversely, processes through which P is released into the water sediment include remobilisation of P-rich sediments and associated release of dissolved P from sediment pore water, desorption of P from sediments, and organic P hydrolysis (Reddy et al., 1999; House, 2003;).

Phosphorus interaction with sediments is a major process in the P cycling in freshwater systems, particularly in rivers and streams where the ratio of bed sediment surface to water volume is relatively high (House and Denison, 2002; House, 2003). In such systems, this interaction of P with sediments is an important factor regulating their productivity (Reddy et al., 1999). The capacity of bed sediments to retain P is influenced by several factors including the overlying waters composition, local redox conditions, water:bed sediment ratio, water residence time, and sediment properties such as particle size, presence of metal oxide coatings, and concentration of exchangeable phosphate adsorbed onto the sediment (Palmer-Felgate et al., 2009; Fox et al., 2016;). Additionally, the availability of other elements, such as carbon (C) and N, and their ratios to P can also strongly influence P immobilisation in sediments (Records et al., 2016). Organic matter can also inhibit P sorption to aluminium and iron oxides, thereby reducing the sediment's capacity to retain P (Records et al., 2016).

The incorporation of P in bed sediments results in changes in P forms in the system, which has implications for P bioavailability (Palmer-Felgate et al., 2009). For example, highly bioavailable soluble inorganic P is converted in less bioavailable particulate forms following uptake by Fe-rich sediments (Palmer-Felgate et al., 2009). In impacted systems, however, the sediments can store large pools of TP, which can be converted into bioavailable forms when, for instance, redox conditions change, or the diffusion gradient across the sediment-water interface is reversed due to a reduction in waters concentrations of available P resulting from pollution control measures or land use changes (Palmer-Felmate et al., 2009; Jarvie et al., 2013; Neidhart et al., 2019). Thus, this stored P can be released from the sediments back to the water column over time, thereby increasing P loadings to downstream waterbodies with a lag time that can be of years or decades (Neidhart et al., 2019). Furthermore, these P reservoirs can be mobilised in particulate forms when sediment disturbance occurs, for example during high flows, with implications to downstream waterbodies (Palmer-Felgate et al., 2009). This 'legacy P' effectively becomes a chronic source of pollution to waterbodies, and can hinder the effectiveness of P pollution mitigation measures (Jarvie et al., 2013; Sharpley et al., 2013), thus representing a challenge for successful nutrient management in agricultural catchments.

2.3.2. Nitrogen

Agriculture is widely recognised as the largest single source of N inputs to the freshwater environment (Birgand et al., 2007). In addition to causing problems in freshwater systems (Follett and Hatfield, 2001), increasing riverine nitrogen loads to coastal waters can altered receiving ecosystems (Jones et al., 2018). Nitrate is a weakly negatively charged ion which is highly soluble and leaches readily from agricultural soils if it is not incorporated into plant biomass (Deakin et al., 2016). It is often delivered to freshwater systems via subsurface pathways, particularly in well-drained systems (Deakin et al., 2016). In streams, the nitrogen cycle is governed by biogeochemical reactions strongly associated with stream sediments and other substrata, which occur through interactions between the surface water, subsurface water and the hyporheic zone (Durand et al., 2011; Trimmer et al., 2012; Corner-Warner et al., 2020). The nitrate concentration in stream waters is influenced by a number of retention-release mechanisms, such as biotic uptake, abiotic adsorption, remineralisation and burial of nitrogen associated with organic matter, and removal mechanisms, including denitrification, ammonia volatilisation and export downstream (Bernot and Dodds, 2005; Trimmer et al., 2012; Welsh et al., 2017). Ammonia volatilisation, however, is generally negligible as the pH of most freshwaters is not high enough (> pH 8) for NH_4^+ to be converted to NH_3 and for this reaction to occur (Bernot and Dodds, 2005).

In agricultural streams, the majority of inorganic nitrogen is in the form of nitrate (van Kessel et al., 2009), although ammonium (and organic nitrogen) can also be abundant in streams where animal slurries are applied (Birgand et al., 2007). Nitrate and ammonium can be taken up directly by aquatic plants and algae, as well as low molecular weight dissolved organic nitrogen (DON) (Durand et al., 2011). Studies investigating the relative contribution of macrophyte assimilation in nitrogen retention in streams have reported that macrophyte uptake can account for between 5 and 70% of nitrogen retention (e.g. House et al., 2001; Howard-Williams et al., 1982; Jansson et al., 1994). The proportion of nitrogen that is removed from the water column by macrophyte uptake is influenced by several factors including macrophyte density and spatial repartition, nitrate affinity and time of the year (Birgand et al., 2007). Few studies have been conducted on nitrogen removal from streams by algae. Microbial biofilms (i.e. microbial communities embedded in a self-produced matrix of extracellular polymeric substances attached on stream substrates) influence the nitrogen cycle in streams by removing nitrogen from the water column via assimilation, and recycling N into the water column by mineralisation. Additionally, biofilms can alter biogeochemical conditions locally thus affecting microbial transformation rates (Bernot and Dodds, 2005).

As with biotic uptake, storage and burial of nitrogen within bed sediments is an important mechanism of nitrogen removal from the water column in agricultural streams (Birgand et al., 2007). Some clays incorporate nitrogen as fixed NH_4 (Bernot and Dodds, 2005), which can act as a mechanism of nitrogen removal from the water column. In sediments, anaerobic conditions tend to exist at different depths, driven by the oxygen demand of decomposing organic matter, and the limited diffusion of oxygen from overlying waters (Birgand et al., 2007). In anaerobic conditions, the mineralisation of buried organic nitrogen does not proceed from the process of ammonification, which produces NH_4 (Kuypers et al,, 2018)..

Thus, organic-rich sediments in agricultural streams are often characterised by an accumulation of NH_4 in interstitial pore water of the anoxic zone. This creates a concentration gradient with overlying waters which drives the upward diffusion of NH_4 from sediments to waters (Birgand et al., 2007). In the aerobic zone of the sediment, however, nitrification can occur, whereby NH_4 is converted to NO_3 by chemoautotrophic organisms.

The nitrification process is an important mechanism in retention and removal of nitrogen in streams because it results in the production of NO_3 from NH_4, which is very soluble and more likely to be mobilised and transported downstream. This is because ammonium can bind to organic and inorganic particles through ion exchange, which decreases its mobility (Bernot and Dodds, 2005). Moreover, nitrification is also often coupled with the denitrification process, whereby nitrate is reduced to gaseous nitrogen oxide which can then be further oxidised to dinitrogen (N_2). Denitrification is carried out by facultative anaerobic microorganisms which utilise nitrate as an electron acceptor in their respiratory chain in the absence of oxygen (Canfield et al., 2010). It is controlled by a number of factors including the availability of and quality of organic carbon (functioning as electron source), redox conditions, temperature and pH (Corner-Warner et al., 2020). The coupling of nitrification – denitrification was first hypothesised by Patrick and Reddy (1976). They suggested that the nitrification of ammonium in the aerobic layer of the sediments created a concentration gradient that caused the upward diffusion of ammonium in the underlying anaerobic layer, which would then undergo nitrification. The resulting nitrate would then diffuse downwards to the anaerobic layer driven by a nitrate concentration gradient between the overlying water, the aerobic surface of the sediments and the anaerobic zone; there, it would be denitrified (Birgand et al., 2007). The existence of adjacent nitrification and denitrification zones in the sediments was later experimentally proven by Sweerts and de Beer (1989) and Jensen et al (1993). Jensen et al (1993) further suggested that the proportion of nitrate produced by nitrification that undergoes denitrification rather than being lost to the water column is influenced by diffusion distances from the aerobic layer of the sediment to the water column and the anaerobic layer. They hypothesised that when oxygen penetrates deeper into the sediment, nitrification occurs further from the surface of the sediment, favouring nitrate diffusion downward to the denitrification layer over diffusion to overlying waters (Jensen et al., 1993). Both nitrification and denitrification therefore take place primarily in the stream sediments (Butturini et al., 2000). Rooted macrophytes can favour the coupling of nitrification and denitrification by increasing organic matter contents and creating aerobic conditions in the rhizosphere (Birgand et al., 2007; Forshay and Dodson, 2011). Aquatic fauna also directly and indirectly influence the nitrogen cycle, particularly those that burrow vertically in the sediment profile (Nickerson et al., 2019).

Other biochemical reactions in the nitrogen cycle include the dissimilatory nitrate reduction to ammonium (DRNA) and anaerobic ammonia oxidation (annamox). DRNA, however, has rarely been measured directly in freshwaters (Trimmer et al., 2012). Annamox is the combination of ammonium with nitrite, in anaerobic conditions, to produce N_2. The process is carried out by chemolithoautotrophic organisms and, as with denitrification, leads to the permanent removal of nitrogen from the aquatic system (Burgin and Hamilton, 2007).

2.3.3. Excess suspended solids

The effects of excess suspended solids pressure on aquatic systems resulting from intensification of agriculture are a global concern (Nader et al., 2016). Suspended solids, defined as fine organic and inorganic particulate matter (<62μm) (Bilotta and Brazier, 2008) can be delivered in excessive quantities to watercourses in areas where these systems and sediments are hydrologically connected and agriculture has resulted in increased soil erosion (Sherriff et al., 2019). This sediment connectivity is controlled by factors such as climate, lithology and land use (Sherriff et al., 2019). Poorly drained catchments, for example, might have higher sediment connectivity because increased surface runoff can result in higher sediment quantities transferred to watercourses (Mellander et al., 2012).

Augmented supplies of suspended solids to freshwater systems can have several detrimental effects. They can cause reduced light penetration, thereby affecting primary producers, cause temperature changes, and smothering of watercourse substrates upon deposition, resulting in habitat loss for benthic taxa (Kemp et al., 2011). Suspended particles can also cause abrasion on aquatic organisms and clog respiratory and feeding organs of invertebrates and fish (Bilotta and Brazier, 2008). Additionally, suspended solids can release contaminants into waters, such as pesticides or phosphorus (Bilotta and Brazier, 2008). Where suspended solids are rich in organic matter, decomposition may lead to oxygen depletion in waters with implications for aquatic organisms (Bilotta and Brazier, 2008).

2.3.4. Contamination of surface waters with faecal material

Faecal contamination is a major cause of water impairment in many countries, including developing nations, but also countries with advanced water treatment systems (Smolders et al., 2015) such as the USA (Rehmann and Soupir, 2009) and New Zealand (Muirhead et al., 2004; Collins et al., 2007). In Ireland, a report for the year 2016 stated that 42% of the groundwater WFD sites were contaminated with faecal indicator organisms, highlighting the need for testing and adequately treating groundwater drinking supplies (EPA, 2018). Indeed, faecal contamination is an on-going issue in drinking water, particularly in small private water supplies (EPA, 2020).

Faecal contamination of water resources is concerning because a large number of infections can be transmitted through the consumption of contaminated water. The main groups of microorganisms that can cause waterborne infection include protozoa, bacteria and viruses (Gray, 2008). Bacteria are the most important group of faecal pathogens, accounting for the majority of waterborne disease outbreaks (Gray, 2008). Faecal pathogenic bacteria include *Salmonella* sp., *Campylobacter* sp., and *Escherichia. coli* (Gray, 2008). *Salmonella* sp. (Gray, 2008) and *Campylobacter* sp. (Evans et al., 2003) are common causes of gastroenteritis in Europe. *Escherichia. coli* is present in the normal human gastrointestinal flora (Kaper et al., 2004). However there are several distinct pathogenic serotypes, including enterohaemorrhagic *E. coli* (EHEC; also referred to as verocytotoxigenic serotypes), of which *E. coli* O157:H7 is considered the most important serotype (Kaper et al., 2004). *E. coli* O157:H7 causes haemorrhagic colitis, haemolytic uraemic syndrome and kidney disease in children. Several outbreaks of *E. coli* O157:H7 have been documented; in the United States; for instance, 4928 cases of infection were reported between 2003 and 2012 (Heiman et al., 2015). In Europe, the average incidence of *E. coli* O157:H7 infection 2018 was 2.4 per 100,000 population, a sharp increase in comparison to the previous four years (1.7 – 1.8) (European Centre for Disease Control [ECDC], 2020).

In addition to potential human and animal health risks, it has been observed that cattle frequently avoid or limit consumption of water contaminated with faecal matter due to poor water palatability (Willms et al., 2002). A decrease in water consumption has been associated with a decrease in forage consumption, and consequently in weight gains (Willms et al., 2002; Lardner et al., 2005), suggesting that failure in providing animals with adequate drinking water sources may lead to economic losses.

In waters, faecal bacteria are usually found in lower concentrations in the water column when compared to bed sediments (Pachepsky and Shelton, 2011; Ling et al., 2012). It has been suggested that, in open waters, bacteria are less able to survive due to nutrient deprivation, predation, inactivation by sunlight and competition with native organisms (Alm et al., 2003). In contrast, association with sediments may favour bacterial persistence as a result of higher nutrient availability and protection from predation (Desmarais et al., 2002) and UV radiation (Kim et al., 2010) Factors influencing bacterial survival in sediments include temperature, salinity and sediment characteristics (Pachepsky and Shelton, 2011). *E. coli* decay rates have been observed to be lower in low salinity (Anderson et al., 2005; Pachepsky and Shelton, 2011) and lower temperature conditions (Pachepsky and Shelton, 2011) and in sediments with high contents of fine particles and organic matter (Desmarais et al., 2002; Craig et al., 2004; Pachepsky and Shelton, 2011). *E. coli* is generally found in the upper sediment layers (0 – 5 cm; Desmarais et al., 2002) and bacterial distribution in the sediments is usually patchy (Pachepsky and Shelton, 2011).

Because sediments favour bacteria accumulation and persistence, they may act both as sinks and sources of water faecal contamination. Bacteria populations in sediments may be mobilised into the water column when sediments are disturbed. Craig et al. (2004) observed a dramatic increase of faecal coliforms concentrations in both waters and sediments at a recreational coastal site in Australia following a significant rainfall event, from 17 ± 11 CFU.100 ml^{-1} and 143 ± 57 CFU.100 mg^{-1} to more than 10^6 CFU.100 ml^{-1} and 10^6 CFU.100 mg^{-1}, respectively. The authors observed that two days after the peak, bacterial concentrations in waters had decreased to 2.2 x 10^3.100 ml^{-1}, whereas bacteria concentrations in sediments remained at 1.2 x 10^5.100 g^{-1}; they suggested that bacteria decay rates in sediments are lower than in the water column and thus microorganisms were able to accumulate in the sediments. Muirhead et al, (2004) reported a 2- to 3-fold increase in faecal indicator bacteria concentrations in waters after storm events when compared to baseflow levels, and it has been suggested that sediment agitation may be a more important mechanism of water faecal bacteria concentrations increase than overland runoff (Davies-Colley et al., 2008; Pachepsky and Shelton, 2011). Similarly, other events that cause sediment disturbance can lead to the resuspension of bacterial cells from sediment reservoirs, such as the disturbance caused by cattle crossing unbridged streams in farming areas (Davies-Colley et al., 2004).

Other faecal pathogenic organisms commonly transmitted through water consumption are *Cryptosporidium* and *Giardia* species, which are protozoa (Gray, 2008). *Cryptosporidium* species are parasitic organisms capable of infecting humans and a wide range of animals (Mendonça et al., 2007). One of the most common species affecting humans and cattle is *C. parvum* (Ryan et al., 2005). It causes a gastrointestinal illness in humans and neonatal livestock (Wells et al., 2015). Neonatal disease in cattle due to cryptosporidiosis can lead to significant economic losses (Mendonça et al., 2007). In waters, *Cryptosporidium* species exist as highly resistant cells known as oocysts (Lucy et al. 2008; Wells et al., 2015), which can remain viable for months (EPA, 2011). Studies have suggested that 1 - 10 oocysts are generally sufficient to cause infection (Gray, 2008). Because infected animals and humans typically excrete large quantities of oocysts (up to 10^{10} cells; Gray, 2008), infection may spread rapidly in farming areas and into the environment (Wells et al., 2015). *Giardia* sp. is also found in the environment as highly resistant cysts and can infect cattle and humans, causing diarrhoeal disease (Mendonça et al., 2007; Lucy et al., 2008) . Several outbreaks of cryptosporidiosis have been documented in the UK, with contamination origins traced to livestock grazing nearby water reservoirs and runoff from fields following slurry application (Gray, 2008). In Ireland, cryptosporidiosis became notifiable in 2004, meaning that any outbreaks of the disease must be notified to the government authorities by law; since then, a total of 3552 cases of infection were recorded (EPA, 2015).

2.4. How does cattle-based agriculture impact on freshwater systems?

By trampling the topsoil of the grazing fields as well as reducing the vegetation at a site, cattle can alter the hydrology and drainage pathways of these areas. This in turn can result in a decrease of the soil water infiltration capacity and a consequent increase in surface runoff (Line, 2003; Kurz et al., 2006). Kurz et al. (2006) observed that this alteration in infiltration capacity led to an increase in the concentration of particulate N and organic P in surface runoff in grazed pastures, and that this effect persisted during winter time, when cattle were absent from the fields. Other studies have shown that faecal matter deposited onto grazing fields can be transported to streams during run-off forming rainfall events, resulting in water contamination with organic matter, particulate forms of nutrients and faecal microorganisms (e.g. Line, 2003; James et al., 2007). Other potential diffuse source of stream water pollution related to cattle farming include the application of cattle slurry and manure on agricultural fields and subsequent wash-off into adjacent streams and rivers (i.e. incidental transfers) (Vidon et al., 2008; Bragina et al., 2017).

Point sources of water pollution related to cattle-based production, such as effluent discharges from farmyards, can also play an important role. For instance, Edwards et al. (2012) measured nutrients, faecal indicator organisms and suspended sediment concentrations loads from field drains as well as from a dairy farm effluent to a small catchment in NE Scotland, and concluded that the farmyard effluent delivered a large proportion of ammonium, phosphate and faecal indicator organisms to the waters.

Additional potential point sources of pollutants include direct livestock access to watercourses, which consist of sites used by the animals to drink, and those used as crossing points between adjacent fields. Some authors have suggested that cattle are characteristically attracted to water (Davies-Colley et al., 2004; Collins et al., 2007) and while they do not seem to spend a disproportional amount of time in the watercourse itself (Bond el at., 2012; Hann et al., 2010), it has been observed that they tend to congregate in the riparian area more than elsewhere (James et al, 2007; Bond et al., 2012; Haan et al., 2010;: Kay et al., 2018). This has been attributed to the fact that riparian areas provide not only drinking water, but also shade and better quality forage to the animals (James et al., 2007) .

2.5. Unrestricted cattle access to watercourses

Cattle access to watercourses has been demonstrated to have the potential to impact water quality in a variety of ways. Cattle grazing in close proximity to watercourses prevent riparian vegetation from growing (O'Callaghan et al., 2018). Riparian vegetation has been shown to have a buffering effect by protecting stream waters from receiving contaminants in run-off from the farmland, and can also stabilise streambanks, reducing bank erosion (Stutter et al., 2012). Frequent traffic of cattle into or across streams causes stream bank trampling and erosion, which leads to increased sediment inputs to the streams at and nearby access points (McKergow et al., 2001; Miller et al., 2011). Elevated loads of inorganic sediment in streams alter stream habitat quality due to infilling of interstitial spaces in streambed sediment, impacting macroinvertebrate communities (Conroy et al., 2016). Increased sediment loads may also lead to associated increases in particulate forms of nutrients, particularly phosphorus (Fox et al., 2016). Finally, excretions in or nearby the watercourses can directly add nutrients and potentially pathogenic faecal organisms to the aquatic system (Larsen et al., 1994). However, the body of literature investigating the extent to which unrestricted cattle access can be detrimental to water quality is limited, and studies assessing the effectiveness of cattle exclusion in preventing negative impacts are often conflicting (O'Callaghan et al.,2018) (Figure 2.1). The following sections discuss how unrestricted cattle access to watercourses can contribute to the deterioration of aquatic systems.

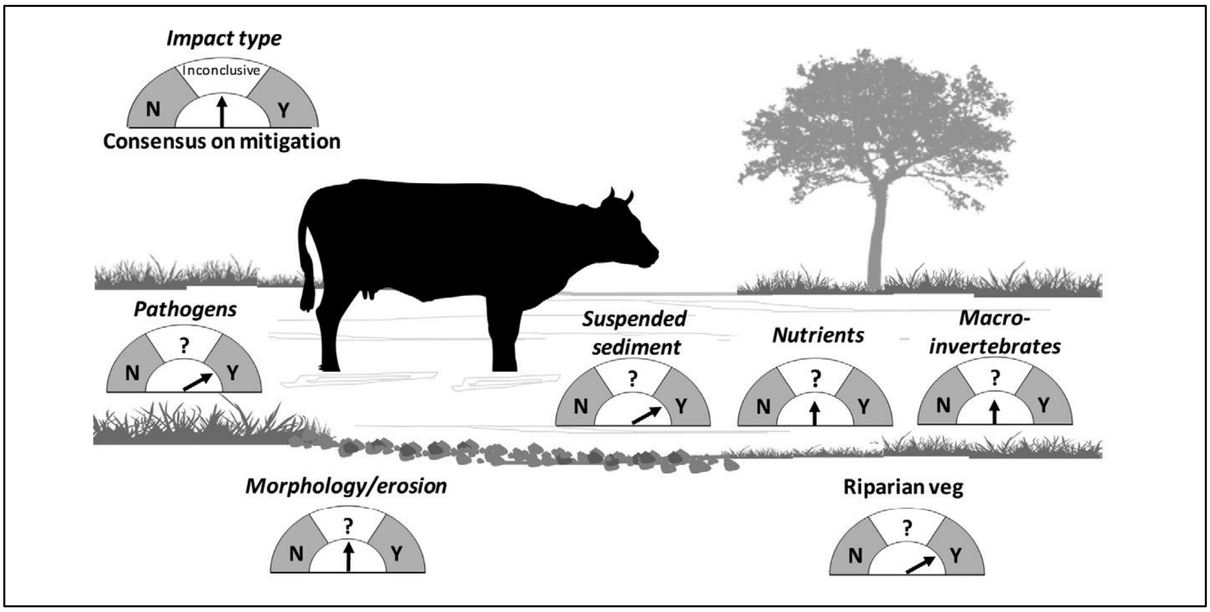

Fig.2.1. Potential impacts of unrestricted cattle access to streams on different physicochemical and biotic parameters, as reviewed by O'Callaghan et al. (2018). Arrows in gauges indicate consensus in the reviewed literature regarding the effectiveness of fencing on the mitigation of cattle access impacts (Y = consensus that there was mitigation; N = consensus that there was no mitigation; ? = the studies were inconclusive). Figure taken from O'Callaghan et al. (2018),

2.5.1. Impacts on stream hydromorphology and sedimentation

Where cattle have direct access to watercourses, their incisional and erosional potential, associated with overgrazing of protective riparian vegetation, can result in reduction of stream bank stability. This can lead to increased erosion and sedimentation at cattle access sites (Zaimes and Schultz, 2012) and alterations to stream channel morphology, with stream channels often becoming shallower and wider (Harrison and Harris, 2002). For instance, in a two year study in NE Oregon, USA, Kauffman et al. (1983) reported significantly greater streambank erosion in stream reaches with uncontrolled cattle grazing than in ungrazed reaches. Similarly, Trimble (1994) observed that unrestricted cattle access to a stream reach in Tennessee, USA, caused approximately six times more gross streambank erosion in comparison to a control protected reach, which was attributed mainly to streambank breakdown by cattle and consequent reduction of the bank's geomorphic resistance.

Parallel to the enhanced streambank degradation and erosion, cattle in-stream movements while crossing or drinking also cause streambed sediment resuspension (Terry et al., 2014). Vidon et al. (2007) monitored water quality upstream and downstream of an area grazed by 25 cows with unrestricted access to the stream over a 12 month period, and reported a dramatic increase in water turbidity (13-fold) and total suspended solids (TSS) (11-fold) from the upstream to downstream points in the summer period, when cattle were often near or in the stream. In their study in in the Sherry River, New Zealand, Davies-Colley et al. (2004) estimated that two crossing events with the total duration of 19 minutes led a to a 54% increase in TSS water concentrations. Elevated inputs of sediment to waters caused by increased erosion of exposed banks and disturbance of the substrate during cattle in-stream activity can lead to habitat alteration as the fine sediment smoothers the substrate or clogs interstitial spaces and the hyporheic zone (Boulton et al., 2003). This can alter the composition of macroinvertebrate communities, and has been highlighted as the main ecological impact of excessive sediment pollution (Braccia and Voshell, 2006). Some studies have reported that cattle activity can cause bank failure, leading to streams becoming shallower and wider (Harrison and Harris, 2010). This in turn can lead to increased water temperatures (Herbst et al., 2012) and impact temperature-sensitive aquatic biota (Braccia and Voshell, 2006).

Fig.2.2. Left: streambank erosion and trampling at a cattle access site. Right: streambank collapse. Both sites are in the Brackan River catchment (Co Wexford).

A number of studies have reported significant improvements in stream morphology and stream bank condition reported improvements in streambank and channel conditions following the adoption of cattle exclusion measures, including increased bank stability and decreased erosion (e.g. Clary, 1999; Laubel et al., 2003; Scrimgeour and Kendall, 2003), decreased channel width (e.g. Magilligan and McDowell, 1997; Harrison and Harris, 2002;), increased stream depth (e.g. Ranganath et al., 2009) and higher riparian vegetation biomass (e.g. Scrimgeour and Kendall, 2003; Ranganath et al., 2009). Significant decreases in TSS concentrations following the adoption of cattle exclusion measures have also been observed (e.g. Line et al., 2000; Georgakakos et al., 2018). Other studies have reported no improvement in stream morphology after the implementation of cattle exclusion measures, and it has been suggested that such benefits might only become apparent after at least a decade following implementation (Kondolf, 1995).

2.5.2. Impacts on stream water and sediment nutrient levels

Cattle access points can act as localised sources of nutrients to watercourses through two main mechanisms: contribution of particulate phosphorus and nitrogen to waters as a result of streambank degradation and erosion (Fox et al., 2016) and excretion directly in waters or in the vicinity of the watercourse (James et al., 2007). Streambank soil P concentrations ranging from 200 $mg.kg^{-1-}$ (Purvis et al., 2016) to 1400 $mg.kg^{-1}$ (Kronvang et al., 2012) have been reported in literature. Fox et al. (2016) postulated that streambanks in catchments which are affected by excessive nutrient inputs should be considered a significant source of TP to waters where streambank erosion and failure are systematic. Thus, streambank TP concentrations and streambank erosion rates are the primary variables controlling P loadings contributed to streams from banks (Fox et al., 2016), both of which can be exacerbated by cattle activity. Indeed, McDowell and Wilcock (2007) have stated that when livestock is allowed access to watercourses, damaged streambanks, rather than topsoil in agricultural fields, may become the primary source of PP in streamflow.

At cattle access sites, frequent defecation and urination directly into stream waters or in trampled and exposed banks can contribute nutrients and organic matter to the aquatic system. A number of studies have reported that, in addition to preferentially congregating in riparian areas (James et al., 2007; Bond et al., 2012) cattle tend to defecate more often when in proximity to water. For instance, in their study in the Sherry River, New Zealand, Davies-Colley et al. (2004), reported that the animals defecated ca. 50 times more per metre when crossing the river than elsewhere. Similarly, while studying the behaviour of four pastured herds of dairy cattle in the Cannonsville Watershed (New York, USA), James et al. (2007) observed that a significant number of animals (average across four herds of 21.4% of the herd) concentrated within 0 – 10 m of the stream at any given time, and tended to defecate more often while in this area.

Cattle faeces are the main pathway of excretion of non-utilised phosphorus (Ternouth, 1990). Faecal TP concentrations for cattle are determined by factors such as the type of diet, feed intake and animal reproductive status (Dou et al., 2002; James et al., 2007), with studies reporting concentrations from 4.9 mg TP.kg dry wt^{-1} (Orr et al., 2012) to 12.65 mg TP.kg dry wt^{-1} (Dou et al., 2002). Dou et al. (2002) reported that a substantial amount of the faecal TP was readily soluble inorganic P, and that this fraction increased with increasing P intake, representing 30.3% to 49.6% of the total. On the other hand, nitrogen is mostly excreted in urine (Selbie et al., 2015). Again, total nitrogen concentrations in cattle urine vary with type of diet, feed and water intake, and even time of the day (Selbie et al., 2015). Studies have reported TN concentrations in cattle urine ranging from 3.0 $g.L^{-1}$ (Spek et al., 2012) to 20.5 $g.L^{-1}$ (Bristow et al., 1992), with the dominant form being urea-N, which in these studies represented 52.1% to 93.5% of TN. Ammonium has been reported to account for a small proportion of urine TN (average 0.9% to 2.9%; Bristow et al., 1992; Gonda and Lindberg, 1994). In a study assessing the defecation and urination behaviour of 24 beef cattle in the UK, Orr et al. (2012) reported average loads of P and N in faeces of 0.7 and 4.1 $g.event^{-1}$, and of 0.003 and 4.5 $g.event^{-1}$ in urine, respectively.

Fig. 2.3. Cow urinating directly in waters at access point (Ireland).

In their study in the Cannonsville Watershed, James et al. (2007) estimated that approximately 10% of the phosphorus loading at the watershed level attributable to agriculture originated from direct deposits of faecal matter by cattle in stream waters. The authors also estimated that the adoption of cattle exclusion measures in approximately one-third of the dairy farms in the watershed had resulted in a reduction of 32% of the P loadings to waters (James et al., 2007). Likewise, Byers at al. (2005) reported significantly higher loads of dissolved reactive phosphorus (DRP) and TP in an unfenced stream where cattle spent 9% of the time in the riparian area, compared to a stream where cattle spent 5% of the time in the riparian area. Vidon et al. (2007) reported increases in average water concentrations of NH_4-N (fourfold), TKN (fourfold) and TP (fivefold) resulting from unrestricted cattle access to the study stream. Similarly, Meals (2001) observed reductions in TP and TKN following the implementation of a number of cattle access mitigation measures, including fencing, in an experimental catchment in Vermont, US, whist observing increases in nutrient levels in a control catchment. Galeone (2000) also observed reductions in N and P exports after introduction of cattle exclusion measures in Pennsylvania, USA. Line et al. (2000) reported statistically significant reductions in TKN (78%), TP (76%) in a stream following the implementation of cattle exclusion measures. In a more recent study, the authors involving paired watersheds, Line et al. (2016) reported significant reductions in TKN (34%), NH_3–N (54%), and total P (47%) in the treatment relative to a control watershed after the implementation of cattle exclusion measures, but no change in oxidised nitrogen (NO_x–N) loads. Sheffield et al. (1997) reported that water column concentrations of total N and total P decreased by 54% and 81%, respectively, when cattle were offered an alternative water source, which in turn led to a reduction of the time cattle spent in the stream causing stream bank erosion to decrease by more than 70%. However, the authors noted that the concentrations of nitrate and orthophosphate were negatively impacted by the mitigation measure (Sheffield et al., 1997). More recently, Georgakakos et al. (2018) observed significant reductions in TP concentrations and loadings in a stream following the implementation of a cattle exclusion area, but not in SRP levels. In contrast, McKergow et al.

(2003) did not find significant reductions in total P or total N loads, or concentrations, in relation to cattle exclusion in a catchment in Western Australia, whereas Davies-Colley et al. (2004) reported only a modest increase (10%) in TN concentrations in their study following two cattle crossing events of a stream.

The impacts of unrestricted cattle access on nutrient levels in the freshwater sediment compartment have received less attention. Palmer-Felgate et al. (2009) reported higher TP sediment concentrations (ranging on average from 1429 to 2480 $mg.kg^{-1}$) in a UK stream located closer to farms where cattle had direct access at a number of points, when compared to a control site within the same catchment in an area grazed by only a small number of animals (ranging on average from 657 to 1060 $mg.kg^{-1}$). The same authors also observed relatively high TP sediment concentrations (ranging on average from 155 to636 $mg.kg^{-1}$) at a control site with low agriculture intensity, which they hypothesised was caused by unrestricted cattle access to the stream (Palmer-Felgate et al., 2009). In contrast, in a study where ion-exchange membranes where used to assess nutrient dynamics in streambanks, no significant impacts of cattle grazing intensity and access to the stream were observed for either NO_3-N or P (Miller et al., 2017).

2.5.3. Faecal contamination of stream waters and sediments

Defecation in or near the streams can add high levels of faecal microorganisms to waters. In their study in the Sherry River (New Zealand), Davies-Colley et al. (2004) estimated one herd to have deposited around 230 billion colony forming units (CFU) of the faecal coliform bacteria *E. coli* to waters in one single stream crossing, following 25 defecation events. Direct deposition of fresh faecal matter in waters is particularly important because animal faeces contain *E. coli* concentrations that may be as high as 10^9 cells g^{-1} (Murphy et al., 2015), and, contrary to diffuse transport of faecal matter, where mechanisms of retention and inactivation operate, during direct defecation there are no opportunities of bacteria immobilisation or die-off before reaching waters (Collins et al., 2007). Cattle are an important reservoir for many pathogens, including *E. coli* O157:H7 (Williams et al., 2008). Although infection with *E. coli* O157:H7 in animals is generally asymptomatic, infected animals excrete large quantities of bacteria, typically 10^2 – 10^5 CFU.g^{-1}, but possibly as high as 10^7 CFU.g^{-1+} (Williams et al., 2008). *E. coli* O157:H7 can survive for prolonged periods in the environment (Williams et al., 2008), and it has been observed to be more resistant to environmental stressors that non-pathogenic *E. coli* strains (Jenkins et al., 2012, 2015), while also having a very low infectious dose (<100 bacterial cells) (Brehony et al., 2018).

Several studies have investigated the impacts of cattle exclusion measures on freshwater faecal contamination. Line (2003) investigated the effects of excluding cattle from a 340 m long stretch of a small stream in North Carolina, USA, which was crossed at half-length by a farm road. In a long term study, the author sampled one point at the start of the fenced stretch (upstream) and at the end (downstream), both before and after fencing. The author reported a reduction in the average concentrations of *E. coli* in waters of 58.6% and 91.0% at the upstream and downstream sites following fencing, respectively, as well as a 95.9% reduction in the difference observed between average levels at the upstream and downstream sites prior to fencing. Smolders et al. (2015) compared faecal coliform levels in waters across a longitudinal gradient in two streams draining a grazing area in the Lake Baroon catchment, Australia, which were both crossed daily by cattle, following installation of a culvert bridge in one of the streams (i.e. treatment stream). The authors demonstrated that concentrations downstream of the crossing site in the unmodified stream (i.e. control stream) were significantly higher than those observed upstream, whereas in the treatment stream, differences between both sites were not significant after cattle exclusion through bridge improvement. However, concentrations in the bridged stream were higher after stream modification, which the authors attributed to a cumulative effect originating at the unmodified stream. McKergow et al. (2001) carried out a 10 year monitoring experiment to investigate the impacts of installing a fence and creating a riparian buffer on sediment and nutrient loadings in an agricultural stream in Western Australia. The authors reported that while there was a noticeable decrease in sediment loadings in the stream, improvements in nutrient exports were limited, which was attributed to the specific geology of the studied site. More recently, Bragina et al. (2017) showed that streamside exclusion fencing effectively reduced stream sediment contamination with *E coli* in a catchment impacted by intensive cattle farming in NE Ireland. The authors reported that sediment average concentrations of *E. coli* in a fenced stream were in the order of 10^2 CFU.g dry wt^{-1} whereas in an unfenced stream of the same catchment, they were significantly higher and up to 10^5 CFU.g dry wt^{-1} (Bragina et al., 2017).

2.6. Water quality protection in European and Irish policy

In 1962, the European Common Agricultural Policy (CAP) was launched with the aim of ensuring food supply in Europe (EC, 2015a). However, although the CAP had been very effective in achieving European Union’s self-sufficiency, its strong productivist approach and the resulting rapid expansion of agriculture led to increased concerns on negative effects of agriculture on the environment. In the mid-1980, this increasing concern on environmental sustainability of agricultural practices led the Community and national governments to work towards implementing agri-environmental measures (Latacz-Lohmann and Hodge, 2003).

One of the first European Commission (EC) environmental laws with potential impacts on agricultural activity was the Drinking Water Directive of 1980 (80/778/EEC), which introduced limits for concentrations of nitrates and pesticides in waters intended for human consumption (Latacz-Lohmann and Hodge, 2003). In 1991, the Nitrates Directive (91/676/EEC; ND) was introduced, which was aimed at specifically protecting water bodies and drinking water quality from nitrate pollution derived from agricultural activity (Latacz-Lohmann and Hodge, 2003; EC, 2010a). The ND required Member States (MS) to identify all surface freshwaters and groundwaters with nitrate concentrations above 50 mg L^{-1} or in risk of exceeding this threshold, as well as targeting all eutrophic water bodies (EC, 2015b). Furthermore, it imposed the identification of areas of land which drain into polluted waters or waters at risk of pollution, thus potentially contributing to nitrate contamination, as Nitrate Vulnerable Zones (NVZ) (EC, 2015b). The Directive required MS to implement compulsory programmes of measures in NVZ to tackle nitrate pollution, and adopt Codes of Good Agricultural Practice (GAP) to be applied by farmers on a voluntary basis elsewhere (EC, 2015b).

The ND became a complimentary measure to the Water Framework Directive (2000/60/EC; WFD) since the introduction of that over-arching directive in 2000. The WFD resulted from the need to address water quality in a more integrated manner, and established the ambitious goal of restoring and protecting the quality of all waters across Europe (EC, 2010b). The Directive introduced water management through an integrated river basin management approach, regardless of political or administrative boundaries (EC, 2010b), and defined a concrete objective: to achieve or maintain at least “good” and non-deteriorating chemical and ecological status in all waters by 2015 (EC, 2010b; Kallis and Butler, 2001).

Following the WFD establishment, the MS defined river basin districts, which would become the basic units of water management. In total, 110 river basin districts were delimited across the EU (EC, 2010b). In addition, water monitoring networks were established (EC, 2010b). In 2009, EU MS presented individual River Basin Management Programmes (RBMPs), which included programmes of measures to achieve the WFD goals, and were required to be fully operational by 2012. The first management cycle of the WFD ended in 2015, and MS presented their second RBMPs, based on the results and lessons learned from their predecessors. The second and third management cycles of the WFD will end in 2021 and 2027, respectively, with the latter being now the revising deadline to the achievement of the WFD goal.

In addition to the ND, the WFD is supplemented by other daughter directives regarding water quality protection, of which the Drinking Water Directive (Directive 98/83/EC) (DWD hereafter), and the Bathing Water Directive (Directive 2006/7/EC) (BWB hereafter) are perhaps more relevant in the context of agricultural pollution. The DWD regulates the quality intended for human consumption and applies to all distribution systems serving more than 50 people or supplying more than 10 $m^3.day^{-1}$, and also distribution systems serving less than 50 people/supplying less than 10 $m^3.day^{-1}$if the water is supplied as part of an economic activity drinking water from tankers, and water used in the food-processing industry (EC, 2021a). It requires a total of 48 microbiological, chemical and indicator parameters to be monitored (EC, 2021a), including *E. coli* and intestinal enterococci (0 CFU.100 ml^{-1}). The directive's latest amendments were adopted in 2020 (Directive (EU) 2020/2184).

The BWD was first introduced in 1976 (Directive 76/160/EEC) and later reviewed and replaced by the current New Bathing Water Directive (2006/7/EC) (EC, 2021b). It is intended to protect human health by preserving and protecting the quality of coastal and inland surface water bodies that are commonly used as bathing areas (EC, 2021b). Under the BWD, the MS are required to monitor bathing areas every year during the bathing season (typically from May to September), mainly for microbial parameters (intestinal enterococci and *E. coli*) although other parameters such as cyanobacteria and microalgae can also be monitored (EC, 2021b). The BWD introduces four categories of bathing waters in relation to numerical standards of bacteriological quality; these values vary for inland and coastal or transitional waters, ranging, for *E. coli*, from 250 – 500 CFU.100 ml^{-1} ("Excellent"), 500 – 1000 CFU.100 ml^{-1} ("Good") (values based upon a 95-percentile evaluation) or 500 – 900 CFU.100 ml^{-1} (values based upon a 90-percentile evaluation) ("Sufficient") (Directive 2006/7/EC). The category of "Poor" refers to failure to comply which such bacteriological standards, in which case the MS must take measures such as banning or advising against bathing, declassifying the area as bathing area (when it has failed to meet mandatory standards for five consecutive years), and taking corrective measures (EC, 2021b).

2.6.1. Agri-environment schemes

The first agri-environmental measures in Europe were initially statutory regulations which focused on controlling pollution from agriculture, mainly by regulating nitrate contamination, intensive livestock farming, the use of pesticides and slurry application on land (Latacz-Lohmann and Hodge, 2003). These regulations were non-compulsory and were defined and applied within individual MS, with little articulation across the European Community. However, government attempts to extend these measures beyond pollution control, aiming at coping with habitat deterioration and habitat loss, were largely contested by farmers, who saw such measures as undue interferences in their property rights and demanded monetary compensations for the loss of opportunities and profits resulting from their implementation (Latacz-Lohmann and Hodge, 2003). In 1981, Britain established the Wildlife and Countryside Act, which worked on the basis of compensating farmers for not undertaking potentially damaging operations in protected land. The scheme was opposed to due to the financial overburden imposed to conservation associations that were responsible for farmers' compensation, eventually leading to the novel idea of offering payments to farmers in protected areas for the provision of environmental goods, rather than for not taking damaging actions. In 1984, the British government launched the Broads Grazing Marshes Scheme in order to protect the threatened area of the Halvergate Marshes (Norfolk Broads, East England), which offered an annual payment to farmers in return for a commitment to farm at low intensities in the area. It marked a shift from a negative compensation policy to a more positive approach while highlighting the wider role of agriculture as a means for both food and fibre production and environmental conservation. The Broads Grazing Marshes Scheme led to the definition of EU agri-environmental schemes as flat-rate payments offered to farmers in return for voluntarily adopt environmentally friendly farming practices. Such schemes were established in all EU MS with the MacSharry reform of the CAP in 1992, and have become a keystone in agricultural and environmental management (Latacz-Lohmann and Hodge, 2003). With the later Agenda 2000 and 2003 reforms, AES become part of the second pillar of the CAP, aimed at improving economic and social conditions of rural areas. Currently, AES are implemented as part of Rural Development Plans (RDP) designed individually by each MS, and are the only mandatory rural development measure for MS.

2.6.1.1. Agri-environment policy in Ireland

In Ireland, the Nitrate Action Programme required by the ND is given legal effect through the European Union (Good Agricultural Practice for Protection of Waters) (GAP) regulations, of which the most recent are GAP Regulations S.I. No. 65/2018. These regulations include amendments to the previous S.I. No. 605/2017. The key components of the Irish NAP include limits on stocking rates, legal limits for nitrogen and phosphorus application rates, prohibition of application of organic and chemical fertilisers at more environmentally vulnerable times (e.g. before periods of heavy rain), minimum livestock manure storage requirements, and set-back distances from waters (Government of Ireland, 2021). Ireland is currently operating the 4^{th} NAP which will run until the end of 2021.

Ireland has also had agri-environmental schemes in place since 1994, when the first Rural Environmental Protection Scheme (REPS) was implemented (Whelan and Fry, 2010). REPS was a voluntary five year programme which was applied horizontally i.e. at a national level (Whelan and Fry, 2010), rather than in specific areas of the country. Its stated goals were to establish farming practices and production methods which reflected a concern for conservation and landscape protection; to protect wildlife habitats and endangered species; and to produce quality food in an environmentally friendly manner (Lenihan and Brasier, 2009). In order to subscribe to REPS, farmers had to implement, among other measures, an individual agri-environmental plan produced by qualified planners which included a Nutrient Management Plan based on the farm conditions (Emerson and Gillmor, 1999; Lenihan and Brasier, 2009). REPS had four incarnations (I-IV), and was superseded by the Agri-Environment Options Scheme (AEOS) in 2010. Both schemes represented a top-down, horizontal approach; however, while REPS involved applying measures to the whole farm with payments made on a per hectare basis, AEOS required farmers to select specific actions for delimited areas in the farm, and, apart from cross-compliance, there was no other requirement regarding the remaining area.

The AEOS was followed by the on-going Green Low Carbon Agri-Environment Scheme (GLAS), launched in 2015. GLAS is a large-scale top-down plan that differs from its predecessors by attempting at a targeted approach towards individual farms in areas of environmental concern (farms with “Priority and Environmental Assets and Actions”) such as Natura 2000 sites and areas with vulnerable catchments or high status waterbodies (Cullen et al., 2018). Famers in these areas had priority access to the scheme. This targeted approach has led, in comparison with the previous AES, to a greater superimposition of regions with high farmer participation rates and regions with high concentrations of environmental public goods, thus contributing to the scheme’s financial effectiveness (Cullen et al., 2018).

2.6.2. Recent policy developments

In June 2018, the EU presented legislative proposals for the post-2020 CAP (2021-2027), aimed at delivering a higher level of climate and environmental ambition while placing greater emphasis in the achievement of results at the regional and national scale, thus moving away from a focus on compliance (EC, 2019a). The new policy comprises nine specific goals to be achieved by each MS, three of which are directly related to protection and environment and climate (EC, 2019a). Each MS is responsible to delineate a CAP Strategic Plan, which will include specific targets and objectives for its territory and present actions to achieve them (EC, 2019a).

The CAP 2021-2027 is interlinked with other EU policies for the protection of the environment and the agri-food sector such as the WFD and the recent Farm to Fork Strategy and Biodiversity Strategy 2030. These are part of the European Green Deal, an action plan presented in 2019 with the over-arching goal of making Europe climate-neutral by 2050 (EC, 2019b). The Farm to Fork Strategy specifically requires the development of integrated nutrient management action plans in MS CAP Strategic Plans to tackle "nutrient pollution at source and increase the sustainability of the livestock sector" and the application of precise fertilisation techniques and sustainable agricultural practices particularly in areas of intensive livestock farming (EC, 2020b).

2.6.3. Measures to restrict cattle access to watercourses for water quality protection

In terms of measures to protect water quality, both REPS and AEOS included, among others, fencing of watercourses to restrict cattle access. The current GLAS, and indeed the Irish RDP 2014-2020 (of which GLAS is the largest scheme) have a strategic focus on water quality objectives (DoHPLG, 2018a). In GLAS, cattle are only allowed access to streams as a means of crossing between parcels of land when no other option is available. Furthermore, in response to the continuous general decline in water quality in Ireland, the 4th Nitrates Action Plan of the Nitrates Directive, launched in 2018, requires farms with an allowance (derogation: hereafter referred to as derogation farms) to farm at a grassland stocking rate over 170 kg N/ha, to prevent cattle from accessing watercourses from January 2021.

2.7. Relevance of the current study

Despite the inclusion of cattle exclusion measures in all Irish AES to date, little is known regarding cattle impacts and exclusion benefits in streams in the Irish or even European context. Most studies conducted on this topic have been carried out in areas of the globe where conditions (e.g. climate conditions, geological features) are much different from the Irish conditions. Such studies suggest that cattle exclusion measures can have beneficial effects on water quality in agricultural fields. However, they have also highlighted that the extent to which such measures can have positive impacts is dependent on both site specific characteristics and farmland management. The current work has therefore focused on quantifying the potential impacts of such practice on the described parameters – freshwater nutrient levels, excess suspended solids, and faecal bacteria - specifically in the Irish context. The study aims at both contributing to the literature describing livestock agriculture pressures on freshwater systems and to substantiate Irish agri-environment policy.

3. Site Selection and Description

Chapter 3. Site selection and description

3.1. Site selection criteria

The selection of the experimental sites studied for this thesis was carried out as part of the COSAINT Project – *Cattle access to watercourses: environmental and socio-economic implications*, funded by the Irish Environmental Protection Agency (EPA) under the Research Program 2014 – 2020, a project which also supported the work presented in this thesis. Specifically, the experimental site selection and characterisation was conducted under Work Package (WP) 2 the of the COSAINT project. The work presented in this thesis was developed under WP3, aimed at analysing geochemical and microbial parameters relative to cattle access and exclusion from watercourses. The project also included the publication of a literature review on the impacts of cattle access to watercourses on freshwater systems and the efficacy of fencing as a water quality protection measure (WP1); the analysis of the effects of cattle access on ecological parameters (WP4); the development of an estimate of on-farm watercourses at a national level (WP5) and an analysis of the cost-effectiveness and socio-economic implications of cattle exclusion measures (WP6).

In Ireland, biological water quality is assessed using the Quality Rating System, developed by the EPA, which relates the abundance of five key groups of macroinvertebrates to water quality. The system uses a five point scale (Q-values, or Q) of water quality rating, with intermediate scores obtainable, with Q1 representing bad water quality and Q5 representing high water quality (McGarrigle et al., 2002) (see Table 3.1). In the current study, five catchments were selected for sampling. In order to reflect the predominantly grass-based agricultural conditions found in the Irish countryside, the majority of the selected catchments (three) were of poor/moderate water quality and represented higher agriculture intensity (see

Table 3.2). Two of the five selected catchments were of high Q-value status and had more extensive agriculture (Table 3.2). Selected sites included a mixture of catchments with waterways fed by ground and surface sources to encompass the different low-flow conditions, sediment dynamics and nutrient pathways represented by each. For all catchments, all sites were on first and second order streams, as these are more commonly used as drinking water sources for cattle, are more vulnerable to the related pressures due to lower dilution rates (Lassaletta et al., 2010), influence downstream water quality (Freeman et al., 2007), are less affected by confounding factors than higher order streams/rivers which may be subject to further unrelated pressures (e.g. wastewater treatment works) and are more easily and safely sampled due to their lower average depths and velocities.

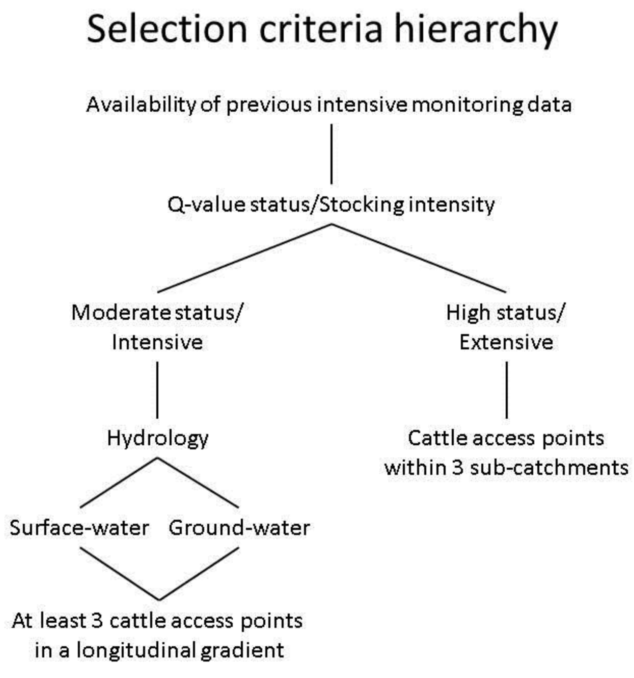

Fig. 3.1. Site selection criteria for the COSAINT project.

Table 3.1. Quality Rating System (Q-value) classification and relationship to the Water Framework Directive (WFD) water quality classification

Q-value	Water quality status	WFD status
Q5, Q4-5	Unpolluted	High
Q4	Unpolluted	Good
Q3-4	Slightly polluted	Moderate
Q3, Q2-3	Moderately polluted	Poor
Q2, Q1-2, Q1	Seriously polluted	Bad

Information taken from http://www.epa.ie/QValue/webusers/.

3.2. Study catchments and experimental design

The five catchments selected for sampling under the COSAINT Project were the Muster Blackwater catchment (BW; Co. Cork); the Douglas River catchment (DG; Co. Laois); the Brackan River catchment (BK; Co. Wexford); the Commons River catchment (CM; Co. Louth); and the Milltown Lake catchment (MT; Co. Monaghan) (see Fig 3.2). Table 3.2 summarises the characteristics of these catchments including ecological status and dominant hydrology pathways (surface flow or subsurface flow, i.e. water flow beneath soil surface (Hu and Li, 2018).

A total of 15 cattle access sites were selected for sampling. Three cattle access sites were selected in the headwaters of the BW and DG catchments, with each cattle access site located on a separate tributary, and labelled A, B and C. These sites represented the uppermost cattle access sites on each of those tributaries. In the BK, CM and MT catchments, three cattle access points were selected that were located longitudinally along a tributary of each catchment, and were labelled 1, 2 and 3. This approach allowed an investigation of the overall effects of cattle access across a range of catchments and aimed at giving insight into the following (see Fig. 3.3):

a. The impacts, if any, of cattle access to watercourses on the selected bed sediment stream microbial (i.e. *E. coli*; Chapter 4) and physicochemical (Chapter 5) parameters at a single access site, i.e. when there are no significant access pressures upstream. To investigate this, nine sites were selected in the headwater zones of the catchments, which had no cattle access upstream (Fig. 3.2). Six of these sites were in the BW and DG catchments with one site on each (the uppermost site) in the BK, CM and MT catchments (see Fig. 3.3);
b. The potential cumulative downstream effects of cattle access sites. Here, the upper sites in BK, CM and MT were used along with two additional sites per stream along a downstream gradient (Fig. 3.2 and 3.3) to investigate potential downstream cumulative effects of cattle access to watercourses on bed sediment freshwater and geochemical parameters.

In Ireland, cattle graze outdoors for the summer months, typically from April to November. For the work presented in this thesis, the stream bed sediments were sampled at each site in April/May and September/October 2016 for sediment characterisation and analysis of sediment nutrient concentrations, June and November/December 2016, for quantification of *E. coli* bacteria sediment concentrations The times of sampling are referred to in this thesis as early grazing season (EG) and late grazing season (LG) in the study investigating the impacts of cattle access on sediment nutrient concentrations (Chapter 5) and as mid-grazing season (MG) and post-grazing season (PG) in the study assessing sediment *E. coli* concentrations (Chapter 4).

Table 3.2. Summary of the characteristics of the study catchments.

Catchment	Munster Blackwater (BW)	Douglas River (DG)	Brackan River (BK)	Commons River (CM)	Milltown Lake (MT)
County	Cork	Laois	Wexford	Louth	Monaghan
Hydrology	Surface	Surface	Sub-surface/Surface	Surface	Surface
Stream Order	1	2	2	2/3	1/2
Predominant soil type[1]	Blanket peat, mineral alluvium	Limestone, sandstone and shale	Poorly drained gleys and some well drained brown earths	Typical and stagnic luvisols, brown earths	Poorly drained mineral soils and peat
Ecological Status (based on Q value)	Good	High/Good	Moderate	Poor	Moderate
Water quality trend (based on Q values)	No change	No change	Deteriorating	No change	No change/Deteriorating
At risk of not meeting WFD objectives?	No	No	Yes	Yes	Yes
Is agriculture a significant pressure?	No	No	Yes	Yes	Yes

Information derived from WFD assessment reports for the study catchments made available by the EPA at https://www.catchments.ie/data/#/?_k=43w5ni.

[1]Information on predominant soil types retrieved from ACP programme for BK and CM catchments; from the National Source Protection Pilot Project (NSPPP) report for the MT catchment; and from Teagasc and GSI maps for the BW and DG catchments.

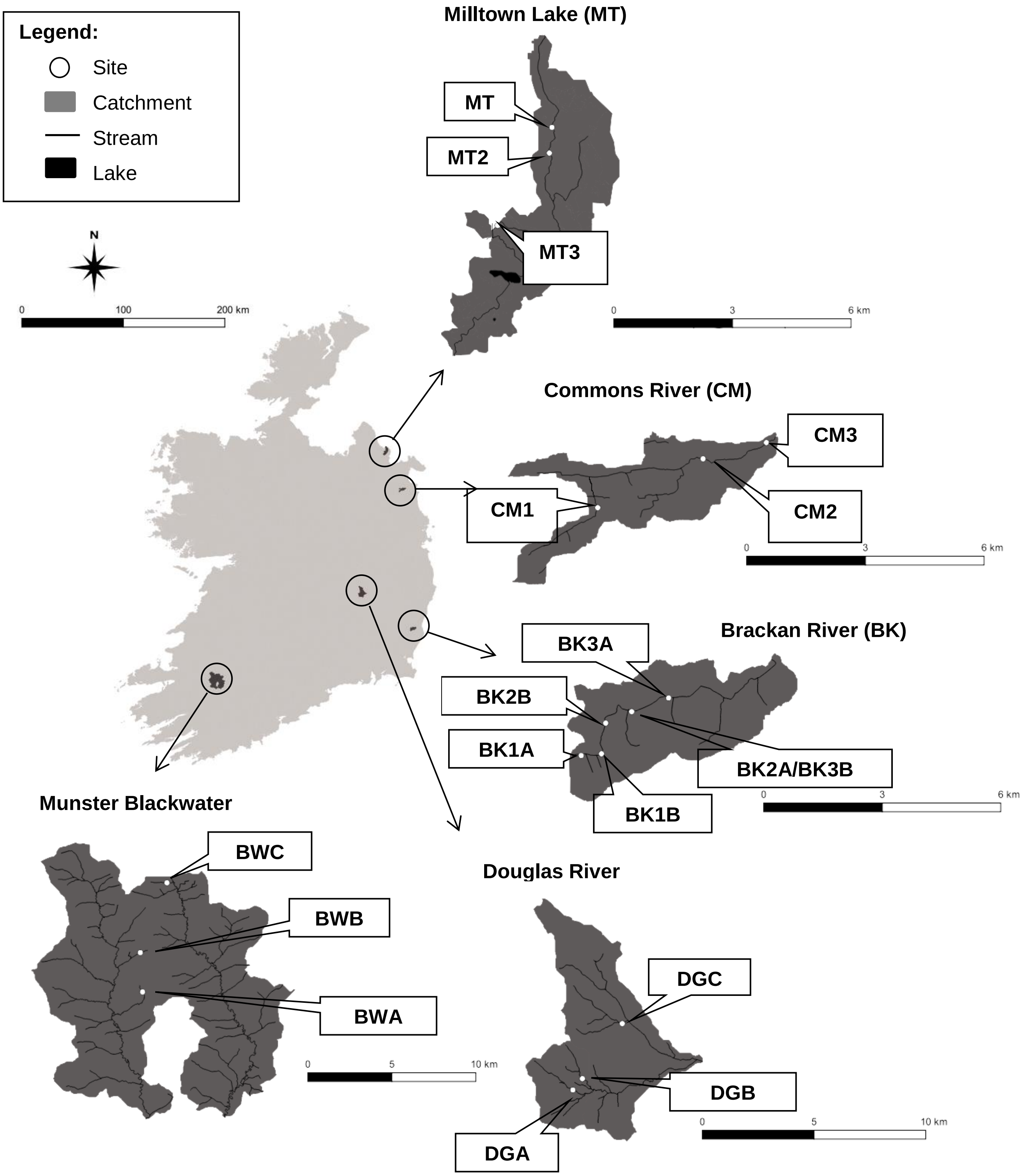

Fig.3.2. Map of the study catchments and selected sites.

Following the first sampling campaign (April – June 2016), sites BK1 and BK3 in the Brackan River catchment became acutely impacted with severe collapse of streambanks such that these sites were no longer suitable for inclusion in the COSAINT project. Therefore, site BK1 and BK2 were replaced by two new sites, while site BK2 in early grazing season became the lower-most site BK3 in late grazing season (Fig.3.2). Given the similarity between the new sites and the replaced sites, it was considered that this substitution did not affect the purposes of this work.

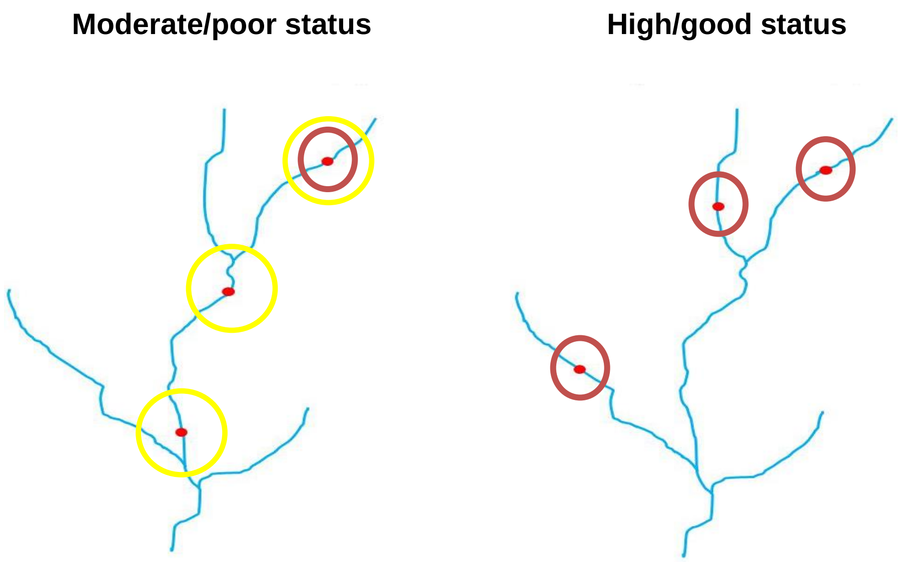

Fig.3.3. Location of the sampled sites in the catchments. Red circles highlight the headwater sites (9 in total), and yellow circles highlight the sites used to assess potential downstream cumulative impacts of cattle access to the streams (9 in total).

Fig 3.4. shows a scheme of the sampling locations at each cattle access site. For the sediment characterisation, samples were collected at the cattle access site (CAS), upstream of the access site at a reach to which cattle had no access, either due to fencing or natural physical barriers (US), downstream (DS) and at the interface (edge) of the stream water level, at the access path used by cattle to enter the stream (INT) (Fig. 3.4). Sampling of this interface area was included (for sediment nutrients only) because it was hypothesis that this area would be subject to a high localised impact of cattle. For assessing sediment faecal contamination with *E. coli*, only US and the cattle access site. At one of the sites, CM3, a high temporal resolution monitoring experiment was conducted between 2016 and 2018 to assess the impacts of direct cattle access to watercourses on water physicochemical and microbial parameters (Chapter 6). Each study catchment and study sites within catchments are described in more detail in the following sections.

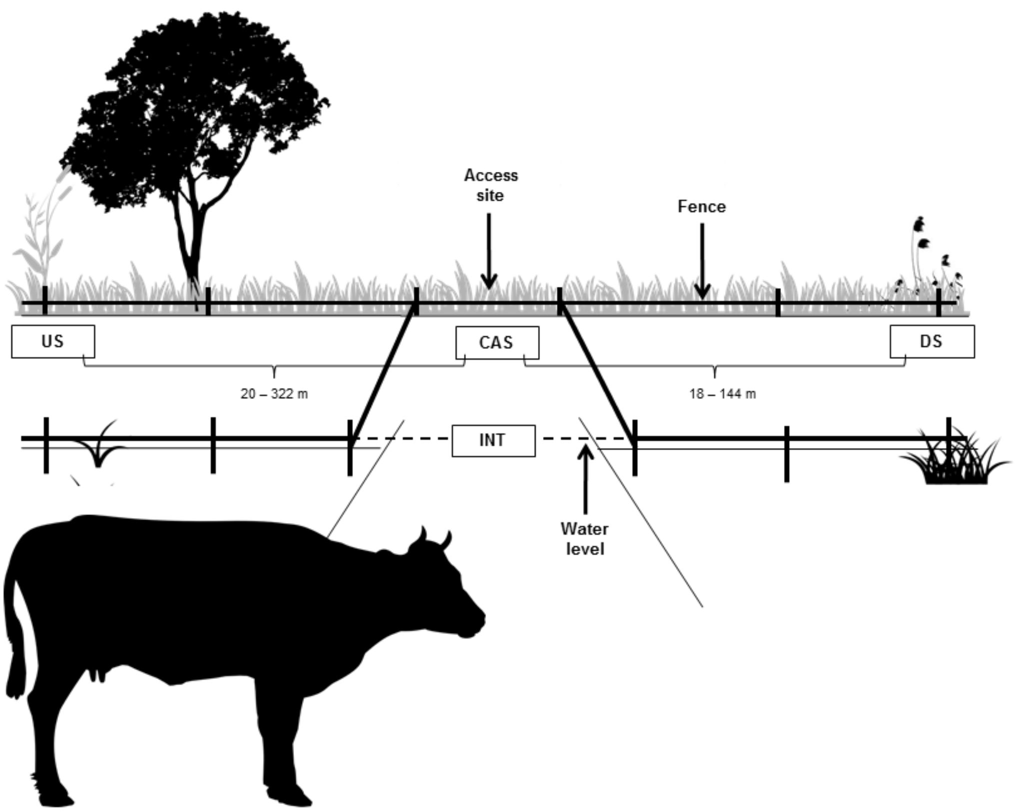

Fig.3.4. Sampling scheme showing the different locations of sample collection at each cattle access site.

3.3. Site description

Catchment land use across sites was principally grassland, although some arable land and plantation forestry were present in the Brackan (Co. Wexford) and Blackwater (Co. Cork) catchments, respectively. Information on the study catchments' ecological, biological and chemical status and stream order were derived from the nearest EPA water quality monitoring stations. Information on flow discharge was retrieved from the nearest flow monitoring station available at https://www.epa.ie/hydronet/#Water%20Levels. Total annual rainfall for each catchment in the year of sampling was calculated using monthly data from the nearest Met Eireann weather station available at the Met Eireann website (https://www.met.ie/climate/available-data). This information is presented in Table 3.3, Table 3.5., Table 3.7, Table 3.9 and Table 3.11.

Stream substrates at all study sites were characterised by visually assessing the mean substrate composition of substrates within each geomorphic unit and standardising these means to the reach scale based on the proportional representation of each geomorphic unit at said reach scale. Stream widths ranged from 0.75 m to 3.5 m. Stream reach gradients at individual sites were calculated using Google Earth tools over a stretch of 600 – 700 m of each stream. These were variable, but there was a tendency towards steeper channels at more upstream sites (particularly in the CM and BW catchments). Data on the type of geological formations and soil type were collected online from the Geological Survey Ireland website (https://www.gsi.ie/en-ie/data-and-maps/Pages/default.aspx) and Teagasc Soil Map (http://gis.teagasc.ie/soils/map.php), as well as from the Agricultural Catchments Programme (ACP) website (https://www.teagasc.ie/environment/water-quality/agricultural-catchments/catchments/) and from the National Source Protection Pilot Project (NSPPP) conducted in the MT catchment.

An attempt to obtain animal numbers and stocking rates for the study sites, was well on duration of grazing season, slurry spreading information and other agricultural management, was made through a survey conducted with the farmers within the COSAINT project (results of the survey are presented in Appendix B1). However, it was not possible to gather clear information for all sites. Hence, for the studies presented in Chapters 4 and 5, total cattle numbers in each catchment were estimated by dividing total cattle numbers per district electoral division (DED), available at the Irish Central Statistics Office website (https://www.cso.ie/en/databases/; figures from the last Agricultural Census, conducted in 2010) by the total pastoral area of each DED. This assumed equal distribution of the cattle within each DED. This is referred to in this study as estimated cattle density (ECD; number of cattle.ha^{-1}). This information is presented in Table 3.4, Table 3.6, Table 3.8, Table 3.10 and Table 3.12.

Cattle access points at the majority of sites were constricted to narrow points of access with fencing or other natural barriers limiting the ability of the cattle to roam within the stream or on stream banks. At some sites however, such as at DGA and CM2, cattle had access to longer stretches of channel and stream bank. All sites were characterised by a Riparian Habitat Index (RHI), developed by O'Sullivan et al. (2019). This index is a metric of the condition of the riparian habitat and can reflect the intensity of cattle access in this study. It was developed by calculating several subindices of qualitative habitat assessment published in literature and adapting the results to reflect the Irish landscape (see O'Sullivan et al., 2019 for details). The maximum achievable RHI score is 80. Here, the RHI of the streams at the cattle access sites and at areas upstream of these sites, to which cattle had no access, are provided to enable a comparison of general habitat quality between the sites.

3.3.1. Munster Blackwater catchment

The selected sites in the Munster Blackwater were part of the River Allow sub-catchment. The total population of the Munster Blackwater catchment is approximately 109 030 with a population density of 33 people per km^2 (EPA, 2020). The River Allow sub-catchment is 310 km^2 and 70% of the soils are poorly drained mineral soils. The dominant hydrology pathway is surface runoff. Blanket peat covers 5% with mineral alluvium being associated with the river channels. The main agricultural land use is grassland for beef and dairy.

River Blackwater and its tributaries, in particular the River Allow, are classed as Special Areas of Conservation (SAC) due to presence of many species and habitats of European importance. These include a number of EU Habitats Directive Annex II listed species, such as the freshwater pearl mussel (*Margaritifera margaritifera*), the salmon (*Salmo salar*) and otter (*Lutra lutra*), and the EU Birds Directive Annex I listed species, the kingfisher (*Alcedo atthis*).

The nearest EPA stations to the study sites were stations RS18B020075 and RS18O090400, which reported good water quality status for the sampling period (Table 3.3). However, pre-sampling (invertebrate kick sampling) of the sites under the COSAINT project indicated that these study sites were in high-status streams.

Table 3.3. Description of the Munster Blackwater (BW) catchment. Water quality status information was derived from Edenireland.ie; information at time of sampling (2016/2017).

Catchment	Munster Blackwater
River	Munster Blackwater (Duhallow Region)
County	Cork
Mean annual flow ($m^3.s^{-1}$)[1]	0.059
Total annual precipitation in 2016 (mm)[2]	1540.9
Hydrology	Surface
Ecological status (Sampling period)	Good
Biological status (Sampling period)	Good
Chemistry conditions (Sampling period)	Pass
Nutrient Condition	Pass
Ortho-P status quality (trend)	High (downwards)
WFD Risk	Not At Risk
Waterbody trend	No change
Significant Pressures	None

[1]Flow data retrieved from EPA Islandbrack station. [2]Precipitation data retrieved from the Ballydesmond Met Éireann station.

Table 3.4. Summary characteristics of the study sites (BWA, BWB, and BWC) in the Munster Blackwater (BW) catchment**.**

<table>
<tr><th colspan="2"></th><th>BWA</th><th>BWB</th><th>BWC</th></tr>
<tr><td colspan="2">Stream order</td><td>1</td><td>1</td><td>1</td></tr>
<tr><td colspan="2">Stream width (m)</td><td>1.95</td><td>1.57</td><td>1.42</td></tr>
<tr><td colspan="2">Reach gradient (%)</td><td>-2.70</td><td>-4.60</td><td>-3.00</td></tr>
<tr><td colspan="2">Soil type</td><td colspan="3">Blanket peat soils with interspersed poorly drained mineral soils</td></tr>
<tr><td colspan="2">Geologic formations</td><td colspan="3">Carboniferous limestone and shale</td></tr>
<tr><td rowspan="4">Substrate (%)</td><td>Boulder</td><td>4</td><td>6</td><td>10</td></tr>
<tr><td>Cobble</td><td>78</td><td>60</td><td>81</td></tr>
<tr><td>Gravel</td><td>16</td><td>32</td><td>8</td></tr>
<tr><td>Sand+Silt</td><td>2</td><td>2</td><td>1</td></tr>
<tr><td colspan="2">Site description</td><td>Open access site, nearby a bridge; ditch draining opposite the site; vegetated banks</td><td>Open access site, other cattle access points downstream; vegetated banks</td><td>Open access site; few ditches draining into the stream; vegetated banks</td></tr>
<tr><td colspan="2">RHI scores (upstream; access site)</td><td>66; 48</td><td>50; 36</td><td>48; 21</td></tr>
<tr><td colspan="2">Estimated cattle density (animals.ha^{-1})</td><td>1.47</td><td>0.92</td><td>1.32</td></tr>
</table>

Fig.3.5. Cattle access sites in the Munster Blackwater (BW) catchment. From the top to the bottom: BWA, BWB, BWC.

3.3.2. Douglas River catchment

The Douglas river catchment is located in the east of Ireland (55°12’N, 6°42’W) (Fig. 3.2). The catchment is a sub-catchment of the Barrow River catchment, which has a total population of approximately 188 117 and population density of 62 people per km^2 (EPA, 2020).

The DGA site is on the Fuer River and is upstream of the EPA monitoring station 14DO30100, which reported a high water quality status (Table 3.5). Sites DGB and DGC are on the Douglas River and are upstream of EPA monitoring station 14DO30040, which reported a good water quality status (Table 3.5). However, pre-sampling (invertebrate kick sampling) of the sites under the COSAINT project indicated that all sites were in high-status streams (O’hUallachain et al., 2020).

Table 3.5. Description of the Douglas River (DG) catchment. Information derived predominantly from Edenireland.ie; information at time of sampling (2016/2017).

Catchment	Barrow
River	Douglas
County	Laois
Flow ($m^3.s^{-1}$)[1]	0.015
Total annual precipitation in 2016 (mm)[2]	669.9
Hydrology	Surface
Ecological status (Sampling period)	Good (DGB, DGC); High (DGA)
Biological status (Sampling period)	High
Chemistry conditions (Sampling period)	Pass (DGB, DGC): NA (DGA)
Nutrient Condition	Pass (DGB, DGC): NA (DGA)
Ortho-P status Quality (trend)	Moderate (Downwards) (DGB, DGC; NA (DGA)
WFD Risk	Not At Risk
Waterbody trend	No change
Significant Pressures	None

[1]Flow data retrieved from the EPA Clonagh station.[2] Precipitation data etrieved from the Athy (Levitstown) Met Eireann station. NA – Not Assigned

Table 3.6. Summary characteristics of the study sites in the Douglas River (DG) catchment.

		DGA	**DGB**	**DGC**
Stream order		2	2	2
Stream width (m)		1.93	2.00	2.00
Reach gradient (%)		-2.10	-2.60	-3.40
Substrate (%)	Boulder	0	0	0
	Cobble	79	67	81
	Gravel	15	28	18
	Sand + Silt	6	5	1
Soil type		Well drained mineral soils	Poorly drained mineral soils	
Geologic formations		Carboniferous limestone, sandstone and shale		
Site description		Access site is a crossing point between fields; vegetated banks	Open access site; multiple access points in the field	Discrete site, steep slope; vegetated banks
RHI scores (upstream; access site)		56; 28	62; 50	80; 69
Estimated cattle density (animals.ha^{-1})		1.81	1.81	1.81

Fig.3.6. Cattle access sites in the Douglas River (DG) catchment. From the top to the bottom: DGA, DGB.

3.3.3. Brackan River catchment

The Brackan River sub-catchment (11.0 km^2) is located in the south-east of Ireland (co. Wexford) (52°36'N, 6°20'W) (Figure 3.2). It is part of the Owenavorragh catchment which has a total population of approximately 27 319 and population density of 69 people per km^2 (EPA, 2020). The catchment is part of the Agricultural Catchments Program (Teagasc, 2017a), which operates a hydrometric station nearby the catchment outlet that collects high temporal resolution data on a variety of water physicochemical parameters.

The soil type is predominantly poorly-drained groundwater gleys in the catchment lowlands with a clay loam texture in A- and B-horizons resulting from a clayey calcareous Irish Sea till subsoil. The uplands contain smaller areas of well-drained brown earths; these soils are underlain by drift deposits with siliceous stones. The underlying geology is permeable, dominated by Ordovician volcanics and metasediments of the Campile formation (Tietzsch-Tyler et al., 1994), which form a productive aquifer with faults (Mellander et al., 2012). Artificial drainage is a key feature including open drains, defined here as ditches, and closed, sub-surface piped drains (predominantly 80 mm diameter). This catchment is considered to be dominated by overland flow pathways (Mellander et al., 2012; Shore et al., 2013) except for areas of well-drained soils featuring sub-surface transport pathways. Land is predominantly grass-based for dairy and beef cattle grazing, and also sheep enterprises (Shore et al., 2013). Arable crops such as spring barley are common on the well-drained soils which are unmanaged between harvest and ploughing for following crop. The stream where the study sites were located is a tributary of the Owenavorragh river, with the nearest downstream EPA monitoring station being 11O010400, reporting a likely water quality status of moderate (Table 3.7)

Table 3.7. Description of the Brackan River (BK) catchment. Information derived predominantly from Edenireland.ie at time of sampling (2016/2017).

Catchment	Owenavorragh
River	Brackan River
County	Wexford
Flow ($m^3.s^{-1}$)[1]	0.200
Total annual precipitation in 2016 (mm)[2]	985.7
Hydrology	Sub-surface/ Surface
Ecological status (Sampling period)	Moderate
Biological status(Sampling period)	Good
Chemistry conditions (Sampling period)	Moderate
Nutrient Condition	Fail
Ortho-P status quality (trend)	Moderate (downwards)
WFD Risk	At Risk
Waterbody trend	Downward
Significant Pressure	Agriculture

[1]Flow data retrieved from Office of Public Works (OPW) station Boleany. [2]Precipitation data retrieved from Met Eireann station Monamolin.

Table 3.8. Summary characteristics of the study sites in the Brackan River (BK) catchment.

		BK1A	BK1B	BK2A/BK3B	BK2B	BK3A
Stream order		2	2	2	2	2
Stream width (m)		0.67	0.93	2.40	2.00	2.34
Reach gradient (%)		-3.83	-2.60	-1.40	-1.50	-0.51
Substrate (%)	Boulder	0	0	2	2	0
	Cobble	26	23	72	55	5
	Gravel	56	60	10	23	80
	Sand + Silt	19	17	16	10	14
Soil type		Mineral soils, mostly well drained with poorly drained soils interspersed				
Geologic formations		Ordovician, rhyolitic grey and brown shales				
Site description		Very shallow and narrow stream; vegetated banks; sheep graze in surrounding fields	Narrow stream; open access site; ditch draining upstream of the access site	Open access site with vegetation impeding access upstream; vegetated banks and ditches draining into the stream	Discrete site, next to bridge; vegetated banks	Open access crossing site; vegetated banks, sheep graze in surrounding fields
RHI scores (upstream; access site)		NA	48; 43	28; 41	68; 54	NA
Estimated cattle density (animals.ha^{-1})		1.68	1.72	1.98	1.72	1.98

A – site sampled during Spring/Summer 2016; B – Sites sampled during Autumn/Winter 2016, following site replacement. NA – Not assigned





Fig.3.7. Cattle access sites in the Brackan River (BK) catchment. From the bottom, clockwise: BK3A, BK2A/BK3B, BK1A, BK1B BK2B.

3.3.4. Commons River catchment

The Commons River catchment (9.5 km^2) is located in north-east Ireland (53°49'N, 6°27'W), and, similarly to the Brackan River catchment, it is monitored under the Agricultural Catchments Program (Teagasc, 2017a). The Commons River catchment is part of the Newry, Fane, Glyde and Dee catchment which is a cross border catchment with a total population in the Republic of Ireland of 115 900 people, with a population density of 83 people per km^2 (EPA, 2020). It has a complex pattern of poor- to moderately-drained soils (Melland et al., 2012) with a loam soil texture dominating the A-horizon whereas clay loams are dominant in the B-horizon. The subsoil is dominated by fine till containing siliceous stones with fluvioglacial sediments located near-channel. Soils are underlain by calcareous greywacke and banded mudstone geology and produce a poorly productive aquifer (Mellander et al., 2012). Hydrologically, surface pathways dominate; however, below-ground pathways may also be important especially during winter (Melland et al., 2012; Mellander et al., 2012). Artificial drainage is dominant, particularly in the poorly-drained catchment areas. Arable land is dominated by winter-sown cereals, but also comprises maize and potatoes. These areas are unmanaged between cropping cycles; however, crop rotation is common. Additional areas of permanent grassland are utilised for dairy cattle, beef cattle, and sheep grazing. The river is a tributary of the White River, with the nearest downstream EPA monitoring station being 06W010400, reporting a likely poor water quality status (Table 3.9).

Table 3.9. Description of the Commons River (CM) catchment. Information derived predominantly from Edenireland.ie; information at time of sampling (2016/2017).

Catchment	Newry, Fane, Glyde and Dee
River	Commons River
County	Louth
Flow (m^3/s)[1]	0.019
Total annual precipitation in 2016 (mm)[2]	715.9
Hydrology	Surface
Ecological status (Sampling period)	Poor
Biological status (Sampling period)	Poor
Chemistry conditions (Sampling period)	Pass/Good
Nutrient Condition	Pass/Fail
Ortho-P status quality (trend)	Moderate/Poor
WFD Risk	At Risk
Waterbody trend	No change/ Deteriorating
Significant Pressure	Channelisation; Agriculture; Urban pressures

[1]Flow data derived from the EPA station Coneyburrow Br.. [2]Precipitation data retrieved from the Met Eireann stationTogher (Barmeath Castle).

Table 3.10. Summary characteristics of the study sites in the Commons River (CM) catchment.

		CM1	**CM2**	**CM3**
Stream order		2	3	3
Stream width (m)		1.52	1.96	2.90
Reach gradient (%)		-5.90	-2.60	-3.20
Substrate (%)	Boulder	0	0	0
	Cobble	77	57	79
	Gravel	13	22	14
	Sand+Silt	10	21	7
Soil type		Poorly drained mineral soils		Deep well drained mineral soils
Geologic formations		Silurian greywacke and mudstone		
Site description		Fenced upstream of the site; vegetated banks; other cattle access points downstream	Open access site; crossing point between two adjacent fields; one access point upstream; vegetated banks	Discrete access site; vegetated banks; only access site in the field
RHI scores (upstream; cattle access)		80; 35	46; 28	64; 45
Estimated cattle density (animals/ha)		2.06	2.12	2.04

Fig.3.8. Cattle access sites in the Commons River (CM) catchment. From the top to the bottom: CM1, CM2, CM3.

3.3.5. Milltown Lake catchment

The Milltown lake catchment (30.6 km^2) is located in north-east Ireland (55°12'N, 6°42'W). It is a sub-catchment of the Newry, Fane, Glyde and Dee catchment. The Drumleek River is the only inflow to Milltown Lake. The catchment total area, excluding Milltown Lake, is 28.8 km^2 (Linnane et al. 2011).

The river branches into three inflowing tributaries 440 m above the lake. Two of the larger tributaries are themselves fed by small lakes: a small lake on the western tributary in Carnagh Forest, Tievenamara, and Gentle Owen's Lake on the middle tributary (Carson, 2010). The area is comprised of small hills with poorly drained alluvial gleys, peaty gleys and inter-drumlin peats with extensive blanket bog. Drainage improvements mean that flashy storm flows and suppressed base flows now characterise the hydrology of the catchment (Wynne, 2012). Land use is predominantly agricultural, with grassland accounting for 90% of agricultural land. However, small areas of other agriculture, forestry and peat bog are also present in the upper section of the catchment. The nearest EPA monitoring station to the study sites is RS06G040080, reporting a likely moderate water quality status (Table 3.11)

Table 3.11. Description of the Milltown Lake (MT) catchment. Information derived predominantly from Edenireland.ie; information at time of sampling (2016/2017).

Catchment	Newry, Fane, Glyde and Dee
River	Gentle Owen's Lake Stream
County	Monaghan
Flow (m^3/s)[1]	0.033
Total annual precipitation (mm)[2]	906.8
Hydrology	Surface
Ecological status (Sampling period)	Moderate
Biological status (Sampling period)	Moderate
Chemistry conditions (Sampling period)	Pass
Nutrient Condition	Pass
WFD Risk	At Risk
Ortho-P status quality (trend)	NA
Waterbody trend	No change
Significant Pressures	Agriculture; wastewater

[1]Flow data derived from the EPA Drumleek station. [2]Precipitation data derived from the Met Eireann Coose – Castleblayney station. NA – Not Assigned

Table 3.12. Summary characteristics of the study sites in the Milltown Lake (MT) catchment.

		MT1	MT2	MT3
Stream order		1	1	2
Stream width (m)		1.90	2.28	2.96
Reach gradient (%)		-2.20	-2.10	-1.40
Substrate (%)	Boulder	4	0	3
	Cobble	85	73	75
	Gravel	10	25	30
	Sand + Silt	1	2	2
Soil type		Poorly drained peaty soils		Shallow mineral soils
Geologic formations		Silurian shale	Ordovician black shale	Silurian shale
Site description		Discrete access site; steep field; vegetated banks, some shrub coverage	Open access site, with other small access points upstream; vegetated banks	Open access site located by bridge; another (partially restricted) access site approximately 20 m upstream; vegetated banks
RHI scores (upstream; access site)		72; 53	37; 18	43; 27
Estimated cattle density (animals.ha^{-1})		2.21	2.21	2.00

Fig.3.9. Cattle access sites in the Milltown Lake (MT) catchment. From the top to the bottom: MT1, MT2, MT3.

4. Impacts of unrestricted cattle access to watercourses on streambed sediment faecal contamination in agricultural streams

Chapter 4. Impacts of unrestricted cattle access to watercourses on streambed sediment faecal contamination in agricultural streams

4.1. Introduction

Agriculture is recognised as a significant contributor to pollution and impairment of surface waters throughout the world (Smith et al., 1999; Malmqvist and Rundle, 2002; Pärn et al., 2012). While many of these impacts arise from diffuse pathways of contamination (e.g Crowther et al., 2002; Collins et al., 2005; Davies-Colley et al., 2008; Douglas et al., 2007; Ulén et al., 2007; Miller et al., 2010), point sources can also play an important role. These include sites where livestock directly access the watercourses for drinking and/or crossing between fields (Vidon et al., 2008; Smolders et al., 2015; O'Callaghan et al., 2018; O'Sullivan et al., 2019). Such activity can result in the removal of riparian vegetation, bank erosion (Kauffman et al., 1983), stream channel degradation (Trimble and Mendel, 1995; Herbst et al., 2012), increased sedimentation (Sheffield et al., 1997; Sovell et al., 2000; O'Sullivan et al., 2019), changes in biotic communities (Conroy et al., 2016) and contamination of waters with potentially pathogenic faecal organisms as a consequence of direct excretion into the stream channel (Eyles et al., 2003; Davies-Colley et al., 2004; Smolders et al., 2015).

A common indicator of faecal contamination is the bacterium *Escherichia coli*, which constitutes part of the normal gastrointestinal flora of humans and warm-blooded animals. *Escherichia coli* (*E. coli*) bacteria are generally commensal, however there strains capable of causing a variety of diseases (Kaper et al., 2004; Croxen et al., 2013), that are generally divided in three clinical syndromes: enteric or diarrhoeal diseases, urinary tract infections and sepsis/meningitis (Kaper et al., 2004). These pathogenic variants, or pathotypes, are an important cause of morbidity and mortality worldwide (Croxen et al. 2013). Among the pathotypes that cause enteric disease is verocytotoxigenic *E. coli* (VTEC) e.g. the O157 serotype (Karmali et al., 2010; Petit et al., 2017). *E. coli* O157 can cause severe illness in humans, particularly young children (Brehony et al., 2018; Pennington, 2010; Rice et al., 2016), and, although it is asymptomatic in cattle (Murphy et al., 2016), infected animals can excrete the microorganism in numbers as high as 10^9 colony forming units (CFU) per g in their faeces (McCabe et al., 2018). The potential severity of illness associated with VTEC, together with its very low infectious dose (10 – 100 bacterial cells) (Brehony et al., 2018; Murphy et al., 2016; Rice et al., 2016) and its reported ability to survive in the environment for prolonged periods of time (Money et al., 2010) make VTEC infection a major health concern associated with cattle-based agriculture (Óhaiseadha et al., 2016; Brehony et al., 2018) (see Table 4.1 for concentrations of *E. coli* bacteria in cow faeces reported in literature).

Table 4.1 Concentrations of *E. coli* bacteria in cattle faeces reported in literature.*figures presented are calculated from reported results.

Reference	***E. coli* concentration in cattle faeces**
Avery et al. (2004)	7.70 $\log_{10}$CFU .g^{-1}
Davies-Colley et al. (2004)	1.2×10^7 CFU.g^{-1} (7.08 $\log_{10}$CFU.g^{-1})
Weavers and Graves (2005)	5.88 $\log_{10}$CFU.g^{-1} (pasture cattle) 7.27 $\log_{10}$CFU.g^{-1} (penned cattle)
Oliver et al. (2010)	7.12 $\log_{10}$CFU.g dry wt^{-1}
Olandeinde et al. (2014)	2.20×10^6 MPN.g dry wt^{-1}
Oliver and Page (2016)*	6.90 $\log_{10}$CFU dry wt^{-1}

In Ireland, agricultural land covers approximately 72% of the total land area (EC, 2016), of which 80% is permanent grassland (EC, 2015c). Bovines constitute approximately 82% of the total livestock units (LSU) (Eurostat, 2019). The Irish Environmental Protection Agency (EPA) has identified agriculture as a major source of pollution of Irish waterbodies (Fanning et al., 2017). Faecal contamination of drinking water supplies in rural areas in Ireland has continuously been recorded (EPA, 2018), and the country has consistently reported the highest incidence rate of VTEC infection in the EU (Garvey et al., 2015; Óhaiseadha et al., 2016; Brehony et al., 2018), which was 20.0 cases per 100 000 population in 2018, compared to an average incidence of 2.4 per 100,000 in the rest of the EU (ECDC, 2020). The consumption of contaminated water from rural household wells, which are exempt from regulations and monitoring, has been identified as the most significant primary transmission pathway of *E. coli* O157 infection (Garvey et al., 2015; Óhaiseadha et al., 2016).

The use of *E. coli* to assess recent environmental faecal contamination (Pachepsky and Shelton, 2011; Cloutier and Mclellan, 2017) is based on the assumptions that these bacteria do not multiply or persist for long periods of time outside of the host (Pachepsky and Shelton, 2011). However, as discussed in previous chapters, it is now widely recognised that *E. coli* can persist in the environment, particularly in sediments. In stream waters, bacteria often become attached to sediment particles which are then deposited on the stream bed, where they can accumulate in concentrations that are generally orders of magnitude higher than those of the overlying waters (Pachepsky and Shelton, 2011) and where they have been observed to persist for weeks to months (Davies et al., 1995; Craig et al., 2004;). The role of sediments in favouring bacterial persistence has been demonstrated in different environments, including coastal sediments (e.g. Craig et al., 2004; Ishii et al., 2007), and freshwater sediments (e.g. Muirhead et al., 2004; Garzio-Hadzick et al., 2010). Thus, bed sediments can act as sinks for faecal contamination, but they can also act as sources (Ishii et al., 2007; Hassard et al., 2016) when disturbance of contaminated sediments as a result of cattle in-stream movements (Collins and Rutherford, 2004) or of high flow conditions (Nagels et al., 2002; Muirhead et al., 2004) causes resuspension of viable faecal organisms into the water column (Jamieson et al., 2005a; Ouattara et al., 2011). Furthermore, studies have indicated that *E. coli* can become naturalised and integrated in environmental indigenous microbial communities (Ishii and Sadowsky, 2008; Perchec-Merien and Lewis, 2012; Jang et al., 2017). Therefore, the bed sediment *E. coli* population would represent a combination of recent contamination and naturalised communities.

Water quality protection measures that limit or restrict cattle access to watercourses (e.g. fencing), have been demonstrated to have positive effects on water quality (McKergow et al., 2001; Agouridis et al., 2005; O'Callaghan et al,, 2018), including reducing faecal contamination (e.g. Line, 2003; Wilcock et al., 2013; Smolders et al., 2015; Bremner et al., 2016; Bragina et al., 2017), and have been frequently included in agri-environmental policy. In Ireland, under the current *Green Low Carbon Agri-Environment Scheme* (GLAS), approximately 20,100 farmers have committed to implement cattle exclusion measures (GLAS Farm Planners [GFP], 2020). Restricting cattle access to watercourses through fencing will also become compulsory in 2021 (DoHPLG, 2018b) for approximately 12 000 derogation farmers under the fourth Nitrates Action Programme. Nevertheless, despite the known impacts of cattle-based agriculture on faecal contamination of surface waters and the wide application of cattle exclusion measures in agricultural policy, the contribution of direct cattle access to stream bed sediment *E. coli* contamination, and how this contamination can persist, has rarely been addressed in a European context. Bragina et al. (2017) recently showed that the levels of *E. coli* in stream sediments in one of the catchments in the current study (Milltown Lake catchment – see Chapter 3) were significantly higher where cattle had access to the stream than where access was restricted through fencing. In that study, average *E. coli* in sediments in a fenced stream ranged from 1.0×10^2 to 3.7×10^2 CFU.g dry wt^{-1-}, whereas in unfenced streams these ranged from 4.8×10^2 to $5.2.\times 10^5$ CFU.g dry wt^{-1} (Bragina et al. 2017). Interestingly, the authors also reported average *E.* coli concentrations of 1.9×10^4 CFU.g dry wt^{-1} at one cattle access site than remained unfenced in their fenced study stream (Bragina et al., 2017). The present study builds on those findings and aims to garner a better understanding of whether unrestricted cattle access can have similar impacts on sediment faecal contamination across Irish catchments generally, and across a range of water quality status and agricultural intensities. It is the first study attempting to quantify this contamination at multiple sites, in Ireland or elsewhere, while also contributing to a general understanding of the effects of unrestricted cattle access on faecal contamination of rural watercourses. The specific objectives of this study were:

a. to quantify background levels (i.e. with no cattle access) of *E. coli* in bed sediments in first to third order streams in five agricultural catchments across Ireland;
b. to investigate the impacts of cattle access on stream bed sediment *E. coli* contamination;
c. to establish whether this contamination varied from mid-grazing season to post grazing season; and
d. to determine whether there was a downstream cumulative effect of sediment *E. coli* contamination in streams with several access sites.

4.2. Methods

4.2.1. Site selection and experimental design

The study on sediment *E. coli* concentrations was separated into two study designs, described in detail in Chapter 3 (Table 4.2). Study 1 aimed to assess the impact of cattle access on *E. coli* levels in sediment in the headwater sites. For this purpose, nine sites were selected in the headwater zones of the catchments. Six of these sites were in the BW and DG catchments (labelled BWA, BWB, BWC and DGA, DGB and DGC), with one site on each of the BK, CM and MT catchments (labelled BK1, CM1 and MT1). The levels of sediment *E. coli* upstream of these sites, where cattle did not have access to the water, were used to assess background levels of *E. coli* contamination. Study 2 investigated potential downstream cumulative effects of cattle access to watercourses on sediment faecal pollution. Here the upper sites in BK, CM and MT were used along with two additional sites per stream along a downstream gradient (sites labelled 1 – 3) (See Chapter 3).

Table 4.2. Allocation of study sites in Study 1 and Study 2 of the experimental design.

Study	Objective	Sites included
Study 1	Investigate the impacts of cattle access to watercourses on stream sediment *E. coli* levels in headwater sites (i.e. uppermost sites)	MT1, CM1, BK1 DGA, DGB, DGC BWA, BWB, BWC
Study 2	Assess potential cumulative effects of cattle access to watercourses on sediment *E. coli* contamination (i.e. downstream gradient along sites)	MT1, MT2, MT3 CM1, CM2, CM3 BK1, BK2, BK3

In Ireland, cattle graze outdoors during the spring and summer months (generally April to October/November) and are housed over the winter months. The 15 sites (Study 1 and Study 2) were sampled in mid-grazing season (June) and in post-grazing season (late November – early December) after cattle had been housed for the winter period, in 2016. These sampling times were selected to ensure that the impacts of cattle access to watercourses on sediment faecal contamination were captured, in accordance with the findings of Bragina et al. (2017), who sampled in three key points of the agricultural management cycle in Ireland (April, July and October) in two consecutive years. The authors reported a recurrent seasonal pattern in which *E. coli* sediment concentrations were the lowest in April, after cattle had been absent from the grazing fields for the winter period, and the highest in July, during grazing season. Thus, June was selected in the current study to assess sediment *E. coli* concentrations when the impact of cattle access would have been apparent for several months. This was to capture faecal contamination during grazing season while combining microbial sample collection with sampling for other studies within the COSAINT project.

4.2.2. Site description

The study sites are described in detail in Chapter 3.

4.2.3. Sediment sampling

On each sampling occasion, three sediment samples were collected randomly at the cattle drinking sites and three were collected upstream (10 – 300 m) of these sites (i.e. a total of six samples per site). The samples were collected into new Petri dishes (1.2 cm depth, 8.5 cm diameter) by placing the dishes upside down onto the stream sediment and lifting them with a help of a metal scrapper that had been introduced under the Petri dish (Bragina el al., 2017). To avoid cross contamination, the metal scrapper was sterilised between samples using a solution of 70% industrial methylated spirit (IMS) and rinsed with deionised water (Bragina et al., 2017). The Petri dishes containing sediment samples were sealed using Parafilm and transported to the laboratory in a cooler box for subsequent analysis.

All samples were stored in the dark at the temperature of 4˚C and analysed within 24 hours where possible; in a small number of instances, where long distance travelling did not allow the completion of analysis within this time period, the analysis was concluded within a maximum of 48 hours. Analysis of *E. coli* densities for samples held at less than 10˚C within 48 hours of collection has been shown to yield comparable results to those obtained in analyses undertaken within 8 hours of sample collection (Pope et al. 2003).

4.2.4. *E. coli* enumeration

For bacterial extraction from sediment, a technique previously described by Boehm et al. (2010) and adapted by Bragina et al. (2017) was used. The sediment samples were thoroughly mixed prior to extraction to ensure homogeneity. Approximately 10 grams of wet sediment were added to 90 ml of Ringer diluent (Oxoid, Hampshire, England) in a sterilised 100 ml Duran bottle. Each bottle was hand shaken for one minute and allowed to settle for a further minute before a set of sequential dilutions was prepared. Each dilution was filtered through a sterile cellulose esters membrane with 0.45 µm pore size with grids (British Standard Institution, 2000). The membranes were placed onto Petri dishes containing Harlequin™ *E. coli/*Coliform medium (LabM, Lancashire, UK) and incubated at 37 ˚C for 18 – 24 hours according to the manufacturer's instructions. All green-blue colonies were counted as presumptive *E. coli* bacteria.

4.2.5. Sediment characterisation

The sediment moisture content was determined as described by Hanlon et al. (2000). Approximately 20 g of fresh sediment from each sample were weighed in crucibles, dried at 105 ˚C for 24 hours and placed in a dried atmosphere (desiccator) to allow the crucibles to cool down. The samples were then re-weighed, with the difference in the sediment sample weight giving the moisture content. Sediments at the 15 study sites were further characterised as part of the study described in Chapter 5. The upper 3 – 5 cm of bed sediment were collected using a core sampler with a diameter of approximately 73 mm at each cattle access site and upstream, with a total of 6 samples collected at each location. The samples were oven-dried at 105˚C for at least 24 hours and sieved to < 2 mm particle size. Composite samples for each location were then made using a quartering technique to allow homogeneous sectioning of each individual sample, and further analysed. Sediment Sediment organic matter content has been reported as a significant factor controlling the survival of *E. coli* in sediment (e.g. Bragina et al., 2017). For sediment characterisation, organic carbon concentrations were determined using an Elementar El Vario Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany), following removal of inorganic carbon by exposing dry sediments to concentrated HCl (37%) fumes (Harris et al., 2001). Samples were calibrated against a standard (acetanilide) (Merck, Darmstadt, Germany) with known concentrations of carbon (71.09%). Sediment characterisation data are presented in Table 4.3.

Table 4.3. Moisture content of the sediment samples and sediment organic carbon at the study sites (mean ± S.E.).

Catchment	Site	Moisture (%)		Organic carbon (mg g dry wt $^{-1}$)	
		Mean ± S.E.	*n*[1]	*Mean ± S.E.*	*n*[1]
BW	BWA	27.86 ± 2.51	12	23.97 ± 1.28	8
	BWB	29.18 ± 4.90	12	28.31 ± 8.29	8
	BWC	32.39 ± 3.34	12	24.01 ± 3.01	8
DG	DGA	29.45 ± 3.42	12	30.69 ± 2.66	8
	DGB	24.93 ± 1.74	12	21.21 ± 1.50	8
	DGC	21.75 ± 0.48	12	21.86 ± 2.46	8
BK	BK1MG	34.79 ± 4.32	6	21.44 ± 5.98	4
	BK2MG / BK3PG	27.70 ± 3.14	12	24.94 ± 1.91	8
	BK3MG	16.49 ± 0.99	6	18.82 ± 1.23	4
	BK1PG	29.93 ± 5.46	6	16.12 ± 2.23	4
	BK2PG	33.15 ± 4.12	6	16.88 ± 4.65	4
CM	CM1	19.66 ± 2.16	12	34.29 ± 3.94	8
	CM2	21.16 ± 1.29	12	31.22 ± 5.68	8
	CM3	29.92 ± 3.71	12	36.28 ± 5.57	8
MT	MT1	41.98 ± 6.88	12	44.83 ± 7.66	8
	MT2	29.03 ± 1.80	11	40.78 ± 4.19	8
	MT3	47.71 ± 7.34	10	29.20 ± 1.97	8

[1]Moisture content (%) was determined in sediment samples used in microbiological analysis (collected in the two sampling times, except in BK sites sampled in only one sampling time); OC was determined in sediment samples for the same sites analysed for nutrients (discussed in Chapter 5).

4.2.6. Statistical analysis

All *E. coli* data were $\log_{10}$ transformed prior to all analyses, to account for the extreme range in the data. All analyses were carried out using R software (version 3.5.1; R Core Team, 2016). Assumptions of equal variance and independence were checked for in all statistical analyses using the methods described in Zuur et al. (2009).

Differences in background sediment bacteria concentrations (i.e. at upstream sites) in each of the nine headwater sites (Study 1) and between the two sampling times were assessed using a two-way analysis of variance (ANOVA). For the remaining analyses where comparisons of bacterial concentrations at cattle access sites (CAS) and at upstream sites (US) were conducted, mean values for bacterial concentrations from the three samples taken (n=3) were used to avoid pseudo-replication. For Study 1, a general assessment of the differences in sediment bacteria concentrations between US and CAS (factor Treatment) and between sampling times (factor Time) was performed using a repeated measures two-way ANOVA, with interactions included. Further comparisons of bacteria concentrations between US and CAS for each sampling time were conducted using a post-hoc test (Tukey test) as well as paired t-tests.

For Study 2, differences in *E. coli* concentrations between US and CAS, sampling times, and sites were assessed using a mixed effects model analysis where factor Catchment was included in the model as a random component, and a variance structure for factor Treatment was added to account for unequal variance in residuals (Zuur et al., 2009). This model was selected by comparing different models using the Akaike Information Criterion (AIC). The model was then optimized by starting with all factors and interactions in the fixed component of the model, systematically dropping non-significant interactions and factors, and comparing nested models according to Zuur et al. (2009). The final model included factors Time, Treatment and an interaction between factors in its fixed component. Post-hoc comparisons were conducted using a Tukey test.

Lastly, generalised additive modelling (GAM) analysis was used to assess the relationship between general site degradation and *E. coli* sediment levels. GAM analysis is a more flexible analysis than traditional methods as it allows for non-linear relationships between explanatory and response variables. The analysis was conducted for all sites separately for each sampling time, using average *E. coli* concentrations and RHI scores for US and CAS. For mid-grazing season data, a variance structure was added to the model to account for unequal variances. In addition, since O'Sullivan et al. (2019) conducted the site assessment in early autumn, the two sites that were later replaced in the BK catchment were not included in the analysis of the data collected in mid-grazing season. GAM analysis was also used to assess potential relationships between the estimated cattle density (cattle.ha^{-1}) at each of the study sites *E. coli* bed sediment concentrations, in each sampling time.

4.3. Results

4.3.1. Background levels of faecal contamination

Sediment *E. coli* concentrations at the five study catchments are shown in Fig, 4.1 and are presented in Table 4.4 (mean and S.E.) for each of the sampled locations. The mean *E. coli* sediment concentrations in the study catchments ranged from 5.7 x 10^{3} CFU.g dry wt^{-1} (BW catchment) to 2.7 x 10^{6} CFU.g dry wt^{-1} (DG catchment) in mid-grazing season, whereas in post-grazing season, mean value ranged from 3.9 x 10^{3} CFU.g dry wt^{-1} (BK catchment) to 3.4 x 10^{4} CFU.g dry wt$^{=1}$ (MT catchment). In bed sediment at the upstream locations (US) of the nine headwater sites, which was considered to represent the background concentration in the absence of cattle, the mean *E. coli* concentrations measured ranged from 6.3 x 10^2 CFU g dry wt^{-1} (site BWA) to 1.1 x 10^4 CFU g dry wt^{-1} (site DGA) in mid-grazing season (Table 4.4). The concentrations in mid-grazing season were significantly higher than those observed in post-grazing season (F-value = 27.875, $p < 0.001$; Fig. 4.2), when site CM1 in the Commons River catchment was the most contaminated site (9.5 x 10^3 g dry wt^{-1}), while at site BWC *E. coli* was not detected (Table 4.4).

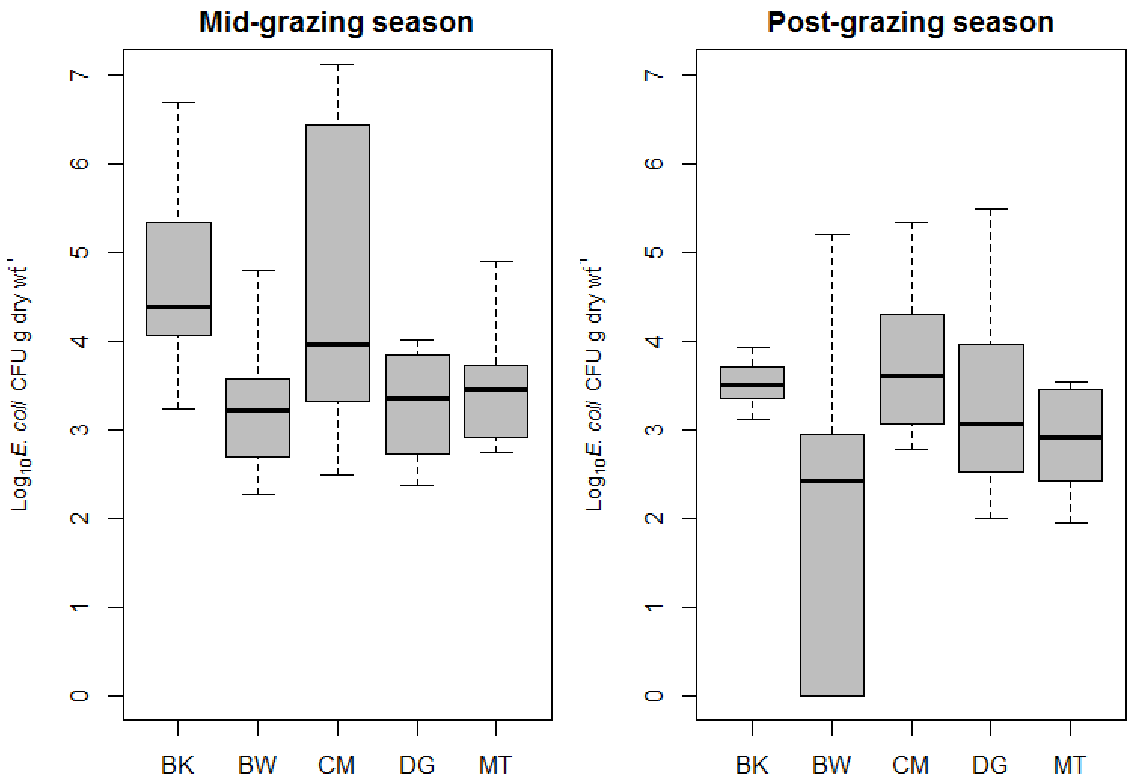

Fig.4.1. Boxplot of overall *E. coli* (log_{10}CFU.g dry wt^{-1}) concentrations in the sediments of the five study catchments in mid-grazing season and post-grazing season (n = 18) (including both US and CAS locations).

Table 4.4. Mean *E. coli* concentrations in sediments at the sites in the five study catchments in both sampling times (n =3).*n = 2. nd - not detected. Grey shading = nine headwater sites.

Catchment	Site	Treatment	***E. coli* (CFU g dry wt^{-1})** Mid-grazing season		Post-grazing season	
			Mean	*S.E.*	*Mean*	*S.E.*
BW	BWA	US	6.3×10^2	3.0×10^2	1.4×10^2	74
		CAS	1.1×10^3	7.6×10^2	6.9×10^2	5.3×10^2
	BWB	US	7.1×10^3	4.0×10^2	7.1×10^2	92
		CAS	2.3×10^4	2.0×10^4	5.5×10^4	5.3×10^4
	BWC	US	1.2×10^3	5.5×10^2	nd	-
		CAS	1.5×10^3	1.1×10^3	1.3×10^2	1.1×10^2
DG	DGA	US	5.9×10^3	2.9×10^3	1.1×10^5	1.0×10^5
		CAS	1.6×10^7	9.0×10^6	2.2×10^4	1.7×10^4
	DGB	US	2.5×10^3	3.9×10^2	4.6×10^2	2.7×10^2
		CAS	4.4×10^3	1.4×10^3	1.5×10^4	8.3×10^3
	DGC	US	1.3×10^3	5.8×10^2	3.7×10^2	55
		CAS	2.5×10^2	1.5×10^2	3.5×10^2	38
BK	BK1MG/ BK1PG	US	1.1×10^4	2.1×10^3	2.2×10^3	1.2×10^3
		CAS	1.9×10^6	1.5×10^6	4.5×10^3	1.9×10^3
	BK2MG/ BKPG	US	1.5×10^4	6.2×10^3	3.8×10^3	7.2×10^2
		CAS	1.3×10^5	7.3×10^4	7.5×10^3	7.9×10^2
	BK3MG/ BK3PG	US	8.5×10^3	6.5×10^3	2.3×10^3	3.0×10^2
		CAS	2.8×10^5	1.4×10^5	3.1×10^3	96

Table 4.4 (continued).

Catchment	Site	Treatment	*E. coli* (CFU g dry wt^{-1}) Mid-grazing season Mean	S.E.	Post-grazing season Mean	S.E.
CM	CM1	US	2.8×10^{3}	8.3×10^{2}	9.5×10^{3}	5.8×10^{3}
		CAS	1.9×10^{6}	4.6×10^{5}	7.9×10^{2}	1.0×10^{2}
	CM2	US	1.0×10^{3}	5.4×10^{2}	1.1×10^{3}	1.6×10^{2}
		CAS	2.5×10^{6}	1.7×10^{6}	8.2×10^{4}	6.6×10^{4}
	CM3	US	2.5×10^{3}	9.0×10^{2}	4.1×10^{3}	9.9×10^{2}
		CAS	7.3×10^{6}	2.7×10^{6}	3.0×10^{4}	1.3×10^{4}
MT	MT1	US	1.3×10^{3}	3.5×10^{2}	2.1×10^{2}	27
		CAS	3.8×10^{4}	2.2×10^{4}	2.0×10^{5}	5.5×10^{4}
	MT2	US	2.8×10^{3}	2.4×10^{2}	7.0×10^{2}	2.6×10^{2}
		CAS	*1.1×10^{5}		2.8×10^{2}	1.8×10^{2}
	MT3	US	1.6×10^{3}	8.2×10^{2}	1.9×10^{3}	5.4×10^{2}
		CAS	1.8×10^{3}	$2.2. \times 10^{3}$	2.0×10^{3}	9.2×10^{2}

4.3.2. Impact of cattle access to streams on sediment faecal contamination and seasonal variation

Study 1

In the nine headwater sites in Study 1, sediments at cattle access sites (CAS) were significantly more contaminated with *E. coli* than those at the upstream sites (Table 4.5). While post-hoc tests did not allow further conclusions, paired t-tests conducted separately for each sampling season indicated that this difference between US and CAS was only marginally significant in mid-grazing season (t = -2.443 , p = 0.0404) and was non-significant in post-grazing season (t = -1.882, p = 0.0966). Nevertheless, it should be noted that the highest *E. coli* concentration observed in this study overall (1.6 x 10^7 CFU g dry wt^{-1}) was found at the cattle access site at site DGA in the mid-grazing season. At this site, the *E. coli* concentration was four orders of magnitude higher that the concentration found immediately upstream (5.9 x 10^3 CFU g dry wt^{-1}). Similarly, concentrations at sites BK1 and CM1 also increased from 1.1 x 10^4 and 2.8 x 10^3 CFU g dry wt^{-1} upstream to both having concentrations of 1.9 x 10^6 CFU g dry wt^{-1} at the access site, respectively.

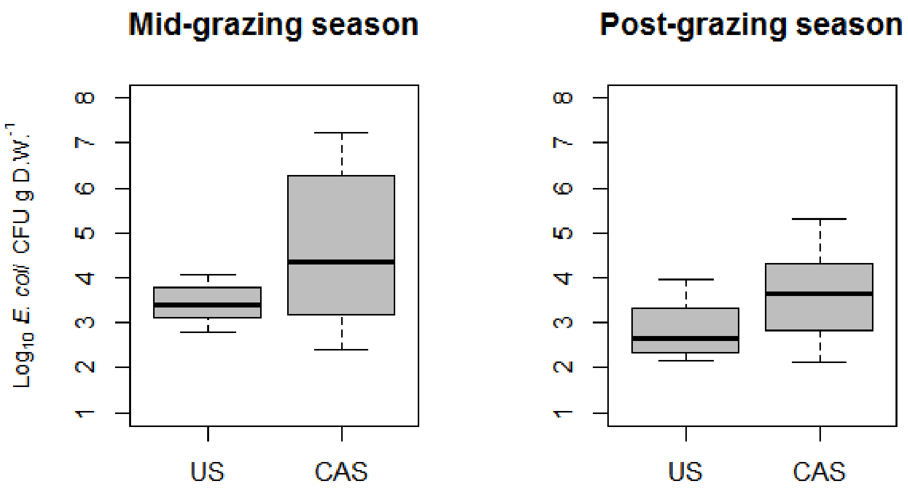

Fig.4.2. Boxplot of average sediment concentrations of *E. coli* at cattle access sites (CAS) and at upstream areas with no cattle access (US) at the nine headwater sites (study 1) (n = 9).

Study 2

Similarly, in Study 2, for 9 sites in the three catchments with moderate water quality status, a significant difference in sediment *E. coli* concentrations was also found between the US and CAS sites (Table 4.5). Post-hoc tests showed that this difference was again only significant in mid-grazing season (Table 4.6), when bed sediment *E. coli* concentrations were generally one to three orders of magnitude higher at cattle access points than at sites immediately upstream (Fig. 4.3). In the post-grazing season sampling period, this effect was not observed, as sediment *E. coli* levels at cattle access sites significantly decreased, while not varying significantly between mid- and post-grazing season at upstream sites (Table 4.6).

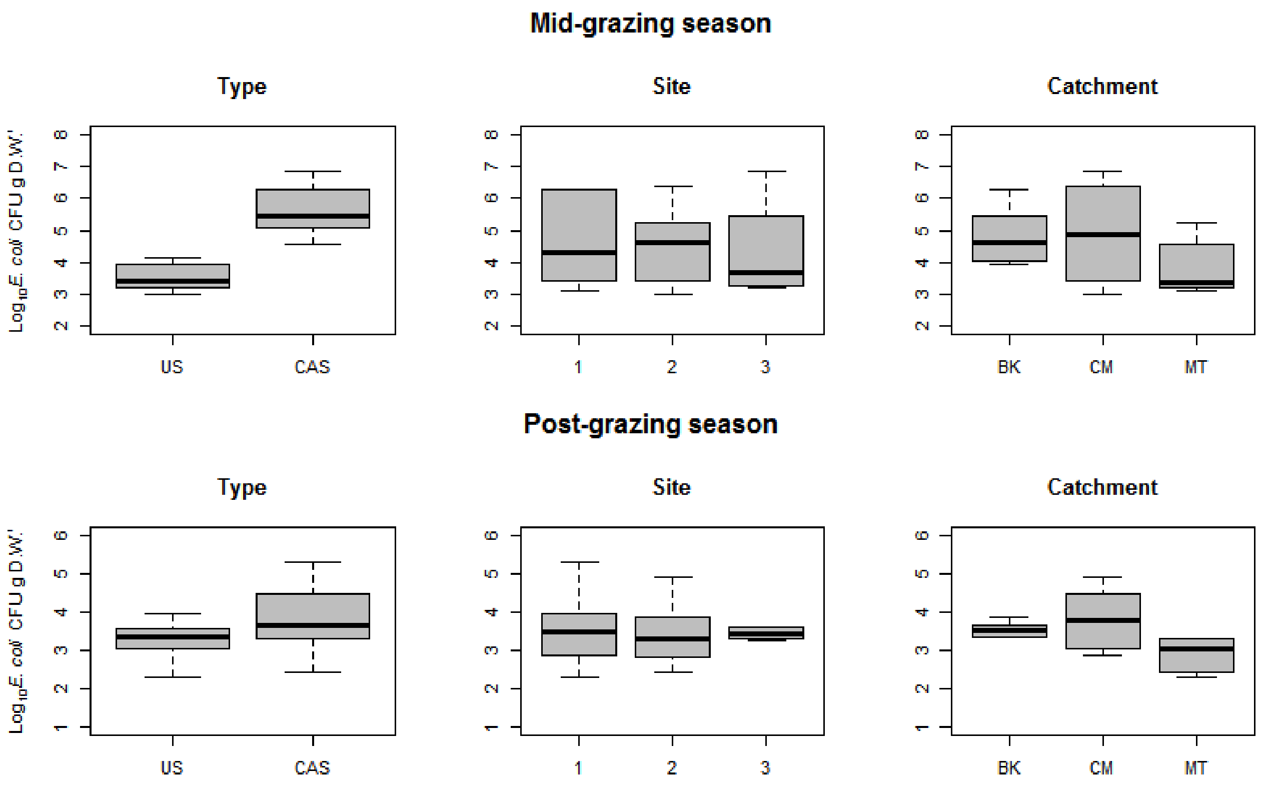

Fig.4.3. Boxplots of average sediment concentrations of E. coli per type (n = 9), site (n = 6) and catchment assessed in study 2 (n = 18), in mid-grazing season and post-grazing season. Note the different scales for different sampling times.

Relationship with RHI and cattle density

Finally, generalised additive model analysis of sediment *E. coli* levels at all sites in mid-grazing season and the RHI scores revealed a significant negative relationship in mid-grazing season (F value = 6.772, p = 0.008*),* with an R-sq. (adj) of 0.24 (Fig. 4.4), indicating that higher bed sediment concentrations of *E. coli* occurred at those sites where a general degradation of the riparian habitat had been recorded. There was no significant relationship between *E.* coli concentrations and RHI for the post-grazing season data (F-value = 0.118, p = 0.668). In contrast, there was as a significant relationship between *E. coli* bed sediment concentrations and estimated cattle density (ECD; cattle.ha^{-1}) in post-grazing season (F-value = 3.727, p = 0.035), with an R-sq. (adj) of 0.26, but not in mid-grazing season (F-value = 1.161, p = 0.291) (Fig. 4.5).

4.3.3. Cumulative downstream gradient

Analysis of *E. coli* sediment levels at the three sampled sites located along a downstream gradient in the BK, CM and MT catchments revealed no significant difference between the sites (F-value = 0.063, p = 0.939), indicating that there was no significant cumulative downstream pattern of faecal contamination of sediments for either sampling times (Table 4.4, Fig. 4.3).

Table 4.5. Analysis of variance of *E. coli* in sediment in Study 1 (repeated measures ANOVA; n = 36) and Study 2 (linear mixed model analysis; n=36). Treatment = US and CAS. Time = mid-grazing season and post-grazing season. Figures in bold denote statistical significance at the 0.05 level.

Significant factors	DF	F-value	*p*
Study 1			
Treatment	**1,8**	**18.406**	**0.003**
Time	**1,8**	**9.636**	**0.0146**
Time*Treatment	8	0.137	0.721
Study 2			
Treatment	**1**	**16.168**	**<0.001**
Time	**1**	**55.896**	**<0.001**
Time*Treatment	**1**	**17.696**	**<0.001**

Table 4.6. Post-hoc comparisons (Tukey test) for the analysis of *E. coli* in sediment in Study 2 (interactions between factor Treatment (US and CAS and Time (mid-grazing season [MG] and post-grazing season [PG]). Figures in bold denote statistical significance at the 0.05 level.

Contrasts	DF	t ratio	*p*
CAS – US, MG	**101**	**8.242**	**<0.001**
CAS – US, PG	101	2.378	0.088
CAS, MG – CAS, PG	**101**	**5.466**	**<0.001**
US MG – US,PG	101	2.096	0.161

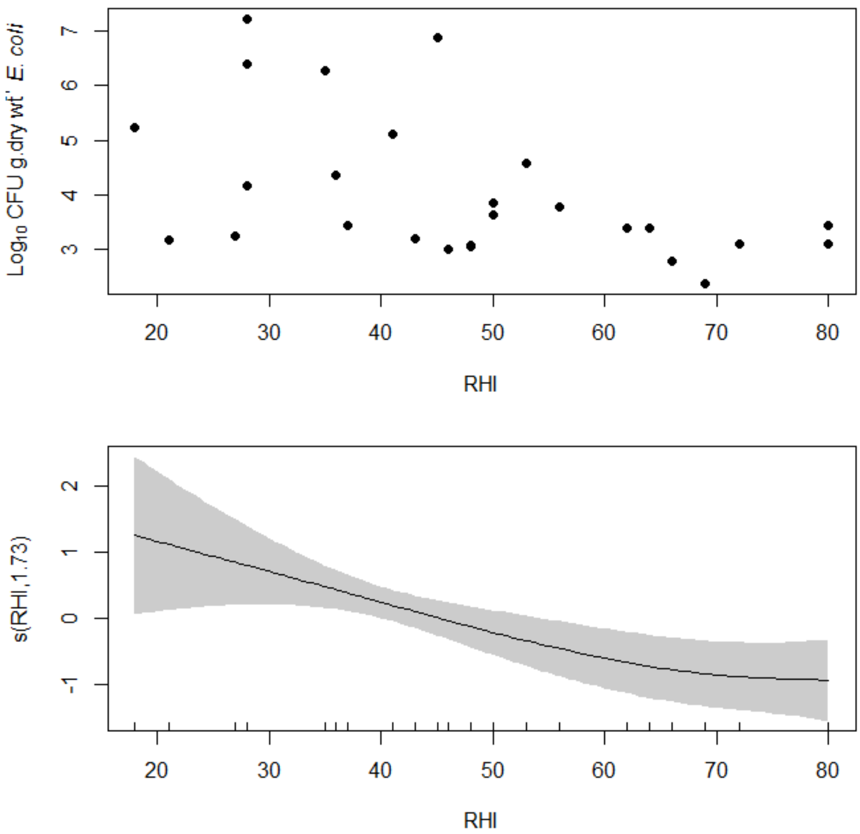

Fig.4.4. Scatterplot and smoother for RHI scores in the GAM with levels of *E. coli* in sediments in mid-grazing season as response variable. The central solid line is the smoother and the grey area is 95% confidence bands. Y axis units are the scaled smoother (s) for RHI with estimated degrees of freedom (edf) of 1.73.

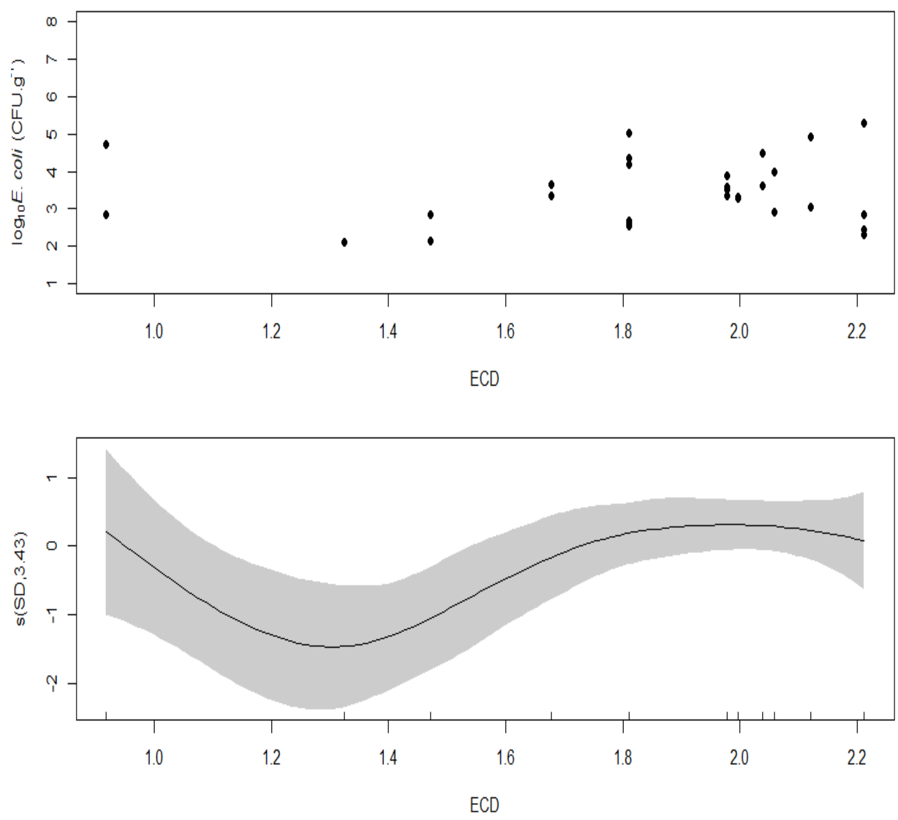

Fig.4.5. Scatterplot and smoother for estimated cattle density (ECD; cattle.ha^{-1}) in the GAM with levels of *E. coli* in sediments in post-grazing season as response variable. The central solid line is the smoother and the grey area is 95% confidence bands. Y axis units are the scaled smoother (s) for RHI with estimated degrees of freedom (edf) of 3.43.

4.4. Discussion

Contamination of freshwater waters and sediments with faecal organisms has implications for both human and animal health. In this study, it was notable that bed sediments at all 15 sites upstream of cattle access points in this study were contaminated with *E. coli*, with only one site where no *E. coli* were detected on one occasion (upstream of cattle access site BWC, in post-grazing season). These results highlight the widespread contamination of stream sediments with *E. coli* in agricultural catchments. Additionally, they show that cattle access to streams clearly exacerbates this contamination.

While studies investigating *E. coli* contamination in sediments of agricultural streams are scarcer in the literature that those on, for example, coastal sands, the concentrations from other studies provide a context for the resulted presented. Hassard et al. (2017) assessed the levels of faecal pollution in estuarine sediments of two areas in the UK impacted by a variety of pollution sources, including agricultural land used for livestock grazing, urban areas and wastewater discharges, and found average *E. coli* levels above 10^4 CFU 100 g dry wt^{-1}. Bonilla et al. (2007) also reported similar average *E. coli* concentrations of 10^4 CFU 100 g^{-1} in sand samples of three recreational beaches in south Florida, USA, over a two-year sampling period. Ishii et al. (2007) observed *E. coli* concentrations of 10^2 to 10^3 CFU g dry wt^{-1} in sediment and sand in a recreational lake beach affected by sewage treatment effluents, while Abdelzaher et al. (2010) reported maximum enterococci concentrations of 10^3 CFU g dry wt^{-1} in sand samples from a recreational beach impacted by diffuse sources of pollution. The levels found in the current study, which ranged from 1.3×10^2 CFU g dry wt^{-1} up to 1.6×10^7 CFU g dry wt^{-1}, are therefore at the higher end of those reported in literature and are likely to have serious implications for the quality of the overlying water if these microorganisms are resuspended.

Bed sediment levels of *E. coli* were highly variable within sites in our study, highlighting the patchy nature of bacteria distribution in sediments (Pachepsky and Shelton, 2011) and also within catchments, suggesting that stream faecal contamination is also governed by field-scale management practices. This would include field-specific factors that were not assessed in the current study, such as stocking rate, period of grazing, grazing rotation practices, existence of other access sites or alternative drinking water sources, and slurry spreading. In this study, however, there was no apparent relationship between estimated cattle density per hectare for each site and the average *E. coli* concentrations measured at the access points, or with the difference between the US and CAS subsites. Unfortunately, despite designing a questionnaire and getting some individual results, it was not possible to collect additional reliable data on agricultural management practices from all farmers and landowners involved in this study to better understand the influence of individual management practices on sediment faecal contamination. The importance of farm-level conditions in understanding faecal pollution dynamics and the difficulties in accessing such information has been highlighted by Winter et al. (2011).

4.4.1. Background levels of stream sediment contamination

Sediment faecal contamination in reaches of the stream with no cattle access at the nine headwater sites, which also did not have significant cattle access pressure upstream or any visible immediate visible influence of point discharges, are assumed to be mostly a result of diffuse pathways of pollution as well as possibly some residual contribution of wildlife faecal contamination. Septic tanks within the catchments can also contribute to faecal contamination; a study by Arnscheidt et al (2007) conducted in three agricultural catchments Ireland reported that although faecal contamination in the sediments was predominantly of herbivore origin, between 7% and 27% of contamination was of human origin. While it is surprising that stream sediments at these headwater reaches (to which cattle had no access) had relatively high concentrations of *E. coli*, these levels of faecal contamination are comparable to those measured by Bragina et al. (2017) in the Milltown Lake catchment, who reported a median *E. coli* sediment concentration of 9.6×10^2 CFU g dry wt^{-1} in a fenced tributary. The same authors also reported a sediment *E. coli* median value of 43 CFU g dry wt^{-1} at a first-order stream in an agricultural area grazed by small numbers of sheep, with no cattle production or human settlement (Bragina et al., 2017). These findings, together with the observation that sediment faecal contamination at the headwater sites was significantly more pronounced in the grazing season, support the evidence that catchment-scale activities can have significant impacts on stream sediment faecal contamination regardless of whether animals have access to the watercourses (Collins et al., 2005; Davies-Colley et al., 2008; Smolders et al., 2015; Bragina et al., 2017). Mechanisms of faecal contamination of watercourses arising from cattle-based agriculture include runoff from grazing fields (Frey et al., 2015; Cho et al., 2016;) and spreading of organic fertilisers such as slurry and farmyard manure (Hodgson et al., 2016), which in Ireland is permitted from approximately mid-January - February to mid-October - November, depending on the region (DAFM and Department of Housing, Planning and Local Government [DHPLG], 2017) and takes place predominantly during the summer months.

4.4.2. Impacts of cattle direct access to watercourses on sediment faecal contamination and seasonal variation

Sediment *E. coli* concentrations in this study were significantly higher at the access sites than at upstream sites in mid-grazing season, with a subsequent general decrease of *E. coli* levels at the access sites in post-grazing season. These findings confirm that direct cattle access to watercourses can exacerbate already high stream sediment faecal pollution. The relationship found in this study between the Riparian Habitat Index scores and the levels of sediment *E. coli* in mid-grazing season indicated that increased faecal contamination is associated with increased general degradation of the sites, which likely also relates to intensity of cattle usage. A similar relationship was found between RHI scores and resuspendable sediment levels at the same sites by O'Sullivan et al. (2019). However, this impact seems to be relatively short-lived. Similarly, in their study in the Milltown Lake catchment, Bragina et al. (2017) observed significantly higher sediment *E. coli* concentrations at sites with unrestricted cattle access in mid-grazing season, but noted that *E. coli* levels decreased at all study tributaries over the winter period and were at their lowest levels in April, with no significant differences observed between any of the study tributaries at this time. In the post-grazing season, in the absence of cattle access and associated frequent direct inputs of faecal contamination, sediment reservoirs of *E. coli* at access sites may be quickly reduced due to a combination of bacteria die-off and episodes of sediment flushing as a result of increased flows in this time of the year (Nagels et al., 2002; Jamieson et al., 2005a) . Bacteria wash-off, along with sediment particles, during high flow episodes is likely to be more pronounced at cattle access sites than at sites with no access due to the general stream channel degradation and typical absence of aquatic vegetation at these sites (O'Sullivan et al., 2019). Nevertheless, it is of note that *E. coli* concentrations at both upstream and cattle access sites in these catchments remained at levels of 10^2 to 10^3 CFU g dry wt^{-1}. Presumably, this sediment *E. coli* contamination is a result of pollution from various sources (e.g. septic tanks and other human wastes, together with slurry spreading and surface run-off) and prolonged persistence of *E. coli* in the sediment matrix. *E. coli* persistence in sediments has been widely reported (Craig et al., 2002; Ishii et al., 2007; Garzio-Hadzick et al., 2010; Pachepsky and Shelton, 2011), and has indeed been shown to be favoured by factors such as lower temperatures (i.e. 4˚C) (e.g. Garzio-Hadzick et al., 2010).

Although faecal contamination was significantly higher at access sites than at sites with no access in both Study 1 and Study 2, in the sites in Study 1 this impact was apparently less pronounced. This might be explained by the fact that these sites are located in areas that typically have less intensive agriculture when compared to the sites in Study 2 (See Chapter 3). Interestingly, in this study, there was a relationship between estimated cattle density at each site and sediment *E. coli* concentrations only in post-grazing season. This might indicate that, during grazing season, cattle access to watercourses has a strong effect on stream bed sediment *E. coli* contamination, whereas in the absence of cattle, diffuse pathways of pollution at the catchment scale dominate. The observation that the highest sediment levels of *E. coli* in this study (1.6 x 10^7 CFU g dry wt^{-1}) were found at the DGA site, which had one of the lowest RHI scores, further supports the evidence that faecal contamination is largely determined by localised agricultural practices when cattle is present.

4.4.3. Downstream cumulative pattern of faecal contamination at the study sites

No evidence of a cumulative effect on sediment *E. coli* concentrations along a downstream gradient was found in this study. Similar results have been reported for the Milltown Lake catchment by Bragina et al. (2017), and indeed for resuspendable sediment levels at the same sampled sites in a study by O'Sullivan et al. (2019). Bacteria can be transported in streams as free floating individuals, but it is widely recognised that in aquatic systems, bacteria are generally associated with sediment particles (Jamieson et al., 2005b); Drummond et al., 2014), which are typically less than 60 µm in diameter (Jamieson et al., 2005b), and that this association influences bacteria transport along the stream channel. Drummond et al. (2014) investigated the transport of synthetic fluorescent fine particles and *E. coli* bacteria in a stream and observed that both fine particles and bacteria migrated similarly in the stream through a series of deposition and resuspension events, with retention occurring mostly near the sediment-water interface in macrophyte strands or at the top 3 cm of the streambed sediments, due to bulk advection, turbulent diffusion and hyporheic exchange processes. The authors observed that macrophyte strands and streambed sediments could act both as short and long-term (i.e. months) reservoirs for microorganisms and fine particles, and that the extent of remobilisation was influenced by the structure of the stream environment, the delivery of water-borne material to depositional areas and the frequency of disturbance events (Drummond et al., 2014). Given that the distance between the sites investigated in the present study is relatively large (approximately 700 to 5000 m), it is likely that, although sediment bacteria resuspension during disturbance events generally play an important role in faecal bacteria dynamics in streams, mechanisms of bacteria deposition in storage zones between sites and of microorganisms die-off can prevent the build-up of a cumulative gradient of contamination along the stream channel.

4.5. Implications of stream sediment faecal contamination

This study shows that where cattle access watercourses, they contribute to reservoirs of *E. coli* in sediments that are able to persist after cattle removal from the grazing fields, with implications for water quality and both human and animal health. This effect can be particularly concerning in small order streams where the low water volume to sediment area ratio and the limited amount of submerged aquatic vegetation make bed sediments the largest available substrate for faecal bacteria accumulation (Badgley et al., 2011). Resuspension of accumulated faecal organisms following sediment disturbance has been widely reported (Jamieson et al., 2005a; Cho et al., 2010; Kim et al., 2010; O'Mullan et al., 2019). Moreover, it has been shown that a significant release of faecal bacteria from sediments to waters can also occur under baseflow conditions via hyporheic exchange (Pachepsky et al., 2017). Moreover, it should be noted that in this study, faecal bacteria were quantified using a culture-based method, however some bacteria, including *E. coli,* have been shown to persist in the environment in a viable but non-culturable (VBNC) state, in which they remain metabolically active but do not grow in microbiological growth media (Hassard et al., 2017, 2016). These bacteria cannot be detected by these conventional methods, but retain the ability to resuscitate if suitable conditions are provided, and are potentially pathogenic (Hassard et al., 2017, 2016). Thus, the sediment concentrations of faecal indicator bacteria reported in this study likely underestimate the real levels of faecal contamination and the animal and human health risk at the study sites.

4.6. Conclusions

The findings of this study show that stream sediments in agricultural catchments can be important reservoirs of *E. coli* and likely therefore other faecal pathogens, and that this contamination can be substantially increased when cattle are allowed access to watercourses. Given that the practice of allowing cattle access to agricultural watercourses is widespread in Ireland, it is likely to have a significant overall impact on water quality and animal and human health, particularly in rural areas. The results of this work emphasise the need to adopt agricultural practices that protect human health and therefore support the integration of cattle exclusion measures in future domestic and international agricultural policy.

5. Impacts of unrestricted cattle access to watercourses on streambed sediment nutrient concentrations in agricultural streams

Chapter 5. Impacts of unrestricted cattle access to watercourses on streambed sediment nutrient concentrations in agricultural streams

5.1. Introduction

Livestock agriculture has been highlighted as an major contributor of excess nutrients (phosphorus and nitrogen) to waterbodies in Ireland (Mockler et al., 2016; O'Boyle et al., 2017). For instance, in a paleolimnological study in Milltown Lake, NE Ireland, Carson et al. (2015) reported indicators in lake sediments of a rapid deterioration of the water quality coinciding with the intensification of livestock agriculture in the catchment since the 1970s. In another paleolimnological study, Foy et al. (2003) reported that the intensification of agriculture and the associated increased loss of phosphorus (P) from agricultural soils were the main drivers of increased P levels in Lough Neagh, Northern Ireland, while Taylor et al. (2006) reached similar conclusions for five out of six lakes in the Irish Ecoregion. The most recent water quality report published by the Environmental Protection Agency (EPA), referring to the period of 2013 – 2018, indicated that 47.2% of monitored surface waterbodies are in less than good ecological status, representing a decrease of 2.6% of waterbodies in satisfactory status in relation to the period of 2010 – 2015, mostly driven by a general decrease water quality in Irish rivers (EPA, 2019). Excess nutrient loadings from agriculture were stated to be the main cause of this surface water pollution (EPA, 2019).

Contamination of watercourses occurs mainly through diffuse mechanisms such as transport of dissolved and particulate nutrients from agricultural soils in surface runoff, subsurface flow and drains and ditches (Douglas et al., 2007). This can represent residual (i.e. release of excess nutrients accumulated in soils) or incidental (e.g. nutrient loss from recently applied mineral or organic fertilisers) nutrient transfers (Shore et al., 2017). However, point source pathways, including points of direct cattle access to watercourses for drinking and crossing between grazing fields, can also play an important role (O’Callaghan et al., 2018).

Cattle can increase water nutrient concentrations by directly defecating and urinating in watercourses (see table 5.1 for nutrient concentrations measured in cattle faeces and urine available in literature) and by causing nutrient release from sediments by grazing and trampling activity (Capece et al., 2007). In addition, where cattle have access to watercourses, they can cause stream banks to become exposed and susceptible to accelerated erosion (Fox et al., 2016), thereby significantly increasing the risk of loss of particulate nutrients to the stream (Fox et al., 2016; McDowell and Wilcock, 2007). Once in the aquatic system, nutrients can accumulate in bed sediments, which can then act as nutrient sources to the water column through resuspension, desorption and remineralisation mechanisms (House, 2003). Within sediments, the finer fraction (<2mm) is generally considered the most reactive due to its higher surface to volume ratio (Lucci et al., 2010). Phosphorus in particular interacts strongly with sediments as P compounds are ‘reactive solutes’ (McGechan et al., 2005) with high affinity for alumino-silicates (clays) and metal (particularly Fe and Al) oxides and hydroxides (Withers and Jarvie, 2008). This causes P to generally accumulate in solid phases in much higher concentrations than in solution phases (Sharpley et al., 2013). The mobilisation of accumulated P from stream sediment reservoirs is determined by geochemical factors such as dissolved P concentrations in the overlying waters and redox conditions, as well as sediment mineralogy and particle size distribution, presence of metal oxides, and the concentration of exchangeable phosphate adsorbed to the sediment particles (Palmer-Felgate et al., 2009; Neidhart et al., 2019). This potential for sediments to serve as both sinks and sources for P has been shown to lead to legacy P effects, whereby stored P is remobilised and acts as a source of contamination for long periods of time (e.g. decades or centuries) after deposition, even after the contamination source has been removed or controlled (Sharpley et al., 2013; Fox et al., 2016). This process is particularly difficult to manage, as the retention and remobilisation processes are slow and ubiquitous along the aquatic system, thus limiting the effectiveness of best management practices and water quality protection measures (Jarvie et al., 2013; Wironen et al., 2018).

The available literature on the contribution of direct cattle access to excess nutrient loadings in watercourses is limited, especially in European sites (see Chapter 2), and very few studies report data on sediment nutrients in the context of livestock impacts (see Table 5.2 for an overview nutrient concentrations in stream banks, sediments and agricultural soils reported in literature). In a study where the effects of cattle activity on nutrient dynamics in streambanks were assessed using ion exchange membranes, Miller et al. (2017) found no significant impacts of cattle access to the stream or cattle grazing intensity on either NO_3-N or P. These findings contrast with those of Palmer-Felgate et al. (2009), who compared streambed sediment TP concentrations in headwater systems with low agriculture intensity (named control systems) and high agricultural intensity within three lowland catchments in the UK. The authors reported higher TP sediment concentrations (ranging on average from 1429 to 2480 $mg.kg^{-1}$) at a site located in the proximity of farmsteads and to which cattle had direct access at a number of points, in comparison to the control site (within the same study catchment) which was in an area grazed by only a few animals (where they ranged on average from 657 to 1060 $mg.kg^{-1}$) (Palmer-Felgate et al., 2009). Additionally, the authors observed relatively high TP sediment concentrations (ranging on average from 155 to 636 $mg.kg^{-1}$) at their control site in one of their study catchments (Wye catchment), which they hypothesised was caused by unrestricted cattle access to the stream (Palmer-Felgate et al., 2009).

Table 5.1. Nitrogen and phosphorus concentrations in cattle urine and faeces reported in literature. *Figures presented here are averaged from the published data.

Reference	Type of cattle	Urine		Faeces	
		N	P	N	P
Lantinga et al. (1987)	Dairy	6.1 – 9.7 $g.L^{-1}$ TN			
Bristow et al. (1992)	Dairy	6.8 – 20.5 $g.L^{-1}$ TN			
Kirchmann and Witter (1992)	Dairy				9.0 g.kg dry wt^{-1}
Gonda and Lindberg (1994)	Dairy	5.8 – 10.7 $g.L^{-1}$ TN			
van Vuuren and Smits (1997)	Dairy	3.9 – 7.6 $g.L^{-1}$ TN			
Dou et al. (2002)	Dairy				5.21 – 12.65 TP g.kg dry wt^{-1}
Sorensen et al. (2003)	Dairy			18.1 – 37.8 g.kg dry wt^{-1} TN	
Kool et al. (2006)	Dairy	9.0 – 10.3 $g.L^{-1}$ TN			
Orr et al. (2012)*	Beef	5.9 $g.kg^{-1}$ TN	0.0039 g.kg dry wt^{-1}	3.2 $g.kg^{-1}$ TKN	4.9 g.kg dry wt^{-1}
Spek et al. (2012)	Dairy	3.0 – 10.4 $g.L^{-1}$ TN			
Dai and Karring (2014)	Beef	261 $mmol.L^{-1}$ TKN 15.9 $mmol.L^{-1}$TAN 152.7 $mmol.L^{-1}$ UN		337.8 $mmol.kg^{-1}$TKN 21.2 $mmol.kg^{-1}$ TAN	
Misselbrook et al. (2016)	Dairy and beef	0.6 – 34.4 $g.L^{-1}$ TN			

Table 5.2. Summary of some of the studies that have reported nutrient concentrations in agricultural field soils, stream bank sediments or streambed sediments in agricultural catchments.TP and TN concentrations are in mg.kg dry wt^{-1} unless stated otherwise.

Reference	Location	Substrate	TP	TN	OC
Murphy et al., 2000	England	Field soils		26 – 37 kg.ha^{-1} (mineral N)	
McDowell and Sharpley, 2001	New Zealand	Bank sediment	259		
McDowell and Sharpley, 2001	New Zealand	Streambed sediment	214		
Thoma et al., 2005	USA	Bank sediment	249 - 452		
Thoma et al., 2005	USA	Field soil	622		
Falkengren-Grerup et al., 2006	Sweden	Forested, formerly cultivated soil		32.0 mol.m^{-2}	
Taylor et al., 2006	Ireland	Lake surface sediments	2000-4000+		100-150 g.kg^{-1} (20-30% LOI)*
McDowell and Wilcock, 2007	New Zealand	Field soil (topsoil; subsoil)	3291; 1578		20.9; 8.1 g.kg^{-1}
McDowell and Wilcock, 2007	New Zealand	Bank sediment	2738		26.1 g.kg^{-1}
McDowell and Wilcock, 2007	New Zealand	Streambed sediment	921		11.1 g.kg^{-1}

*OC concentration is based on reported % loss-on-ignition (LOI) as 50% LOI (Dean, 1974).

Table 5.2 (continued)

Reference	Location	Substrate	TP	TN	OC
Zaimes et al., 2008	USA	Bank sediment	303 - 555		
Palmer-Felgate et al., 2009	UK	Streambed sediment	394 - 2678		
Tufekcioglu, 2010	USA	Bank sediment	246 - 349		
Kronvang et al., 2012	Denmark	Bank sediment	400-1400		
Carson et al., 2015	Ireland	Lake surface sediment	940 - 1690		110 $g.kg^{-1}$ (22% LOI)*
Ishee et al., 2015	USA	Bank sediment	138 - 1140		
Shore et al., 2016	Ireland	Ditch sediment	200-1800		47 $g.kg^{-1}$ (9.40% LOI)*
Shore et al., 2016	Ireland	Bank sediment	200-400		21 $g.kg^{-1}$ (4.20% LOI)*
Shore et al., 2016	Ireland	Field soil	500-1400		62.5 $g.kg^{-1}$ (12.50% LOI)*
Neidhart et al., 2019	Germany	Streambed sediment	449 - 1392		

*OC concentration is based on reported % loss-on-ignition (LOI) as 50% LOI (Dean, 1974).

Most of the literature available to date referring to cattle access to watercourses has focused on assessing the effectiveness of mitigation measures, rather than quantifying the impact of unrestricted cattle access to watercourses on nutrient levels within the aquatic system. Additionally, few studies have considered the stream sediment compartment and its potential role as a sink and source of excess nutrients. Notably, to the knowledge of the authors, there is no study specifically investigating the influence of cattle density on stream sediment nutrient concentrations. This factor is particularly relevant in Ireland as the size of the national herd increases to respond to changes in agricultural policy (e.g. the abolition of EU's milk production quotas in 2015) and agricultural development plans aimed at expanding the Irish agri-food sector such as Food Wise 2025 (O'Boyle et al., 2017; DAFM, 2020).

The present study aimed to contribute to these gaps in the literature. The aims of the study were:

a. To determine whether concentrations of total phosphorus, total nitrogen and organic carbon in stream bed sediments were higher at reaches of the stream where cattle had access (cattle access points) compared to upstream reaches of the stream not used by cattle;
b. To assess whether nutrient concentrations were greater the smaller silt + clay fraction (<63μm) of the sediment than in the <2mm fraction;
c. To evaluate whether cattle access points in areas with higher cattle density (expressed as estimated animal numbers per hectare, calculated at the locality level) had higher sediment nutrient concentrations than cattle access points in areas with lower cattle density.

5.2. Methods

5.2.1. Site selection and experimental design

A total of 15 active cattle access sites located in five agricultural catchments in Ireland were selected for this study. These sites and the study catchments are described in detail in Chapter 3.

5.2.2. Sediment sampling

At each site, the stream bed sediment was sampled at four locations: 1. at the cattle access site, i.e. where cattle actively used the stream (CAS); 2. immediately upstream) of the access site, where animals had no access to the stream either due to fencing or natural physical barriers (this could be 20 – 322 m depending on the site (US); 3. immediately downstream of the cattle access site, where cattle did not have access (18 – 144 m depending on the site) (DS); and 4. at the interface (edge) of the stream water level, at the access path used by cattle to enter the stream (INT) (see Chapter 3). Sampling of this interface area was included because this area was hypothesised to be subject to a higher localised impact of cattle as they stand and drink from the stream.

Six sediment samples were collected randomly at each of the four locations using a clear Plexiglas corer with a bevelled edge (73 mm diameter) (Hedrick et al., 2013). The corer was used to ensure consistent sample size within and between sites. A metal scrapper, which had been previously acid-washed and rinsed with stream water to prevent sample contamination, was placed under the core base once inserted to the desired depth to aid in core removal. The upper 3 to 5 cm of the bed sediment were collected depending on the sediment depth, giving an approximate sample of 126 cm^3 to 209 cm^3. Sediment samples were placed in individual plastic bags and transported to the lab in cool boxes, where they were kept in the dark at 4˚C until further processing.

In Ireland, cattle graze outdoors during the spring and summer months (generally April to October/November) and are housed over the winter months. The 15 sites were sampled in early grazing season (mid-April and May) and in late grazing season (October), before cattle were housed for the winter period, in 2016. This was to capture potential differences in sediment nutrient concentrations related to the agricultural management cycle. i.e. following the winter/spring period when cattle would be absent from grazing fields, and following the summer months during which cattle would have been grazing and therefore using the study cattle access site.

5.2.3. Sediment analysis

Sediment samples were oven-dried at 105˚C for a minimum of 24 hours and sieved to <2mm particle size fraction. The weights of both the >2mm and <2mm fractions of each sample were recorded before discarding the former fraction. Composite samples for each of the four locations at each site were then prepared using a quartering technique to ensure homogenous sectioning of each <2mm original sample, yielding a total of 120 composite samples. Each composite <2mm sample was then subsampled to a <63µm particle size sample using the same quartering technique. Both fractions were subsequently analysed to determine sediment particle size distribution, total phosphorus (TP), organic carbon (OC), and total nitrogen (TN) concentrations.

Sediment particle size distribution was determined in each sample using the hydrometer method, as described in Carter and Gregorich (1993). The samples were soaked overnight in a solution containing 100 ml of 50 $g.L^{-1}$ sodium hexametaphosphate (Fisher Scientific, UK), a dispersing agent, and 300 ml of ultrapure water, and shaken for one hour on a mechanical shaker, to prevent flocculation and ensure dispersion of particles.

The samples were then transferred to graduated cylinders and the volume was brought to 1 L by adding ultrapure water. The suspensions were allowed to equilibrate to room temperature (20 - 25˚C) and shaken thoroughly to mix the contents. The density of each suspension was then determined after 40 seconds using a hydrometer (ASTM E100 152H, GH Zeal Ltd.). This reading gives the percentage of sand in each sample according to the equation

$$Sand\ (\%) = 100 - (R40s - RL)x\ \frac{100}{oven - dried\ soil\ weight\ (g)}$$

where *R40s* is the reading taken after 40 seconds and *RL* is the reading obtained from a suspension of sodium hexametaphosphate (ThermoFisher Scientific) (5 $g.L^{-1}$). The percentage of <63µm particle size sediment (silt+clays) was then determined as the remaining portion of each sample.

Sediment total phosphorus was extracted separately for the <2mm fraction and the < 63µm fraction using a microwave assisted acid digestion method (adapted from the USEPA 3051a method (USEPA, 2007). Using this method, 0.5 ± 0.01 g of well mixed dry sediment was placed in Teflon Xpress vessels (CEM Corporation, USA) and sequentially predigested with 1 ml of concentrated sulphuric acid (98%) for 15 minutes and 10 ml of concentrated nitric acid (70%) for further 15 minutes. The samples were then digested using a Mars 230/60 Microwave Digester (CEM Corporation, USA). Each digestate was then diluted to a 100 ml solution in acid-washed volumetric flasks and left overnight to allow residual sediment particles to settle. Total phosphorus was then measured in each sample using a manual procedure adapted from the colorimetric method described by Murphy and Riley (1962) as modified by Watanabe and Olsen (1965). Calibration standards and quality control standards were prepared using a phosphate certified standard solution (1000 mgL^{-1}; Inorganic™ Ventures) and a stock solution prepared using an analytical grade KH_2PO_4 salt (1000 mgL^{-1}; ThermoFisher Scientific), respectively. The limit of detection for this analysis was calculated as 10 µg $P.g^{-1}$.

Sediment organic carbon and total nitrogen were determined simultaneously using an Elementar El Vario Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany), following removal of inorganic carbon by exposing dry sediments to concentrated hydrochloric acid (37%) fumes (Harris et al., 2001). The instrument was calibrated against a standard (acetanilide) (Merck, Darmstadt, Germany) with known concentrations of carbon (71.09%), nitrogen (10.36%) and hydrogen (6.71%). Drift calibration standards were also included every ten samples to account for any drift over each run. The limit of detection for this analysis was estimated as < 40 ppm (0.004%) for both TN and OC.

5.2.4. Estimation of stream nutrient loadings

To estimate the mass sediment per metre squared for each site, the average mass of <2mm sediment contained in each composite sample was extrapolated up to a square meter by multiplying by 238.9 (number of Plexiglas corer areas in 1 m^2). The mass of the <63μm fraction was then calculated using the percentage of silt+clay in each composite sample (obtained in the particle size distribution analysis). To give a more accurate estimate of sediment cover, the mass of each fraction was then corrected for the heterogeneous composition of the substrate at each location using estimations of the coverage of fine sediment at each site (% of substrate). These were visually assessed by O'Sullivan et al. (2019) during site characterisation for the determination of Riparian Habitat Index values. Nutrient bed sediment loadings at each sampling location were then calculated by multiplying the sediment nutrient concentrations by the estimated mass of sediment ($g.m^{-2}$). Since O'Sullivan et al. (2019) conducted their assessment of percentage of fine sediment coverage in the late grazing season, nutrient loadings were estimated for this sampling time only.

5.2.5. Statistical analysis

All analyses were carried out using R software (version 3.5.1; R Core Team, 2016), and the packages *nlme* (Pinheiro et al., 2019) and *mgcv* (Wood, 2015). Differences in sediment nutrient concentrations between the <63μm and the <2mm particle size fractions were assessed individually for each nutrient using generalised least squares (GLS) analysis. A compound symmetry correlation structure was included to account for correlation between observations obtained from the same composite samples, and a variance structure was included for the factor Catchment to account for heterogeneity in the model residuals (Zuur et al., 2009). Potential correlations between the concentrations of all nutrients in both fractions were assessed using Spearman rank correlation test. Prior to the assessment of potential differences in sediment nutrient concentrations due to direct cattle access, the independence of the 15 sites was confirmed through spatial correlation analysis (Zuur et al., 2009).

The impact of direct cattle access on sediment nutrients in each of the sediment fractions was assessed separately for each nutrient using linear mixed effects modelling. Linear mixed models allow for the inclusion of variables as random components of the linear models and are useful for nested or hierarchical designs (Zuur et al., 2009). The analysis included factors Time (two levels: EG (early grazing season), LG (late grazing season), Location (four levels: US, CAS, INT, DS) and an interaction between these factors (Time x Location) as fixed terms, with the factor Catchment modelled as a random intercept. This approach allows the intercept of the model to change per catchment, and induces a correlation structure for all observations from the same catchment (Zuur et al., 2009). The factor Catchment was included as a random term in all models except for the analysis for OC concentrations in the <2mm particle size fraction, where the addition of a random intercept did not improve the model in comparison to a GLS model with only fixed terms.

The analysis was performed on log-transformed data for OC and TN in the <2mm fraction to account for heterogeneity problems that were not appropriately resolved using various variance structures in previous attempts. Additionally, different variance structures were added to the models to eliminate issues caused by heterogeneity of variances. The addition of a random intercept and of a compound symmetry correlation structure for factor Site was also tested but did not improve the models.

Finally, the effect of estimated cattle density (ECD) (described in Chapter 3) for each sampled site on sediment nutrient concentrations as well as on nutrient loadings was assessed separately for each nutrient using generalised additive modelling, which allows for the modelling of non-linear relationships between variables. In this analysis, a smoother for ECD and the factor Location were included, as well as an interaction between the two variables. Variance structures were applied as necessary. Additionally, GAM analysis was used to assess the relationship between general site degradation and nutrient sediment levels. The analysis was conducted for all sites separately for each sampling time, using sediment nutrient concentrations, nutrient loadings, and RHI scores for the US and INT subsites. Since O'Sullivan et al. (2019) conducted the site assessment in early autumn, the two sites that were later replaced in the BK catchment were not included in the statistical analysis of the data collected in early grazing season. All models were selected validated and optimized according to the protocols described in Zuur et al. (2009). The optimum model was that with the lowest AIC (Akaike Information Criterion) value.

5.3. Results

5.3.1. Particle size distribution of the samples

The particle size distribution of streambed sediment at the study sites is presented in Table 5.3. The relative proportions of sand and silt+clay varied across the sites. However, the sediments were dominated by sand size particles across all catchments and sites, with average silt+clay fraction proportions of 9.38% ± 1.17% for BW, 5.42% ± 094% for DG, 10.30% ± 2.19% for BK, 5.82% ± 0.86% for CM and 9.23% ± 2.02% for MT (mean ± S.E.). Although the DG and CM catchments had lower average values for the silt+clay fraction, differences between the five catchments were not statistically significant (F-value = 0.654, *p*

= 0.637). There was also no statistically significant difference for the particle size distribution between early grazing season and late grazing season (F-value = 0.137, p = 0.713). The average percentage for the silt+clay fraction at the different locations were 10.35% ± 2.07% for US, 6.93% ± 1.64% for DS sites, 5.70% ± 0.75% for CAS and 9.34% ± 0.92% at the interface areas (INT) between the stream and the field. Particle size distribution, however, did vary significantly with Location (F-value = 7.019, p < 0.001). Pairwise tests revealed that the silt+clay proportion was significantly lower at CAS areas than at INT areas (t-ratio = -4.197, p < 0.001).

5.3.2. Nutrient concentrations in the <63 µm and <2mm particle size sediment fractions

Total phosphorus concentrations in the <63 µm fraction ranged from 137 to 2191 µg.g dry wt^{-1} (median value of 683.5 µg.g dry wt^{-1}), organic carbon concentrations ranged from 2.90 to 85.05 mg.g dry wt^{-1} (median value of 25.99 mg.g dry wt^{-1}) and total nitrogen concentrations ranged from 0.39 to 7.54 mg.g dry wt^{-1} (median value of 2.73 mg.g dry wt^{-1}). In the <2mm particle size fraction (which it should be noted included the <63µm fraction) concentrations ranged from 98 to 1540 µg.g dry wt^{-1} for TP (median value of 464 µg.g dry wt^{-1}), 2.87 to 57.17 mg.g dry wt^{-1} for OC (median value of 15.12 mg.g dry wt^{-1}) and 0.33 to 5.11 mg.g dry wt^{-1} for TN (median value of 1.51 mg.g dry wt^{-1}).

The <63 µm sediment fraction had significantly higher concentrations of all three nutrients than the <2mm fraction (Fig. 5.1.; Table 5.4). In the former fraction, consistent patterns were observed when comparing across the five study catchments, with sediments in the CM and the MT catchments having the highest nutrient concentrations, and sediments from the BW catchment generally having the lowest concentrations. This pattern of difference between the five catchments was not apparent in the <2mm fraction (Fig. 5.1).

TN and OC concentrations were significantly correlated in both the <63 µm and <2mm fraction ($p < 0.001$, r = 0.93; $p < 0.001$, r = 0.81, respectively) (Fig. 5.2), indicating that most TN present in the sediment was incorporated in organic material. TP concentrations were also correlated with both TN and OC for the <63µm fraction but with a lower Peason's coefficient value ($p < 0.001$, r = 0.54; $p < 0.001$, r = 0.49), but were not correlated for the <2mm fraction (Fig. 5.2).

Table 5.3. Particle size distribution and coverage of sand and silt+clay particles at the study sites.

Catchment	Site	Location	%Sand	%Silt+clay	%Estimated fine sediment coverage	Sand ($g.m^{-2}$)	Silt+clay ($g.m^{-2}$)
BK	1	US	93	8	10	1196.8	97.0
		CAS	83	18	52	6934.4	1470.9
		INT	73	28	89	11014.1	4177.8
		DS	93	8	58	10173.6	824.9
	2	US	58	43	48	4233.5	3129.1
		CAS	95	5	59	17248.7	907.8
		INT	93	8	50	11919.7	966.5
		DS	95	5	48	7118.1	374.6
	3	US	95	5	29	3802.2	200.1
		CAS	97	3	70	16574.6	512.6
		INT	88	13	92	12434.0	1776.3
		DS	99	1	42	5209.3	77.4
BW	A	US	83	18	4	248.0	52.6
		CAS	95	5	25	7712.2	405.9
		INT	85	15	80	15937.0	2812.4
		DS	100	0	2	158.7	0.0
	B	US	90	10	37	5438.9	604.3
		CAS	95	5	9	1084.2	57.1
		INT	93	8	20	1338.9	108.6
		DS	94	6	7	1113.6	67.9
	C	US	70	30	<1	41.8	17.9
		CAS	95	5	<1	19.5	1.0
		INT	93	8	74	8551.0	693.3
		DS	98	3	<1	36.2	0.9
CM	1	US	95	5	25	1798.8	94.7
		CAS	90	10	64	8085.5	898.4
		INT	89	11	55	2978.9	369.2
		DS	88	12	100	2821.6	369.2
	2	US	89	11	62	3031.7	360.3
		CAS	95	5	24	4299.8	226.3

Table 5.3 (continued).

Catchment	Site	Location	%Sand	%Silt+Clay	%Estimated fine sediment coverage	Sand ($g.m^{-2}$)	Silt+clay ($g.m^{-2}$)
CM	2	INT	93	8	85	8232.8	667.5
		DS	90	10	52	7262.7	807.0
	3	US	99	1	15	1870.4	25.5
		CAS	95	5	55	8142.6	428.6
		INT	93	8	90	12641.8	1025.0
		DS	98	3	54	6590.2	169.0
DG	A	US	95	5	15	1856.1	97.7
		CAS	97	3	45	4206.6	128.9
		INT	94	6	50	5472.5	364.8
		DS	97	3	30	3656.3	113.1
	B	US	76	24	17	3595.2	1119.8
		CAS	98	3	53	7112.6	182.4
		INT	94	6	60	7151.7	476.8
		DS	98	2	37	5038.4	122.7
	C	US	97	3	32	8233.6	232.8
		CAS	98	2	42	4231.3	82.3
		INT	93	7	NA	NA	NA
		DS	98	2	7	984.5	22.6
MT	1	US	88	13	6	553.4	79.1
		CAS	95	5	8	673.0	35.4
		INT	93	8	89	7635.4	619.1
		DS	50	50	8	1216.3	1216.3
	2	US	98	3	14	235.6	6.0
		CAS	96	4	19	5030.2	196.0
		INT	98	3	20	1976.5	50.7
		DS	88	13	<1	47.8	6.8
	3	US	96	4	11	2045.2	79.7
		CAS	98	3	31	5340.9	136.9
		INT	95	5	68	7544.9	397.1
		DS	96	4	8	2004.4	78.1

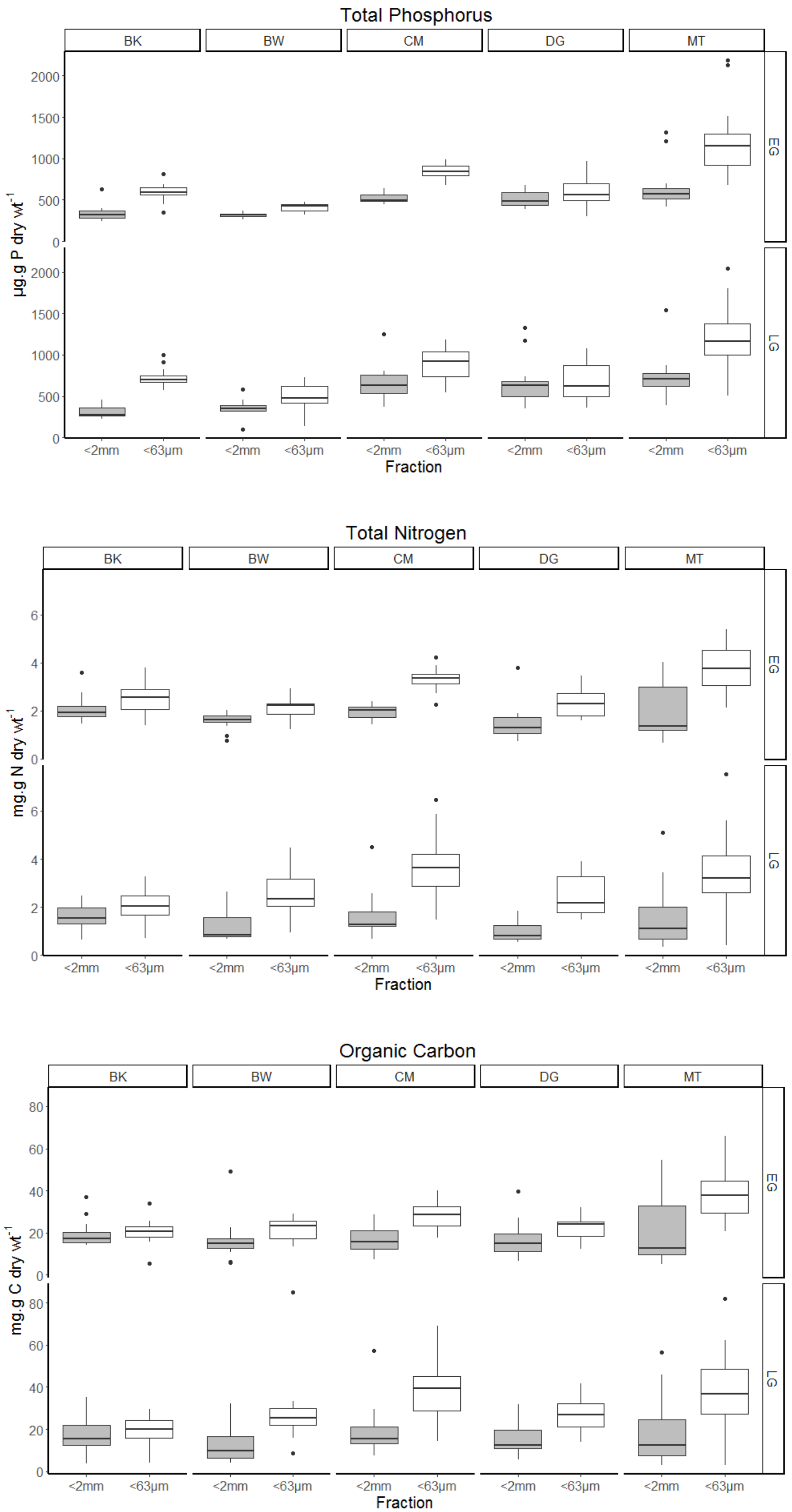

Fig.5.1. Boxplots of sediment concentrations in the <63 µm and <2 mm sediment fractions in the five study catchments, in early grazing season (EG) and late grazing season (LG) (n = 12).

Table 5.4. Sediment nutrient concentrations in the <2mm and <63µm particle size fraction for each catchment for each sampling time (n = 60), location sampled (n = 15) and catchment (n = 12) (mean ± S.E.).

	TP (µg.g dr wt^{-1})		**TN (mg.g dr wt^{-1})**		**OC (mg.g dr wt^{-1})**		**C:N ratio**	
	<2mm	*<63µm*	*<2mm*	*<63µm*	*<2mm*	*<63µm*	*<2mm*	*<63 µm*
Early grazing season								
Catchment								
BW	317±8	409±15	1.575±0.110	2.150±0.141	16.973±3.226	21.820±1.491	10.6±1.9	10.2±0.4
DG	511±29	596±52	1.522±0.233	2.369±0.186	17.328±2.704	22.341±1.621	11.8±1.1	9.5±0.3
BK	342±29	603±38	2.079±0.169	2.555±0.199	19.820±1.992	21.333±2.211	9.5±0.5	8.2±0.5
CM	524±19	844±24	1.936±0.091	3.318±0.148	16.496±1.703	28.366±1.856	8.4±0.7	8.5±0.3
MT	666 ± 84	1233±142	2.041±0.337	3.819±0.299	21.837±4.719	40.199±3.943	9.9±0.6	10.4±0.3
Location								
US	440±38	663±68	1.843±0.192	2.699±0.258	17.179±2.412	25.398±3.320	9.1±0.6	9.2±0.5
CAS	435±27	691±66	1.848±0.219	2.697±0.234	18.327±3.269	25.749±3.173	9.5±0.8	9.4±0.4
INT	561±80	903±152	1.906±0.221	3.083±0.271	21.514±3.336	28.031±2.590	11.3±1.5	9.2±0.3
DS	453±34	691±63	1.726±0.131	2.889±0.199	16.943±1.428	28.069±2.115	10.2±0.7	9.7±0.3
Average for EG	472±25	737±48	1.831±0.095	2.842±0.120	18.491±1.348	26.812±1.393	10.0±0.5	9.4±0.2

Table 5.4 (continued)

	TP ($\mu g.g$ dr wt^{-1})		TN ($mg.g$ dr wt^{-1})		OC ($mg.g$ dr wt^{-1})		C:N ratio	
	<2mm	*<63µm*	*<2mm*	*<63µm*	*<2mm*	*<63µm*	*<2mm*	*<63µm*
Late grazing season								
Catchment								
BW	359±33	496±48	1.184±0.173	2.519±0.279	12.770±2.398	29.042±5.462	10.2±0.6	11.1±0.8
DG	681±83	682±68	0.972±0.121	2.487±0.252	15.450±2.196	26.830±2.526	16.3±1.8	10.9±0.4
BK	310±22	735±34	1.582±0.149	2.048±0.203	17.391±2.480	19.714±2.152	10.5±0.8	9.4±0.4
CM	669±64	895±56	1.658±0.300	3.744±0.418	20.031±3.791	39.491±5.017	12.3±0.8	10.3±0.3
MT	745 ±82	1195±135	1.645±0.424	3.551±0.533	19.671±5.194	39.313±6.074	11.4±0.5	10.7±0.4
Location								
US	514 ±72	776±96	1.310±0.173	2.691±0.384	15.823±2.670	29.394±4.628	12.3±1.8	10.5±0.4
CAS	547 ±67	798±98	1.128±0.104	2.789±0.245	13.552±1.504	28.919±3.063	12.0±0.7	10.2±0.3
INT	617 ±79	892±106	1.798±0.297	3.401±0.453	21.222±3.393	37.664±5.855	11.9±0.6	10.9±0.6
DS	533 ±72	737±58	1.396±0.297	2.598±0.294	17.653±3.931	27.535±3.413	12.3±0.8	10.4±0.3
Average for LG	553±36	801±45	1.408±0.118	2.870±0.177	17.063±1.513	30.878±2.195	12.1±0.5	10.5±0.2

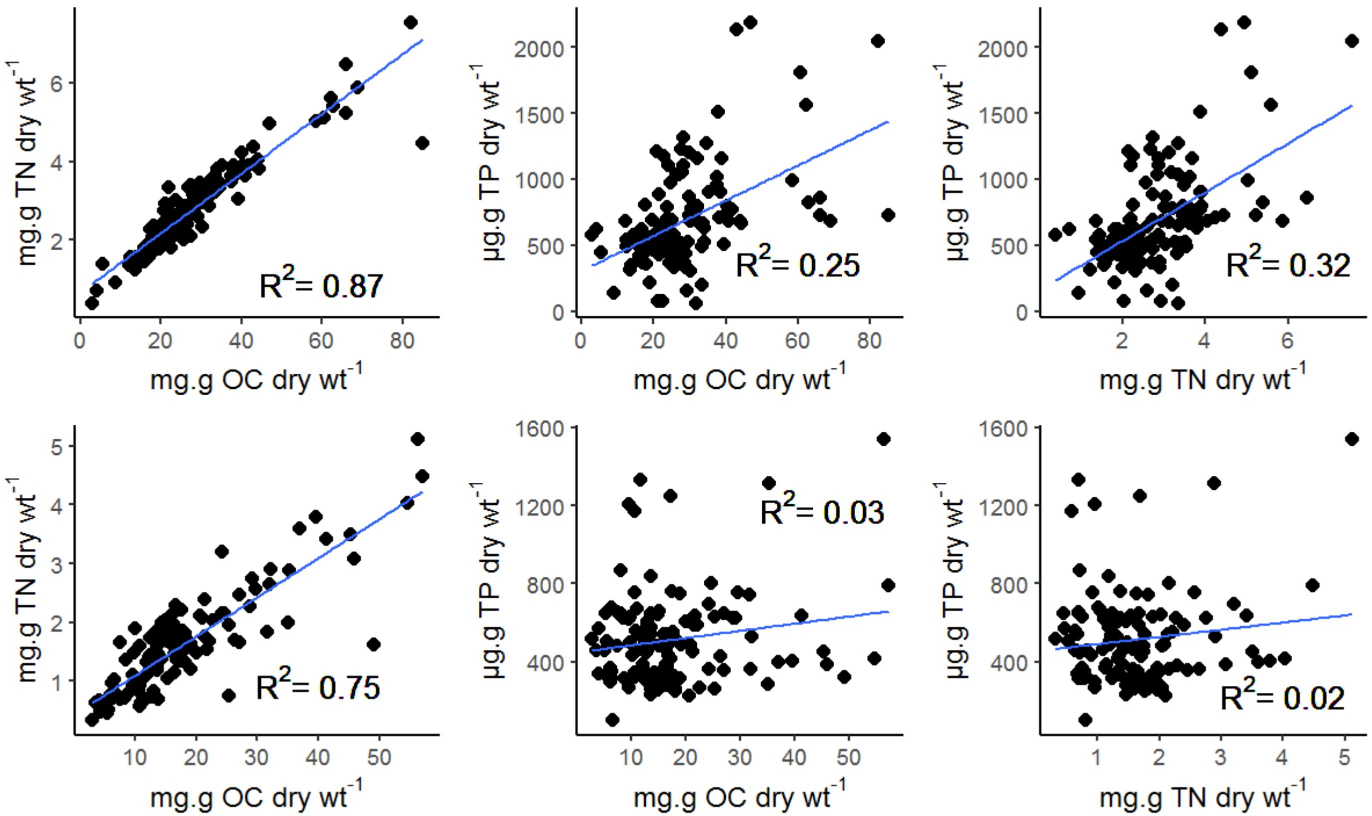

Fig. 5.2. Scatterplots showing the relationships between nutrients in the <63µm (top row) and the <2mm (bottom row) particle size fractions.

5.3.3. Effects of direct cattle access on stream sediment nutrient levels

For the <63µm fraction, all nutrient concentrations were generally higher at INT sites than at the remaining Locations (US, CAS and DS) (Fig. 5.3), however the factor Location was only statistically significant for TN (Table 5.5). Pairwise tests revealed that overall TN concentrations at INT areas were significantly higher than at US areas (t-ratio = 2.734, p = 0.036). There was also a significant increase in sediment TP concentrations in late grazing season compared to early grazing season, but no significant differences were observed for OC and TN between the two sampling times (Table 5.5). No significant interactions between factors Time and Location were found (Table 5.5)

For the <2mm fraction, no significant differences were found between the four different levels of Location for any of the three nutrients (Fig. 5.4, Table 5.6). However, p values for this comparison were on the boundary of significance for all three nutrients (Table 5.6). In contrast with the <63µm fraction, TP concentrations in this fraction did not change significantly between the early grazing season and the late grazing season. Conversely, TN sediment concentrations decreased significantly from early grazing season to late grazing season (Table 5.6). Organic C concentrations did not vary significantly between sampling times. Similarly to what had been observed for <63µm fraction, there were no significant interactions between the factors Location and Time for any of the nutrients (Table 5.6).

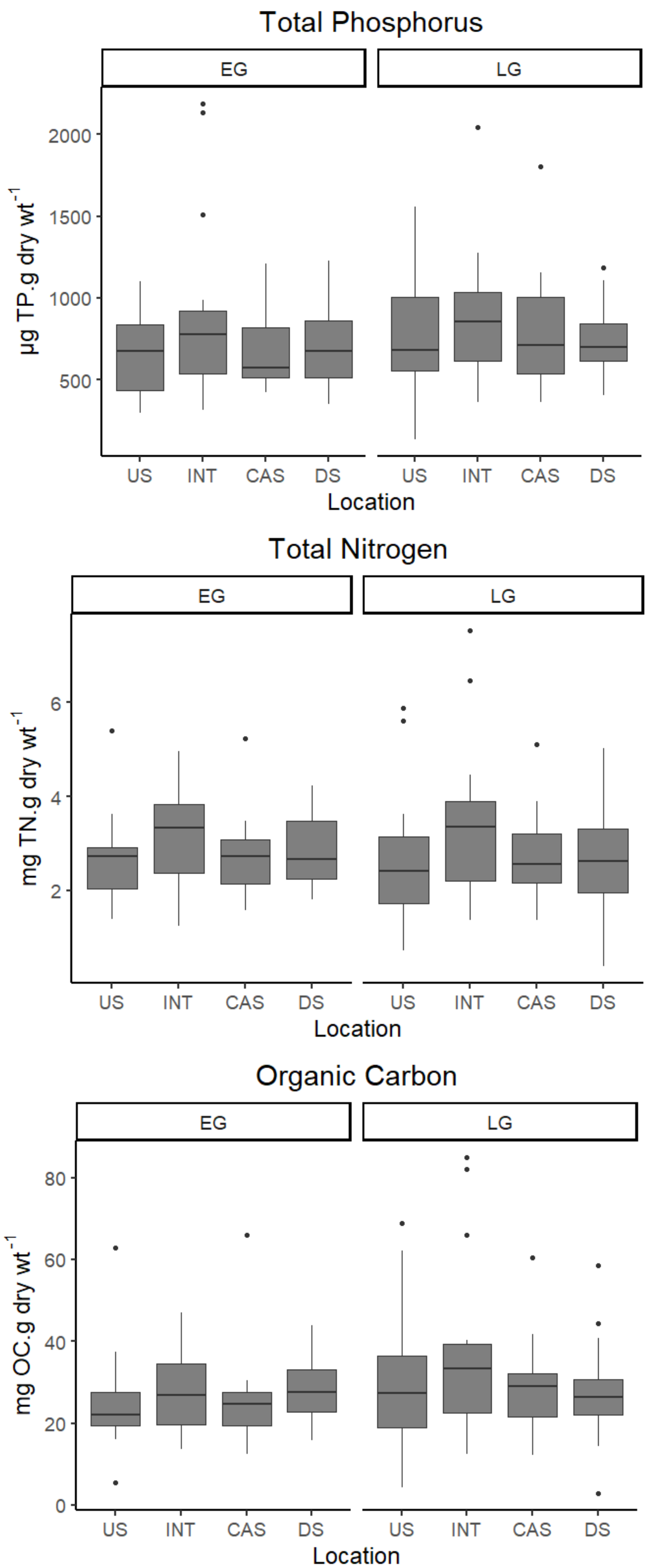

Fig.5.3. Boxplots of sediment concentrations in the <63µm particle size sediment fractions at each of the locations sampled at the cattle access sites, in early grazing season (EG) and late grazing season (LG) (n = 15).

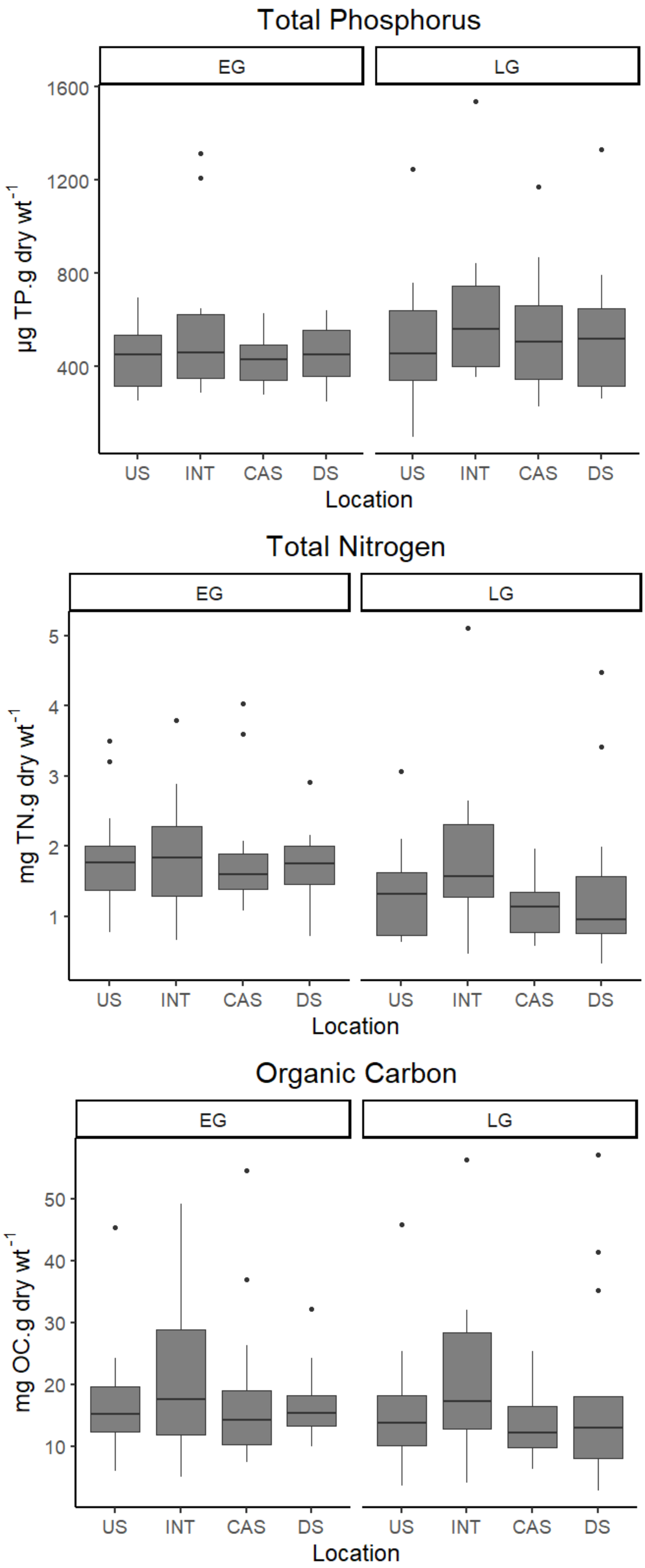

Fig.5.4. Boxplots of sediment concentrations in the <2mm particle size sediment fractions at each of the locations sampled at the cattle access sites, in early grazing season (EG) and late grazing season (LG) (n = 15).

Table 5.5. Statistical parameters for the effect of sediment fraction (GLS analysis) and for the effect of Location, Time and interaction between the two factors (mixed effects modelling) on sediment nutrient concentrations in the < 63µm fraction. Significant effects are shown in bold.

Analysis	TP		TN		OC	
	F-value	*p*	F-value	*p*	F-value	*p*
Fraction	**158.138**	**< 0.001**	**118.412**	**< 0.001**	**48.157**	**< 0.001**
Location (<63µm)	1.997	0.119	**2.722**	**0.048**	1.857	0.141
Time (<63µm)	**6.231**	**0.014**	1.997	0.119	2.700	0.103
Location x Time (<63µm)	0.469	0.704	0.405	0.750	0.162	0.922

Table 5.6. Statistical parameters for the effect of Location, Time and interaction between the two factors (mixed effects modelling) on sediment nutrient concentrations in the <2mm fraction. Significant effects are shown in bold.

Analysis	TP		TN		OC	
	F-value	*p*	F-value	*p*	F-value	*p*
Location (<2mm)	2.673	0.051	2.392	0.073	2.525	0.061
Time (<2mm)	1.956	1.645	**22.501**	**<0.001**	1.298	0.257
Location x Time (<2mm)	0.067	0.977	1.016	0.389	0.126	0.945

5.3.4. Sediment nutrient loads

Nutrient loads in the stream bed sediment based on the particle size distribution of the sediment at the study sites and the fine sediment coverage estimates are presented in Table 5.7 (for late grazing season only). For TP, TN and OC, sediment nutrient loads in the silt+clay fraction were significantly higher at INT sites than at the three remaining locations (TP: F-value = 7.590, $p < 0.001$; TN: F-value = 6.629, $p = 0.001$; OC: F-value = 6.630, $p < 0.001$) (Fig 5.5; Table 5.8). In the <2mm fraction, sediment loads of all three nutrients varied significantly with Location (TP: F-value = 7.482, $p < 0.001$; TN: F-value = 7.226, $p < 0.001$; OC: F-value = 8.214, $p < 0.001$). Pairwise comparisons showed that sediment nutrient loads were higher at INT sites than at US and DS sites, but not significantly higher than sediment loads at CAS (Fig.5.5; Table 5.8).

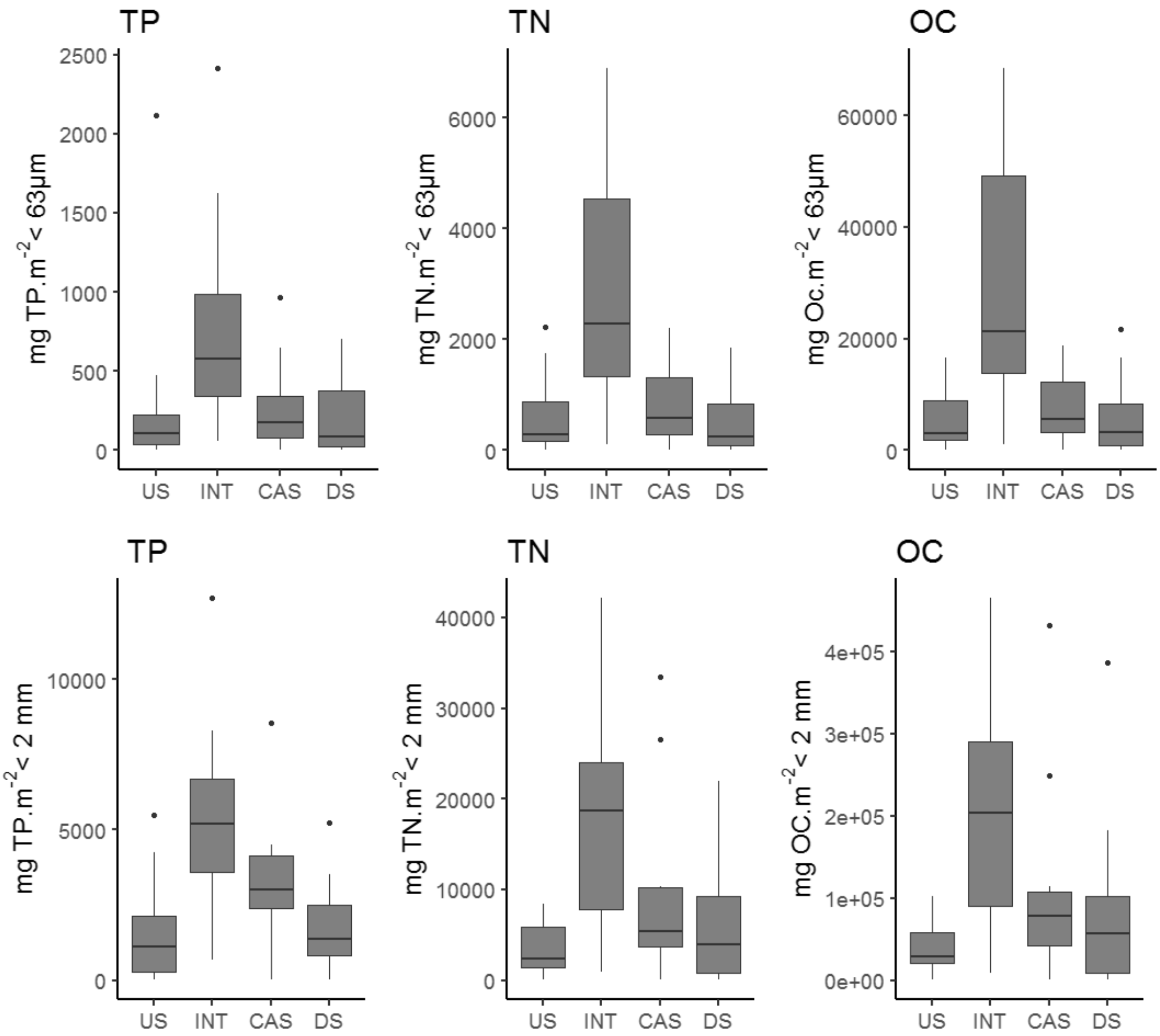

Fig. 5.5. Nutrient loads in the silt+clay fraction (top row) and <2mm fraction (bottom row) in late grazing season at each study location.

Table 5.7. Sediment nutrient loads in each study catchment and at each location in late grazing season (mean ± S.E.).

	TP ($mg.m^{-2}$)		**TN ($mg.m^{-2}$)**		**OC ($mg.m^{-2}$)**	
	<2mm	*<63µm*	*<2mm*	*<6 µm*	*<2mm*	*<63µm*
Catchment[1]						
BW	1449.8 ± 610.8	163.6 ± 84.9	6078.1 ± 2937.7	1066.4 ± 575.6	67541.5 ± 33039.7	11060.0 ± 5681.6
DG	3322.5 ± 664.3	162.0 ± 44.5	4929.0 ± 800.7	586.2 ± 159.8	75935.1 ± 12789.3	6079.3 ± 1488.0
BK	3222.6 ± 524.1	830.1 ± 230.8	16494.6 ± 3222.9	1924.5 ± 486.0	188272.0 ± 42421.4	18016.2 ± 4844.2
CM	3828.0 ± 614.7	383.9 ± 71.5	9710.5 ± 1934.1	1600.8 ± 387.9	116942.0 ± 24487.5	16361.6 ± 3964.7
MT	2787.9 ± 1066.2	268.7 ± 109.6	5947.0 ± 3393.8	714.7 ± 374.4	66461.3 ± 37397.3	7611.2 ± 4075.4
Location[2]						
US	1606.7 ± 424.2	266.2 ± 1 36.5	3490.8 ± 734.9	597.7 ± 178.9	39244.6 ± 7815.2	5718.5 ± 1539.2
CAS	3131.5 ± 534.6	262.3 ± 68.9	8553.2 ± 2430.5	852.8 ± 186.5	98852.4 ± 28522.7	8053.9 ± 1592.8
INT	5279.8 ± 818.0	755.1 ± 174.9	17739.7 ± 3347.6	2883.9 ± 568.4	206801.0 ± 36286.0	29552.1 ± 5623.4
DS	1801.1 ± 378.3	202.6 ± 62.1	5597.7 ± 1657.3	532.9 ± 155.0	75948.0 ± 26348.4	5543.0 ± 1704.3

[1]n = 12; [2]n = 15

Table 5.8. Pairwise comparisons within factor Location for sediment nutrient loadings in late grazing season. Significant factors are shown in bold.

Comparison		TP		TN		OC	
	df	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*
Silt+clay fraction							
CAS – DS	51	0.160	0.999	1.866	0.255	1.505	0.442
CAS – INT	**51**	**-4.019**	**0.001**	**-3.591**	**0.004**	**-3.852**	**0.002**
CAS – US	51	-0.456	0.968	1.104	0.688	1.131	0.672
DS – INT	**51**	**-4.171**	**0.001**	**-4.200**	**0.001**	**-4.293**	**<0.001**
DS – US	51	-0.616	0.926	-0.301	0.990	-0.083	0.100
INT - US	**51**	**3.585**	**0.004**	**3.940**	**0.001**	**4.163**	**0.001**
<2mm fraction							
CAS – DS	51	2.495	0.073	1.201	0.629	0.604	0.930
CAS – INT	51	-2.281	0.116	-2.354	0.099	-2.366	0.097
CAS – US	51	2.421	0.086	2.281	0.116	2.061	0.180
DS – INT	**51**	**-4.083**	**0.001**	**-3.412**	**0.007**	**-2.497**	**0.024**
DS – US	51	0.413	0.976	1.363	0.528	1.363	0.528
INT - US	**51**	**4.015**	**0.001**	**4.198**	**0.001**	**4.525**	**<0.001**

5.3.5. Effects of cattle density and intensity of impact on stream sediment nutrient levels

The generalised additive modelling (GAM) analysis revealed a highly significant effect of estimated cattle density (ECD) on sediment concentrations for all three nutrients in the <63µm particle size fraction across the 15 sites (Fig. 5.6, Table 5.9). ECD explained 23%, 28% and 41% of the variance for OC, TN and TP, respectively. When similar analysis was undertaken for the <2mm particle size fraction, ECD had a significant effect on TP only, explaining 23% of the variance (Table 5.9). Interestingly, a model which included ECD and factor Location revealed a significant effect of factor Location for TP concentrations in the <63µm fraction. This model indicated a significant interaction there between INT and factor ECD when different variance structures where applied. However this interaction became non-significant at the 5% level when applying the variance structure that gave the lowest AIC value ($p = 0.079$; Table 5.9). Figure 5.7 shows the smoother for the effect of ECD on sediment TP concentrations in this fraction at US areas (set as a reference), while the remaining three plots show the additional change in TP concentrations explained by each of the other levels of factor Location. TP concentrations show a further increase at INT areas compared to US areas, whereas no further changes were observed for CAS and DS sites. The same analysis conducted on nutrient loadings at each sampled locations did not reveal any significant effect of ECD on these parameters.

In contrast, no effect of the intensity of impact at cattle access sites, as expressed by the RHI scores attributed by O'Sullivan et al. (2019) were found in this study for bed sediment nutrient concentrations or loads.

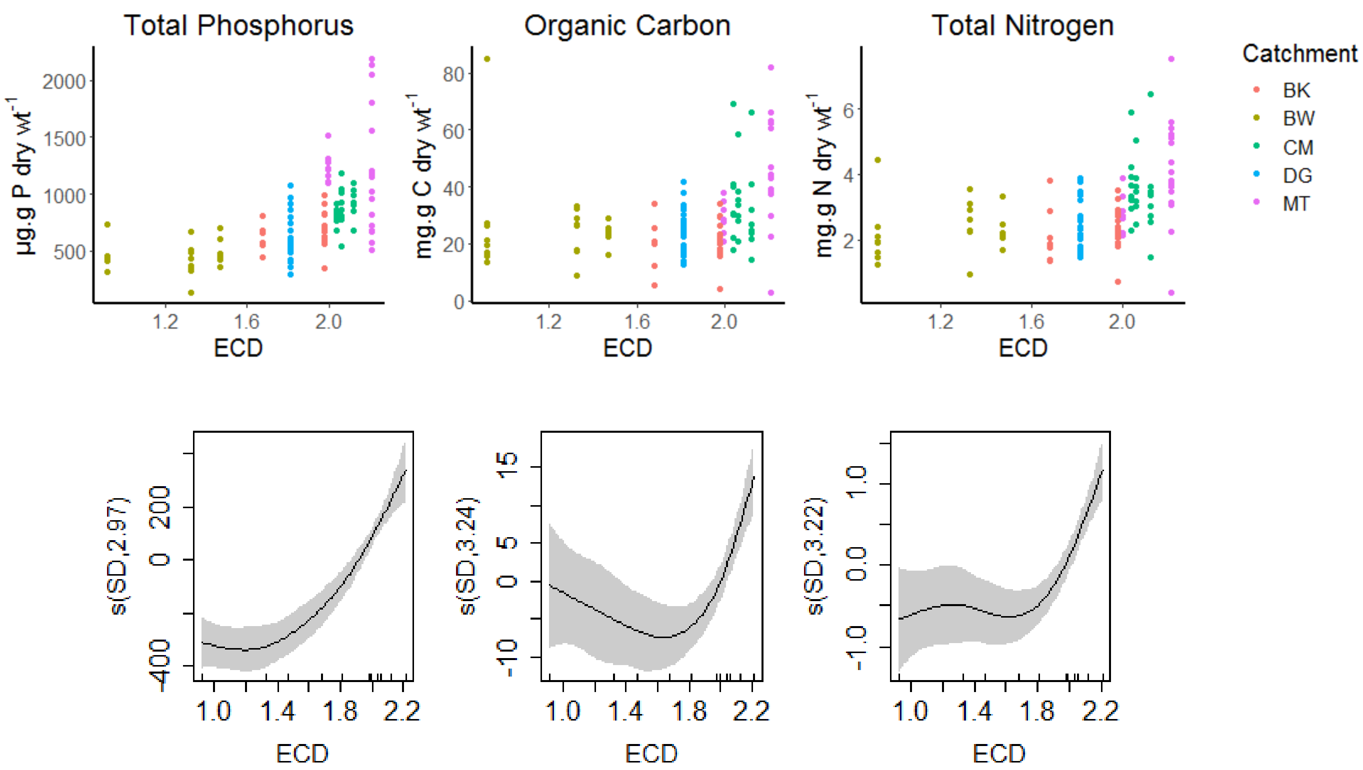

Fig.5.6. Top row: fine sediment (<63µm) total phosphorus (TP) organic carbon (OC) and total nitrogen (TN) concentrations versus estimated cattle density (ECD; animals.ha^{-1}) (including data for the early and late grazing season and for upstream, interface, cattle access points, and downstream locations); bottom row: generalised additive models using these data. The central solid line is the smoother, the grey area is the 95% confidence bands, and the Y axis units are the scaled smoother (s) for ECD with estimated degrees of freedom (efd).

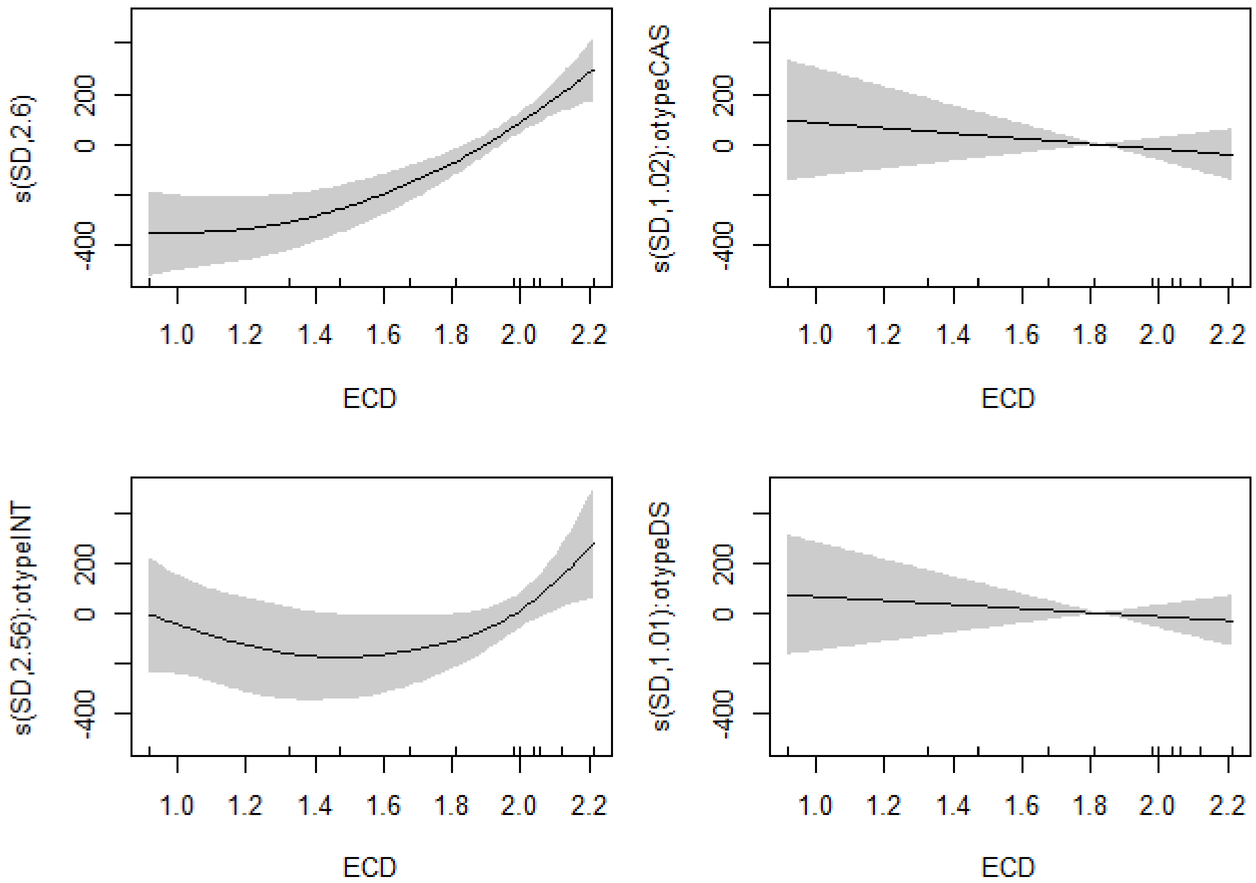

Fig 5.7. A. smoother for the effect of estimated cattle density (ECD) on TP for upstream (US) sites (reference level), and difference smooths reflecting the estimated differences between the upstream sites and the CAP sites (CAS, top, right), interface sites (INT, bottom, left), and downstream (DS, bottom, right) respectively. The central solid line is the smoother, the grey area is the 95% confidence bands, and the Y axis units are the scaled smoother (s) with estimated degrees of freedom (efd).

Table 5.9. Generalised additive model parameters for the effect of cattle density on sediment nutrient concentrations (n = 120) (ECD = estimated cattle density; edf = estimated degrees of freedom). Note that the optimum model for TP <63µm used an ordered factor approach, and therefore includes a parametric term for the factor Location, where upstream (US) was set as the reference level. Only values for significant effects are shown except for ECD*Location (INT) effect.

Fraction	Nutrient	Driver	edf	Statistics	*p*	R^2 adj
<2mm	TP	ECD	2.65	F = 16.70	<0.0001	0.23
<63µm	OC	ECD	3.23	F = 10.81	<0.0001	0.23
<63µm	TN	ECD	3.21	F = 14.81	<0.0001	0.28
<63µm	TP	ECD	2.97	F = 32.32	<0.0001	0.41
		Location (INT)		t = 2.51	0.0136	
		ECD* Location (INT)	2.56	F = 2.15	0.079	

5.4. Discussion

The practice of allowing cattle access to watercourses has been shown to negatively impact freshwater systems in a number of studies (Trimble, 1994; Vidon et al., 2008; Herbst et al., 2012; Terry et al., 2014); however, its effects on sediment nutrient levels have been largely overlooked in literature. Furthermore, while high stocking intensity has been linked to exacerbated diffuse nutrient pollution (Chalar et al., 2017), it has rarely been considered in the context of cattle access to watercourses and indeed in relation to contamination of streambed sediments. In the present study, although direct cattle access did not cause a measurable accumulation of phosphorus or organic matter at a local level, with only TN varying significantly with Location within cattle access sites, ECD was a significant driver of stream sediment nutrient concentrations in the <63μm particle size fraction. For TP sediment concentrations only, this effect was accentuated at the interface locations at the cattle access site.

5.4.1. Enrichment of the <63μm fraction

Overall, the nutrient concentrations measured in this study fell within the range of what has been observed by other authors that have examined streambed sediment, stream bank and soils nutrient concentrations in areas impacted by agriculture (Table 5.2). The current study also found a strong nutrient enrichment effect for the smaller <63μm (silt+clay) proportion of the sediment in comparison to the <2 mm particle size fraction. This was expected as this fraction is considered to be the most chemically reactive due to its higher surface to volume ratio (Sharpley et al., 2013), and would be particularly apparent for phosphorus, due to its affinity for aluminium-silicate minerals (Fox et al., 2016). This enrichment likely played a role in the overall pattern that was observed across the five study catchments that was not apparent in the <2mm fraction and that was consistent for all three nutrients. More importantly the exploration of the effect of cattle numbers on streambed sediment nutrient levels showed that this pattern was strongly related to cattle density for the sites, as shown by the GAM analysis. The highest nutrient concentrations were found for the five study catchments were in the CM and MT catchments, which are characterised by intensive agriculture, while the lowest nutrient concentrations were the BW catchment, which has more extensive agriculture. To the knowledge of the authors, such relationship between cattle agriculture intensity and bed sediment nutrient concentrations has not been previously reported.

5.4.2. Impacts of cattle access on sediment nutrient concentrations

Sediment nutrient concentrations did not differ significantly at stream reaches used by cattle compared to areas not used by cattle in the <2mm size fraction whereas in the <63µm particle size fraction, only TN differed significantly with Location, with concentrations at INT areas being significantly higher than at US areas. However, *p* values obtained for all three nutrients in the <2mm particle size fraction were on the boundary of the significance limit, which suggests that there is a weak effect of cattle access on sediment nutrient levels in this fraction.

Increased sediment nutrient concentrations at access sites would result from in-stream defecation and urination, addition of faecal matter attached to the animals' legs and washing-off of faecal material and urine deposited nearby the stream. Bond et al. (2014) estimated that cattle faeces are mostly composed of water (89.4%), containing on average 0.79% nitrogen and 0.43%% phosphorus by wet mass. The authors suggested that cattle faeces would be particularly soluble and susceptible to transport and dispersion within the stream following deposition into stream waters. Furthermore, within the stream, cattle faeces can be quickly colonized and utilized by benthic invertebrate taxa (Mesa et al., 2016). Indeed, a study carried out at the same sites sampled in the present work based on isotope analysis of organic carbon sources indicated that invertebrate taxa may consume cattle faeces at cattle access sites (Ó hUallacháin et al., 2020). Cattle urine contains mainly N in the form of urea, which hydrolises rapidly upon excretion (Dijkstra et al., 2013). Following deposition, urine is subject to dilution and transport within the stream. Both major dissolved forms of N, NO_3^- and NH_4^+, can be readily absorbed by microoorganisms, periphyton and macrophytes or can be transported downstream before it becomes incorporated in the bed sediment (Kronvang et al., 1999; Butturini et al, 2000). This may help to explain the observation that OC and TP did not accumulate significantly in sediment at areas used by cattle despite frequent in-stream defecation and urination. Furthermore, the systematic disturbance of the sediments by cattle while using the stream would cause its resuspension and subsequent transport downstream, particularly for the smaller particles (Naden et al, 2010; Sharpley et al., 2013), which can also prevent the accumulation of nutrient-enriched sediments at access sites.

When looking at nutrient levels at cattle access sites in terms of loads, all three nutrients where present in significantly higher loads at interface areas. This is related to the fact these areas had higher fine sediment coverage than the remaining areas. These results indicate that these interface areas that are created with cattle repeatedly accesses a watercourse, and where animals likely congregate to drink, and which are rich in nutrients and fine sediment, likely to act as critical source areas of these contaminants to the watercourse (Thompson et al., 2013).

5.4.3. Impacts of cattle density on sediment nutrient concentrations

One of the most important results from this study was that there was a significant effect of estimated cattle density on streambed sediment concentrations of all three nutrients in the <63µm fraction, and for TP in the <2mm fraction. For both OC and TN, there seemed to be a threshold at an estimated 1.6 animals per ha, after which sediment nutrient concentrations increased sharply (Fig. 5.6). For TP, this threshold seemed to be lower at 1.2 animals per ha (Fig. 5.6). In Europe, dairy cattle densities are highest in Western Germany, Bavaria, the Netherlands, Northern Italy, Ireland and the Brittany region in France, whereas beef cattle densities are highest in Scotland and North England, Ireland and Central France (Neumann et al., 2009). An estimation of cattle density in ten European countries with strong cattle-based agriculture is shown in Table 5.10. According to this estimation, only two of these EU Member-States have cattle densities lower than 1.6 cattle.ha^{-1} (Table 5.9). Thus, the findings of the current study have implications in the context of livestock agriculture management in Europe.

Table 5.10. Estimation of cattle density in EU Member States. Cattle density was calculated by dividing an estimation of the total number of cattle by the area devoted to permanent grassland in each country.

Country	Total bovine population[1]	% EU bovine population[1]	Number of cattle	Permanent grassland (ha)[5]	Cattle.ha^{-1}
France	87000000	0.212	18444000	9593990	1.92
Germany		0.137	11919000	4713400	2.53
UK		0.11	9570000	11277000	0.85
Ireland		0.075	6525000	4064210	1.61
Spain		0.074	6438000	7037370	0.91
Italy		0.072	6264000	3659630	1.71
Poland		0.071	6177000	3149870	1.96
The Netherlands		-	3721000[2]	763790	4.87
Luxembourg		-	196127[3]	67710	2.90
Belgium		-	2500000[4]	1356080	1.84

[1]Information retrieved from the most recent EU Agriculture, Forestry and Fishery Statistics report (Eurostat, 2019). [2]Data retrieved from Statistics Netherlands (www.cbs.nl). [3]Data retrieved from https://statistiques.public.lu. [4]Data retrieved from https://www6.inrae.fr/sustainbeef/Publications/Beef-production-in-the-EU/Beef-production-in-Belgium. [5]Data retrieved from https://ec.europa.eu/eurostat/web/products-datasets/-/tag00025.

In addition to the effect of cattle density on sediment nutrient concentrations in the silt+clay fraction, there was also an increase in TP concentrations at interface (INT) areas of the cattle access sites with estimated cattle density, indicating a possible effect of direct cattle access at these more impacted areas and cattle density on stream bed sediment phosphorus reservoirs. The fact that this effect was only apparent for TP again illustrates the higher chemical reactivity of phosphorus with sediments particles (McGechan et al., 2005) in comparison to other nutrients. This impact of diffuse contamination likely reflects the nature of the soils at the study sites, which are predominantly poorly drained, and thus would favour the transport of particulate forms of P to the aquatic streams in surface pathways (Deakin et al., 2016).

5.4.4. Variation in sediment nutrient concentrations between sampling times

In general, sediment nutrient concentrations might be expected to have increased in late grazing season compared to early grazing season as a result of cattle activity. However, there was only a marginal increase in TP in the overall sediment concentrations in the silt+clays fraction between early and late season, whereas TN significantly decreased in the late grazing season in the <2mm particle size fraction. A closer examination of the sediment nutrient concentrations at each sampled location within access sites showed that the general increase in TP levels in late grazing season was mainly seen at interface areas. Conversely, the decrease in TN sediment concentrations in late grazing season occurred at all locations within access sites, but remained high in comparison at interface areas. The higher TN concentrations in early grazing season may have been associated with slurry spreading, which has been reported to occur mostly in the spring months (roughly from January to end of April) (Hennessy et al, 2011). The higher TN concentrations at INT sites in late grazing season suggest that cattle access to watercourses influences TN sediment levels at access sites. The fact that this was not reflected in the statistical analysis of the data could be related to the small size of the sample when examining interactions between sampling time and location.

5.5. Implications

The results of this study highlight the role that cattle-based agriculture plays in streambed nutrient contamination. Although diffuse pollution was not specifically monitored in this study, results indicate that streambed nutrient contamination is influenced by diffuse pathways of pollution. Cattle-based agriculture can result in increased nutrient losses from agricultural fields via the following mechanisms: 1) wash-out of nutrients from application of animal slurries, usually associated with intensive livestock production, on grazing fields, 2) wash-out of nutrients from inorganic fertilisers applied to pastoral areas, and 3) nutrient loss associated with runoff from fields where cattle faeces and urine have been deposited. Diffuse nutrient transfers from grazing fields are primarily controlled by rainfall runoff events and can be separated in residual transfers, whereby nutrient losses are derived from non-utilised nutrients stored in agricultural soils, and incidental transfers, whereby nutrients are lost from recently applied slurries or fertilisers (Shore et al., 2017). Cattle can also contribute to diffuse pollution indirectly by causing changes in soil structure, particularly in wet soils, which can promote surface runoff generation (Bilotta et al., 2007). The mechanic action of cattle hooves on soils can cause soil treading, pugging (i.e. livestock treading on wet soft soil creating deep hoof imprints) and poaching, resulting in reduced soil porosity and increased soil bulk density, and associated reduced water infiltration capacity (Bilotta et al., 2007). This deformation of agricultural soils has been shown to increase with stocking density (Willatt and Pullar, 1983; Mulholland and Fullen, 1991), due to the cumulative impact of the animals on the soils, and to the lower protective vegetation cover that is generally available at higher stocking rates (Bilotta et al., 2007).

In addition to this larger scale, diffuse pollution caused by cattle-based agriculture, the findings of this study suggest that this effect can be further exacerbated by direct cattle access to watercourses as cattle creates nutrient-rich, erodible areas in streams, that can act as critical source areas of pollution (Thompson et al., 2013). This contamination will negatively impact water quality, while also contributing to potential legacy phosphorus issues (Sharpley et al., 2013). This can hinder the effect of mitigation measures and water quality protection practices (Jarvie et al., 2013), with potential implications for the achievement of the Water Framework Directive requirement of achieving at least good ecological status of all waterbodies by 2027 (Government of Ireland, 2018). The results of this study are particularly important in the context of the current agricultural and agri-environmental policies, such as Green Low Carbon Agri-Environment Scheme (GLAS) which contains measures to restrict cattle access to watercourses. However, adherence to this scheme and the implementation of such measures is voluntary, and limited by financial and other practical constrains. Further research should therefore focus on the cost-effectiveness of cattle exclusion measures on reducing excess nutrient inputs to waterbodies, and measures aimed at engaging the farmer community on water quality protection are recommended.

6. A near-real time assessment of the effects of cattle in-stream activity on water physicochemical and microbial parameters

Chapter 6. A near-real time assessment of the effects of cattle in-stream activity on water physicochemical and microbial parameters

6.1. Introduction

Cattle seek watercourses and riparian areas for drinking water, shade and palatable vegetation (McKergow et al., 2003; James et al., 2007;), and may also use them as crossing points between grazing fields (Davies–Colley et al., 2004; O'Callaghan et al., 2018). It has been reported that where access to watercourses is unrestricted, cattle tend to preferentially congregate in the riparian area (e.g. James et al, 2007; Haan et al., 2010; Bond et al., 2012: Kay et al., 2018), although some studies have reported that cattle do not favour the watercourse itself (e.g. Hann et al., 2010; Bond et al., 2012). Nevertheless, as discussed in previous chapters, unrestricted cattle access to watercourses has been shown to have a wide range of direct detrimental effects including streambank deterioration (Braccia and Voshell, 2007; Zaimes and Schultz 2011), increased sedimentation (O'Sullivan et al., 2019), loss of riparian vegetation and habitats (Belsky et al., 1999) and streambed faecal contamination (Bragina et al., 2017).

A number of studies have reported that cattle tend to defecate more often when in proximity to water. For example, in their study of the water quality impacts of a dairy herd (246 animals) crossing the Sherry River in New Zealand, Davies-Colley et al. (2004) estimated that the herd defecated c. 50 times more per metre of stream then elsewhere. Similarly, Bond et al. (2012), studying a herd of 68 bullocks in the UK, observed that the animals defecated in waters five times more frequently than the average frequency of defecation overall. A study of four dairy cow herds in the US also reported that the animals defecated 1.3 to 7.8 times more often in the stream and riparian area than elsewhere (James et al., 2007). There is no similar study investigating the frequency of urination by cattle in or nearby streams, however it has been observed that the spatial incidence of urination is positively correlated to the time cattle spend in certain areas (White et al., 2001; Draganova et al., 2015). Thus, given the spatial preference of cattle for riparian areas (James et al, 2007; Haan et al., 2010; Bond et al., 2012; Kay et al., 2018), it is likely that cattle urinate more often in these areas, potentially increasing the risk of nutrient loss to waters. Additionally, as highlighted in previous chapters, stirring up of stream bed sediments during cattle activity within the stream channel can also increase concentrations of nutrients (House, 2003) and faecal organisms in the water column (Collins and Rutherford, 2004).

The volume of cattle excretal output is determined by factors such as type of diet, feed and water intake, animal liveweight and reproductive status (Dijkstra et al., 2013; Smith and Frost, 2000). Studies have reported average numbers of urination events per animal per day ranging from 6.5 (dairy cattle) to 13.5 (beef cattle) (Oudshoorn et al., 2008; Orr et al., 2012; Selbie et al., 2015; Misselbrook et al., 2016), but typically 10 urinations.day^{-1},. Defecation events are typically around 10 per day (Oudshoorn et al., 2008; Orr et al., 2012). Urination events consisting of 0.4 L to 6.4 L (mean of 1.8 L) have been reported (Misselbrook et al., 2016), whereas defecation events typically consist of deposits of 1 – 2 kg (fresh weight) (Davies-Colley et al., 2004; James et al., 2007; Bond et al., 2014). Dry matter in cow faeces generally makes up between 11% (Bond et al., 2012; Orr et al., 2012) to 18% (James et al., 2007) of the fresh faeces.

Urine is the main pathway of excretion of nitrogen in cattle, containing more readily available forms of N than faeces (Selbie et al., 2015). Studies have reported TN concentrations in cattle urine ranging from 3.0 g.L^{-1} to 20.5 g.L^{-1} (see Table 5.1). The dominant form of nitrogen in urine is urea-N, which in these studies represented 52.1% to 93.5% of TN. Ammonia was measured in two of these studies, corresponding to an average of 0.9% (Gonda and Lindberg, 1994) to 2.9% (Bristow et al., 1992) of the TN in urine. Phosphorus, on the other hand, is mostly excreted in faeces and is present in cattle urine in much lower concentrations, with Orr et al. (2012) reporting an average of 0.0039 $mg.kg^{-1}$ urine TP in their study. TP concentrations in cattle faeces ranging from 4.9 mg.kg dry wt^{-1} to 12.65 mg.kg dry wt^{-1} have been reported in literature (see Table 5.1). Furthermore, in a study investigating the effects of dietary P on faecal P excretion by dairy cows, Dou et al. (2002) observed that a substantial amount of the faecal TP was readily soluble inorganic P, and that this fraction increased with increasing P intake, representing 30.3% to 49.6% of the total. The addition of phosphorus to stream waters is particularly relevant as phosphorus is generally the limiting nutrient for plant and algal growth in freshwater systems (Reddy et al., 1999; Jennings et al., 2003). Nitrogen concentrations in faeces ranging from 18.1 g.kg dry wt^{-1} to 37.8 g.kg dry wt^{-1} have been reported (Table 5.1), with 3 g.kg dry wt^{-1} to 28 g.kg dry wt^{-1} corresponding to NH_4-N (Sorensen et al., 2003).

Concentrations of the faecal bacteria *E. coli* in fresh cattle faeces reported in literature are typically in the range of 10^6 - 10^7 CFU.g dry wt^{-1} (see Table 4.1), with some studies suggesting that *E. coli* populations in faeces can grow in the days following faecal deposition (Oliver et al., 2010; Oliver and Page, 2016). Furthermore, as discussed in Chapters 2 and 4, it has been widely reported that once bacteria are deposited in the aquatic system, they can be incorporated into the sediment, where they can persist for prolonged periods of time (Garzio-Hadzick et al., 2010; Ishii et al., 2007; Ishii and Sadowsky, 2008; Badgley et al., 2011; Shelton et al., 2014). Indeed, significantly higher concentrations of *E. coli* bacteria have been reported in sediments at sites with unrestricted cattle access compared with sites with no access by Bragina et al. (2017), and have been observed in the study described in Chapter 4 of this thesis.

As discussed in earlier chapters, studies have shown that unrestricted cattle access to watercourses can lead impact water concentrations of nutrients (e.g. Davies-Colley et al., 2004; Byers et al., 2005; Vidon et al., 2007). Demal (1982) conducted an early pilot real-time study on the impacts of cattle access to watercourses and in-stream activity on water concentrations of nutrients and faecal bacteria in the Avon River basin, Canada, and observed increases in TSS, TP, free ammonium and faecal coliform concentrations during cattle access, but not in SRP or nitrate concentrations. More recently, Terry et al. (2014) conducted a high-temporal resolution study using sensors to investigate the impacts of direct cattle access on water TSS concentrations, and concluded that 57.9% of the events that caused TSS concentrations to exceed a threshold of 25 $mg.L^{-1}$ were attributable to cattle activity. However, the authors also noted that these events corresponded to only 3.6% of the total SS exports in the stream for the period of study (Terry et al., 2014). Wilson and Everard (2017) also reported cattle in-stream activity to be strongly correlated with increases in water turbidity and faecal coliforms concentrations, but the study was inconclusive regarding its effects on SRP concentrations.

The present study aimed at addressing the general paucity in previous research regarding the impacts of direct cattle access to watercourses on the aquatic system by providing a more in-depth analysis of the impacts of direct cattle access on a set of water quality parameters (i.e. SRP, TP, NH_4-N, NO_3-N, TSS, and *E. coli* bacteria), in the Irish context. To the knowledge of the authors, only three other studies have aimed at assessing the impacts of cattle in-stream activity on water quality at a high temporal resolution (Demal, 1982; Terry et al., 2014: Wilson and Everard, 2017). The early study by Demal (1982) was published as a project report, but measured a comprehensive set of water quality parameters, including nutrients (SRP, TP, TKN, nitrate and nitrite, and free ammonium), suspended solids and faecal contaminants (faecal coliforms, faecal streptococci, *Pseudomonas aeruginosa*, *Salmonella* sp.). However, this study was based on a rather small number of manual samples (four upstream and downstream of the site for physicochemical analysis, one at each location for microbiological analysis) collected for only two cattle access events although in five access sites with varying land use and physical characteristics. Terry et al. (2014) and Wilson and Everard (2017) conducted studies with more intensive sampling, however Terry et al., (2014) focused their study on suspended solids concentrations, whereas Wilson and Everard (2017) reported turbidity, SRP and *E. coli* only, at a lower temporal resolution (5 minutes – 30 minutes interval samples)and in a rather limited number of events (on 8 cattle access events data was collected on turbidity, on 7 events for SRP, and on 4 events for *E. coli*).

The specific aim of the current study was to quantify upstream to downstream concentrations changes in the selected variables at a single cattle access site at a high temporal frequency and for multiple events. These data were then used to estimate the changes in loads both when cattle where in the stream and when no cattle were present. The research described in this chapter is the first study that has measured a wide range of water quality parameters during multiple events at a fine temporal resolution, and that has also included events of no cattle access, thereby acknowledging the background variability of the measured parameters at the study site. The study hypothesis was that in-stream cattle activity would result in increased concentrations of total suspended solids, faecal bacteria and nutrients resulting from streambed sediment disturbance and excretion directly to and nearby waters.

6.2. Methods

6.2.1. Study site

This study was conducted in the Commons River catchment, Co. Louth (in site CM3, see Chapter 3). The cattle access site was selected due to the site fulfilling a number of required criteria (including that is used utilised by cattle, isolated from other cattle access points in the near vicinity and there was access permission from the landowner). This site also had availability of high temporal resolution water quality and discharge data, collected by a hydrometric station located approximately 280 m downstream from the site (Fig 6.1) as part of the Agricultural Catchments Programme (ACP) (Teagasc, 2017a). Meteorological data for this site were also available from the ACP which operates a weather station located centrally in the catchment. The study site was on a third order stream. It had a width of approximately 2.90 m, and consisted of a stretch of approximately 8 m in the stream with electric fencing at the upstream and downstream boundaries preventing cattle from further dwelling in the stream (Fig. 6.1). The streambanks were dominated by alder (*Alnus* sp.), ash (*Fraxinus excelsior*) and sycamore *(Acer pseudoplatanus*) trees. Fencing as well as riparian vegetation prohibited the animals of crossing the stream to the adjacent field. The site was the only access site to the stream in a field of approximately 5.46 ha, grazed by between 20 and 25 beef heifers during each grazing season. The stream was the only drinking water source available to the animals, i.e. there were no drinkers provided. Additionally, there were no feeders or other structures located in proximity to the access site that could have had an added influence on the amount of time the animals congregated in the area. In Ireland, the grazing season generally takes place between April and October - November. In 2017, the period when most of the sampling for the current study took place, animals were first seen in the fields on 15th April, and their presence was last registered on the 8th October. Prior to the start of this study, stream water and stream bed sediment samples were collected at the site in Spring and Summer and repeated in Autumn 2016, at the same time as the work described in the previous chapters (Tables 6.1 and 6.2). This provided the general ranges for the study parameters prior to the current study.

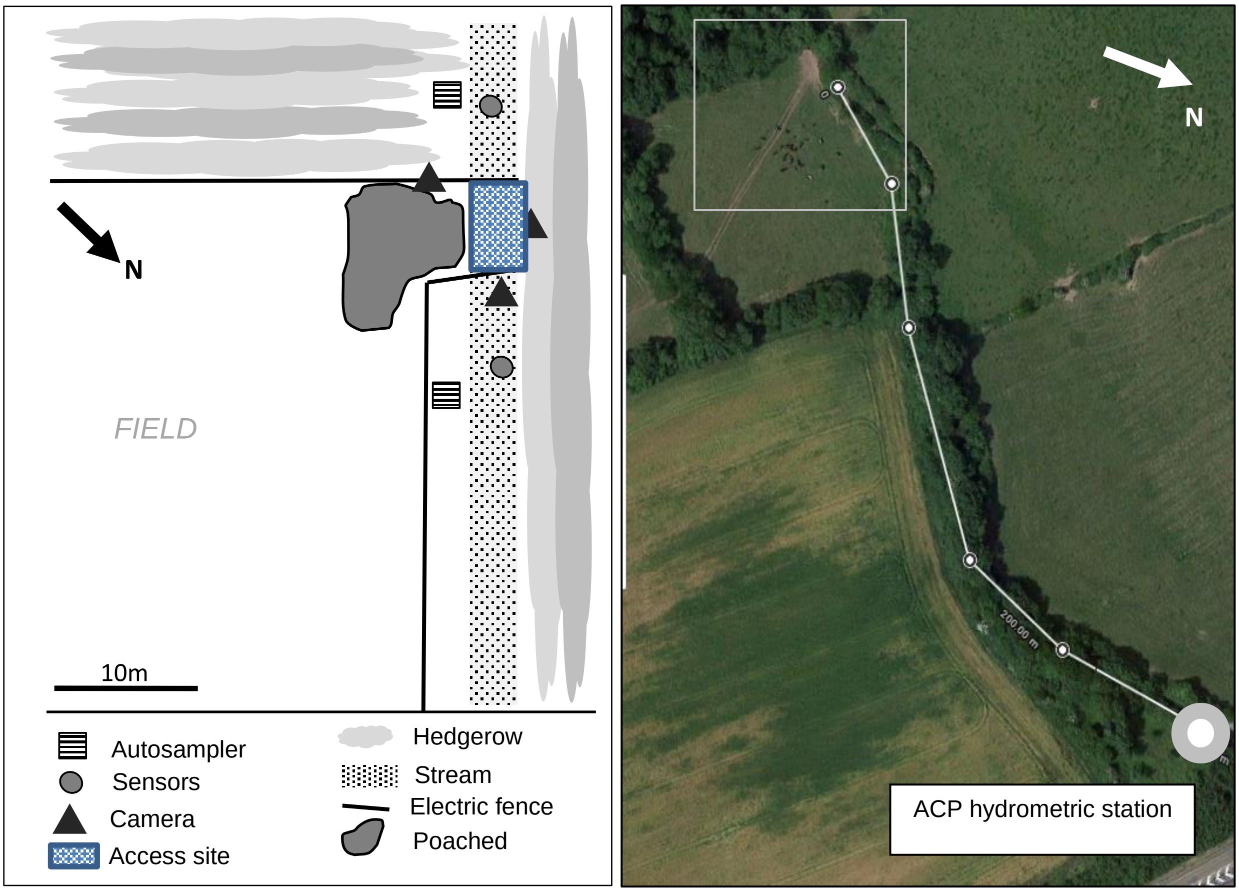

Fig 6.1 Schematic representation of the experimental site and position relatively to the ACP hydrometric station.

Table 6.1. Water quality parameters at the study site at Dunleer (CM3) in 2016 (data for one composite sample based on five subsamples).

Time	Temperature (˚C)	DO ($mg.L^{-1}$)	DO (%)	Conductivity ($\mu S.cm^{-1}$)	pH	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NH_4-N ($mg.L^{-1}$)	TON ($mg.L^{-1}$)
Spring 2016	12.17	10.23	97.86	317	8.24	44	91	0.04	3.14
Autumn 2016	11.87	9.62	87.83	637	8.14	174	224	0.03	2.50

Table 6.2. Characteristics of the bed sediment at the study site at the study site (CM3) in 2016.

Time	TN ($mg.g$ dry wt^{-1})	OC ($mg.g$ dry wt^{-1})	TP ($\mu g.g$ dry wt^{-1})	*E. coli* (CFU.g dry wt^{-1})	Particle size
Spring – Summer 2016	2.15	19.26	521	7.3×10^{6}	90% sand, 10% silt+clay
Autumn 2016	1.34	16.72	556	3.0×10^{4}	94% sand, 6% silt+clay

TN – Total Nitrogen, OC – Organic Carbon, TP –Total Phosphorus. Particle size and nutrients analysed on composite samples based on 6 subsamples; n = 3 for microbiological analysis).

6.2.2. Water sampling

Two autosamplers (HACH AS950 Portable Autosamplers, with 24 x 1 L capacity bottles) were placed approximately 20 m upstream and 20 m downstream of the boundaries of the cattle access site (Figure 6.1). Water quality monitoring was carried out during set time periods referred to as “events”. During each of these events, both autosamplers were set to collect composite samples every 15 minutes, with 200 ml of stream water collected every 3 minutes to yield one 1 L composite sample for each 15 minute interval. This sampling continued for between 2.5 and 5 hours, with most events lasting 3.7 hours (222 min). There was a 3 minute delay between the start time used for the upstream autosampler and the start time for the downstream autosampler, with the aim of capturing the same stream water body at both sampling sites. This time interval was calculated based on an average stream flow rate at the site. Three motion-activated cameras (Bushnell Trophy Cam™ HD) were placed at the cattle access site to register cattle presence in the stream as well as to allow estimation of animal numbers and in-stream defecation and urination events (see Figure 4.1 for locations). The cameras were set to take three consecutive images following triggering of the motion-activated sensors, with intervals of 4 to 13 seconds in between shots. The cameras had been trialled on a number of occasions to investigate possible patterns in the timing of cattle access and to estimate the best time of the day for sampling. After each sampling event, the autosampler water bottles and the camera data were collected and immediately transported to the DkIT laboratory for analysis. Flow measurements were taken manually upstream and downstream of the site at the start and the end of each sampling event using a flow meter (Global Water, Xylem Inc.).

6.2.3. Water analysis

In preparation for each sampling event, the autosampler bottles and water collection tubes were acid-washed with a 10% HCl solution, sterilised with a solution of 70% industrial methylated spirit (IMS) and rinsed several times with Milli-Q water. Following collection, the samples were analysed for concentrations of dissolved nutrients (SRP, NH_4-N, NO_3-N), total reactive phosphorus (TRP), total phosphorus (TP), total suspended solids (TSS), *E. coli,* Other Coliforms and conductivity. Chloride concentrations were also measured to rule out possible influences of other sources e.g. septic tank discharges or dairy washings, on changes in concentrations of dissolved nutrients and faecal bacteria.

6.2.3.1. *Analysis of physicochemical parameters*

All glassware used in nutrient analysis was acid-washed with 10% HCl, rinsed with Milli-Q water and dried prior to the analysis. An aliquot of each sample was filtered through a nitrocellulose 0.45 µm filter (Sartorius Stedim Biotech, Germany) for analysis of dissolved nutrients. SRP was determined on the filtrate using a manual colorimetric method based on phosphate ascorbic acid reduction (Murphy and Riley, 1962). NH_4-N was determined using a manual colorimetric method based on ammonia reaction with salycilate and dichloroisocyanurate in alkaline solution (HMSO, 1981). NO_3-N and chloride were determined simultaneously in a high performance integrated ion chromatography system (DIONEX®, ICS-2000, ThermoFisher Scientific). An unfiltered aliquot of each sample was used to determine TRP and TP using a colorimetric method (Murphy and Riley, 1962) directly and following sample acid persulfate digestion at 120˚C, respectively. All nutrient analyses were carried out in duplicates. Calibration standards and quality control standards for phosphorus analysis were prepared using stock solutions (1000 $mg.L^{-1}$) prepared with analytical grade chemicals (ThermoFisher Scientific) (potassium phosphate, KH_2PO_4, and sodium phosphate Na_3PO_4, respectively). In the case of TP analysis, all standards were digested in the same manner as the samples. Calibration standards and quality control standards for ammonium analysis were prepared using a certified standard solution (1000 mg.L^{-1}; Inorganic™ Ventures) and a stock solution prepared with analytical grade ammonium chloride (NH_4Cl; ThermoFisher Scientific) (1000 mg.L^{-1}), respectively. Calibration standards and quality control standards for nitrate analysis were prepared using certified standard solutions (1000 mg.L^{-1}) of sodium nitrate ($NaNO_3$) purchased from different manufacturers (Inorganic™ Ventures and Reagecon, respectively). The limits of detection for nutrient analysis were 3 µg.L^{-1} for SRP and TRP, 4 µg.L^{-1} for TP, 0.017 mg.L^{-1} for NH_4-N and 0.015 mg.L^{-1} for NO_3-N. These were calculated for each method according to Armbruster and Pry (2008).

To determine TSS concentrations, 300 to 500 ml of each composite sample were filtered through previously dried and weighted 1.2 µm pore size glass microfiber filters (G/FC Sartorius, Germany), which were then dried for 24 hours at 105 ˚C, reweighted, and the differences in weights recorded. Finally, pH and conductivity were determined in each sample using a multiparameter probe (YSI Professional Plus, YSI Inc.).

6.2.3.2. Microbiological analysis

Due to time and material constraints, not all samples collected at each event were analysed for *E. coli* concentrations. In such cases, the camera images were inspected upon arrival to the laboratory to determine times of cattle in-stream activity and the corresponding samples were analysed. An aliquot of each sample was transferred into a sterile Duran bottle and used to determine water concentrations of *E. coli* using a membrane filtration technique. One to two 1:10 sequential dilutions were prepared from each original sample using Ringers solution as diluent. Each dilution was then filtered through a sterile cellulose esters membrane with 47 mm diameter and 0.45 µm pore size with grids as described in ISO 9308-1:2000. The membranes were placed onto Petri dishes containing HarlequinTM *E.*

coli/Coliform medium (LabM, Lancashire, UK) and incubated at 37 ˚C for 18 – 24 hours according to the manufacturer's instructions. All green-blue colonies were counted as presumptive *E. coli* bacteria, and all purple/pink colonies were counted as Other Coliforms.

6.2.4. Data processing

Data on stream discharge ($m^3.s^{-1}$, averaged hourly) and daily precipitation (mm day^{-1}) in the 48 hours preceding the sampling events were provided by the Agricultural Catchments Programme where possible (see Table 6.4).

The camera images were inspected manually for periods of cattle in-stream activity, cattle numbers and direct excretion into stream waters. Due to the physical characteristics of the site and the limited possibilities for positioning the cameras, at times when a high number of animals (>5) accessed the site simultaneously, visibility within the group of cattle was limited despite having three angles; thus the total number of cattle in the stream on those occasions is the best estimate that was possible to obtain by inspecting the images collected from the three angles. Additionally it is possible that on such occasions, a small number of defecation or urination episodes may have not been recorded.

TSS concentrations were consistently disproportionally higher in the first sampling intervals of the events. It was most likely an indication of human disturbance of the stream sediment while preparing and starting the autosamplers, therefore the first observations for all parameters for both upstream and downstream (corresponding to 12 minutes from start) were excluded from analysis. Nutrient concentrations below the limit of detection for the method used were replaced with ½ of the limit of detection value for data analyses.

The differences in nutrient, TSS and *E. coli* concentrations downstream of the access site relative to the upstream site were calculated for each upstream-downstream sample pair. For this purpose, periods of cattle in-stream activity included the times at which cattle were recorded accessing the stream, and also the 15 minutes period immediately following (i.e. the next sample), in order to account for the falling phase of the changes in freshwater parameters. The loads of nutrients, TSS and *E. coli* during and following periods of cattle in-stream activity were calculated by multiplying the change in concentration between the upstream and downstream sampling sites (which could be negative) by the average value of the two stream discharge (available as hourly averages) measured in the two points preceding and following each sample time interval. In the cases where the sampling interval coincided with the hour and thus with stream discharge measurement, the corresponding hour stream discharge was used in the calculations.

6.2.5. Statistical analysis

The difference in the distributions of the dataset for all calculated 15 minute loads of nutrients, TSS and *E. coli* for the downstream site compared to the same data for the upstream site was assessed using a two-sided Kolmogorov–Smirnov test. This test determines whether two distributions are significantly different from each other. Since it was likely that the calculated loads for sequential timesteps at the sampling locations and between sampling locations were not independent, bootstrap resampling was used to assign significance. The analysis was performed in R software (version 4.0.0; R Core Team, 2016) using the packages *Matching* (Sekhon, 2013) and *boot* (Canty and Ripley, 2020) and the ks.boots function, with nboots (number of resamplings) set to 1000. Empirical cumulative density functions (ECDF) were plotted for each pair of distributions.

6.3. Results

In total, 16 events were sampled between August 2016 and June 2018 (Table 6.3). Eleven of these events are described in this chapter, selected based on the availability of data for a broader range of parameters and adequate camera data. Of these, seven events captured cattle in-stream activity (Table 6.4). All events with cattle activity also included periods before and after cattle activity when there was no cattle activity in the stream. The stream discharge in these 11 events varied from 0.003 $m^3.s^{-1}$ to 0.129 $m^3.s^{-1}$ (25^{th} percentile = 0.013 $m^3.s^{-1}$; 75^{th} percentile = 0.148 $m^3.s^{-1}$) (Table 6.4).

6.3.1. Background variation in water quality parameters

Background concentrations (i.e. those measured at the sampling point upstream of the access site) of nutrients, TSS and *E. coli* bacteria for the sampling events included in this chapter are shown in Table 6.5. Data for TRP, chloride, conductivity and Other Coliforms are not included in this chapter and are presented in Appendix A. Event 8, which took place on May 24, 2017 (Fig. 6.5), is presented here as an example of the variation in water quality parameters at the study site in the absence of cattle in the stream. During this event, although cattle were present in the fields, they did not visit the stream during the period of sampling based on the camera data.

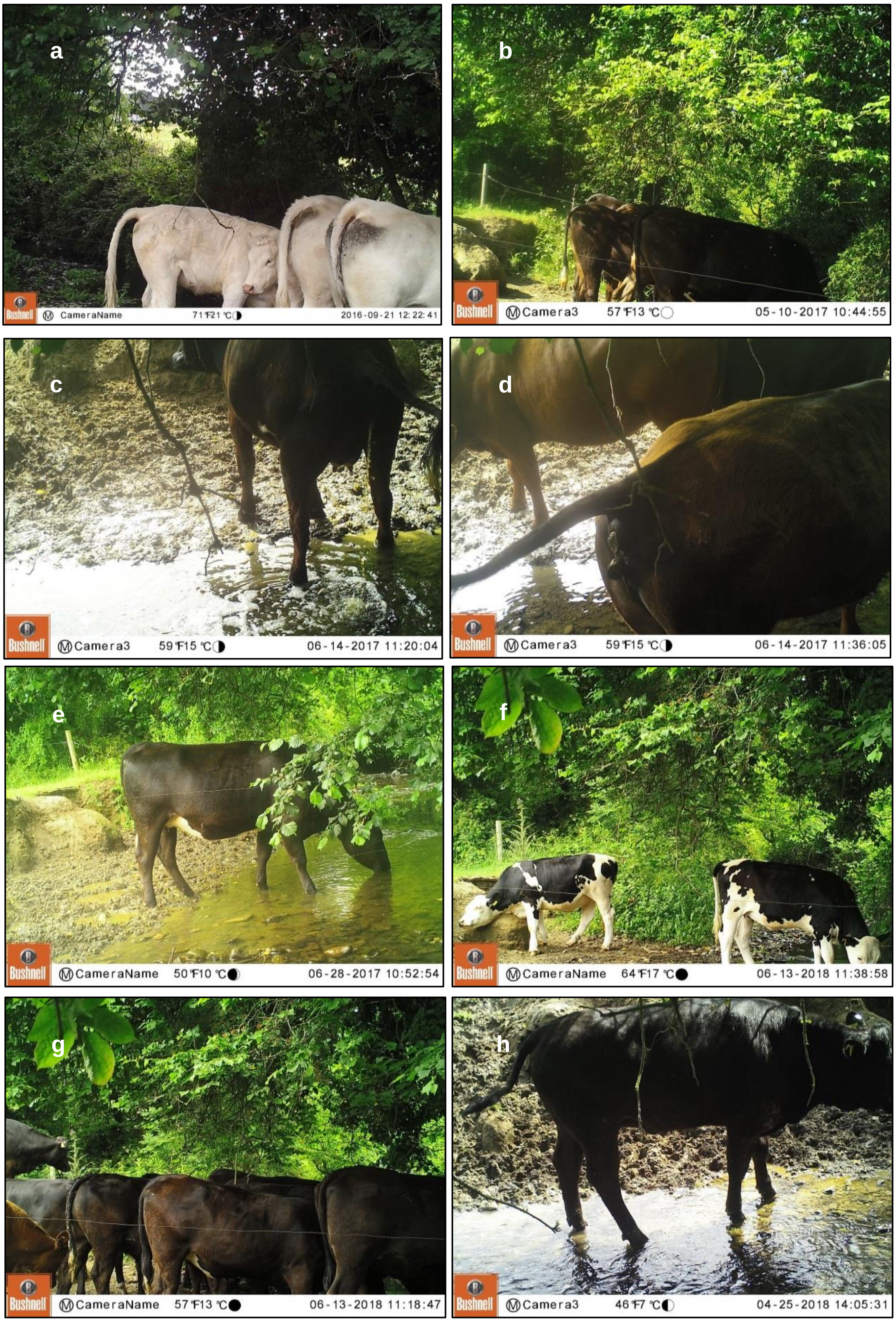

Fig.6.2. Images taken during sampling events. a) fence pole view showing 3 animals in the stream in Event 2; b) side view of at least 4 animals in the stream in Event; c) in-stream urination and d) defecation in Event 9; e) one animal in Even10; f) side view of two animals and g) several animals in the stream in Event 16; h) front view of one animal in Event 15, after the sampling period.

Table 6.3. List of sampling events conducted in this study. Events discussed in this chapter are in bold.

Event	Date	Available data	Cattle access	Included	Reasons for exclusion/Comments
1	24/08/2016	SRP, NH_4-N, TON, TSS	Yes	No	Different methodology (longer periods of time between samples)
2	**21/09/2016**	SRP, NH_4-N, TON, TSS	**Yes**	**Yes**	
3	16/11/2016	NH4-N, TON, TSS	No	No	Fewer parameters
4	30/11/2016	NH_4-N, TSS	No	No	Fewer parameters
5	06/04/2017	TSS, *E. coli*	No	No	Fewer parameters
6	26/04/2017	SRP, TP,TSS, *E. coli*	Yes	No	Poor quality of camera images
7	**10/05/2017**	SRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms	**Yes**	**Yes**	
8	**24/05/2017**	SRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms	**No**	**Yes**	
9	**14/06/2017**	SRP, TP, NH_4-N, NO_3-N , TSS, Cl^-, *E. coli*, Other Coliforms	**Yes**	**Yes**	
10	**28/06/2017**	SRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, conductivity	**Yes**	**Yes**	Not possible to use data for *E. coli*

Table 6.3. (continued)

Event	Date	Available data	Cattle access	Included	Reasons for exclusion/Comments
11	**08/08/2017**	SRP, TP, NH_4-N, NO_3-N TSS, Cl^-, *E. coli*, Other Coliforms, conductivity	**Yes**	**Yes**	
12	**30/08/2017**	SRP, TRP, TP NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms	**Yes**	**Yes**	
13	**07/02/2018**	SRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms, conductivity	**No**	**Yes**	
14	**21/03/2018**	SRP, TRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, *E.coli*, conductivity	**No**	**Yes**	
15	**25/04/2018**	SRP, TRP, TP, NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms, conductivity	**No**	**Yes**	Not possible to use data for TP
16	**13/06/2018**	SRP, NH_4-N, NO_3-N, TSS, Cl^-, *E. coli*, Other Coliforms, conductivity	**Yes**	**Yes**	Not possible to use data for TP

Table 6.4. Description of the sampling events presented in this chapter.

Event	Date	Cattle in field	Access	Max cattle	Total access (min)	Cattle-minutes[3]	Defecations recorded	Urinations recorded	Daily mean stream discharge ($m^3.s^{-1}$)[1]	Precipitation in previous 48 hours (mm)[2]	Precipitation on day (mm)
2	21/09/2016	Yes	Yes	5	18.8	41	1	1	0.013	0.0	7.0
7	10/05/2017	Yes	Yes	6	26.5	76	1	2	0.012	0.0	0.0
8	24/05/2017	Yes	No	-	-	-	-	-	0.007	0.4	0.0
9	14/06/2017	Yes	Yes	4	32.9	81	1	1	0.015	0.0	1.8
10	28/06/2017	Yes	Yes	1	2.0	2	0	0	0.021	32.1	6.8
11	08/08/2017	Yes	Yes	3	23.0	46	nd	1	0.005	1.0	0.0
12	30/08/2017	Yes	Yes	3	13.1	25	nd	nd	0.003	1.6	0.2
13	07/02/2018	No	No	-	-	-	-	-	0.129	1.8	0.0
14	21/03/2018	No	No	-	-	-	-	-	0.123	0.0	0.0
15	25/04/2018	Yes	No	-	-	-	-	-	0.056	8.0	0.0
16	13/06/2018	Yes	Yes	5	41.2	99	nd	nd	0.005	1.9	1.9

[1]Data provided by the Agricultural Catchments Programme, except for Event 16, for which this was calculated using a regression line based on ACP data and field flow measurements. [2]Data provided by the ACP except for Events 14 to 16, for which they were retrieved from data from Met Eireann's nearest meteorological station.[3]Cattle-minutes are calculated multiplying the number of minutes of access by the number of animals in the stream in each minute. nd – not detected

Stream nutrient concentrations measured in this event upstream of the access site varied from 125 µg.L^{-1} to 131 µg.L^{-1} for SRP, 169 to 194 µg.L^{-1} for TP, <0.02 to 0.05 mg.L^{-1} for NH_4-N and 4.12 to 4.24 mg.L^{-1} for NO_3-N. At the downstream site, nutrient concentrations ranged from 124 µg.L^{-1} to 129 µg.L^{-1} for SRP, 166 to 195 µg.L^{-1} for TP, <0.02 to 0.04 mg.L^{-1} for NH_4-N and 3.93 to 4.18 mg.L^{-1} for NO_3-N. Upstream TSS concentrations ranged from 3.4 to 8.6 mg.L^{-1}, while downstream TSS concentrations varied from 2.6 to a maximum of 31.0 mg.L^{-1}, which was a peak value observed in the composite sample for the time interval 162 to 177 minutes at the downstream site. For the upstream site, *E. coli* concentrations ranged from 2.6 x 10^3 CFU.100 ml^{-1}. to 1.8 x 10^4 CFU.100 ml^{-1}, while downstream they ranged from 2.7 x 10^3 to 1.3 x 10^4 CFU.100 ml^{-1}. These changes in freshwater parameters concentrations reflect the background variability at the site both at each sampling point and between sampling points.

6.3.2. Changes in water quality parameters during cattle access to the stream

Events 7 and 12 are presented here as examples of the changes across all water physicochemical and microbiological parameters during periods of cattle in-stream activity of different intensities.

In Event 12 (Fig 6.4), which took place on August 30, 2017, cattle accessed the stream on five shortly spaced occasions for an average duration of 2.6 minutes and a total duration of 13.1 minutes. A maximum of three animals were observed in the stream simultaneously. During the period of cattle access, increases in the concentrations of TSS, TP, NH_4-N and *E. coli* were observed downstream of the access site in comparison to upstream concentrations, in particular in the interval corresponding to 162 to 177 minutes from start time of sampling, after three animals accessed the stream simultaneously. TSS concentrations then increased to a maximum of 11.0 mg.L^{-1} downstream of the access site, while concentrations upstream for the equivalent 15 min sampling interval were 3.8 mg.L^{-1}.

Similarly, TP concentrations peaked at 349 µg.L^{-1} at the downstream site during cattle access, compared to a TP concentration upstream of 211 µg.L^{-1}. Both NH_4-N and *E. coli* also showed upstream to downstream increases when cattle were in the stream. NH_4-N concentrations peaked at 0.10 mg.L^{-1} downstream at the sampling period of 162 to 177 minutes, compared to 0.02 mg.L^{-1} upstream. *E. coli* concentrations reached a maximum of 5.1 x 10^3 CFU.100 ml^{-1} downstream of the access site during in-stream activity, while upstream concentrations for the equivalent time were 1.7 x 10^3 CFU.100 ml^{-1}. In contrast, both SRP and NO_3-N concentrations did not increase downstream of the access site relative to upstream concentrations during or following cattle access to the stream. It is of note, however, that TSS concentrations downstream at the site peaked at 11.0 mg.L^{-1} in the time interval 12 – 27 minutes from start, when no cattle were in the stream, while TSS concentrations upstream for the equivalent sample were 3.0 mg.L^{-1}.

In Event 7 (Fig 6.5), which took place on May 10, 2017 cattle accessed the stream almost continuously for a period of 25.6 minutes (from minute 77 to minute 104), with a maximum of six animals standing in the stream simultaneously (Table 6.4). One in-stream defecation at (minute 94) and two in-stream urinations (minute 94 and 103) were registered during this period. During the period of cattle in-stream activity, concentrations of TSS and TP reached maximum values of 27.4 mg.L^{-1} and 148 µg.L^{-1}, respectively, downstream of the site, while concentrations measured upstream for the same period were much lower at 3.4 mg.L^{-1} and 84 µg.L^{-1}. Downstream concentrations of SRP showed only a modest increase during and following cattle in-stream activity, reaching a maximum of 81 µg.L^{-1} at interval 102 – 117 minutes from start, whereas the SRP concentration measured upstream at the same period of time was 73 µg.L^{-1}. NH_4-N concentrations increased to 0.04 mg.L^{-1} and 0.08 mg.L^{-1} downstream in the sampling intervals of 102 – 117 and 117 – 132 minutes from start, which followed the two episodes of in-stream urination observed in this event. These changes in NH_4-N corresponded to increases of +0.02 mg.L^{-1} and +0.06 mg.L^{-1} in comparison with upstream concentrations (0.02 mg.L^{-1} for both). However, there was also an increase in downstream NH_4-N concentrations at the start of the sampling event (interval of 12 – 27 minutes from start) that could not be explained by cattle activity, when concentrations at the upstream and downstream sites were 0.02 mg.L^{-1} and 0.06 mg.L^{-1}, respectively. There was also a clear increase in *E. coli* concentrations that coincided with the time period when cattle were in the water (Fig.6.5). *E. coli* concentrations downstream of the access site were 1.0 x 10^3 CFU.100 ml^{-1} at interval 62 - 87 minutes from start, during which cattle entered the stream, while upstream concentrations for the equivalent time were 4.9 x 10^2 CFU.100 ml^{-1}. *E. coli* downstream concentrations peaked at 2.4 x 10^4 CFU.100 ml at interval 102 – 117 minutes from start, corresponding to an increase in two orders of magnitude relative to concentrations observed upstream of the access site (4.2 x 10^2 CFU.100 ml^{-1}) at the same time.

Table 6.5. Concentrations of nutrients, total suspended solids (TSS) and *E. coli* bacteria measured upstream of the cattle access site during the sampling events (mean ± S.D.).

Event	n	SRP (µg.L^{-1})	TP (µg.L^{-1})	NH_4-N (mg.L^{-1})	NO_3-N (mg.L^{-1})	TSS (mg.L^{-1})	*E. coli* (CFU.100 ml^{-1})
2	14	163 ± 3	NA	0.02 ± 0.02	2.14 ± 0.08*	0.4 ± 0.2	NA
7	14	74 ± 2	82 ± 8	0.02 ± 0.01	4.44 ± 0.11	4.0 ± 2.0	4.3×10^2 ± 56 (n=5)
8	14	127 ± 2	181 ± 7	0.02 ± 0.01	4.19 ± 0.03	5.2 ± 1.7	8.8×10^3 ± 5.6×10^3 (n=7)
9	19	101 ± 3	217 ± 50	0.02 ± 0.01	3.09 ± 0.26	4.5 ± 0.9	9.4×10^2 ± 1.4×10^2 (n=7)
10	18	244 ± 14	293 ± 27 (n=17)	0.04 ± 0.02	2.72 ± 0.10	9.9 ± 12.1	NA
11	14	244 ± 7	276 ± 20	0.15 ± 0.07	2.75 ± 0.06	6.4 ± 4.1	7.4×10^2 ± 1.4×10^2 (n=13)
12	14	209 ± 3	227 ± 10	0.04 ± 0.06	2.73 ± 0.03	2.6 ± 0.9	1.9×10^3 ± 4.0×10^2 (n=9)
13	14	32 ± 1	64 ± 4	0.09 ± 0.02	8.31 ± 0.11	4.3 ± 1.5	3.5×10^2 ± 1.2×10^2 (n=13)
14	9	48 ± 3	77 ± 6	0.07 ± 0.01	7.50 ± 0.07	9.2 ± 1.6	1.7×10^3 ± 1.2×10^3
15	14	103 ± 4	NA	0.33 ± 0.07	6.79 ± 0.10	17.2 ± 22.4	2.8×10^3 ± 6.7×10^2
16	14	135 ± 2	NA	0.05 ± 0.03	5.44 ± 0.10	5.4 ± 1.6	2.8×10^3 ± 2.4×10^3

*Measured as TON. n = number of 15 minute sampling times. NA – not available due to issues during sample collection or processing.

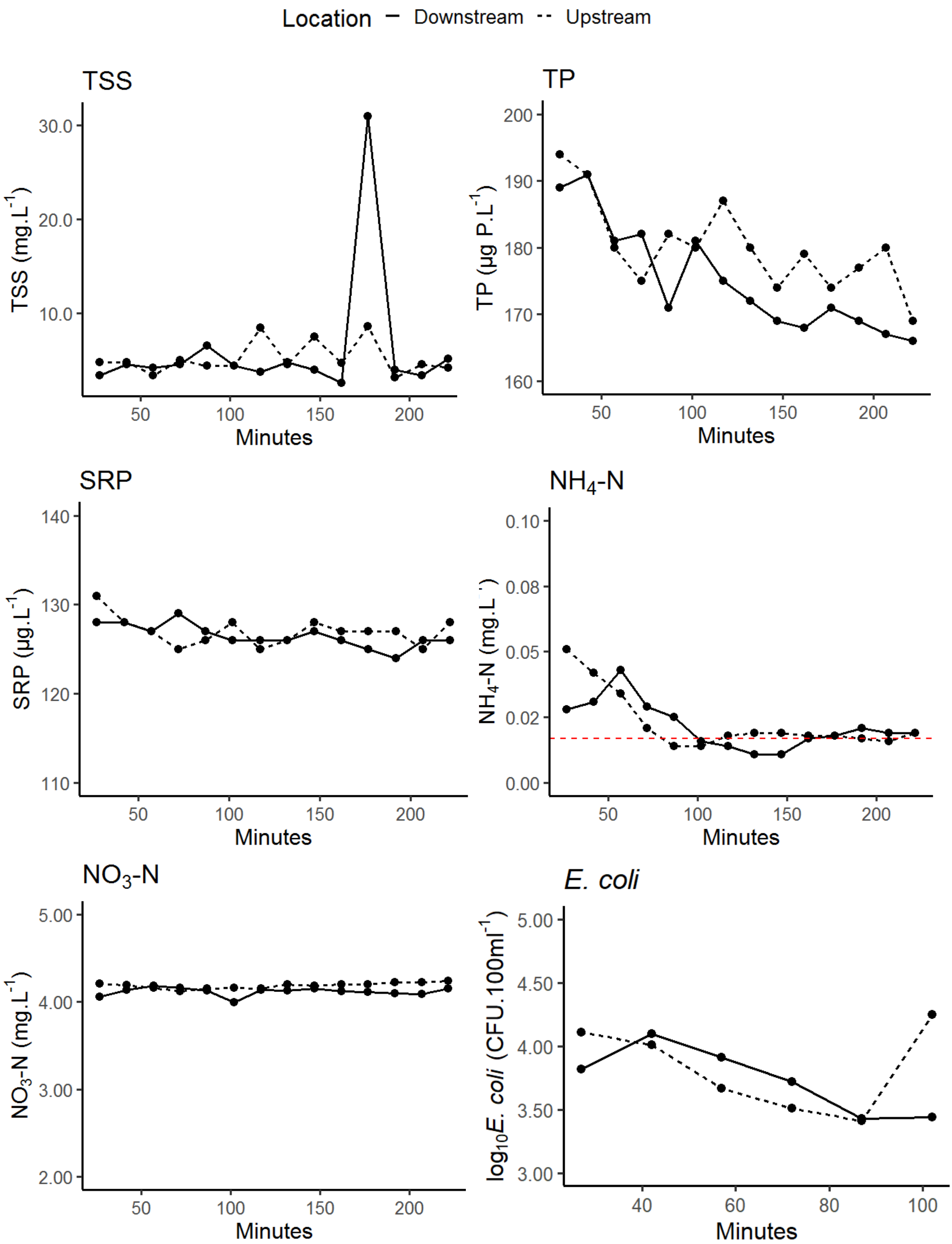

Fig.6.3. Variation in mean nutrients, TSS and *E. coli* bacteria concentrations upstream and downstream of the cattle access site during Event 8 (May 24, 2017), when cattle were present in the fields but did not access the stream during the sampling period. The dashed red line represents the limit of the detection for the analysis of the parameter.

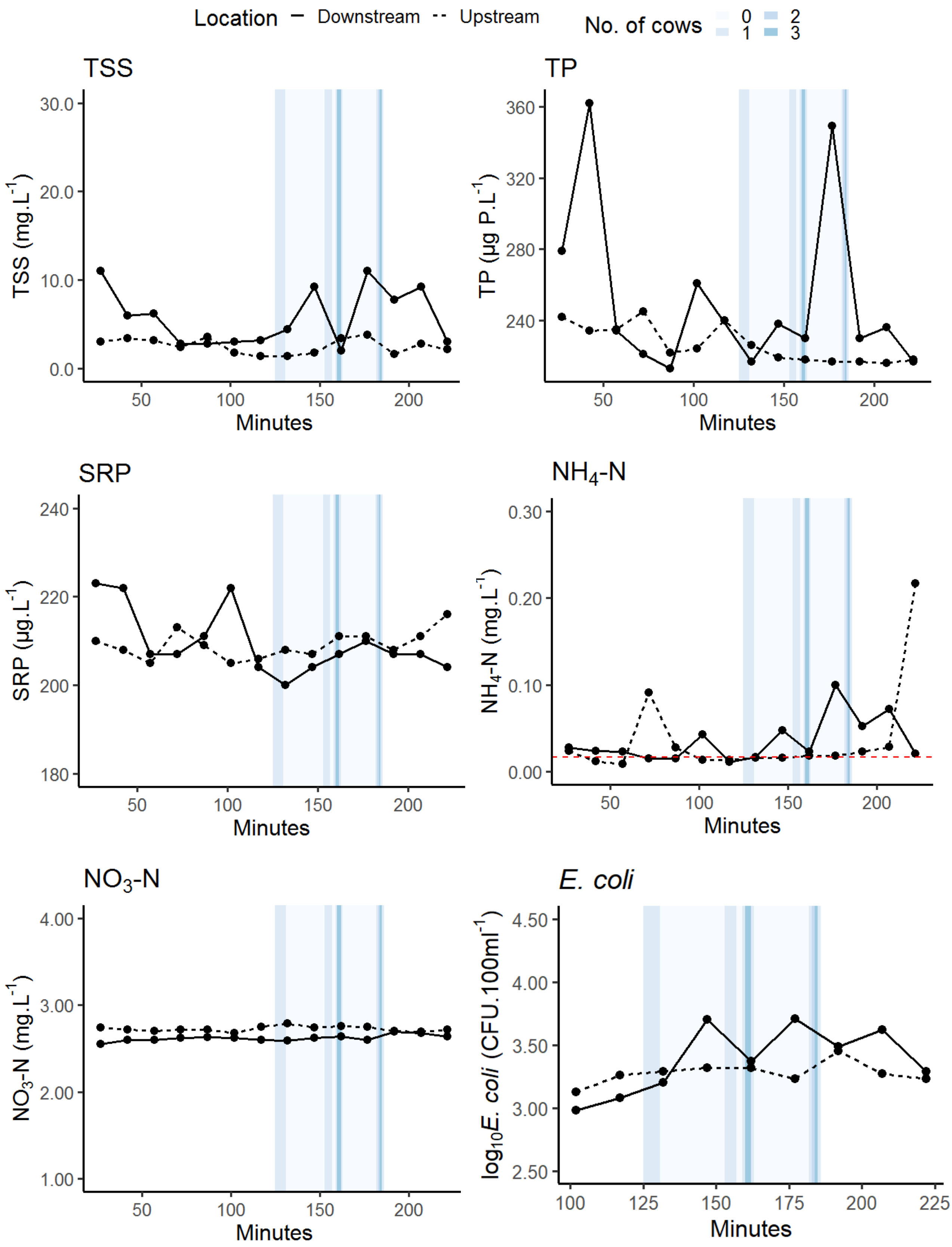

Fig.6.4. Variation in nutrients, TSS and *E. coli* bacteria concentrations upstream and downstream of the cattle access site during Event 12 (August 30, 2017), when a maximum of 3 animals accessed the stream simultaneously. The dashed red line represents the limit of the detection for the analysis of the parameter.

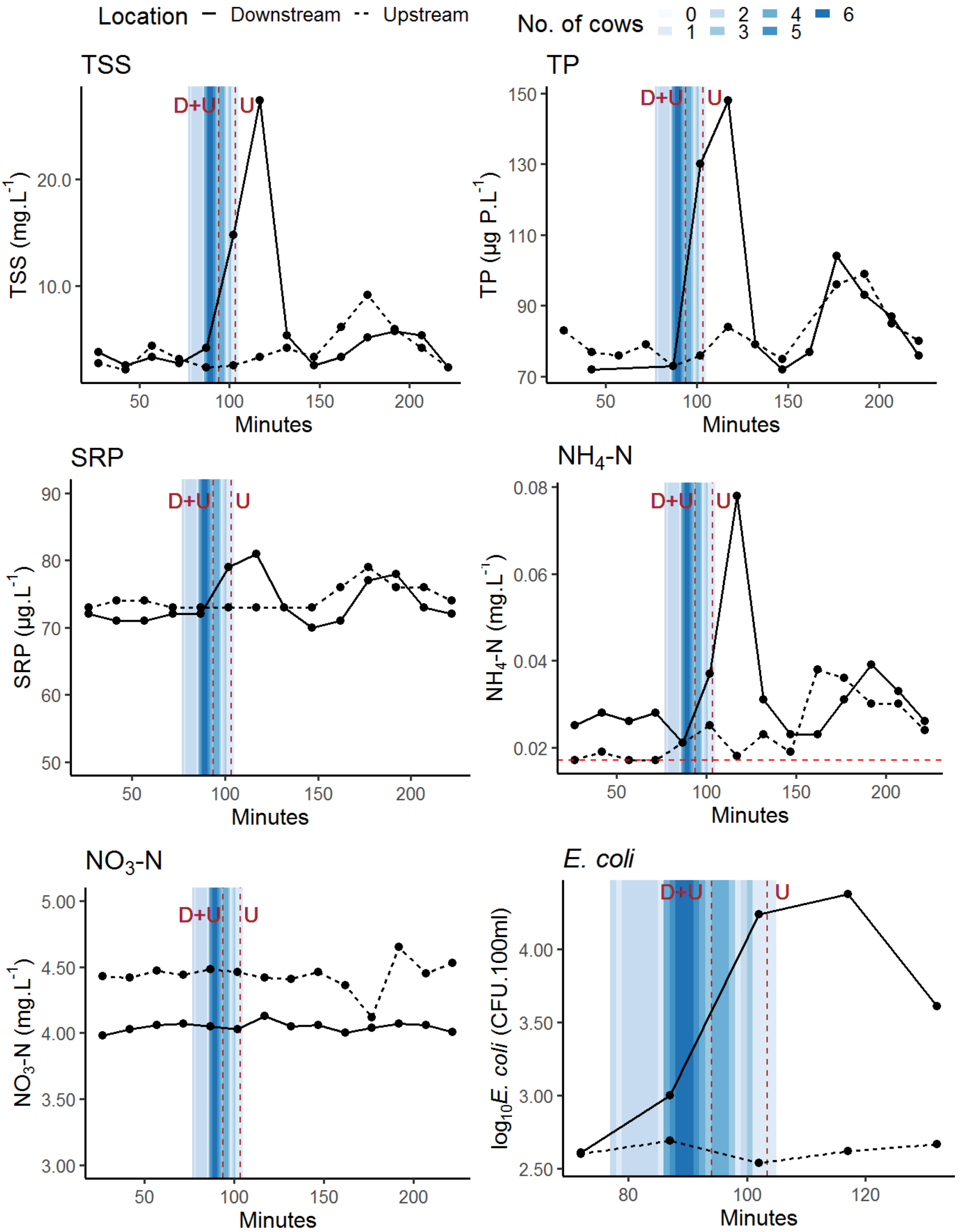

Fig.6.5. Variation in nutrients, TSS and *E. coli* bacteria concentrations upstream and downstream of the cattle access site during Event 7 (May 10, 2017), when a maximum of 6 animals accessed the stream simultaneously. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. The dashed red line represents the limit of the detection for the analysis of the parameter.

Table 6.6. Peak concentrations of nutrients, total suspended solids and *E. coli* bacteria measured downstream (DS) of the access site during periods of cattle access to the stream, and concentrations measured upstream (US) for the same sampling interval (15 minute composite samples).

Event	**SRP ($\mu g.L^{-1}$)**		**TP ($\mu g.L^{-1}$)**		**NH_4-N ($mg.L^{-1}$)**		**NO_3-N ($mg.L^{-1}$)**		**TSS ($mg.L^{-1}$)**		***E. coli* (CFU.100 ml^{-1})**	
	US	DS	US	DS	US	DS	US	DS	US	DS	US	DS
2	165	192	NA		0.05	0.14	2.04*	2.15*	0.3	5.5	NA	
7	73	81	84	148	0.02	0.08	No increase		3.4	27.4	4.2×10^2	2.4×10^4
9	93	99	155	309	< 0.02	0.05	No increase		4.4	18.4	1.2×10^3	8.5×10^3
10	No increase		299	306	0.06	0.07	No increase		6.2	9.4	NA	
11	240	244	268	367	0.08	0.19	2.77	3.01	5.8	42.4	8.0×10^2	1.3×10^4
12	No increase		217	349	0.02	0.10	No increase		1.8	9.2	1.7×10^3	5.1×10^3
16	132	143	NA		0.03	0.24	5.49	6.20	4.3	34.2	5.5×10^2	7.9×10^4

*Measured as TON. NA – Not available due to issues during sample collection or processing.

6.3.2.1. *Changes in total suspended solids (TSS) concentrations across all 11 events*

Changes in total suspended solids (TSS) concentrations upstream and downstream of the access site during the sampling events are shown in Fig. 6.6 and Fig. 6.7. TSS concentrations were highly variable both upstream and downstream of the site, with occasional increases observed in events when there were no cattle present to access the stream (e.g. Events 8, 13 and 15) (Fig 6.6), as well as at times when cattle were present in the field but did not access the stream (Events 9 - 12) (Fig 6.7). These occasional increases ranged from +2.2 $mg.L^{-1}$ (Event 13) to +74.3 $mg.L^{-1}$ (Event 15) relative to TSS concentrations measured in the time interval immediately preceding increases. However, TSS concentrations also showed increases downstream of the access site during and following cattle in-stream activity.

In Event 10 (June 28, 2017), when one animal entered and remained in the stream for 2.0. minutes, downstream TSS concentrations peaked at 9.4 $mg.L^{-1}$ while concentrations measured upstream were 6.2 $mg.L^{-1}$. However, these changes are not substantial in the context of the natural variation in TSS concentrations measured on this event. Similarly, in Event 12 (August 30, 2017), (maximum of three animals in the stream simultaneously; in-stream activity lasted 2.6 minutes on average and 13.1 minutes in total), TSS concentrations increased by a maximum of +7.2 $mg.L^{-1}$, from 3.8 $mg.L^{-1}$ upstream to 11.0 $mg.L^{-1}$ downstream. Although these changes coincided with the period of cattle in-stream activity, they fell within the overall range of TSS concentrations measured for that event. For all other events when the intensity of cattle access to the stream was higher (i.e. higher number of animals visiting the stream and/or longer periods of in-stream activity), TSS concentrations increased downstream of the cattle access site during cattle in-stream activity (Table 6.6, Fig. 6.7). In Event 7 (May 10, 2018), when the highest number of animals observed in the stream simultaneously in this study was registered (6 animals), TSS concentrations reached a maximum of 27.4 $mg.L^{1}$, eight times higher than the concentration measured upstream at equivalent time (3.4 $mg.L^{-1}$*).* However, the highest increase in TSS concentrations coinciding with cattle accessing the stream was measured during Event 11 (August 8, 2017), when a maximum of three animals visited the stream simultaneously, and the total duration of access was 22.0 minutes. On this occasion, an increase of +36.6 mg $TSS.L^{-1}$ was recorded, with a concentration of 5.8 $mg.L^{-1}$ at the upstream site and 42.4 $mg.L^{-1}$ at the downstream site.

6.3.2.2. Changes in total phosphorus (TP) water concentrations across all 11 events

Changes in TP water concentrations generally followed a similar trend to changes in TSS concentrations (Fig. 6.8 and 6.9). Although TP concentrations showed a relatively high variation in the absence of cattle (Table 6.5, Fig. 6.8), they consistently increased during or following cattle in-stream activity, with the exception of Event 10. In this latter event, TP concentrations increased and decreased markedly both upstream and downstream of the cattle access site, an observation that was not related to cattle access to the stream. , Although an increase of +7 µg $TP.L^{-1}$ was observed downstream following cattle access, this was however negligible in the context of TP general variation in this event.

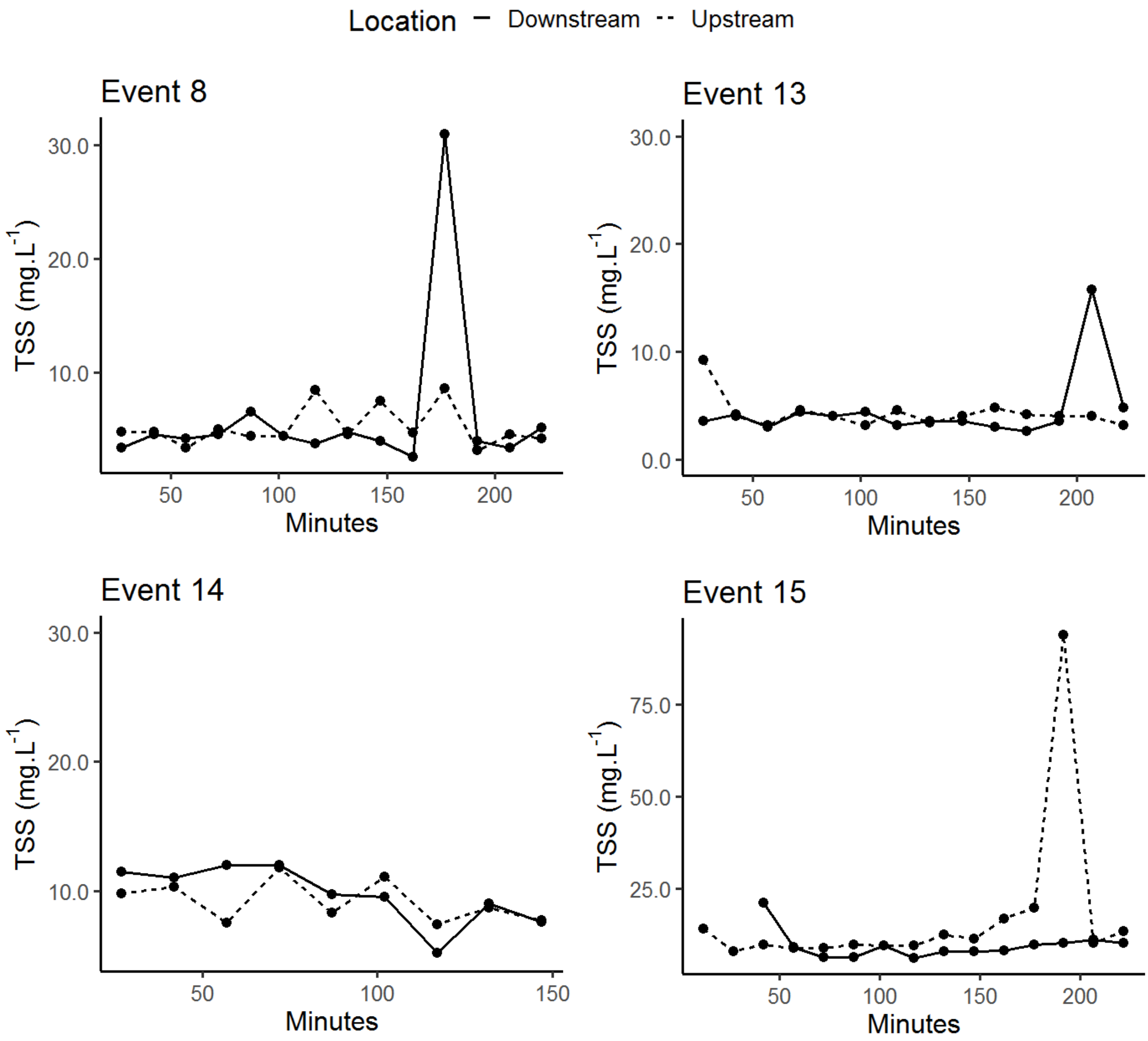

Fig.6.6. Variation in TSS concentrations upstream and downstream of the cattle access during events with no cattle access to the stream. Note the differences in scales.

In the remaining sampling events, there were more substantial changes in TP concentrations coinciding with or following periods of cattle access to the stream (Table 6.6, Fig. 6.9). In Event 7 (May 10, 2017), in which a maximum of 6 animals accessed the stream for a total of 25.6 minutes, TP concentrations downstream of the site peaked at 148 µg.L^{-1} L, an increase of +64 µg.L^{-1} in comparison to upstream concentrations at the same time interval (84 µg.L^{-1}). In Event 9 (June 14, 2017), during which a maximum of 4 animals accessed the stream

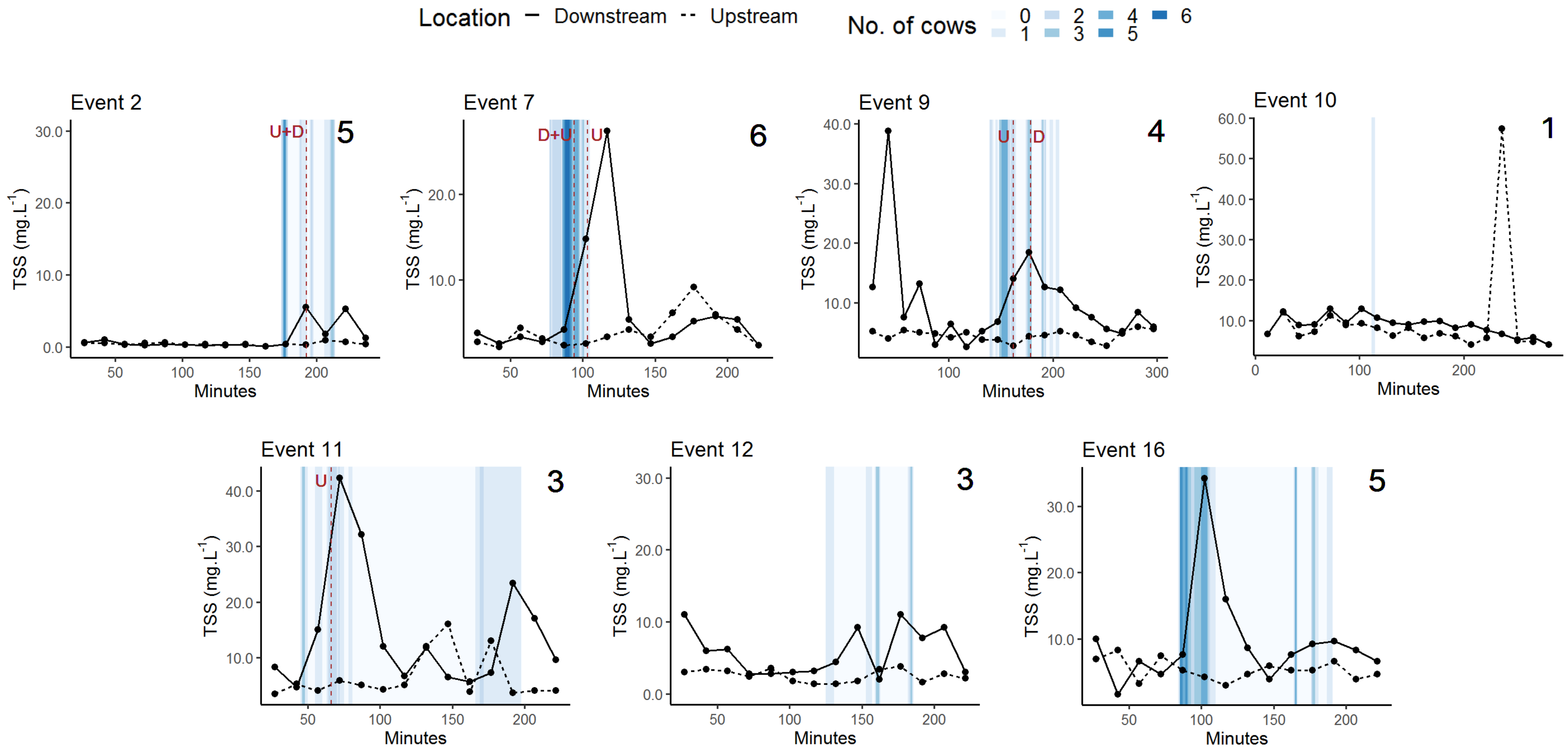

Fig.6.7. Variation in TSS concentrations upstream and downstream of the cattle access during events that captured cattle access to the stream. Note the differences in scales. Blue shading indicates times of in-stream activity. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

and in-stream activity lasted on average 4.1 minutes and 32.9 minutes in total, TP concentrations downstream of the site peaked at 309 $\mu g.L^{-1}$ whereas concentrations measured upstream in the same time interval were 155 $\mu g.L^{-1}$. This was the highest difference in concentrations for the downstream site relative to the upstream site measured during cattle in-stream activity in this study (+154 $\mu g.L^{-1}$),. It should be noted, however, that this was also due to a decrease in upstream concentrations which had been higher at 206 $\mu g.L^{-1}$ in the previous sampling interval. In Event 11 (August 8, 2017), when a maximum of 5 animals visited the stream simultaneously and in-stream activity lasted on average 2.6 minutes and in total 13.1 minutes, downstream TP concentrations peaked at 320 $\mu g.L^{-1}$, while upstream concentrations at the same time period were 278 $\mu g.L^{-1}$. In Event 12 (August 30, 2017), TP downstream concentrations peaked at 349 $\mu g.L^{-1}$, a difference of +132 $\mu g.L^{-1}$ relative to concentrations measured upstream (217 $\mu g.L^{-1}$).

6.3.2.3. Changes in soluble reactive phosphorus (SRP) concentrations across all 11 events

The variation in SRP concentrations during the sampling events is shown in Fig. 6.10 and Fig. 6.11. Contrary to the patterns observed for TSS and TP, water concentrations of SRP did not show a consistent pattern of change during periods of cattle in-stream activity (Table 6.6, Fig. 6.11). During Events 10 (when one animal accessed the stream for 2.0 minutes) and 12 (when a maximum of 3 animals accessed the stream for a total of 13.1 minutes), SRP concentrations did not increase during or following periods of cattle in-stream activity. In Events 9 (June 14, 2017, when a maximum of 4 animals visited the stream simultaneously and access lasted 32.9 minutes in total) and 11 (August 8, 2017, with a maximum of 3 animals simultaneously in the stream and duration of access of 23 minutes), small increases of +6 $\mu g.L^{-1}$ and +4 $\mu g.L^{-1}$, respectively, were registered. In Events 7 (May 10, 2017, when there was a maximum of 6 animals in the stream and the total access duration was 25.6 minutes) and 16 (June 13, 2018, a maximum of 5 animals simultaneously in the stream with a total duration of access of 41.2 minutes), SRP concentrations downstream of the access site increased by a maximum of +11 µg.L^{-1} and +8 µg.L^{-1}, respectively (Table 6.6), following cattle in-stream activity.

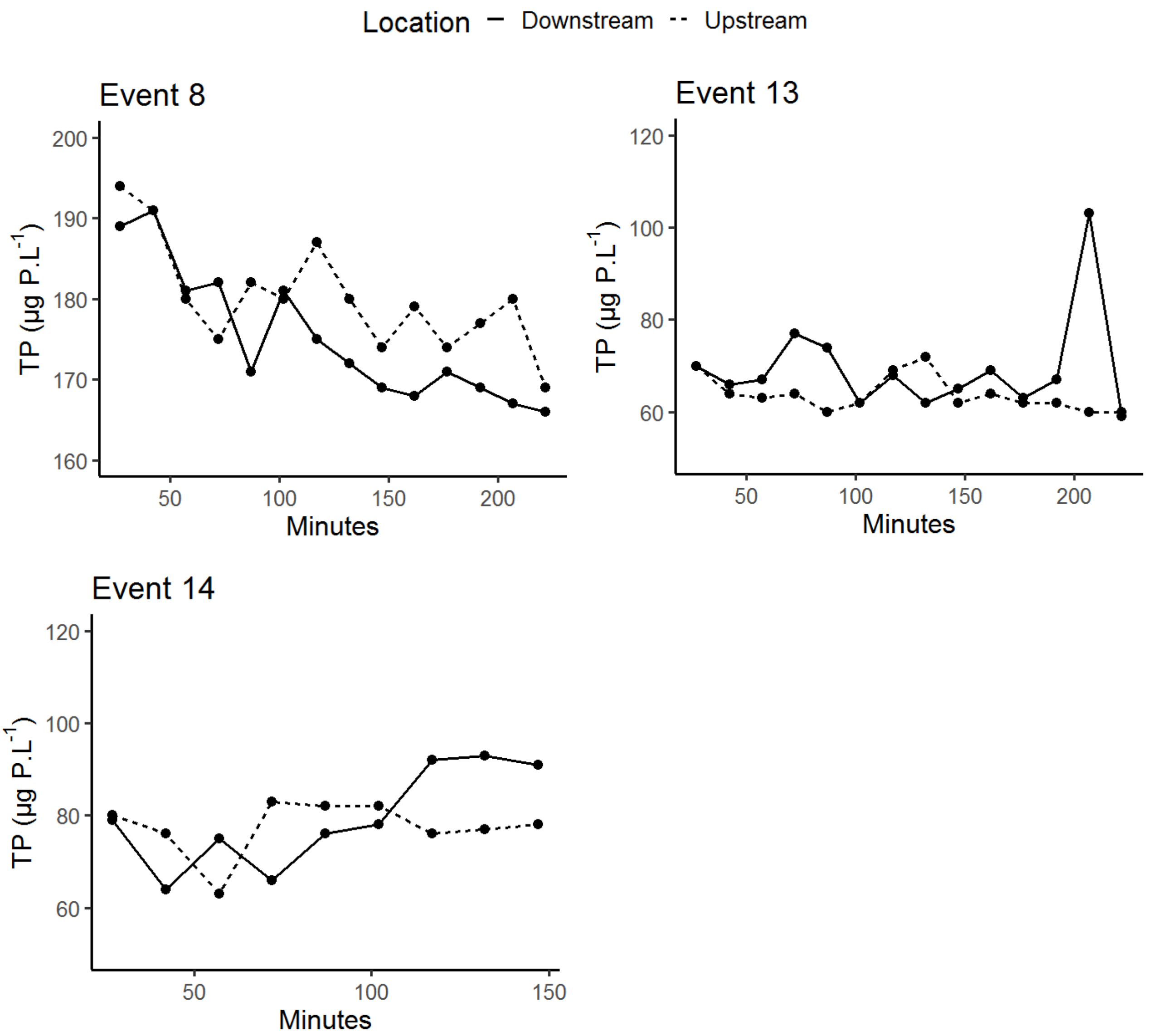

Fig.6.8. Variation in TP concentrations upstream and downstream of the cattle access during events with no cattle access to the stream. Note the differences in scales.

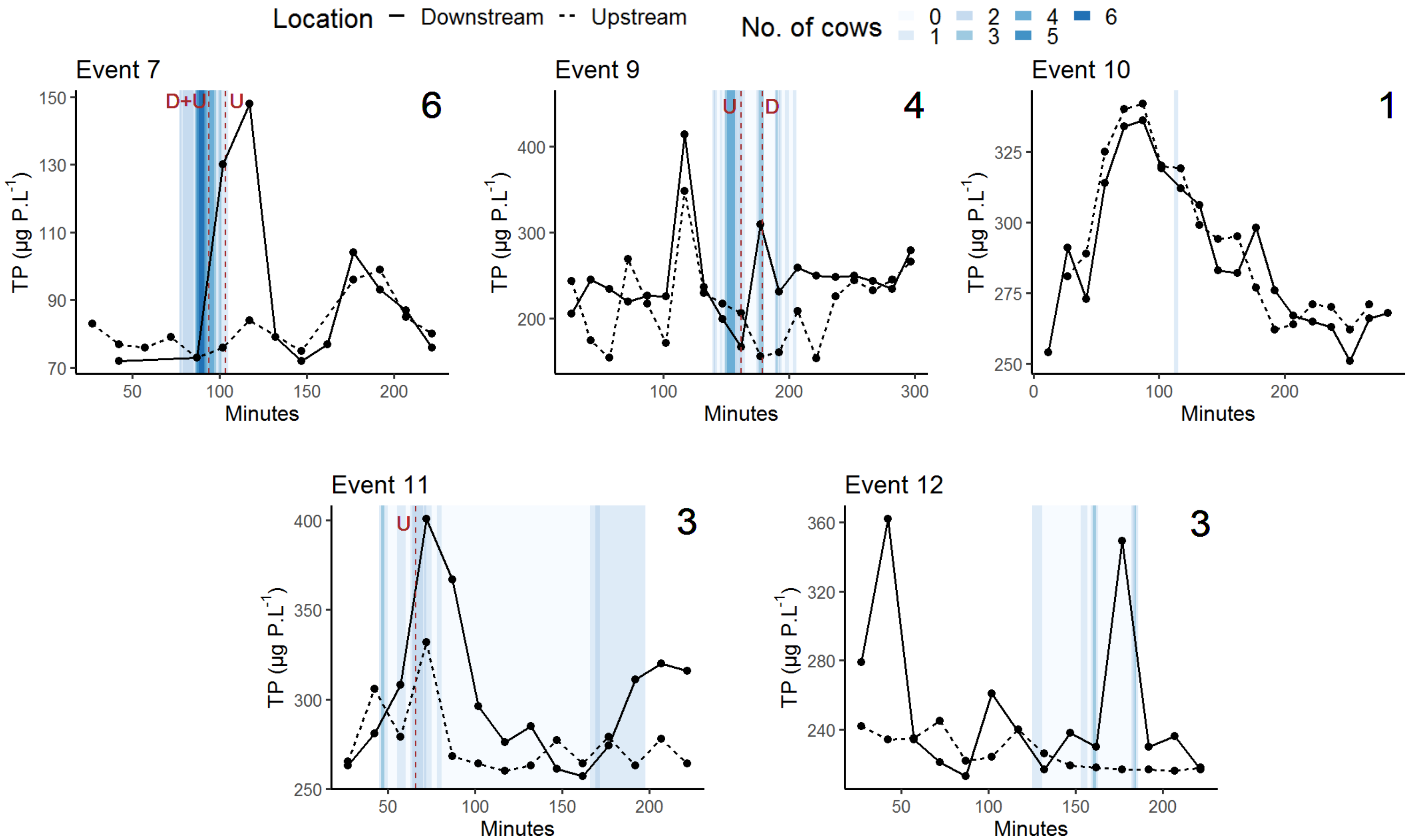

Fig.6.9. Variation in TP concentrations upstream and downstream of the cattle access during events that captured cattle access to the stream. Note the differences in scales. Blue shading indicates times of in-stream activity. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

6.3.2.4. *Changes in ammonium (NH_4-N) water concentrations across all 11 events*

Ammonium (NH_4-N) concentrations in stream water at the study site were highly variable both in time and within sampling events (Table 6.5, Fig. 6.12 and 6.13). Nevertheless, while some increases in NH_4-N concentrations downstream of the access site were registered at periods when cattle did not access the stream, changes in NH_4-N concentrations were more pronounced during and following cattle in-stream activity. For example, NH_4-N concentrations increased downstream of the access site during cattle activity during Event 12 (August 30, 2017), peaking at a concentration of 0.1 $mg.L^{-1}$, an increase of +0.08 $mg.L^{-1}$ in relation to concentrations measured upstream. However, downstream concentrations that were higher than those measured upstream, by +0.03 $mg.L^{-1}$, were also observed during the same sampling event during periods of no cattle access. In Event 11, NH_4-N concentrations measured downstream of the site were higher than those measured upstream both during and following cattle in-stream activity, by a maximum of +0.11 $mg.L^{-1}$ (corresponding to 0.19 $mg.L^{-1}$ downstream), however this difference was also caused by a decrease in NH_4-N concentrations upstream of the access site (0.08 $mg.L^{-1}$, from 0.13 $mg.L^{-1}$ in the previous sampling interval). In the same sampling event, NH_4-N concentrations at the downstream site increased once again in comparison to upstream concentrations during a second period of cattle in-stream activity, but to a much lower extent (differences of +0.03 and +0.02 $mg.L^{-1}$). During Event 9 (June 14, 2017), ammonium concentrations increased and peaked at 0.05 $mg.L^{-1}$ during cattle in-stream activity, but also were higher than those measured upstream by +0.01 $mg.L^{-1}$ and +0.03 $mg.L^{-1}$ at sampling times both before and after the period of cattle access when no cattle were in the stream. In Event 16, NH_4-N concentrations

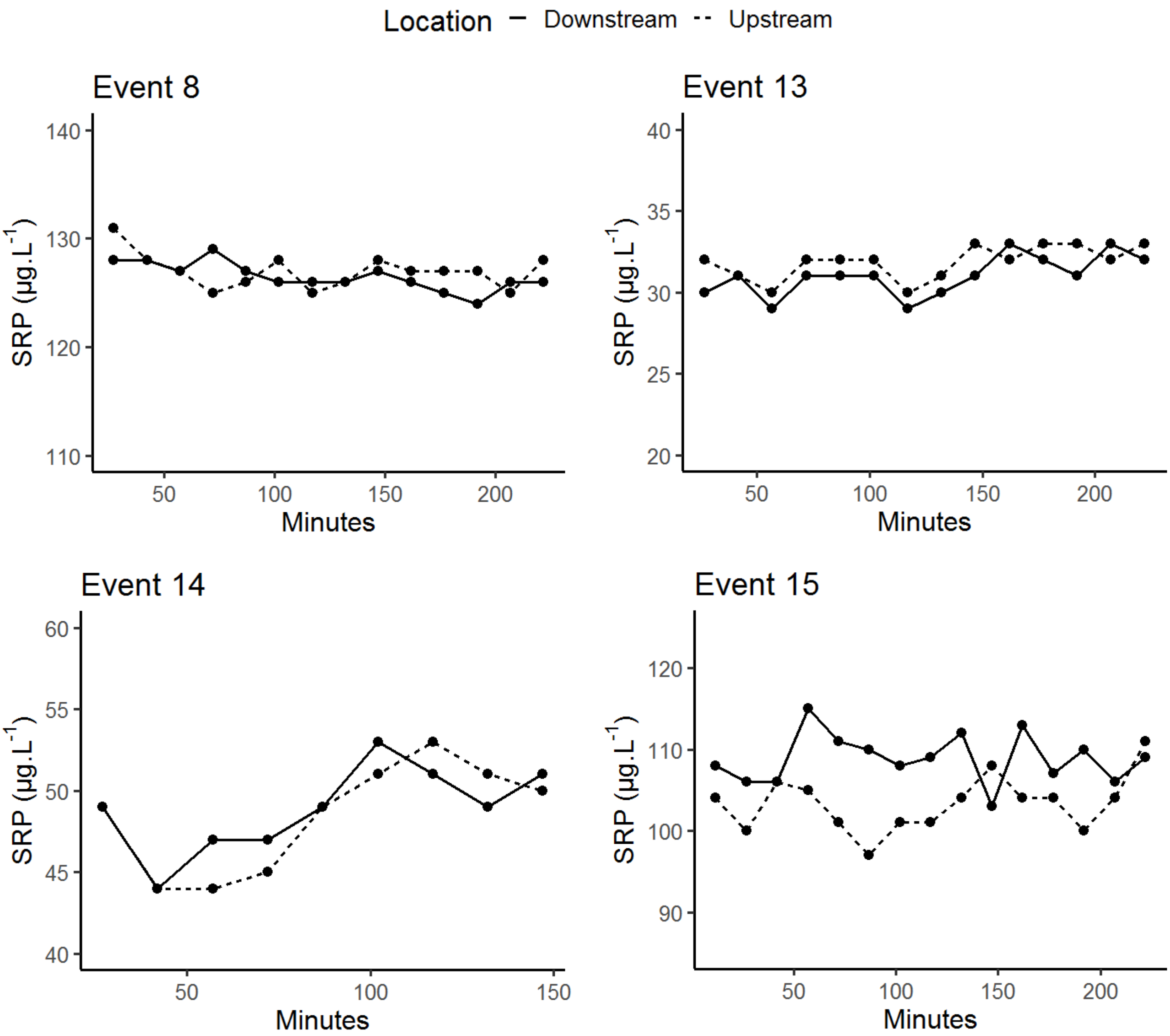

Fig.6.10. Variation in SRP concentrations upstream and downstream of the cattle access during events with no cattle access to the stream. Note the differences in scales.

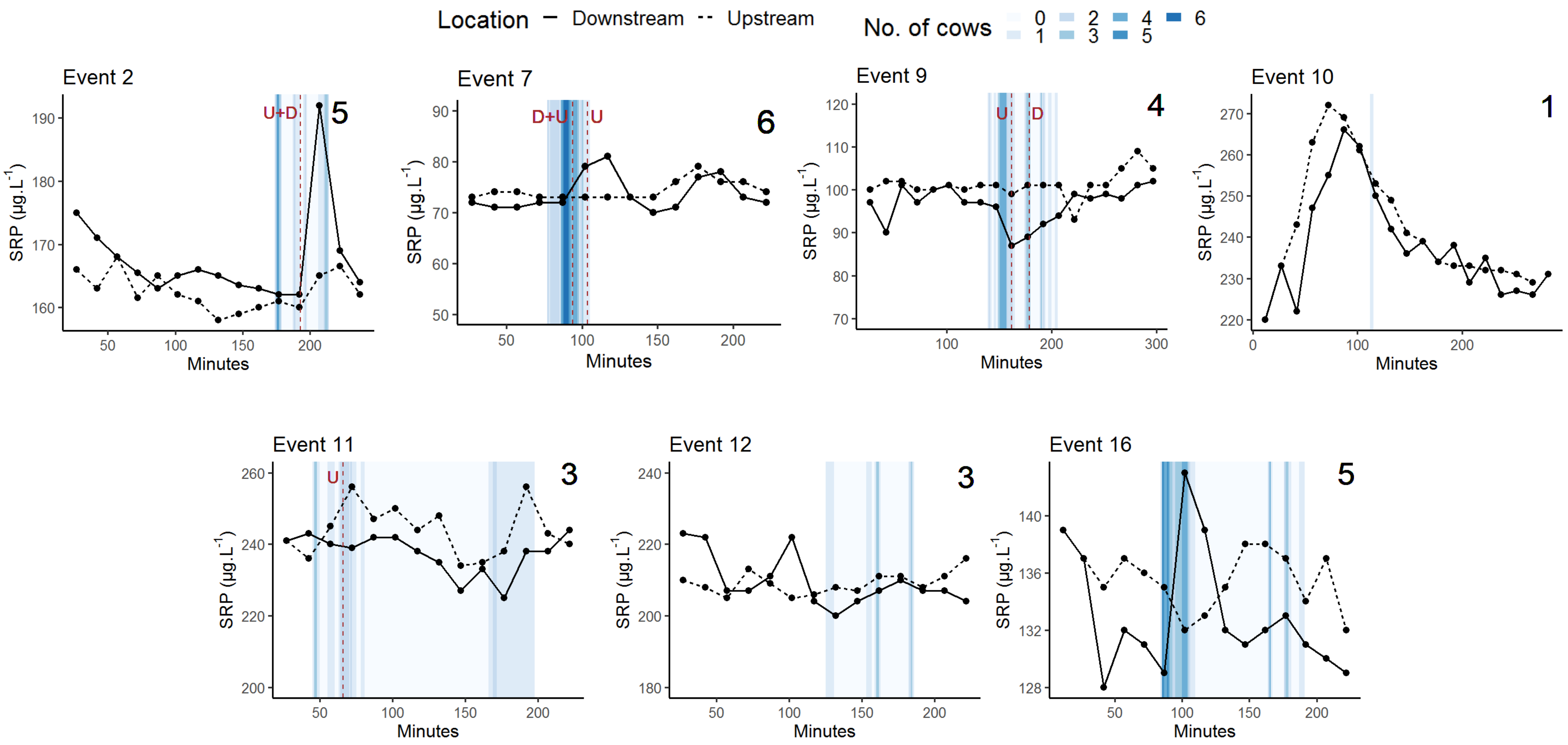

Fig.6.11. Variation in SRP concentrations upstream and downstream of the cattle access during events that captured cattle access to the stream. Note the differences in scales. Blue shading indicates times of in-stream activity. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

increased markedly following a period of cattle in-stream activity and in-stream defecation, from 0.02 mg.L^{-1} upstream to 0.21 mg.L^{-1} downstream, and were again higher than upstream levels following a second period of cattle access (at minute 165) to the stream by +0.05 mg.L^{-1}. In Event 7 (May 10, 2017), an increase in NH_4-N concentrations was observed at the downstream site during cattle access and following two urinations and one defecation event, from 0.02 mg.L^{-1} upstream to 0.08 mg.L^{-1} downstream. NH_4-N concentrations were at times higher upstream of the site than downstream in almost all sampled events, during both periods of cattle access and periods of no access, highlighting the high variability of this parameter. The highest difference in upstream concentrations relative to downstream concentrations was registered in Event 11, when NH_4-N concentrations were 0.22 mg.L^{-1} upstream at the sampling interval of 207 to 222 minutes from start, while downstream concentrations were 0.02 mg.L^{-1} downstream.

6.3.2.5. Changes in nitrate (NO_3-N) water concentrations across all 11 events

Nitrate nitrogen (NO_3-N) concentrations did not demonstrate any consistent response to cattle in-stream activity during the sampling events (Table 6.6., Fig. 6.14 and 6.15). For all seven events that captured cattle access to the stream, two events (Events 11 and 16) registered very small increases in NO_3-N concentrations during and following cattle in-stream activity. In Event 8, NO_3-N concentrations downstream of the site peaked at 3.01 mg.$^{-1}$ during cattle access to the stream, an increase of +0.24 mg.L^{-1} in relation to upstream concentrations at the same time interval. In Event 16, NO_3-N concentrations downstream of the site reached 5.62 mg.L^{-1} during a first period of cattle access to the stream, an increase of +0.20 mg.L^{-1} in relation to upstream concentrations (5.42 mg.L^{-1}). The downstream concentrations then peaked at 6.20 mg.L^{-1} during a second period of cattle in-stream activity, an increase of +0.71 mg.L^{-1} relative to upstream concentrations for the equivalent time interval (5.49 mg.L^{-1}) (Table 6.6, Fig. 6.7). In this last sampling event, however, there was also a decrease of -0.34 mg.L^{-1} in in downstream NO_3-N concentrations (5.11 mg.L^{-1}) in comparison to upstream concentrations (5.45 mg.L^{-1}) following the first period of cattle in-stream activity.

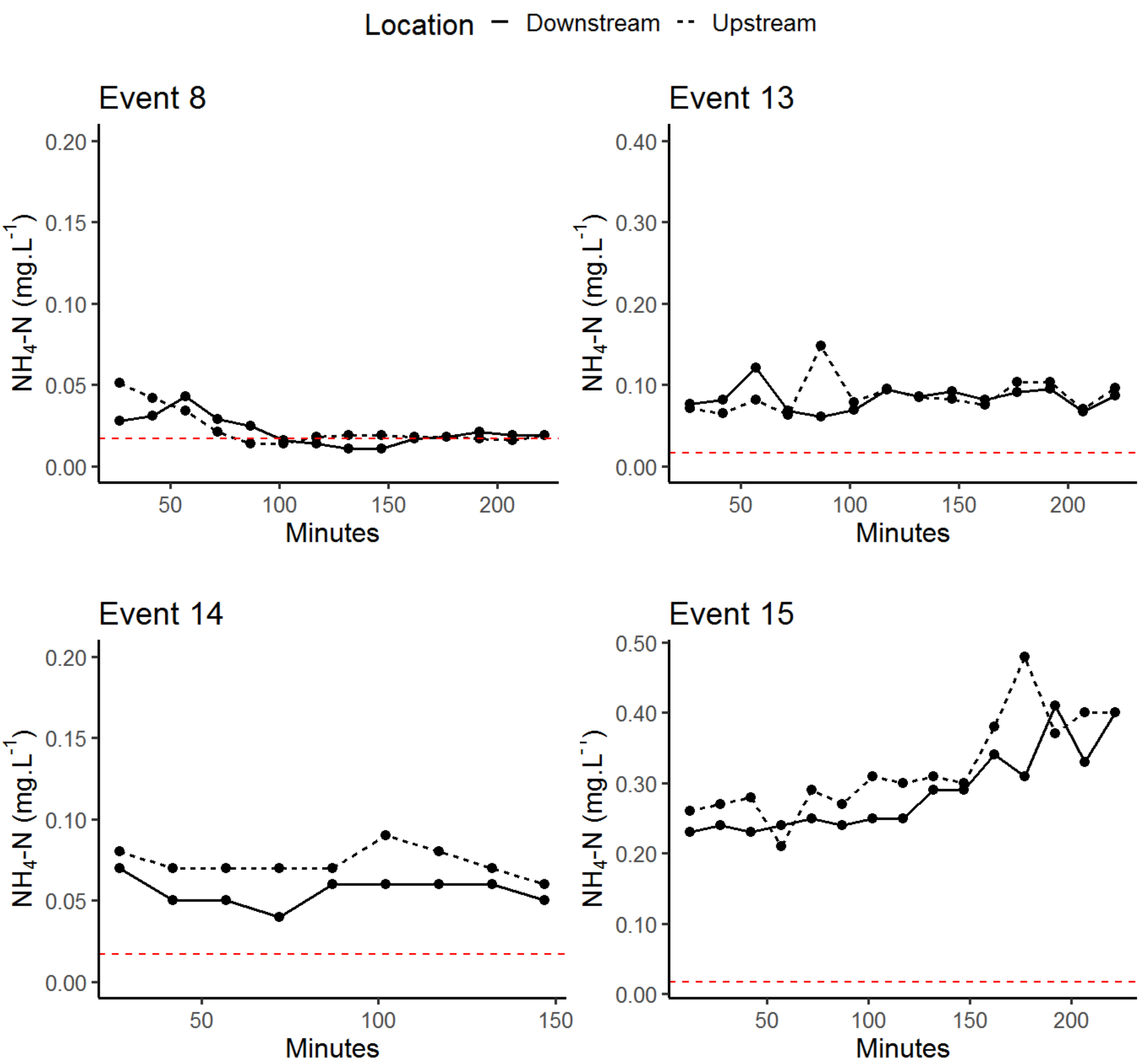

Fig.6.12. Variation in NH_4-N concentrations upstream and downstream of the cattle access during events with no cattle access to the stream. Note the differences in scales. The dashed red line represents the limit of the detection for the analysis of the parameter

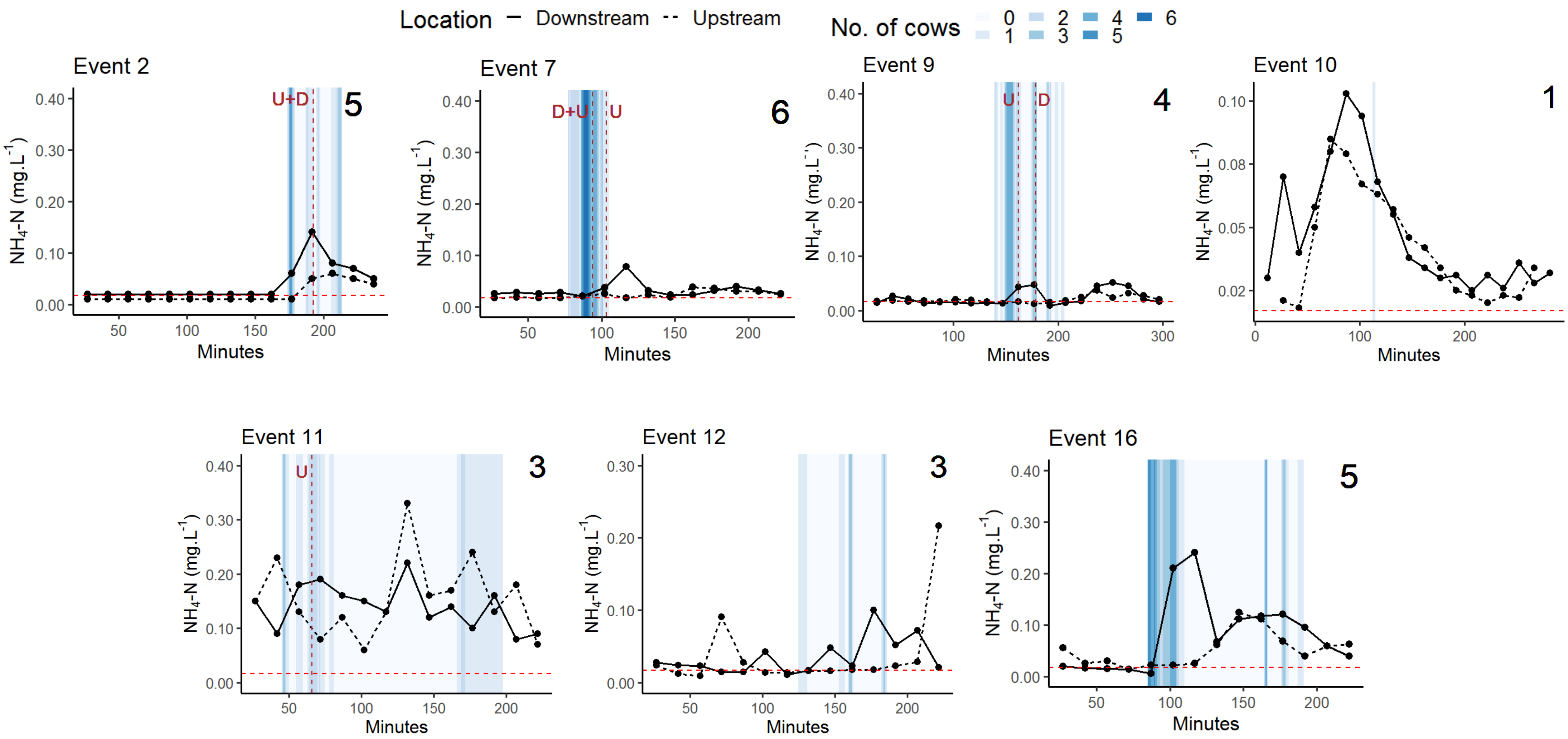

Fig.6.13. Variation in NH_4-N concentrations upstream and downstream of the cattle access during events that captured cattle access to the stream. Note the differences in scales. Blue shading indicates times of in-stream activity. The dashed red line represents the limit of the detection for the analysis of the parameter. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

6.3.2.6. Changes in E. coli bacteria concentrations in stream water across all 11 events

Changes in *E. coli* concentrations during the sampling events are shown in Fig. 6.16 and Fig. 6.17. Concentrations of *E. coli* in stream waters showed a clear and consistent pattern of increase during periods of cattle in-stream activity (Table 6.6., Fig. 6.17). Increases in *E. coli* concentrations downstream of the access site were observed in all events that captured cattle in-stream activity, including those where no in-stream defecation was recorded. In Event 12, *E. coli* concentrations downstream of the site increased during and following three episodes of cattle access to 5.0 x 10^3 CFU.100 ml^{-1}, 5.1 x 10^3 CFU.100 ml^{-1} and 4.2 x 10^3 CFU.100 ml^{-1} while upstream concentrations at the equivalent time intervals were 2.1 x 10^3 CFU.100 ml^{-1}, 1.7 x 10^3 CFU.100 ml^{-1} and 1.9 x 10^3 CFU.100 ml^{-1}, respectively (Fig. 6.8). In Event 11 (August 8, 2017), *E. coli* concentrations peaked at 9.1 x 10^3 CFU.100 ml^{-1} and 1.3 x 10^4 CFU.100 ml^{-1} downstream of the access site during two periods of cattle in-stream, while upstream of the site concentrations measured for the corresponding sampling periods were 9.9 x 10^2 CFU.100 ml^{-1} and 8.0 x 10^2 CFU.100 ml^{-1}, respectively, representing a difference of approximately one order of magnitude. During Event 9, *E. coli* bacteria concentrations also increased during cattle in-stream activity, reaching a maximum of 8.5 x 10^3 CFU.100 ml^{-1}, a near tenfold increase in comparison to upstream concentrations, which were at 1.2 x 10^3 CFU.100 ml^{-1} for the same time period. These increased *E. coli* concentrations at the downstream site were sustained for approximately 60 minutes, during which cattle visited the stream on several occasions. In Event 7, an increase of two orders of magnitude in *E. coli* bacteria concentrations from 4.2 x 10^2 CFU.100 ml^{-1} upstream to 2.4 x 10^4 CFU.100 ml^{-1} at the downstream site was observed during cattle access (maximum 6 animals) to the stream. Finally, in Event 16, downstream *E. coli* bacteria concentrations increased dramatically

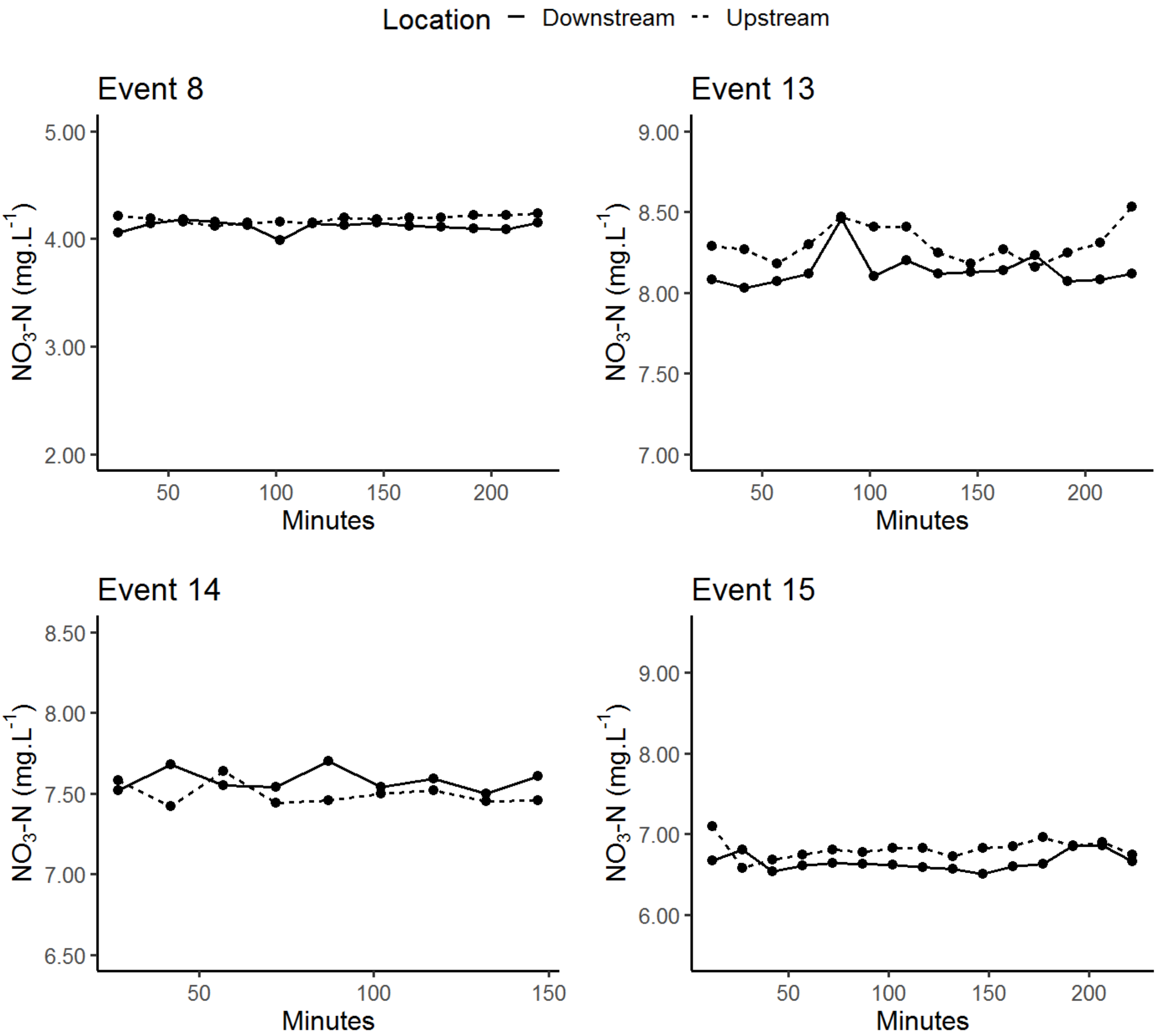

Fig.6.14. Variation in NO_3-N concentrations upstream and downstream of the cattle access during events with no cattle access to the stream.

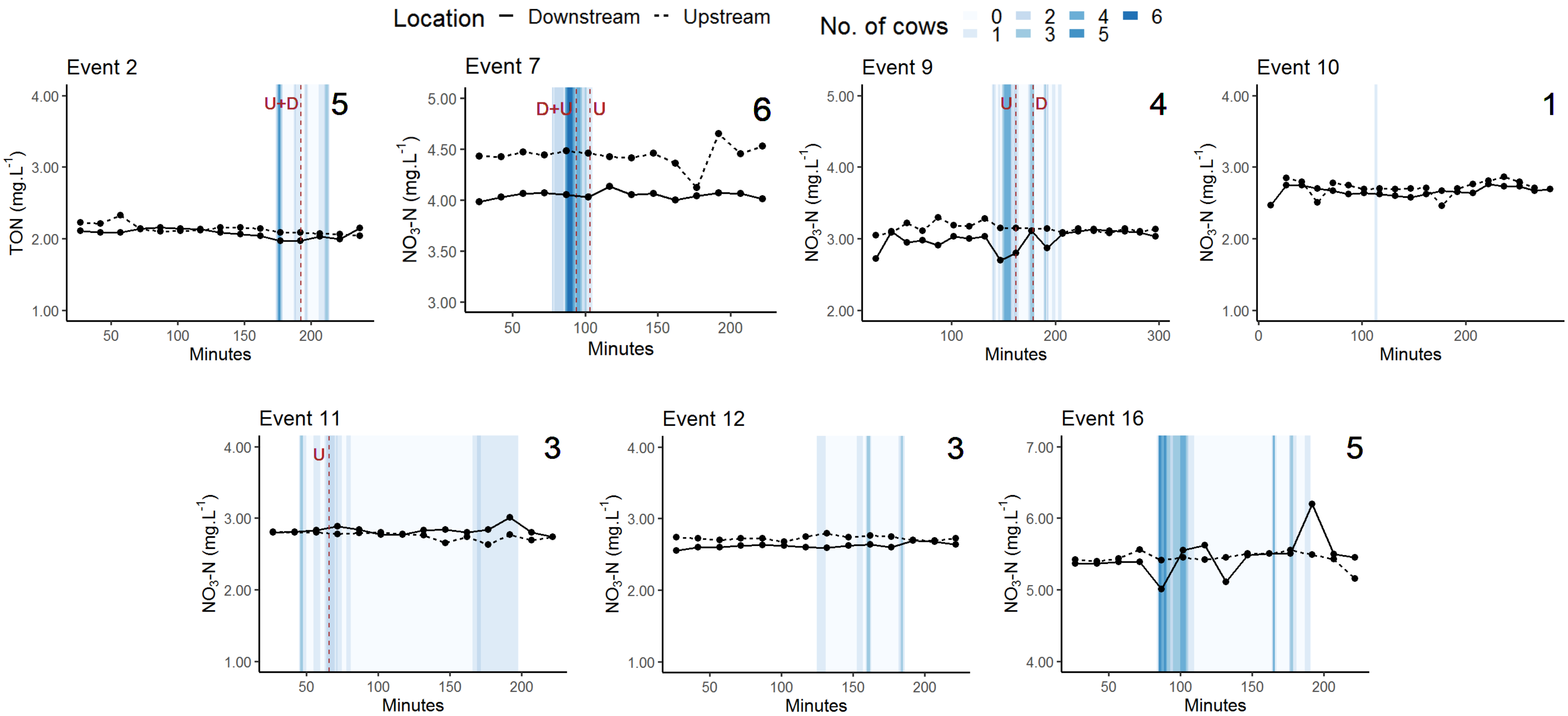

Fig.6.15. Variation in NO_3-N concentrations upstream and downstream of the cattle access during events that captured cattle access to the stream. Note the differences in scales. Blue shading indicates times of in-stream activity. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

following cattle access to the stream, peaking at 7.9 x 10^4 CFU.100 ml^{-1} from values of 5.5 x 10^2 CFU.100^{-1} upstream of the access site, corresponding to a difference of more than two orders of magnitude. Following the period of stream disturbance by cattle during this event, downstream *E. coli* concentrations decreased and approached upstream concentrations, at 9.7 x 10^3 CFU.100 ml^{-1-} after approximately 38 minutes. However, they increased again following a second period of cattle access (maximum of 4 animals) to the stream with a maximum concentration of 1.9 x 10^4 CFU.100 ml^{-1}, a period when upstream concentrations remained one order of magnitude lower at 4.6 x 10^3 CFU.100 ml^{-1}.

6.3.3. Loads of nutrients, TSS and *E. coli* bacteria during cattle access

The upstream-downstream difference in loads (g.15 $minute^{-1}$) of phosphorus (SRP and TP), NH_4-N, NO_3-N, TSS and *E. coli* bacteria at peak concentrations, as well as the net upstream - downstream difference in loads during periods of cattle access to the stream are shown in Table 6.7.

The additional TSS loads during cattle in-stream activity corresponding to the time of the peak in recorded concentrations downstream of the access site ranged from +20.34 g.15 $min.^{-1}$ (Event 12, August 30, 2017, when there was a maximum of 3 animals accessing the stream for a total of 13.1 minutes) to +249.48 g.15 min^{-1} (Event 7, May 10, 2017, which had a maximum of 6 animals and a total duration of access of 25.6 minutes). Additionally, there was a net increase in TSS loads downstream relative to upstream during cattle access for all access events, with the highest net difference registered in Event 9 (June 14, 2017 when a maximum of 4 cows accessed the stream for a total of 32.9 minutes) (+675.90 g TSS).

The additional TP loads at the downstream site at the time of peak concentrations ranged from +0.150 g.15 min^{-1} (Event 10) to +2.177 g. 15 min^{-1} (Event 9). The total added TP loads

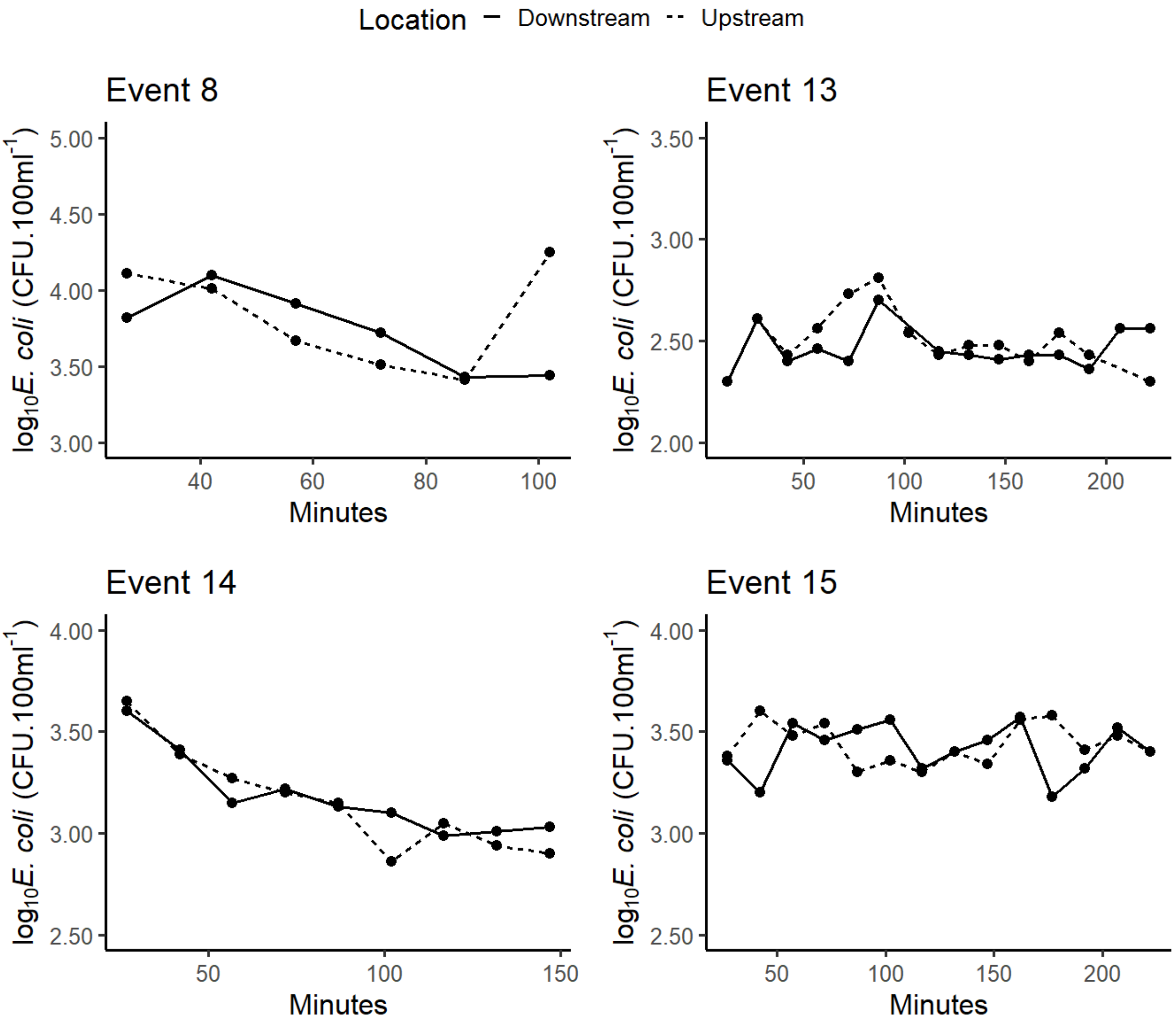

Fig.6.16. Variation in *E. coli* bacteria concentrations upstream and downstream of the cattle access during events with no cattle access to the stream.

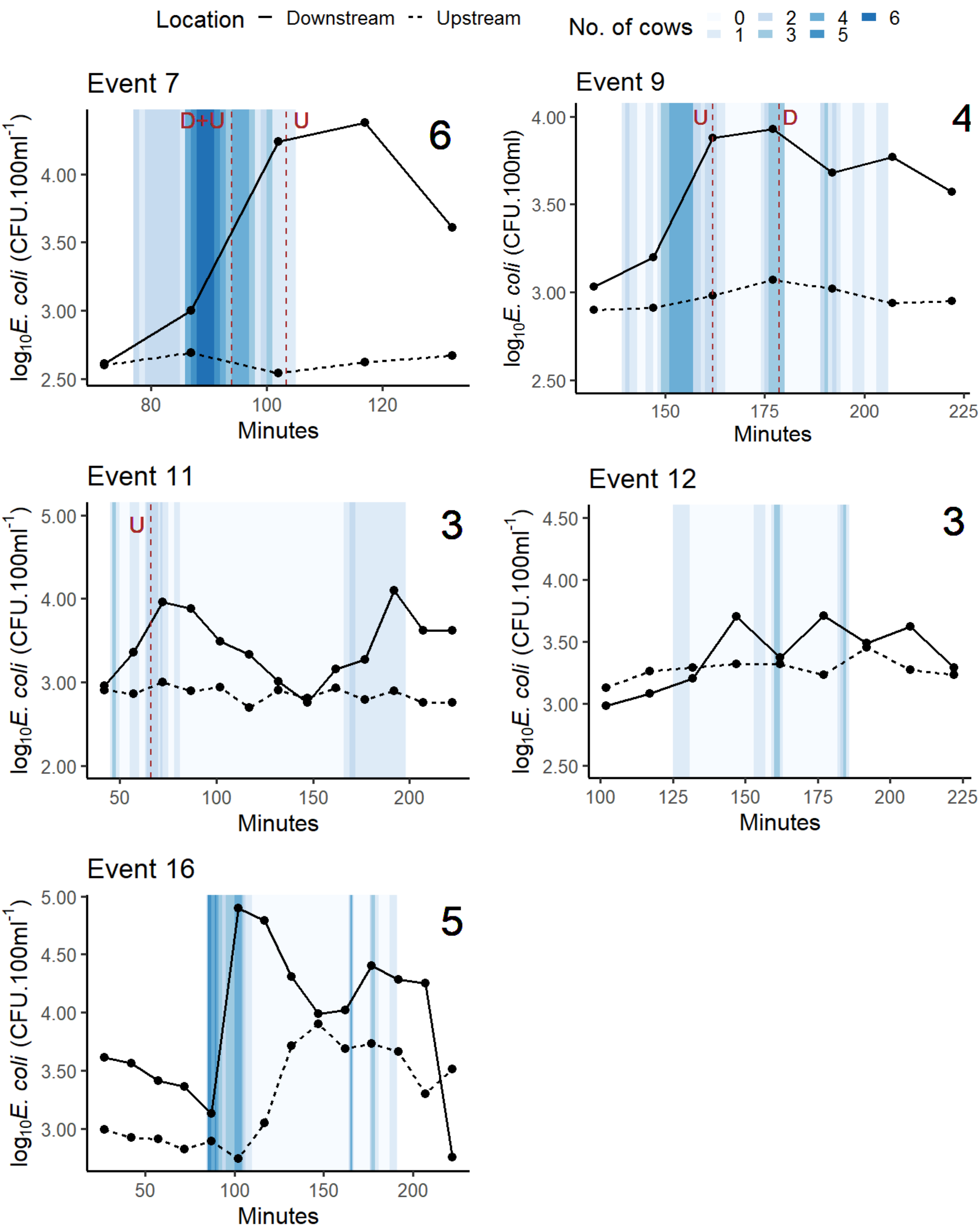

Fig.6.17. Variation in *E. coli* bacteria concentrations upstream and downstream of the cattle access during the sampling events. Note the differences in scales. Blue shading indicates times of in-stream activity. Brown dashed lines indicate times of defecation (D) and urination (U) episodes. Numbers indicate the maximum number of animals observed simultaneously in the stream for each event.

during cattle access ranged from +0.013 g (Event 10), to +4.442 g (Event 9). Maximum additional loads of NH_4-N during cattle access to the stream varied from +0.099 g. 15 min^{-1} (Event 10) to +1.017 g. 15 min^{-1} (Event 16, June 13, 2018, with a maximum of 5 animals and a total duration of 41.2 minutes), with total net loads ranging from +0.055 g to +2.394 g corresponding to the same events. Again, the net variation in loads was lower than the added load at peak concentration for Event 10.

An increase in SRP loads downstream of the access site relative to upstream was estimated in all events with the exception of Events 10 and 12, events during which the intensity of cattle access to the stream was the lowest. However these increases were generally very small and the net variation in downstream SRP loads was actually negative for 5 out of 7 sampling events, when SRP loads were lower downstream of the access site than upstream during periods of cattle access. The exceptions were Event 2 (September 21, 2016, with a maximum of 5 animals accessed the stream simultaneously and access lasting 18.8 minutes in total) and Event 7. In these events, the additional SRP loads at the downstream site at the time of maximum concentrations were +0.316 g. 15 min^{-1} and +0.083 g. 15 min^{-1}, and net differences were +0.404 g and +0.135 g, respectively.

Nitrate loads downstream of the access site were actually lower than those measured upstream during cattle access for 4 out of the 7 sampling events when cattle were present, including at the time of maximum downstream concentrations. The exceptions were Events 2, 11 (August 8, 2017, with a maximum of 3 animals simultaneously in the stream and which had access that lasted 23.0 minutes) and Event 16, when the downstream loads at peak concentrations of +1.287 g. 15 min^{-1} NO_3-N, +1.197.g 15 min^{-1} g and +3.384 g. 15 min^{-1}, respectively.

Finally, there was a net increase in the load of *E. coli* bacteria (i.e. total additional CFU) for all events where cattle accessed the stream (Table 6.7). The additional *E. coli* loads at peak concentrations ranged from +9.3 x 10^7 CFU in Event 12 to +3.7 x 10^9 CFU in

Table 6.7. Calculated differences in nutrient, TSS and *E. coli* bacteria loads at the downstream of the site relative to upstream loads during periods of cattle in-stream activity.

Event	SRP (g)		TP (g)		NH_4-N (g)		NO_3-N (g)		TSS (g)		*E. coli* (CFU)	
	Max	*Net total*	*Max*	*Net total*	*Max*	*Net total*	*Max*	*Net total*	*Max*	*Net total*	*Max*	*Net total*
2	0.316	0.404	NA		1.008	2.294	1.287	-2.867	61.830	135.946	NA	
7	0.083	0.135	0.665	1.227	0.576	0.800	-3.015	-15.696	249.480	407.469	2.5×10^9	4.7×10^9
9	0.085	-0.551	2.177	4.442	0.558	0.678	-0.288	-0.688	197.910	675.898	1.0×10^9	3.7×10^9
10	-0.059	-0.209	0.150	0.013	0.099	0.055	-1.566	-3.504	68.850	115.882	NA	
11	0.020	-0.333	0.493	1.823	0.567	0.621	1.197	-3.576	182.160	574.135	5.9×10^8	1.9×10^9
12	-0.003	-0.058	0.362	0.514	0.225	0.570	-0.027	-1.674	20.340	79.275	9.3×10^7	2.4×10^8
16	0.052	-0.029	NA		1.017	2.394	3.384	1.429	142.470	288.737	3.7×10^9	9.7×10^9

Max = difference in loads downstream relative to upstream corresponding to the highest concentration downstream during cattle access during that event. *Net total* = sum of differences in downstream loads relative to upstream loads during the total duration of cattle access to the stream. NA – data not available due to issues during sample collection or processing.

Event 16, and net differences in loads varied from +2.4 x 10^8 CFU and +9.7 x 10^9 CFU for the same events.

Despite these increases in loads when cattle accessed the stream, for all parameters with the exception of *E. coli*, the upstream-downstream difference in loads for periods of cattle access fell within the range of the upstream-downstream difference calculated for those times when no cattle were present (Fig. 6.18 and 6.19). Moreover, the difference in loads for *E. coli* during periods of access was always positive, while in periods of no access, they were close to zero (Fig. 6.18). *E. coli* was also the only parameter for which the magnitude of increase in loads coinciding with cattle access generally increased with the increasing intensity of access when quantified in cattle-minutes (Fig 6.19). Finally, the distribution of the dataset for the upstream-downstream difference in loads during periods of cattle access was significantly different from that during periods with no cattle access for three parameters only: *E. coli,* TSS and NH_4-N (Table 6.8, Fig. 6.20). In contrast, there were no significant differences found for SRP, TP or NO_3-N loads (Fig. 6.19).

Table 6.8. Statistical parameters for the Kolmogorov-Smirnov test with bootstrap resampling (n =1000). Significant effects are shown in bold.

Parameter	n (Access, No access)	D	*p*
E. coli	**21, 58**	**0.485**	**< 0.001**
TSS	**26, 121**	**0.488**	**< 0.001**
TP	17, 84	0.276	0.232
SRP	26, 122	0.146	0.754
NH_4-N	**26, 122**	**0.391**	**0.003**
NO_3-N	26, 122	0.189	0.431

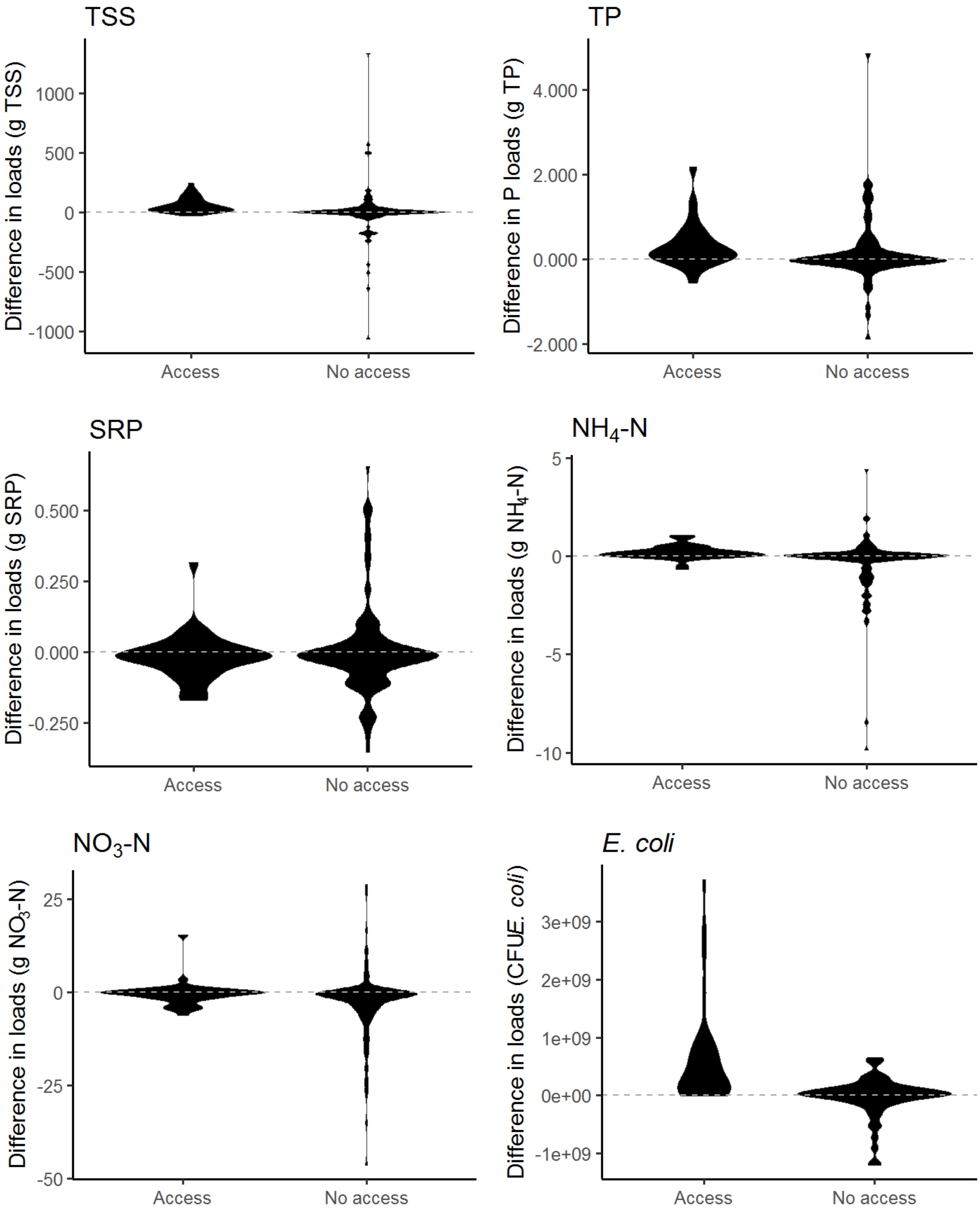

Fig.6.18. Violin plots for the differences in loads downstream in relation to upstream of nutrients, TSS and *E. coli* bacteria during cattle access to the stream and during periods of no access.

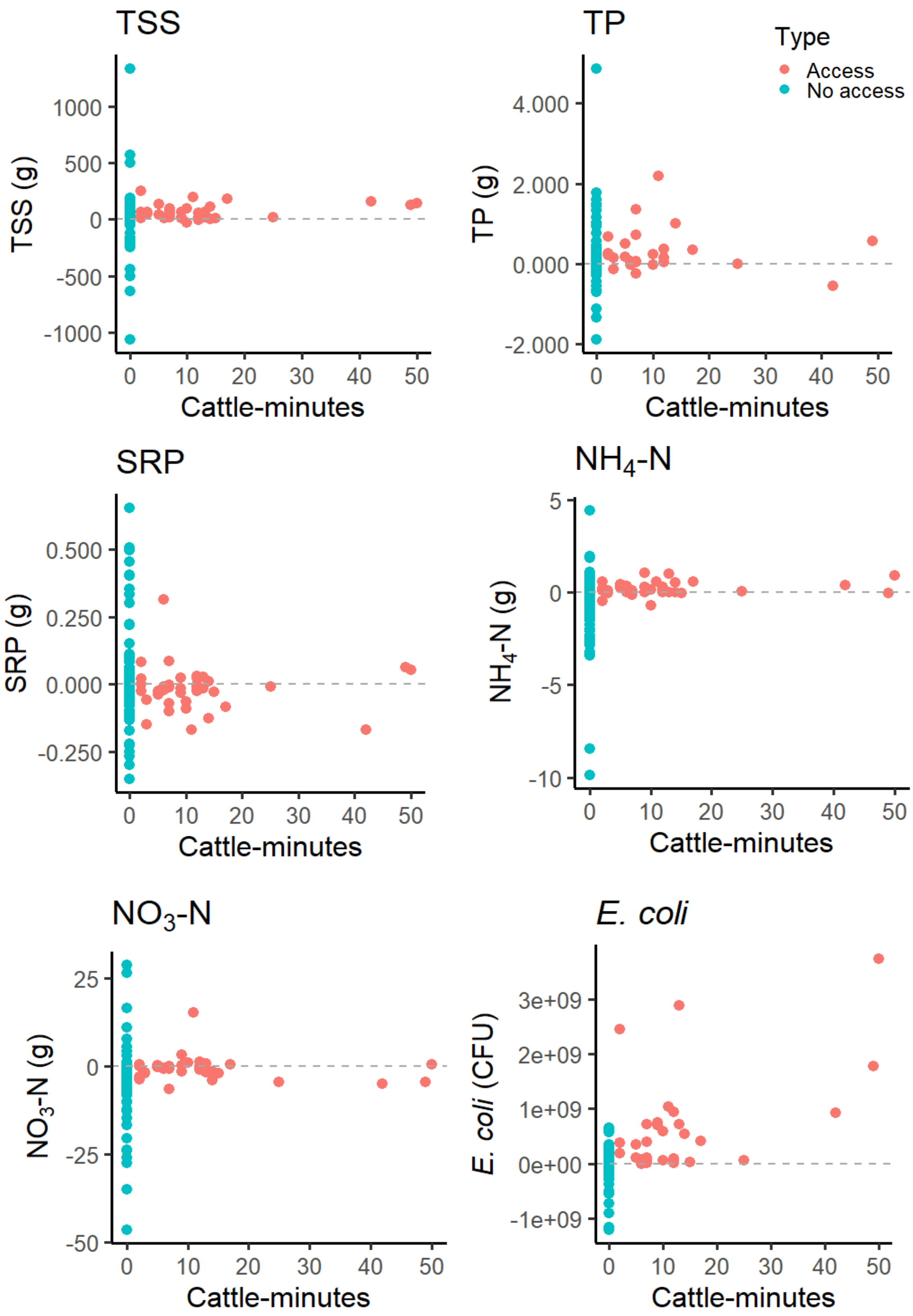

Fig.6.19. Scatterplots showing the relationship between the differences in loads of nutrients, TSS and *E. coli* bacteria downstream relative to upstream during periods of cattle access to the stream (orange) and periods of no access (blue), intensity of access expressed in cattle-minutes.

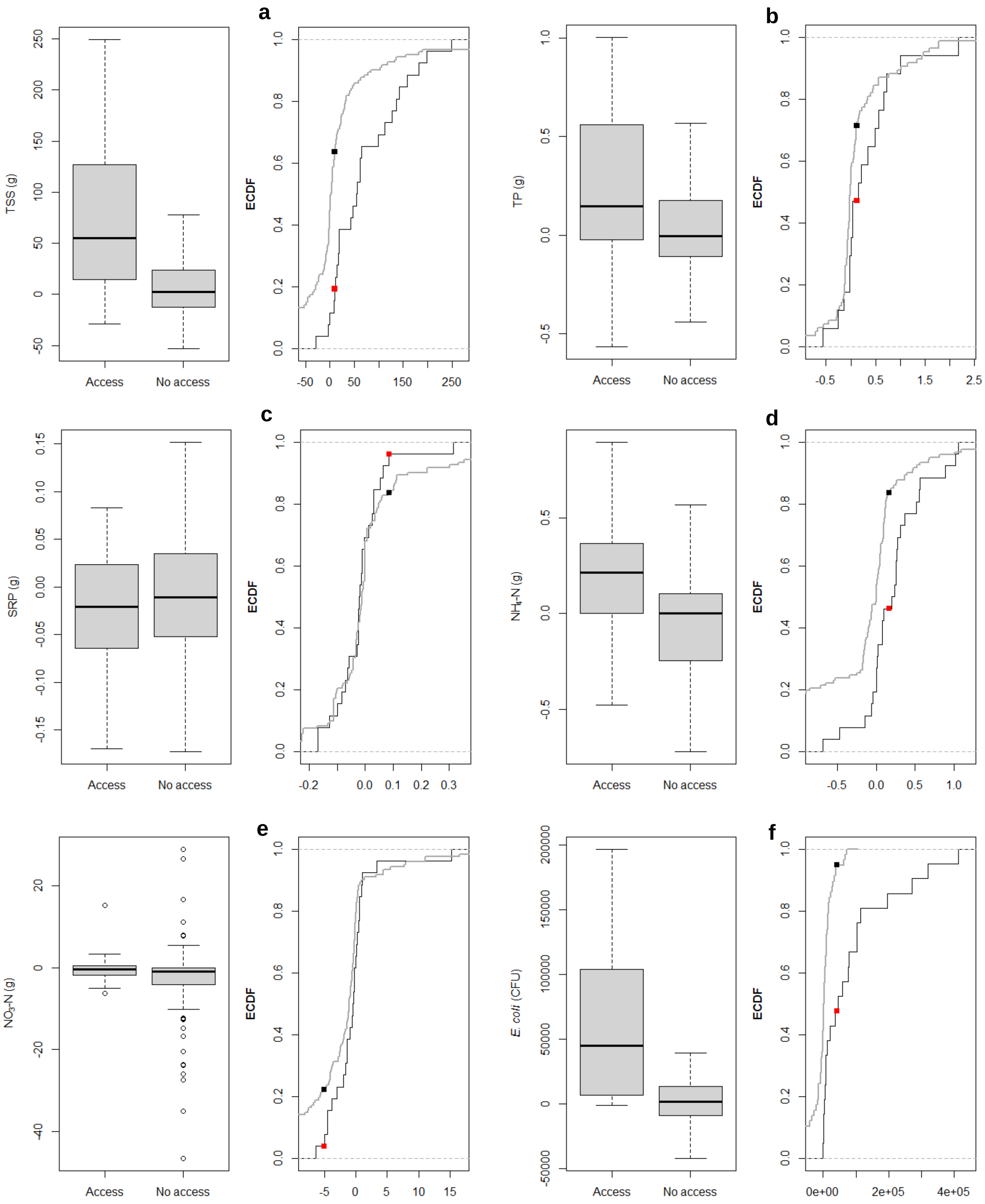

Fig.6.20. Boxplots and empirical cumulative distribution functions (ECDF) for the difference in loads of a) TSS, b-e) nutrients and f) *E. coli* bacteria downstream of the access site relative to upstream during periods of cattle access to the stream and periods of no access (grey line on ECDF = Access, black = No Access); points are the values where there is a maximum difference between the two distributions.

6.4. Discussion

Unrestricted cattle access to watercourses has been associated with water quality deterioration as a result of excess nutrient and fine sediment inputs and faecal contamination. However, there is a general paucity of research seeking to assess and quantify such impacts, in particular in the European context (O'Callaghan et al., 2018), and especially those that quantify these impacts at a high temporal resolution and/or concurrent with cattle access. The present study aimed at assessing these immediate impacts of cattle access to watercourses on a number of water quality parameters in a near real-time experiment (i.e. at a high temporal resolution that captured cattle activity). The study found that cattle in-stream activity consistently led to increased water concentrations of faecal bacteria (*E. coli*), even when no direct defecation in stream waters was registered. Moreover, these increases in concentration resulted in significantly higher loads to the stream. Additionally, cattle in-stream activity led to an increase in water total suspended solids concentrations and loads, and similarly significant but more variable increases in ammonium loads. In contrast, the study found that cattle access did not have a significant effect on water loads of SRP, TP or nitrate, a result which is consistent with the few other studies that are available in near-real time (e.g. Demal, 1982; Wilson and Everard, 2017).

6.4.1. Impact of cattle access on total suspended solids in waters

The study hypothesised that total suspended solids would increase downstream of access sites whenever cattle entered the stream and the streambed sediment was disturbed. Factors potentially influencing the magnitude of such impacts include the number of animals in the stream at a given time, the intensity of activity (i.e. how frequently the animals move in the stream), as well as the particle size distribution of the sediment and the amount available for resuspension (Terry et al., 2014). The deposition of faeces in the stream water will also contribute to total suspended solids concentrations. Cattle faeces are mostly composed by water (Bond et al., 2012), with 11% - 18% of the faeces fresh weight consisting of solids (Bond et al., 2012; James et al., 2007). Thus, a single deposit of 1.9 kg (James et al., 2007) would add to the stream waters roughly between 206 g and 342 g of fresh organic solids.

In the current study, TSS concentrations consistently increased downstream of the access site during cattle in-stream activity. However, the relationship between the magnitude of this increase and the intensity of cattle access in cattle-minutes was not clear, and occasional increases were also observed when no cattle were present. The total added TSS load at the downstream site ranged from 79.3 g in Event 12 (over 25 cattle-minutes) to 675.9 g in Event 9 (over 81 cattle-minutes), whereas the events with the lowest and highest intensity of access, Events 10 (2 cattle-minutes) and 16 (99 cattle-minutes), had total added loads during access of 115.3 g and 288.7 g, respectively, although in the former event, this was not substantially different from the TSS variation at the study site on that occasion. Although immediate increases in TSS water concentrations during cattle access have been reported in the limited number of studies where this has been quantified (e.g. Demal, 1982; Terry et al., 2014; Wilson and Everard, 2017), Terry et al. (2014) did not find a clear relationship between suspended solids concentrations and the total number of cattle feet in the stream. The authors hypothesised that this resulted from a combination of the sporadic nature of episodes of cattle in-stream activity together with varying intensities of access during the study, with in-between periods of minutes to days, and irregular flow patterns, leading to fluctuating sediment stores available for resuspension (Terry et al., 2014). Such processes will likely also be in operation in the current study.

It is of note that the variation in TSS loads downstream in relation to upstream during cattle access fell within the background range of variation observed during periods when cattle were absent from the stream, which included sporadic episodes of large increases in TSS concentrations either upstream or downstream of the site. In their study, Terry et al. (2014) also reported that 57.9% of the occasions when TSS concentrations exceeded the guideline threshold of 0.025 $mg.L^{-1}$ defined by the EU Freshwater Fish Directive were caused by cattle in-stream activity, but that these only represented 3.6% of the total TSS exports, with flow being the main agent of sediment loss in the area. Additionally, wildlife visiting the stream could also have caused sporadic increases in TSS concentrations at either sampling locations, although in the current study such instances would have been captured on the cameras but were not.

6.4.2. Impact of cattle access on *E. coli* bacteria in waters

Stream water concentrations of *E. coli* increased downstream of the cattle access site in all sampled events that captured cattle in-stream activity. Peak downstream concentrations were generally between one and two orders of magnitude higher than the *E. coli* concentrations registered upstream of the site at the equivalent time. The highest concentration of *E. coli* bacteria in waters downstream of the site during cattle access was observed in Event 16 (7.9×10^4 CFU.100 ml^{-1}), which also corresponded to the highest added load (3.7×10^9 CFU) of *E. coli* relative to bacteria levels measured upstream. Wilson and Everard (2017) also reported significant increases in faecal coliforms concentrations downstream of their study site in relation to upstream during cattle access, however, their increases were rather modest (+85%) in comparison to the current study, whereas Demal (1982) observed increases in faecal coliforms downstream of access sites during cattle access to the stream in 5 out of 9 cattle access events sampled at 5 different sites, which also corresponded to increases of one to two orders of magnitude compared to upstream concentrations.

Increases in faecal bacteria concentrations downstream of the access site during cattle access are expected due to either in-stream defecation, and to disturbance of streambed sediments that are enriched in faecal matter as a result of cattle activity (Bragina et al., 2017). In addition, cattle often defecate at the edge of the stream, and it is possible that some of the freshly deposited faecal matter is transported to the stream in cattle hooves due to cattle movement at the site, potentially leading to increased *E. coli* concentrations. Assuming cattle faeces contain roughly 10^7 CFU.g dry wt^{-1} (Table 4.1) and an average weight of a faecal deposit of 1.9kg containing an average of 15% solids (James et al. 2007), a single in-stream defecation would deposit in the stream roughly 2.9×10^9 CFU *E. coli*. In Event 10, when an in-stream defecation episode was registered at minute 94, *E.* coli concentrations increased to 1.7×10^4 and 2.4×10^4 CFU.100 ml^{-1}, between minutes 87 and 117, when upstream concentrations at equivalent times were 3.5×10^2 and $4.2.\times 10^2$ CFU.100 ml^{-1}. When water flow is taken into account, these increases represented an added load of 4.2×10^9 CFU *E. coli* in 30 minutes. Additionally, the highest increase in *E. coli* in water in Event 16 coincided with an episode of defecation at the edge of the stream (at minute 95), where the faecal deposit possibly reached the stream water due to the cattle movement.

Direct defecation in-stream waters or at the stream edge also causes the sediment to become enriched in faecal matter, and it has been widely demonstrated that faecal organisms can persist in sediments (Garzio-Hadzick et al., 2010; Ishii et al., 2007; Ishii and Sadowsky, 2008; Badgley et al., 2011; Shelton et al., 2014). Given the high concentration of *E. coli* in the stream sediment at the access site (Table 6.2), disturbance of the sediment by cattle is likely to cause a substantial increase in *E. coli* concentrations downstream of the site. For instance, in Event 12, when no direct defecations in stream waters were registered, the highest concentration of *E. coli* bacteria measured downstream of the access site was 5.1×10^3 CFU.100 ml^{-1}, which represented an increase in bacterial loads of 9.3×10^7 CFU in relation to upstream at the equivalent sampling time. Downstream TSS concentrations at the same sampling interval were 11.0 mg.L^{-1}, corresponding to an increase in TSS loads of 19.8 g in relation to upstream loads. Based on the average *E. coli* concentration in stream sediments at the study site (7.3×10^6 CFU.g dry wt^{-1} sediment), this increase in TSS loads would correspond to an added bacterial load of 1.4.x 10^8 CFU, which is very similar to the observed increase in *E. coli* loads at that time.

In this study, similar total added loads of *E. coli* bacteria during cattle access were seen across sampling events, with the highest total loads observed with the access was most intense, as expressed by total cattle-minutes (Event 16, 99 cattle-minutes), and the lowest when access was the least intense (Event 12, 25 cattle-minutes). In the study conducted by Demal (1982) in Canada, the increases in faecal coliform levels did not appear to be correlated to the intensity of access or the number of in-stream defecations. For example, the author reported similar increases in faecal coliform levels in two different cattle access events at two different study sites, one in which 30 animals accessed the stream for 31 minutes and 7 defecations were registered, and the other with 9 animals in the stream, total duration of 11 minutes and no in-stream defecations. However, that study was conducted in sites that were very heterogeneous in physical characteristics (e.g. length, depth, degree of bank erosion), stocking density and type of access (e.g. unlimited versus limited and improved), thus it is difficult to draw conclusions from it.

6.4.3. Impact of cattle access on NH_4-N in waters

Cattle access also had a significant impact on NH_4-N loads downstream of the access site, but the pattern of variation of NH_4-N concentrations during access was less clear. During both periods of cattle access and non-access to the stream, NH_4-N concentrations fluctuated at both sampling points. Ammonium is readily assimilated by macrophytes, algae and microbial biofilms, removing NH_4-N from the water column (Birgand et al., 2017). Additionally, it can bind to organic and inorganic particles through ion exchange (Bernot and Dodds, 2005), and can be then removed from the water column through sedimentation. Conversely, NH_4-N retained in sediments resulting from organic matter remineralisation can be returned to the water column upon sediment disturbance (Bernot and Dodds, 2005) or diffusion. In this study, the differences in NH_4-N loadings downstream of the access site in relation to upstream values during periods of cattle activity fell within the natural range of variation seen at periods of cattle absence from the stream. Nevertheless, positive differences, i.e. increases in loadings downstream of the site, were more frequent during cattle in-stream activity. Moreover, the largest increases in downstream loadings tended to coincide with times of in-stream urinations. Considering an average urine volume of 1.8 L (Misselbrook et al., 2016) and an average urine TN concentration of 8.2 $g.L^{-1}$ (Shelbie et al., 2015), a single urination episode would deposit in the stream 0.36 g of NH_4-N. Urea, which is the predominant form of nitrogen in cattle urine (Selbie et al., 2015), will also yield free ammonium ions as a result of its decomposition in the aquatic system. However, in the absence of the enzyme urease, urea is a very stable molecule, with a half-life time of about 3.6 years (Ray et al., 2018). Of seven events that captured cattle access to the stream, direct urination episodes occurred in four events. In these events, the highest increases in NH_4-N concentrations downstream of the site relative to upstream concentrations generally coincided with the intervals in which urinations occurred, corresponding to increases in loads ranging from 0.62 g NH_4-N (Event 11) to 1.18 g NH_4-N (Event 2). Additionally, a peak in NH_4-N downstream concentrations coinciding with an episode of urination in the edge of the stream (at minute 91), which may have reached the stream waters, was observed during Event 16, corresponding to an increase of 1.13 g of NH_4-N downstream of the site. Demal (1982), reported increases in free ammonia concentrations in streamwater resulting from cattle in-stream activity in three of nine events sampled, of +0.13 $mg.L^{-1}$, +0.16 $mg.L^{-1}$ and +0.34 $mg.L^{-1,}$ the latter two sampled at the same site. However, episodes of in-stream urination (10) were only registered on one of those events (when there was an increase of +0.13 $mg.L^{-1}$) (Demal, 1982).

6.4.4. Impact of cattle access on SRP and TP in waters

Cattle access to the stream did not cause significant increases in loadings of SRP or TP. Both SRP and TP might be expected to increase during cattle in-stream activity mainly due to in-stream defecation and resuspension of nutrient- and organic matter-enriched sediment. In cattle faeces, phosphorus concentrations can be highly variable depending on type of diet, animal liveweight and reproductive status. Roughly, using the data reported by James et al. (2007) for heifers/dry cows, a single defecation episode would add 1.9 kg of fresh faeces into the stream, containing 1.9 g of TP, of which approximately 60% (Dou et al., 2002), or 1.14 g, would be in the form of readily soluble P. Increases in SRP loadings downstream of the access site during or following times of in-stream defecation were observed in Event 2 (+0.316 g SRP) and Event 7 (+0.083 g of SRP), but not in Event 9. Additionally, the differences in SRP loads observed at times of cattle in-stream activity fell within the range of differences observed at times of cattle absence. Similarly, loadings of TP downstream of the access site showed increases coinciding with times of cattle defecation, of +0.665 g in Event 7 and +2.177 g in Event 9. However, similar changes were also observed at times of no access, and, although the differences in loadings downstream of the site tended to be positive more often at cattle access times than at times of cattle absence, this effect was found to be nonsignificant. Demal (1982) reported only slight or negligible increases in filtered reactive phosphorus during and following cattle in-stream activity, with the exception of one of their study sites, where concentrations increased from 0.048 $mg.L^{-1}$ to 0.134 $mg.L^{-1}$ and 0.039 $mg.L^{-1}$ to 0.079 $mg.L^{-1}$ in two access events. In the same events, TP concentrations increased from 0.071 to 0.804 $mg.L^{-1}$ and 0.077 to 0.791 $mg.L^{-1}$. In-stream defecations were recorded in the first of these events (2 defecations over a 6 minutes period), however events sampled at other sites with a higher number of defecations and a longer period of in-stream activity did not result in increases in SRP or TP concentrations. The author attributed this to the sediment characteristics at the site, which was largely composed by fine sediment, and the low percentage of vegetative coverage, causing the sediment to be more prone to resuspension of particulate P and release of interstitial soluble P (Demal, 1982). Wilson and Everard (2017) also did not find a consistent relationship between cattle in-stream activity and increases in SRP concentrations downstream of the access site, with changes in SRP concentrations falling within the range of the background variation between sampling points. Phosphorus is a highly particle-reactive ion, undergoing reactions of sorption and desorption with particulate material and sediments, and, in its dissolved inorganic forms, it is readily assimilated by plants, algae and microorganisms, (Reddy et al., 1999; Jennings et al., 2003). Such characteristics may hinder the observation of measurable differences in water phosphorus concentrations that might result from cattle in-stream activity.

6.4.5. Impact of cattle access on NO_3-N in waters

Nitrate concentrations did not show a pattern of variation in response to cattle in-stream activity. Similar to this study, Demal (1982) reported negligible changes in nitrate concentrations in all monitored cattle access events. Nitrogen in cattle faeces is predominantly in organic forms, whereas urine nitrogen is predominantly urea-N (Selbie et al., 2015), which may explain the lack of an immediate effect of cattle in-stream activity on water nitrate concentrations. Nevertheless, decomposition of cattle faeces in sediments at the access site can contribute to nitrate water levels when organic nitrogen is remineralised (Birgand et al., 2007).

6.5. Implications

This study revealed that cattle access to watercourses has an immediate clear and significant impact on loads of *E. coli* bacteria and a less obvious but still significant impact on

TSS and NH_4-N loads. Faecal bacteria such *as E. coli* are directly added to the water column during in-stream defecation, but *E. coli* would also become available through resuspension of viable faecal organisms from the stream sediment based on the concentrations described in Chapter 4. This contamination of water resources with potentially pathogenic faecal organisms represents a potential risk for human and animal health, including for *E. coli* O157, which has its main reservoirs in ruminant animals and in cattle in particular (Óhaiseadha et al., 2016). Furthermore, it is well established that faecal bacteria can survive and potentially grow in stream sediments, which have also been considered as genetic reactors where bacteria routinely exchange genetic material, including genes for antibiotic resistance (Jang et al., 2017). Additionally, excess sediment resulting from increased bank erosion and resuspension of bed sediment during cattle in-stream activity can also have important ecological impacts. Excess fine sediment can smother the substrate and clog substrate interstices (Wright and Berrie, 1987), leading to habitat loss and less diverse macroinvertebrate communities (Braccia and Voshell, 2006). Fine sediment can also clog the gills (Relyea et al., 2012) or filter-feeding organs of aquatic organisms (Lemly, 1982). Increased turbidity conditions can also reduce the feeding efficiency of predatory organisms (Jones et al., 2012) and have detrimental impacts on primary producers (Izagirre et al., 2009). Cattle access to watercourses therefore represents a route of systematic contamination of freshwaters with potentially pathogenic organisms and fine sediments, and exclusion of cattle from agriculture streams and riparian zones is desirable.

7. A short study investigating the effectiveness of fencing as mitigation measure

Chapter 7. A short study investigating the effectiveness of fencing as mitigation measure

7.1. Introduction

Streamside fencing to exclude livestock from watercourses has been suggested as an effective method to mitigate the impacts of cattle access on freshwater ecosystems (Line, 2003; Scrimgeour and Kendall, 2003; Miller et al., 2010). One of the main effects of streamside fencing is preventing livestock from directly defecating and urinating within the stream channel (e.g. Miller et al., 2010). An additional result of fencing is reduction of inputs from runoff and erosion from those areas of un-vegetated or sparsely vegetated stream banks caused by livestock trampling and excess grazing (O'Callaghan et al., 2018). Exclusion fencing also promotes the establishment of the vegetation in riparian buffer areas which further aids in reduction of particulate input to streams through filtering overland discharge and retention in vegetation (Liu et al*.,* 2008).

As described in Chapter 3, fencing to exclude livestock from watercourses as a water quality protection measure has been included in most European agri-environment schemes (AES) (Dworak et al., 2009), including in Ireland, where it has been part of the measures of all AES since the implementation of the first scheme in 1994. However, despite this common inclusion in policy, relatively few studies have evaluated the effectiveness of such measures. This is particularly apparent in Europe, and indeed in Ireland (Finn and Ó hUallacháin, 2012).

In a study in USA, Larsen et al. (1994) determined that a 0.61 m riparian fenced buffer had the potential to reduce faecal coliform concentrations entering a stream by 83%, while bacterial loads were reduced by 95% with a 2.31m buffer. In their study in North Carolina (USA), Line et al. (2000) reported reductions in stream water nitrate, total Kjeldahl nitrogen

(TKN) and total phosphorus (TP) concentrations of 33%, 78% and 76%, respectively. In a later study, Line (2003) observed statistically significant reductions in water levels of faecal coliforms and enterococci, of 65.9% and 57% respectively, following streamside fencing. Galeone (2000) also observed reductions in water nitrogen and phosphorus loads following the installation of livestock fencing in Pennsylvania (USA). Similarly, in a study in Vermont (USA), Meals (2001) observed reductions TKN and TP following the installation of measures to mitigate cattle access including fencing, improved stream crossings and bank stabilisation measures in the experimental catchment while observing increases in the control catchment. Additionally, the author detected decreases in *E. coli*, faecal coliform and faecal streptococcus levels in waters after cattle exclusion in the experimental catchment, while reporting an increase in faecal organism counts in the control catchment during the same period. Similarly, although not strictly related to fencing, Vidon et al. (2008) sampled above and below a stream section to which cattle had unrestricted access and observed a 36-fold increase in *E. coli* concentrations in stream waters and suggested that restricting access would result in improvements in water quality. Likewise, in Scotland, Kay et al. (2007) saw between a 66% and a 81% reduction in faecal indicator levels during high flows following remediation measures. While the effects of cattle exclusion on water levels of contaminants have been widely investigated, less attention has been given to the sediment compartment. In one such study, conducted in the Milltown Lake catchment in NE Ireland, Bragina et al. (2017), reported significant lower *E. coli* sediment concentrations in a stream that had been fenced to exclude cattle in comparison to those observed in an unfenced stream with similar characteristics in the same catchment.

Other studies, in contrast, have reported no significant improvement in water quality parameters following the installation of streamside fencing. For instance, Miller et al. (2010) found no difference in water quality variables (TP, TN, DO, temperature) in response to cattle exclusion. Similarly, despite the improvements regarding water faecal contamination, Line (2003) observed no significant changes in upstream to downstream ratio levels of dissolved oxygen, pH, temperature and specific conductivity in cattle exclusion areas. In a study in the Milltown Lake catchment, Veerkamp (2019) reported that the estimated annual export rates of TP and TN were lower in the fenced stream (0.57 kg $TP.ha^{-1}.yr^{-1}$ and 5.99 kg $TN.ha^{-1}.yr^{-1}$) than in the unfenced stream (0.77 kg $TP.ha^{-1}.yr^{-1}$ and 7.24 kg $TN.ha^{-1}.yr^{-1}$). However, the author observed that this fencing was only effective during the grazing season, when the riparian vegetation was denser. Conversely, in the winter and early spring periods, when the riparian vegetation had died back, the TP loads in the fenced stream exceeded those in the unfenced stream (Veerkamp, 2019). Overall, it is apparent that while streamside fencing can have positive effects on freshwater faecal contamination, the effectiveness of such measures reducing livestock impacts on water physicochemical parameters remains unclear.

As discussed previously, despite the lack of empirical evidence as to their cost-effectiveness, provisions for preventing cattle access have been included in many European agri-environment schemes, as well as in every Irish agri-environment scheme, to date (i.e. REPS, AEOS, GLAS). It has been acknowledged that in the absence of empirical evidence on the actual impact of cattle access and on the effectiveness of cattle exclusion, it is difficult to justify full riparian fencing of watercourses as a cost-effective approach to maintain or enhance freshwater ecosystems (Terry et al. 2014). Thus the aims of this short study were to:

a. Assess changes in sediment physicochemical and microbial parameters in the bed sediment compartment at cattle access sites after ~ one year of fencing (short-term fencing), in a *before-after* comparison study;
b. Assess the impact of longer term (nine years) cattle exclusion fencing on water physicochemical and microbial parameters (i.e. SRP, NH_4-N, NO_3-N, *E. coli*) using data from the Milltown Lake catchment in a *paired treatment-control* study. In this study, two similar streams (Table 7.3), one of which was fenced to exclude cattle in 2008, were sampled at monthly intervals during

approximately one year. A second aim of this study was to gather complementary data for a study on the effects of excluding cattle on macroinvertebrate communities under the COSAINT project.

7.2. Site description and methods

7.2.1. Site description

7.2.1.1. Before-after study on the impacts of fencing on short-term

Six sites were assessed where cattle exclusion fencing had been installed by the famer or installed by the project team. This approach facilitated *before and after* analysis in these sites for sediment nutrient and sediment *E. coli* levels. A larger number of sites had been initially selected for this *before*-after comparison study, however due to non-compliance with fencing by a number of farmers at the time of re-sampling, study site selection had to be re-evaluated during the project. This resulted in a limited number of sites suitable for the study as well as microbiological data not available for one of the six sites.

The six cattle access points were located in five catchments (Fig. 7.1 and 7.2) in the east and south of Ireland and were sampled for bed sediment prior to, and one year following exclusion of cattle from streams via fencing, in October of 2016 and October of 2017, respectively. Four of the six sites are described in Chapter 3 and were part of the studies described in Chapters 4 and 5; these were sites MT1, CM1, BWA and BWC. Two extra sites, SN1 and SN2, were included in this study, which were located along the moderate status, second order Blacklion stream in Co. Carlow (Tables 7.1 and 7.2). The area is poorly drained and low lying lands are prone to flooding in the winter. The main soil group found is

Peaty Gley, and the geology primarily consists of limestone with some sandstone and granite. The streams are tributaries of the River Slaney. Fencing of the access sites (two strand barbed-wire fencing for cattle exclusion, typically 1.5 m from the bank edge as per GLAS regulations) allowed for some visible recovery of the cattle access sites (reduction in visible fine sediment, some vegetation growth).

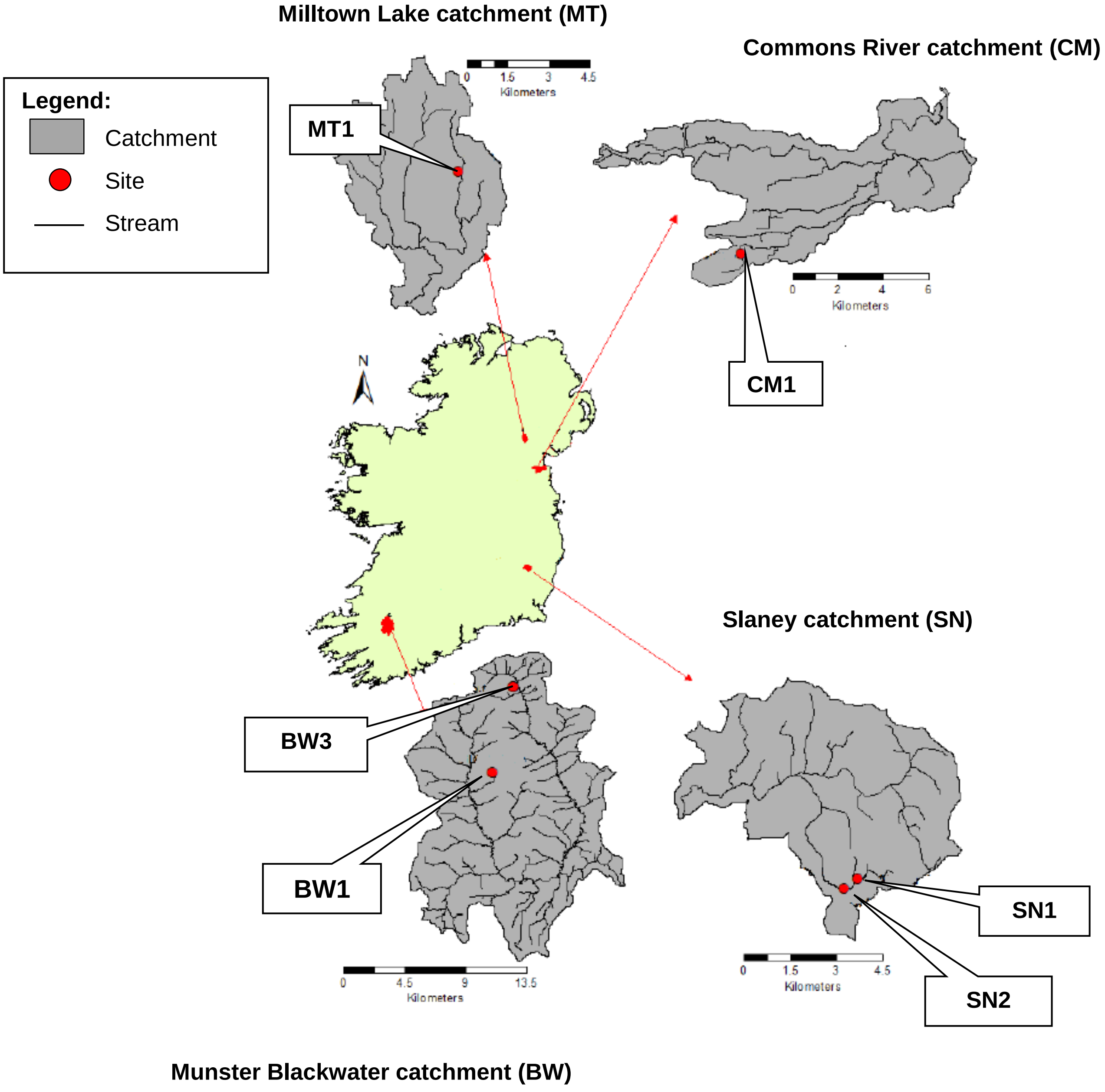

Fig.7.1. Map of the study sites in the *before-after* study of fencing effectiveness as a water quality protection measure.

Table 7.1. Study sites in the Slaney catchment (not described previously in Chapter 3). *Information derived predominantly from Edenireland.ie; information at time of sampling (2016/2017).

Catchment	Slaney
River	Blacklion Stream
County	Carlow
Total annual precipitation (mm)[1]	591.6 (2016) 616 (2017)
Ecological status (Sampling period)	Moderate
Biological status(Sampling period)	NA
Chemistry conditions(Sampling period)	Pass
Recent chemistry trend(Sampling period)	Pass
Nutrient Condition	Pass
Ortho-P status quality (trend)	High (upwards)
WFD Risk	At Risk
Waterbody trend	No change
Significant Pressure	Agriculture

[1]Precipitation data derived from Tullow (Ardoyne Glebe) Met Eireann station.

Table 7.2. Summary characteristics of the study sites in the Slaney catchment.

	SN1	SN2
Stream order	2	2
Stream width (m)	1.72	1.71
Reach gradient (%)	-4.70%	-3.10%
Soil type	Poorly drained	
Geologic formations	Granite	
Site description	Open access site, vegetated banks, steep banks	Open access site, vegetated banks

7.2.1.2. Paired treatment-control study on the effects of fencing on long-term

The longer-term efficacy of fencing as a means of improving some water quality parameters was also tested in the Milltown Lake catchment of Co. Monaghan. Here, one tributary in the catchment had been completely fenced in 2008, as part of a previous project that ran from 2005 - 2010 (the National Source Protection Pilot Project, NSPPP: Linnane et al. 2011) (see Fig. 7.2 and Tables 7.3 and 7.4). This western tributary, which is located in a sub-catchment fed from the Carnagh Lake in Tievnamara (TV), had fencing installed at a distance of 1.5 m from the stream edge at that time, excluding livestock from the water as well as allowing the re-establishment of streamside vegetation, thus providing a buffer strip. The other two tributaries in the Milltown Lake catchment, Gentle Owen (GO) (middle), and Tullycaghney (TH) (eastern) still had cattle access to the stream water. A survey conducted in 2010 by Veerkamp (2019) identified one unfenced cattle access site in the TV tributary, which was relatively small in comparison to the remaining sites, serving as a crossing point. In the TH tributary (unfenced), 18 cattle access sites were identified. In total, 12 cattle access sites had been sampled in October 2008, six in the TV tributary and six in the TH tributary, prior to fencing, for a limited set of water column parameters (TRP, NH_4-N and *E. coli*). These sites were re-sampled in the current study at approximately monthly frequency from December 2017 to November 2018, approximately nine years post fencing Data on discharge of the two tributaries were collected from the EPA Drumleek station approximately 500 m upstream (54°8'48"N; 6°42'15"W) from the inflow to Milltown Lake. Continuous stream discharge data (15 minute intervals) are available at this site from the EPA Hydronet website (hydronet.epa.ie).

Table 7.3. Characteristics of the streams used in the paired control-treatment study, in the Milltown Lake catchment (from Veerkamp, 2019).

Characteristics	Catchment	Fenced tributary (TV)	Unfenced tributary (TH)
Total area (km^2) excluding Milltown Lake	28.8	9.6	10.1
Stream length (km)	24.6	8.4	6.3
Dwellings per tributary area (2010)	748	232	247
Cattle per tributary area (2010)	5554	1722	1833

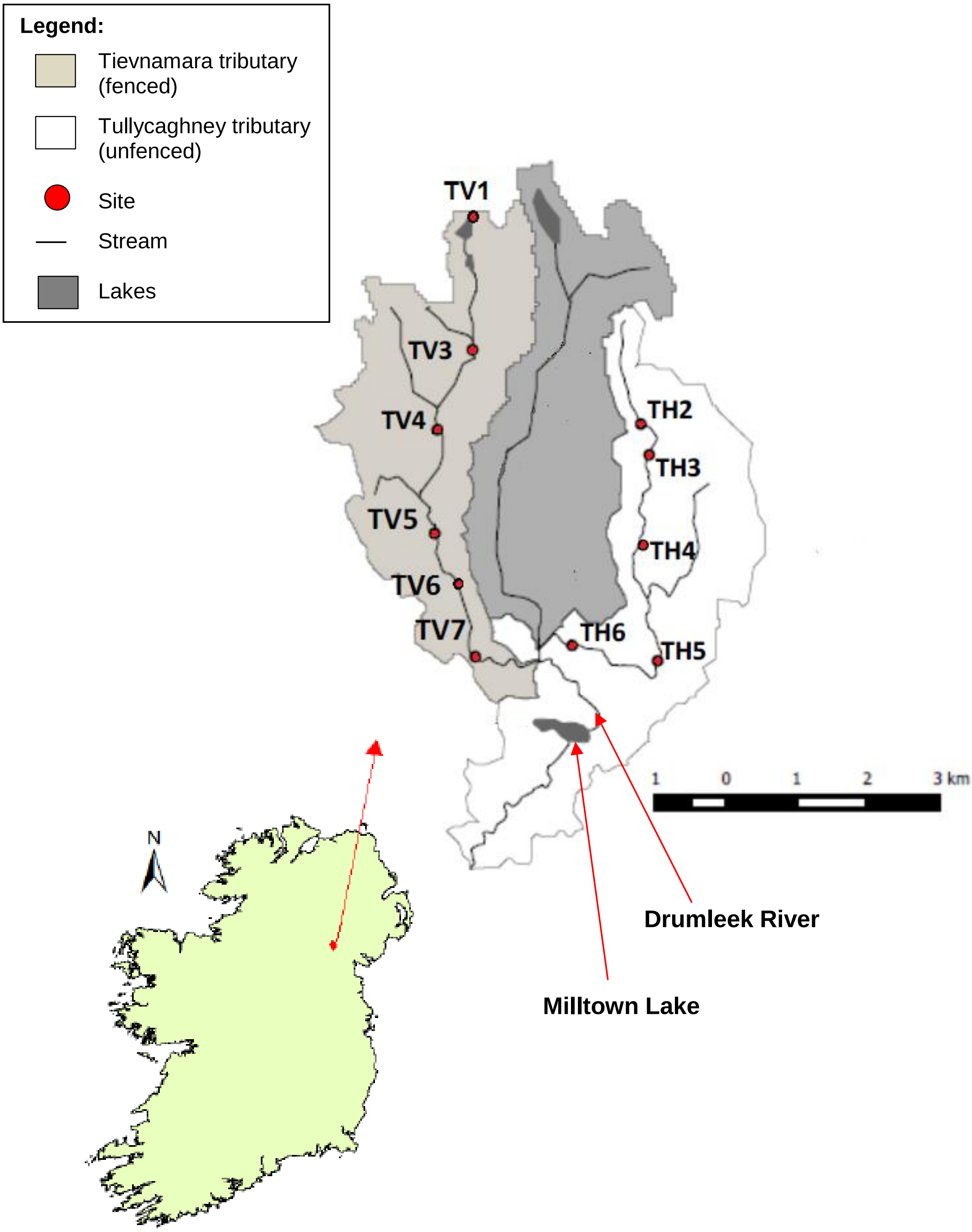

Fig.7.2. Map of the study sites in the paired *treatment* - *control* study of fencing effectiveness as a water quality protection measure.

Table 7.4. Description of the sites sampled in the fenced (TV) and unfenced (TH) tributaries in the Milltown Lake catchment (from Bragina, 2017).

Name	Site description	Location	Stream order	Width (cm)		
				Mean	*Max*	*Min*
TV1	No cattle access	54.199751 -6.728785	1	NA	NA	NA
TV3	Cattle access site on fenced stream	54.186766, -6.735303	1	152	180	110
TV4	No cattle access	54.179181, -6.737842	2	226	305	200
TV5	No cattle access	54.172003, -6.738826	2	258	300	165
TV6	No cattle access	54.162057, -6.733004	2	NA	NA	NA
TV7	No cattle access	54.155433, -6.731612	2	251	285	200
TH1	Cattle access site	54.197612, -6.696669	1	NA	NA	NA
TH2	No cattle access	54.187583, -6.692207	1	167	285	62
TH3	Access site, steep field	54.183814, -6.692985	1	228	300	180
TH4	Access site	54.168668, -6.695717	1	189	240	97
TH5	Access site	54.157916, -6.690028	2	278	390	130
TH6	Access site	54.157000, -6.714875	2	221	253	140

7.2.2. <u>Sample collection</u>

7.2.2.1. Before-after study on the impacts of fencing on short-term: sediment nutrient and E. coli sampling

Sediment sampling and analysis techniques for nutrient and *E.* coli concentrations were similar to those described in Chapters 4 and 5. At each site, however, the stream bed sediment was sampled at three locations (location DS was not considered in this study): at the cattle access site, i.e. where cattle actively used the stream (CAS); upstream (20 – 322 m) of the access site, where animals had no access to the stream either due to fencing or natural physical barriers (US); and at the interface (edge) of the stream water level, at the access path used by cattle to enter the stream (INT). For microbiological analysis, three sediment samples were collected randomly at the cattle access sites (CAS) and three were collected upstream (US) (10 – 300 m) of these sites (i.e. total of six per site).

7.2.2.2. Paired treatment-control study on the effects of fencing on long-term

One grab sample was taken at six sampling sites on the TV tributary and six sites on the TV tributary. Note that these sites were previously used for water sampling in the NSPPP (Linnane et al, 2010). Sample were analysed for SRP, NH_4-N and NO_3-N.

The samples were collected using a polyethylene 0.5 L beaker previously disinfected with 70% industrial methylated spirit (IMS), acid-washed with a 70% HCl solution and rinsed several times with ultrapure Milli-Q water. Water samples for nutrient analysis were transferred into polyethylene bottles previously acid-washed in the same manner, whereas water samples for microbiological analysis were transferred to previously autoclaved 100 ml Duran bottles. The sampling beaker was rinsed with IMS, acid-wash solution, ultrapure Milli-Q water and finally three times with stream water in between samples. Samples were placed in cool boxes and transported to the laboratory where they were kept in the dark at 4˚C until analysis.

7.2.3. Laboratory analysis

7.2.3.1. Before-after study on the impacts of fencing on short-term

Sediment samples were processed and analysed for *E. coli* and nutrient concentrations as described in Chapters 4 and 5, respectively.

7.2.3.2. Paired treatment-control study on the effects of fencing on long-term

The methods for analysis of water nutrient and *E. coli* concentrations were similar to those described in Chapter 6. Additionally, microbiological samples were also analysed using the Colilert Quanti-tray method (IDEXX Laboratories, 2012) to replicate the data collected for the NSPP project.

7.2.4. Data analysis

All *E. coli* data were log transformed before statistical analysis. Significant differences were assessed between the US and CAS locations (and between the US and INT locations) using a paired t-test in R (R Core Team, 2018). A Shapiro-Wilk test was used to test for the assumption of normality. Where this assumption was breached, a paired Wilcoxon text was used as an alternative to the paired t-test.

7.3. Results

7.3.1. Before-after study on the impacts of fencing on short-term

There were no significant differences in sediment OC, TN or TP concentrations, or in OC:TN ratios, between the upstream sites (US) and the cattle access site (CAS) before fencing across all six sites (Fig. 7.3 and Table 7.5). However, prior to fencing, sediment concentrations for OC, TN and TP were significantly higher at the stream interface (INT) sites, where cattle would have been most likely to congregate, when compared to the US locations for all six sites (Table 7.6 and Fig. 7.4). These increases in concentration from US to INT locations were generally consistent across all six sites. In contrast, post-fencing, there was no significant difference between the US and INT sites for any of the three sediment nutrients assessed (Fig. 7.3, Table 7.6) It should be noted that no fine sediment (<2mm) was found at the INT site for one site (MT1) post-fencing.

Sediment *E. coli* levels did show an increase between the US and CAS locations at three of the five study sites in the Autumn of 2016 (prior to fencing) ($n = 3$), but the differences were not significant when all five sites were considered (Fig.7.5). This site-specific difference was most pronounced for the Milltown MT1 site, where the mean *E. coli* concentration increased from 2.1×10^2 CFU g.dry wt^{-1} to 2.0×10^5 CFU g.dry wt.$^{-1}$ ($t = -22.1$, $p < 0.001$). At BWA, *E.coli* was not detected at the US location but had a mean value of 6.9×10^2 CFU g.dry wt.$^{-1}$ at the CAS location. Concentrations at the US and CAS locations for the MT1 site were also not significantly different from each other post-fencing, with mean values of 6.2×10^2 CFU g.dry wt^1 and 5.3×10^2 CFU g.dry wt.$^{-1}$ respectively.

Table 7.5. Bed sediment concentrations of nutrients (mean ± S.E., n = 6) and *E. coli* (mean ± S.E., n = 5) at the study sites between upstream (US) and cattle access site (CAS): pre-fencing (2016) and post- fencing (2017).

	Pre-fencing		Post-fencing	
Parameter	**US**	**CAS**	**US**	**CAS**
OC (mg.g dry wt^{-1})	11.997 ± 6.177	8.794 ± 2.571	12.180 ± 4.440	10.466 ± 3.719
TN (mg.g dry wt^{-1})	1.023 ± 0.392	0.796 ± 0.198	1.358 ± 0.537	1.021 ± 0.289
OC:TN	10.0 ± 1.4	10.8 ± 0.7	9.3 ± 1.0	9.4 ± 1.0
TP (mg.g dry wt^{-1})	275 ± 80	412 ± 115	351 ± 65	375 ± 105
E coli (CFU.g dry wt^{-1})	$2.0 \times 10^3 \pm 1.4 \times 10^3$	$4.1 \times 10^4 \pm 2.3 \times 10^4$	$9.9 \times 10^2 \pm 4.4 \times 10^2$	$1.4 \times 10^3 \pm 6.5 \times 10^2$

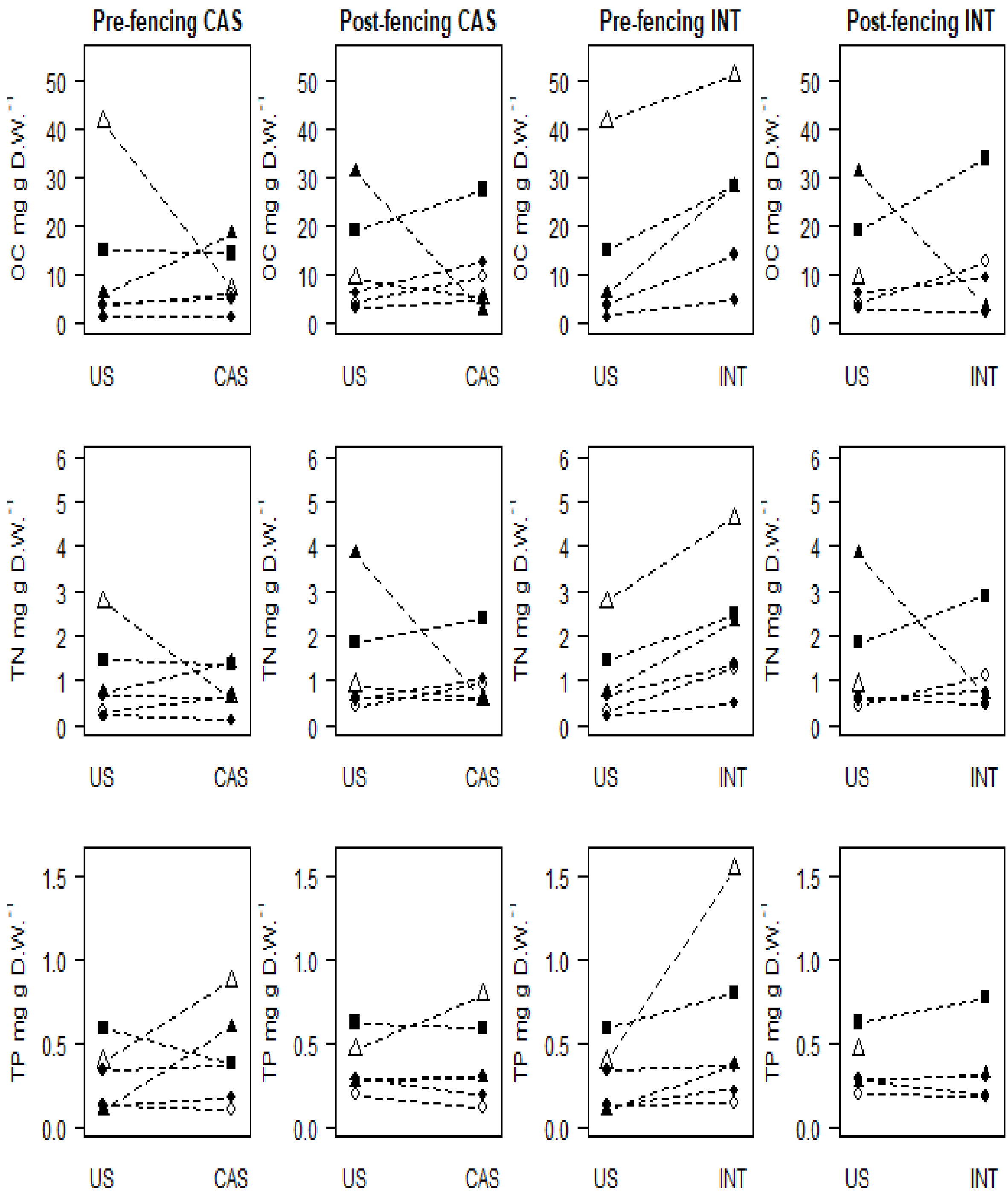

Fig.7.3. Change in the mean sediment organic carbon (OC, row 1), total nitrogen (TN, row 2), and total phosphorus (TP, row 3) concentration at the upstream (US) control sites and at the cattle access site (CAS) and from the US sites versus the interface (INT) for the six sites (columns 1 and 3 = pre-fencing (2016) and columns 2 and 4 = post-fencing (2017).

Table 7.6. Bed sediment (mean ± S.E., n=6) data for the study sites at the upstream control (US) and the interface (INT) at the cattle access site: pre-fencing (2017) and post- fencing (2018); data in bold were significantly different US versus INT. *significant at the 0.05 level; **significant at the 0.001 level.

	Pre-fencing		**Post-fencing**	
Parameter	**US**	**INT**	**US**	**INT**
OC (mg.g dry wt^{-1})	**11.997 ± 6.177**	**23.416 ± 6.637****	12.180 ± 4.440	12.410 ± 5.088
TN (mg.g dry wt^{-1})	**1.023 ± 0.392**	**2.086 ± 0.589****	1.358± 0.537	1.181 ± 0.440
OC:TN	10.0 ± 1.4	11.0 ± 0.3	9.3 ± 1.0	9.2 ± 1.6
TP (mg.g dry wt^{-1})	**275 ± 80**	**572 ± 215***	351 ± 65	349 ± 110

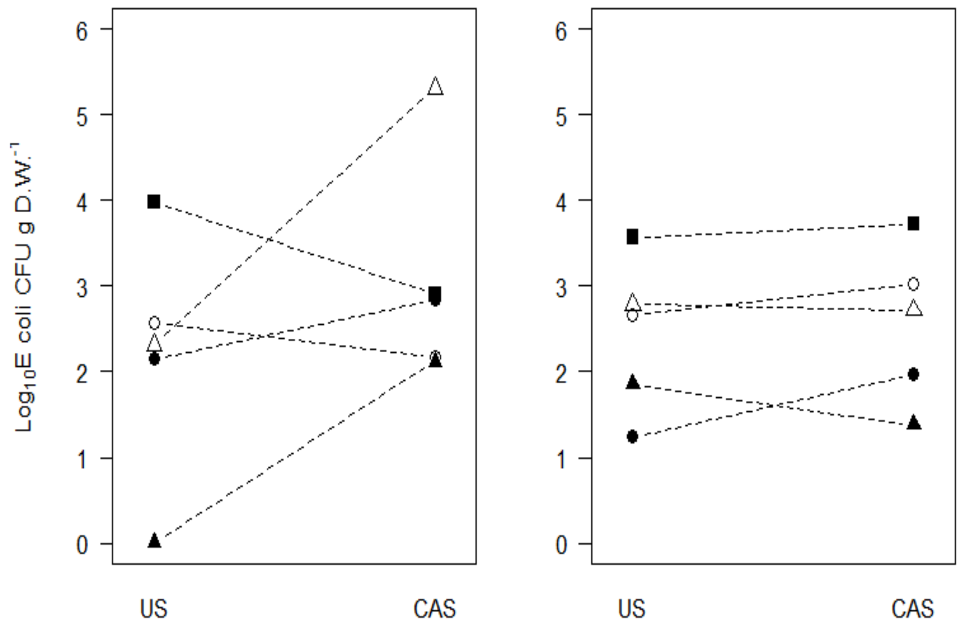

Fig.7.4. Change in the mean sediment *E. coli* concentration at the upstream (US) control sites and at the cattle access site (CAS) for the five sites (left = pre-fencing (autumn 2016) and right = post-fencing (autumn 2017).

7.3.2. Paired *treatment-control* study on the effects of fencing on long-term

Stream water concentrations of nutrients and *E. coli* in the fenced and unfenced tributaries of the Drumleek River are presented in Table 7.7. For the stream water data collected over one annual cycle in the Milltown Lake catchment, there was no significant difference between the values for the TV tributary (fenced) and those for TH tributary (unfenced) for the concentrations of SRP, TP, NO_3-N and NH_4-N when all data were included, or when only data for months when cattle would be in the field (Apr - October) were included (Fig. 7.5, Fig. 7.6). Notably, nutrient concentrations in both tributaries followed a very similar pattern, and SRP, TP and NH_4-N concentrations reached a peak in June 2018 (Fig 7.5). For water column *E. coli* concentrations which were measured (using the membrane filtration technique) four times over the annual cycle (December 2017, January/May/November 2018), there was again no significant difference between the fenced and unfenced tributaries

(Fig. 7.7). Similarly there was no significant difference for stream water *E. coli* concentrations measured using the Colilert method (Fig. 7.7).

Table 7.7. Stream water nutrients and *E. coli* concentrations in the fenced and unfenced streams in the Milltown Lake catchment (mean ± S.E.). Mean monthly discharge is calculated from daily mean discharge available from the EPA monitoring station in the Drumleek river.

Stream	Date	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NH_4-N ($mg.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	*E.coli* (CFU.100 ml^{-1})	*E. coli* (MPN.100 ml^{-1})	Mean monthly discharge ($m^3.s^{-1}$)
Fenced	16/11/2017	-	-	-	-	$2.5 \times 10^2 \pm 52$	-	0.802
	04/12/2017	47 ± 5	77 ± 10	0.07 ± 0.02	0.59 ± 0.03	$1.4 \times 10^2 \pm 28$	149.2 ± 34.0	1.136
	24/01/2018	73 ± 4	158 ± 13	0.06 ± 0.02	0.47 ± 0.07	$1.3 \times 10^3 \pm 2.2 \times 10^2$	1238.8 ± 267.1	2.359
	16/03/2018	28 ± 2	87 ± 7	0.10 ± 0.02	0.71 ± 0.05	-	-	0.952
	20/04/2018	39 ± 3	96 ± 3	-	-	-	-	0.701
	10/05/2018	44 ± 4	115 ± 16	0.09 ± 0.03	0.47 ± 0.06	$3.7 \times 10^2 \pm 82$	351.6 ± 135.2	0.219
	28/06/2018	107 ± 20	211 ± 27	0.32 ± 0.15	0.76 ± 0.15	-	-	0.064
	27/07/2018	21 ± 7	48 ± 10	0.07 ± 0.03	0.66 ± 0.13	-	-	0.058
	30/08/2018	21 ± 1	38 ± 2	0.06 ± 0.03	0.45 ± 0.08	-	-	0.144
	10/10/2018	48 ± 8	104 ± 13	0.13 ± 0.06	0.41 ± 0.07	-	-	0.144
	20/11/2018	76 ± 2		0.07 ± 0.02	1.55 ± 0.08	$2.2 \times 10^2 \pm 69$	216.1 ± 75.9	1.283
Unfenced	16/11/2017	-	-	-	-	$2.0 \times 10^2 \pm 1.0 \times 10^2$	-	0.802
	04/12/2017	30 ± 4	50 ± 7	0.03 ± 0.01	0.44 ± 0.08	$1.9 \times 10^2 \pm 1.1 \times 10^2$	236.2 ± 122.1	1.136
	24/01/2018	40 ± 3	110 ± 5	0.04 ± 0.01	0.63 ± 0.10	$5.2 \times 10^2 \pm 68$	552.2 ± 149.2	2.359
	16/03/2018	25 ± 3	66 ± 9	0.14 ± 0.05	0.68 ± 0.13	-	-	0.952
	20/04/2018	26 ± 2	58 ± 4	-	-	-	-	0.701
	10/05/2018	25 ± 4	80 ± 25	0.04 ± 0.01	0.28 ± 0.09	$7.1 \times 10^2 \pm 4.9 \times 10^2$	701.7 ± 386.1	0.219
	28/06/2018	73 ± 10	217 ± 32	0.29 ± 0.13	0.86 ± 0.26	-	-	0.064
	27/07/2018	13 ± 3	57 ± 13	0.05 ± 0.02	0.63 ± 0.25	-	-	0.058
	30/08/2018	20 ± 2	40 ± 10	0.02 ± 0.004	0.45 ± 0.09	-	-	0.144
	10/10/2018	36 ± 8	161 ± 84	0.07 ± 0.05	0.26 ± 0.07	-	-	0.144
	20/11/2018	66 ± 3		0.03 ± 0.003	1.50 ± 0.31	$1.5 \times 10^2 \pm 41$	141.5 ± 29.8	1.283

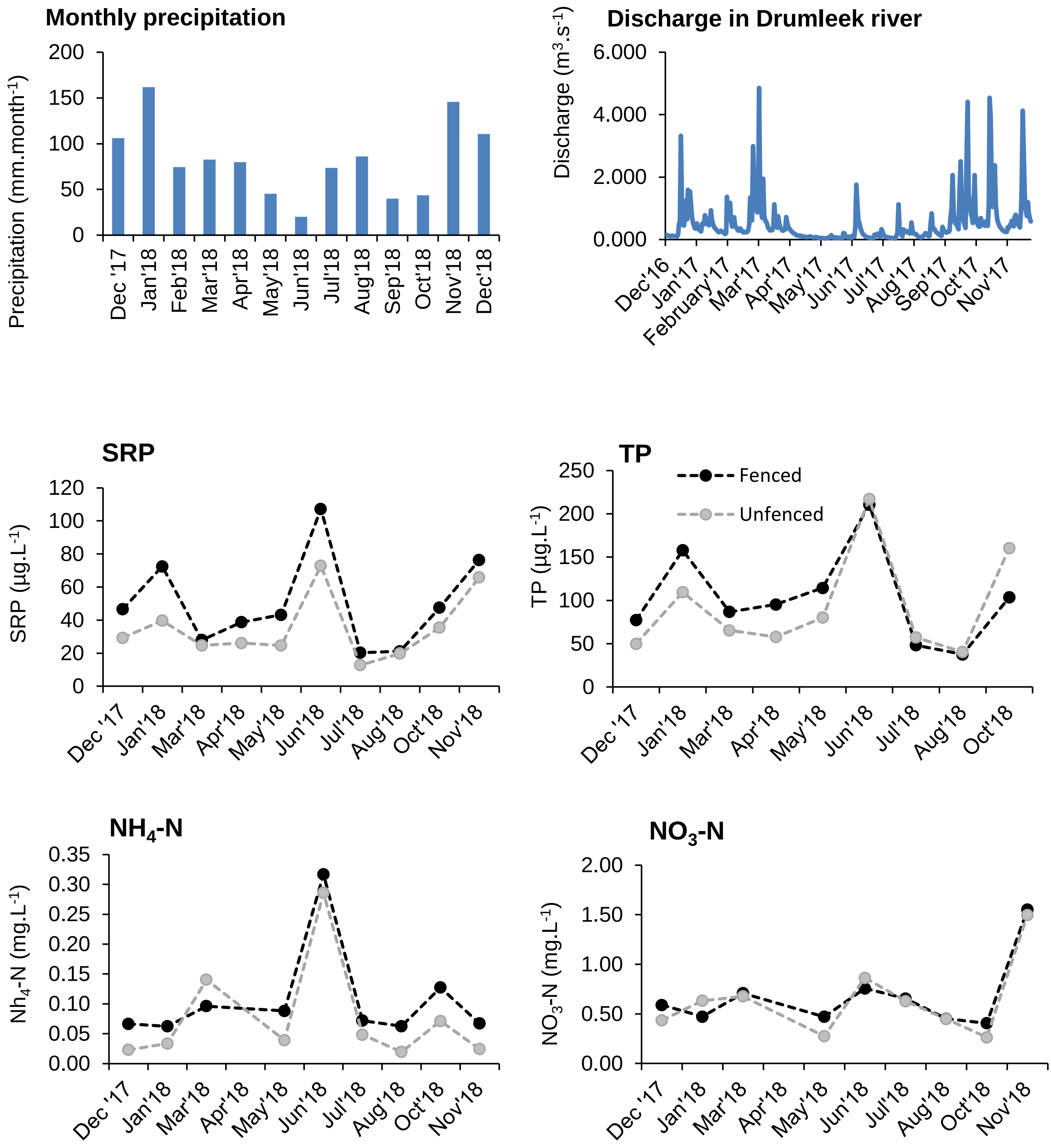

Fig.7.5. Monthly total precipitation (mm) in the Milltown Lake catchment from December 2017 to December 2018 (data from the Met Eireann station Coose – Castleblayney), mean daily discharge ($m^3.s^{-1}$) in the Drumleek River (data from the nearest EPA monitoring station) and nutrient concentration variation in the TV tributary (fenced) (black) and TH tributary (unfenced) (grey).

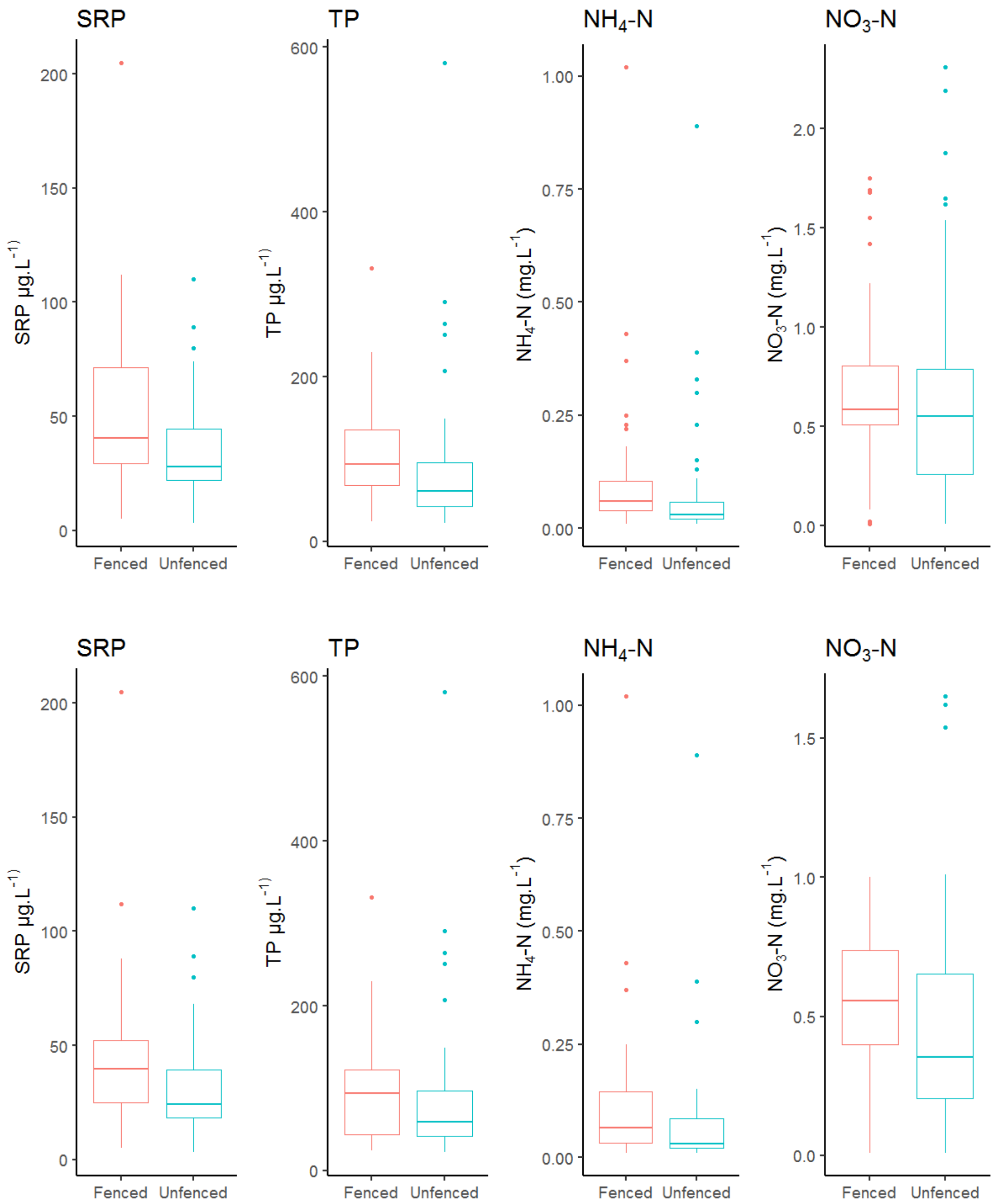

Fig.7.6. Stream water nutrient concentrations in the fenced and unfenced streams. Top row: (December 2017 to November 2018, no sample in February and September 2018) (SRP: n = 60; NH_4-N: n = 48; TP and NO_3-N: n = 47). Bottom row: during the period of cattle grazing outdoors (April to October 2018, no sample in February 2018) (SRP: n = 36; TP: n = 35; NH_4-N and NO_3-N: n = 24).

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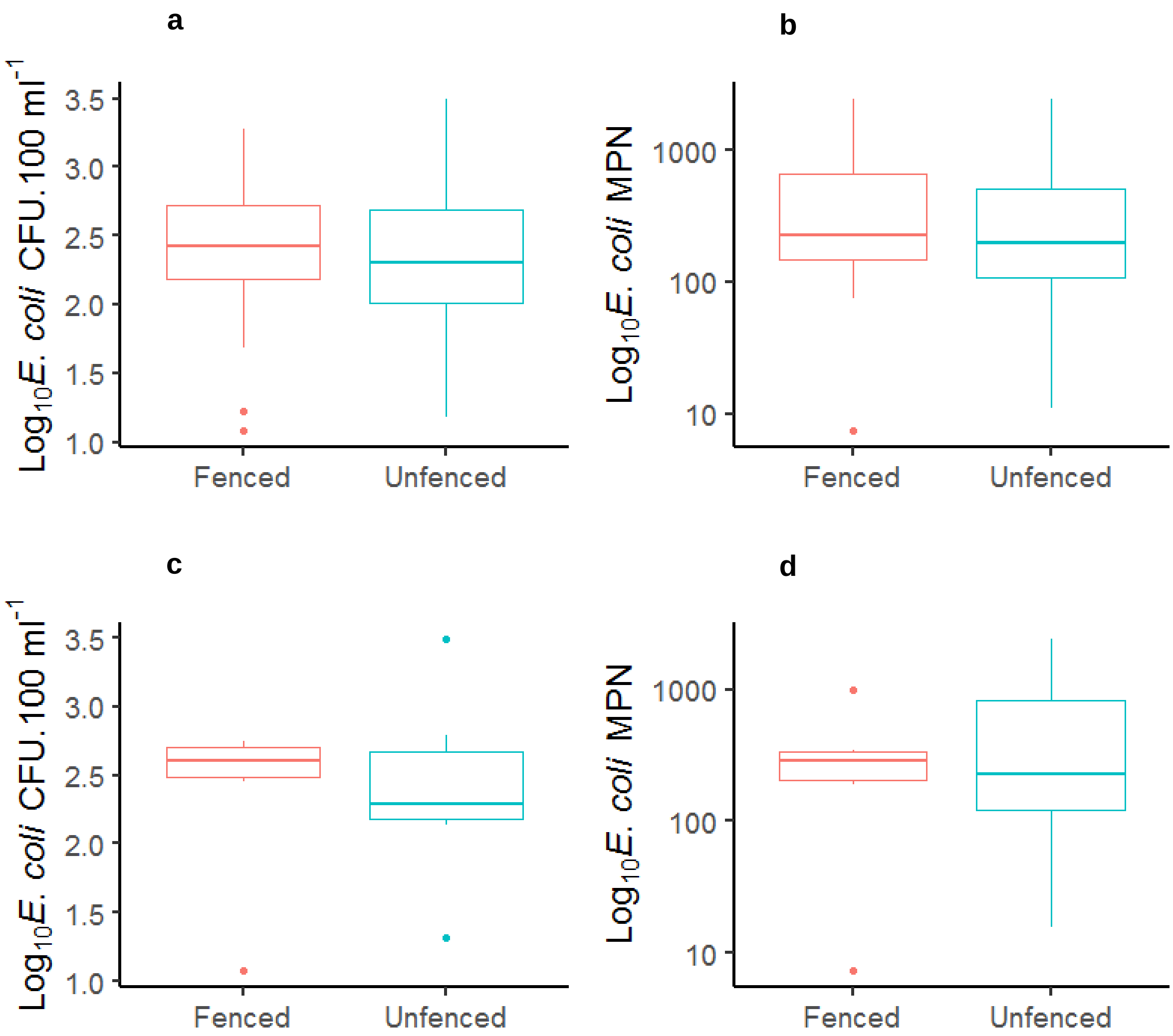

Fig.7.7. Stream water *E coli* concentration the fenced and unfenced streams. a) and c) data obtained using the membrane filtration technique (log_{10} CFU.100 ml^{-1}); b) and d) data obtained using the Colilert method (Most Probable Number (MPN).100 $ml^{-1-)}$; a) and b) data collected in November and December 2017, January, May and November 2018 (MF: n = 59; Colilert method: n = 48, no data for November 2017); c) and d) data for May 2018 when cattle were grazing outdoors (n = 12).

7.4. Discussion

While uncontrolled livestock access to watercourses can negatively impact the aquatic system and the riparian area, it has been suggested that such effects are localised and site-specific (Wilcock et al., 1999). Streamside fencing to exclude livestock from watercourses has been adopted as a water quality protection measure, or best management practice (BMP), in many regions globally; however the body of literature assessing its effectiveness is limited and studies have been predominantly undertaken in the USA and New Zealand (O'Callaghan et al., 2018). Furthermore, while many studies have focused on the effects of cattle exclusion fencing on stream water quality (O'Callaghan et al., 2018), few to date have assessed either the impacts of cattle access on nutrient and microbial contamination of stream bed sediment or assessed the effects on excluding cattle on these sediment characteristics. This short study aimed at providing a screening assessment of the effectiveness of fencing in the Irish context.

As seen in Chapter 5, prior to fencing bed sediment nutrient concentrations at edge of the stream, where cattle would congregate to drink (i.e. interface sites) were significantly higher than those for the bed sediments upstream of these sites. Interestingly, the sediments in the main channel (i.e. CAS) did not have significantly higher nutrient concentrations either in Chapter 5 or in this short study, suggesting an ongoing flushing of material downstream (Eder et al., 2014; Terry et al., 2014). These differences, however, were not apparent for the same comparison undertaken approximately one year post-fencing. While other factors may play a role in year to year variability, this likely reflects the effects of cattle absence from the stream during the grazing season of 2017.

The mechanisms by which these sediments at access sites can become nutrient-enriched include both the incorporation of additional organic matter generally from cattle faeces (e.g. Davies-Colley et al., 2004), and the adsorption of phosphate to ion exchange sites of fine particles (McDowell and Sharpley, 2001). While the number of sites and the assessment time in the current study was limited, the results indicated that excluding cattle reduced the bed sediment nutrient concentrations in these access point stream margins. It is also possible, however, that in a longer study, increased vegetation in the stream margins of the fenced cattle access points could also act as a trap for fine sediment thus potentially increasing concentrations in these areas (Reddy et al., 1999; Sand-Jensen, 1998; Walling and Collins, 2016).

Regarding *E. coli* contamination*,* bed sediments were sampled in the main channel only. While the overall change across the six sites was not significant, there were substantially higher sediment *E. coli* concentrations at the cattle access site than upstream before fencing at individual sites, in particular the MT1 site (2.0×10^5 and 2.1×10^2 CFU.g dry wt^{-1}, respectively). Again this difference was not apparent after one year of fencing, indicating that cattle exclusion likely had a positive impact on the levels of contamination of the bed sediment at the sites. This effect was similar to that reported for a larger two year study in the Milltown Lake catchment by Bragina et al*.* (2017) who observed similar concentrations and found significantly higher levels of *E. coli* in the bed sediments of the fenced TV tributary compared to the unfenced TH and GO tributaries. While high levels of *E. coli* in bed sediment can also persist over time, Jamieson et al. (2005b) pointed out that *E. coli* in bed sediments will be flushed and transported downstream. Bragina et al. (2017) also reported a decrease in bed sediment concentrations between October and April, which they suggested was due to flushing over the winter. Nevertheless, it is worth noting that the levels of *E coli* contamination even post-fencing and in the unfenced sites in the study by Bragina et al. (2017) were much higher (>10^2 CFU g.dry wt)' than those at a non-agricultural upland site that was also assessed in the latter study, suggesting on-going contamination of the stream bed from other sources of animal and/or human wastes.

While the stream water concentrations differed between sites and between times as would be expected for dynamic stream systems in agricultural catchments (e.g. Mellander et al., 2015), there were no significant differences in the stream water concentrations of nutrients between the upstream reaches at the six fenced sites. There was also a high level of synchrony between the temporal patterns in the two sites. However, there was no significant difference between the nutrient concentrations for the fenced and unfenced streams over the annual cycle or over the months when cattle would have been in the field (April to October) for the two tributaries in the Milltown Lake catchment, which indicates a predominant role of diffuse pollution from the catchment area in determining the nutrient water levels. The impacts of intensification of agriculture in the Milltown Lake catchment since the 1950s and associated increased livestock numbers and application of fertilisers and slurries have been highlighted as the main drivers of water quality deterioration in paleolimnological studies by Carson et al. (2015) and Chique et al. (2018).

This short study does suggest, however, that streamside fencing can be beneficial in reducing sediment reservoirs of nutrients. Fencing eliminates nutrient contributions to watercourses resulting from cattle excretion within the stream channel, and would also promotes streambank recovery and vegetation establishment, thereby reducing bank erosion and the wash-out of particulate material into stream waters. In other words, cattle access sites in agricultural streams can be viewed as critical source areas of contamination (due to their potentially high reservoirs of contaminant that are susceptible to erosion/resuspension and transport downstream) (Thompson et al., 2013) which are reduced following cattle exclusion through fencing implementation. Reducing sediment nutrient reservoirs at these sites can reduce the potential for sediments to act as sources of contaminants to the water column, therefore mitigating potential legacy issues. In this light, although fencing may offer limited effectiveness in controlling diffuse pollution, particularly in winter periods when, in Ireland and other western European locations, rainfall is typically higher and vegetation in riparian buffer strips dies back, its implementation combined with other diffuse pollution measures can contribute to the success of such measures.

In other studies undertaken in the same sites under the COSAINT project, short-time streamside fencing was also demonstrated to reduce sediment deposits downstream of the access sites and site specific changes in the macroinvertebrate communities, potentially resulting from reduced impacts of excess fine sediment at the sites (Ó hUallacháin et al., 2020). Additionally, Ó hUallacháin et al. (2020) reported that long-term fencing in the Milltown Lake catchment had positive impacts on freshwater ecology with improvements in the abundances of Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa observed in the fenced tributary in comparison to the unfenced tributary.

7.5. Conclusions

This short study has highlighted the potential benefits of implementing cattle exclusion measures in reducing nutrient and *E. coli* concentrations in sediment reservoirs. Fencing can help mitigating future legacy effects through a reduction of the reservoirs of nutrients and faecal bacteria within the stream channel. However, this study also highlights the need to implement cattle exclusion measures and diffuse pollution mitigation measures in a concerted matter to successfully reduce agricultural pressures in aquatic systems. Notwithstanding the limitations of fencing as water quality protection measure, its reported benefits on freshwater nutrient and faecal contamination, along with the positive impacts it can have on freshwater ecology (e.g., Ó hUallacháin et al., 2020), favour its implementation. Thus, albeit limited, this short study provides relevant information to stakeholders, policy makers, and the local community suggesting fencing can be implemented as part of a concerted effort to manage nutrient and faecal contamination at the catchment scale.

8. Final Discussion

Chapter 8. Final Discussion

Freshwater systems constitute a vital natural resource, providing a wide range of ecosystem services. (Dudgeon et al., 2006: Pham et al., 2019). Moreover, they support approximately 6% of all plant and animal species described so far (Dudgeon et al., 2006). Yet, they represent one of the most endangered systems in the world, with declines in biodiversity surpassing those observed in most impacted terrestrial systems (Dudgeon et al., 2006; Schmidt-Kloiber et al., 2019). Freshwater systems have been insidiously impacted by urbanisation, damning and water abstraction, agriculture intensification, mining and industry activities, introduction of invasive species and climate change (Malmqvist and Rundle, 2002). As noted earlier, in the last decades several bodies of legislation have been introduced to protect freshwater systems, including the Clean Water Act in the US and several policies in the EU, in particular the over-arching Water Framework Directive, which aligns with the UN Sustainable Development Goals (Rouillard et al., 2017).

Agriculture has been identified as a major pressure on freshwater systems worldwide (Vorosmarty et al., 2010). Much of this pollution, which includes excess nitrogen and phosphorus from fertilisers, excess sediment loadings and microbial contaminants, reaches inland waters through diffuse pathways of contamination (Heathwaite, 2010; Muirhead and Monaghan, 2012; Deakin et al, 2016). Such pathways have been extensively studied and are well documented in literature (e.g. McDowell et al., 2006; McDowell and Wilcock, 2007; Mellander et al., 2012; Bowes et al., 2015; Lloyd et al., 2019) . Less consideration has been given, however, to agricultural point sources of pollution, for example the role of unrestricted access of livestock to watercourses and the potential contribution of contaminants to the aquatic system.

There has been a general paucity of research investigating the potential of cattle access and instream activity to contribute to water quality deterioration. This is particularly true in the European context, as most studies on this topic to date have been conducted in the US and Australasia, with a smaller number of contributions from the UK (e.g. Collins et al., 2010; Bond et al., 2012, 2014) and Ireland (e.g. Conroy et al., 2016; Bragina et al., 2017; Madden et al., 2019; O'Sullivan et al., 2019). The recent review by O'Callaghan et al. (2018) highlighted the uncertainty around the impact of cattle access to watercourses and the efficacy of fencing as water quality protection measure.

The focus of the research presented in this thesis was to contribute to an understanding of the effects of unrestricted cattle access to watercourses on freshwater systems, expanding the body of literature conducted on the topic globally. Specifically, the study aimed to assess the extent to which such practice impacts water and sediment levels of contaminants such as excess nutrients, suspended sediment, and *E. coli*. This topic is of paramount importance in Ireland, where 67.4% of the land is dedicated to agriculture (CSO, 2020) and beef and dairy production predominate (Teagasc, 2017b), and where, despite the paucity of empirical evidence, restricting cattle access to watercourses has been included in agri-environmental policy since the introduction of the Rural Environmental Protection Scheme (REPS) in 1994. This study is equally relevant to other European countries where beef and dairy production are important economic sectors, e.g. France, Germany, the UK and Luxembourg (Eurostat, 2019; Eurostat, 2020). An overview of how cattle access to watercourses may affect such parameters is presented in Fig. 8.1.

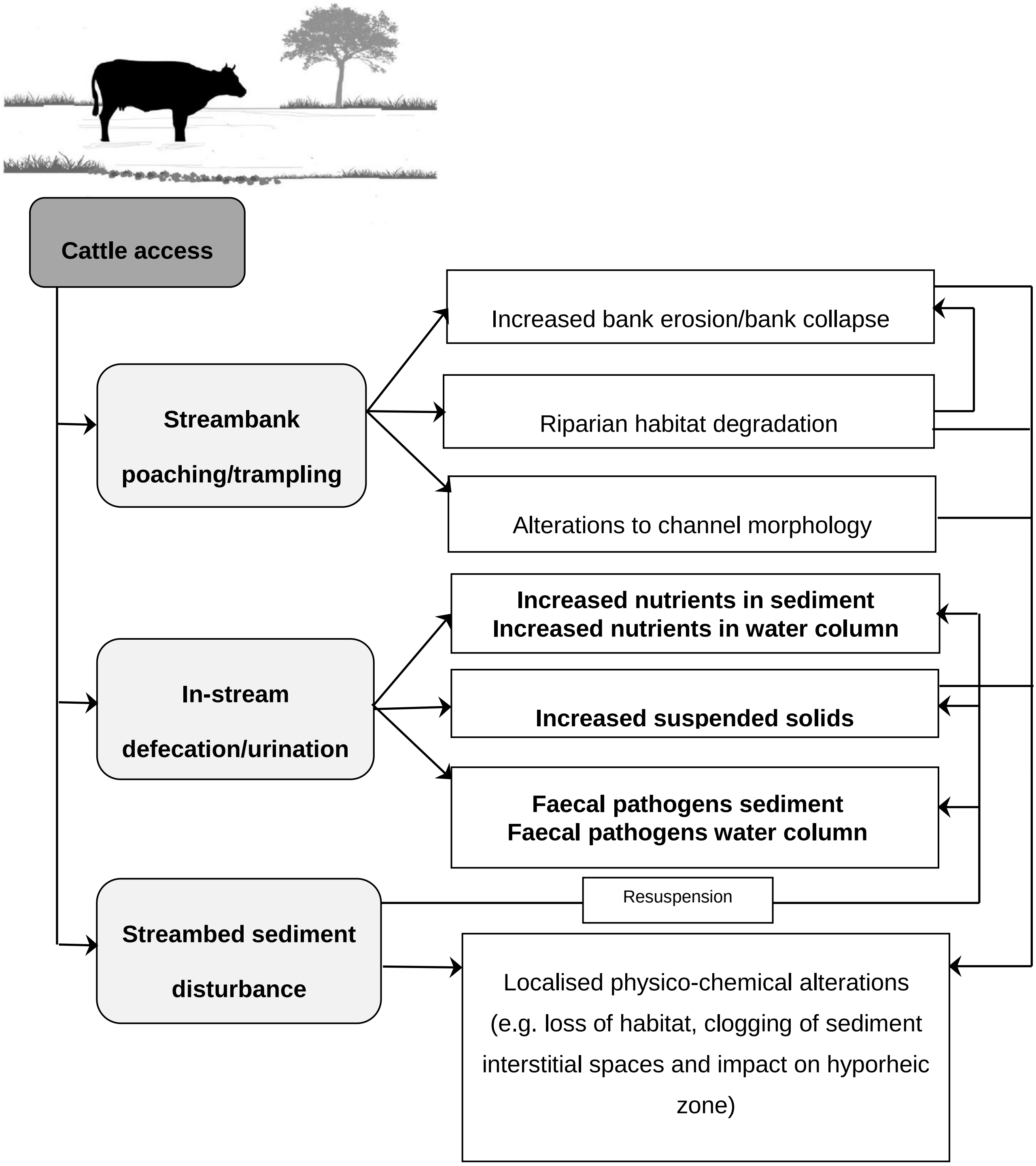

Fig.8.1. Diagram showing how unrestricted cattle access to watercourses can affect stream geochemical and microbial parameters. Parameters in bold are impacts demonstrated in this study.

8.1. Key findings

The present study revealed new information on how unrestricted cattle access to watercourses can impact water quality in terms of pollutants such as sediment, excess nutrients and faecal contaminants. The results from this study are particularly important because as noted previously there is a very limited body of research conducted on the topic in Europe in particular, Moreover, in spite of the widely recognised role of bed sediments as sinks and sources of pollution in aquatic systems, the majority of research addressing freshwater pollution associated with cattle access to watercourses or the effectiveness of fencing as a mitigation measure tends to focus on concentrations of contaminants in the water column. This study specifically addressed the potential impacts of cattle access on stream bed sediment reservoirs of contaminants. In addition, the study investigated and quantified the changes in a broad range of water quality parameters in response to cattle access to stream waters in near-real time, which, to the knowledge of the authors, no other study has addressed.

The key findings of the present research can be summarised as follows:

I. Unrestricted cattle access to watercourses can contribute to higher concentrations of *E. coli* of bed sediment
   a. High levels of *E. coli* in stream sediments were found at all study sites indicating that this is widespread in Irish agricultural catchments
   b. Cattle access to watercourses was found to significantly increase these stream bed sediment *E. coli* levels
   c. Sediment *E. coli* contamination was reduced (but persisted) in post-grazing season when cattle had been removed from the fields

d. Sediment contamination *E. coli* concentrations appeared to be governed by local (i.e. field-scale) management during grazing season, and catchment-scale factors in the absence of cattle

II. Unrestricted cattle access to watercourses was found to influence nutrient concentrations in the stream sediment but to a lesser extent than *E. coli*

a. Cattle access to watercourses contributed to stream sediment reservoirs of phosphorus, but had less impact on reservoirs of total nitrogen and organic carbon

b. The impact of nutrient contamination was more apparent in the smaller silt+clay fraction (< 63µm), as opposed to the < 2mm fraction

c. Sediment nutrient levels for all three nutrients were more likely to be influenced by catchment-scale management i.e. cattle density, rather than local-scale (field-scale) management

d. Where there was an indication of cattle access effect, this was only apparent at the interface between stream and land, suggesting a rapid flushing of nutrients downstream in the main channel of the streams

III. Cattle in-stream activity consistently resulted in increased the loads of total suspended solids and *E. coli* bacteria in the stream water while also increasing ammonium loads

a. Although increases in total phosphorus loads were observed during cattle in-stream activity, these were not significantly different from background variation at the site

b. Soluble reactive phosphorus loads were unaffected by cattle in-stream activity

c. Cattle in-stream activity had resulted in no increase in stream nitrate loads

8.1.1. Unrestricted cattle access to watercourses contributed to sediment faecal contamination

Pastoral agriculture can cause faecal contamination of watercourses through diffuse pathways, whereby high concentrations of faecal organisms are delivered to the watercourse (generally in surface run-off during storm events) (Kay et al., 2007; Muirhead and Monaghan, 2012). Nevertheless, previous research by Nagels et al. (2002) suggested that stream sediment reservoirs of faecal bacteria resulting from direct excretion in the stream channel by livestock can be of similar or greater importance than diffuse pathways of contamination in the overall faecal contaminant yield in agricultural streams.

The study described in Chapter 4 aimed at assessing the extent to which unrestricted cattle access to watercourses contributed to reservoirs of faecal contaminants in sediments of agricultural streams in Ireland. The study revealed that *E. coli* was present in all sediments in these Irish agricultural streams, and was detected at all but one of the sampled sites (the exception being site BWA and in post-grazing season only), including those sites where there was little or no upstream cattle activity. During the grazing season, *E. coli* concentrations were in the order of 10^2 – 10^4 CFU.g dry wt^{-1} at stream reaches in headwater sites not used by cattle. As is typical for agricultural activity in Ireland these catchments were dominated by cattle-based farming, thus the findings highlight the potential significant impacts that agriculture (cattle-based) can have on stream sediment *E. coli* levels and possible faecal contamination, regardless of whether animals actually have access to the watercourses. *E. coli* contamination may result from wildlife and human septic tank wastes, but in the context of cattle-based agriculture, wash-out from slurry spreading and runoff from grazing fields where faeces have been deposited are the main diffuse pathways through which faecal contaminants are delivered to watercourses (Fenlon et al., 2000; Vinten et al., 2004; Murihead and Monaghan, 2012). It should also be noted that, as it is now widely accepted, *E. coli* can become naturalised in sediments (Perchec-Merien and Lewis, 2012;

Jang et al., 2017). Thus, the faecal contamination measured in this study does not necessarily reflect recent pollution events.

Additionally, stream reaches accessed by cattle were, in general, significantly more contaminated than reaches immediately upstream with no animal access. Sediment concentrations of *E. coli* bacteria at cattle access sites were one to three orders of magnitude higher, and four orders of magnitude in one instance, than concentrations at sites with no access. Although the faecal contamination reduced in the post-grazing season, the concentrations remained at 10^2 – 10^3 CFU.g dry wt^{-1}, suggesting some level of bacteria persistence in the sediment matrix. These results indicate that unrestricted cattle access to watercourses has the potential to significantly exacerbate already high faecal contamination of agricultural streams, and that the threat to human and animal health should not be considered restricted to the summer months.

The increase in sediment *E. coli* contamination at cattle access sites (relative to upstream sites) was found to be associated with the degradation of the sites, as shown by the inverse relationship between *E. coli* bacteria concentrations and river habitat index (RHI) scores. These RHI scores, calculated at a local-scale, reflected the sites' general degradation, with lower RHI scores indicating more impacted sites (O'Sullivan et al., 2019). However, the current study found a relationship between stream sediment *E. coli* contamination and estimated cattle density per locality (estimated number of animals per ha at each sampled site) only in post-grazing season. This suggests that during grazing season, local-scale stream sediment *E. coli* contamination is governed by localised (i.e. field-scale) factors – for example animal behaviour, faecal bacterial concentrations, and stream flushing effects – which dominate over diffuse contamination pathways. Faecal bacteria in agricultural soils to which animal slurries and manures have been applied have been shown to have longer decay rates when UV and temperature values are lower (Hodgson et al., 2016), which could contribute to the observation that cattle density per locality influenced *E. coli* bed sediment concentrations in post-grazing season (i.e. early winter) but not in mid-grazing season (i.e. summer).

Faecal contamination of stream waters has implications not only for human health, due to the risk of zoonotic infection, but to animal health and performance (Willms et al., 2002). Where infected animals defecate into waters, the stream can serve as a vehicle of disease or parasite transmission to animals downstream. For instance, calves infected with the parasite *Cryptosporidium* sp. excrete large numbers of oocysts (a highly resistant spore-like stage of the parasite) in faeces, which may remain infective in waters for many months, particularly in norther climates where surface water temperatures can be low but remain above freezing (Fayer, 2004).

Another public health issue that has been repeatedly found to be associated with cattle density is human verocytotoxigenic *E. coli* (VTEC) infection (e.g. Brehony et al., 2018; Óhaiseadha et al., 2016). Ireland, in particular, has consistently reported the highest rate of VTEC infection in the EU, several times higher than the EU average (Garvey et al., 2016; Ohaiseadha et al., 2017; Brehony et al., 2018). Previous research has linked high cattle density farming and consumption of water retrieved from private wells, which are exempt of regulations, to increased incidence of VTEC infection (Garvey et al., 2016; Ohaiseadha et al., 2017; Brehony et al., 2018). In this light, the findings of this present study are of importance, as they indicate that unrestricted cattle access to watercourses plays a role in this public health issue, contributing to the pathways for animal and human infection, thus favouring the implementation of cattle restriction measures.

8.1.2. Cattle access to watercourses potentially contributes to stream sediment reservoirs of phosphorus

Chapter 5 investigated the extent to which unrestricted cattle access to watercourses contributed to the sediment reservoirs of total phosphorus (TP), total nitrogen (TN) and organic carbon (OC). Nutrient concentrations in sediments at all study sites were assessed in the fine fraction (<2mm particle size) and in the silt+clay fraction only (<63µm particle size), as this latter fraction is generally considered the most chemically reactive fraction. The silt+clay fraction was enriched in all three assessed nutrients, and analysis of this fraction revealed a pattern whereby the highest nutrient concentrations were observed in the catchments located in regions with traditionally more intensive cattle agriculture, i.e. the Commons River (CM) and the Milltown Lake (ML) catchments and a significant effect of estimated cattle density per region on sediment concentrations of all three nutrients in this fraction. This is one of the major results from this thesis and has implications for issues related to legacy phosphorus (Jarvie et al., 2013; Sharpley et al., 2013) in streams and for livestock management in relation to water quality.

Contrary to what was observed for sediment faecal bacteria concentrations, sediment nutrient concentrations did not vary significantly within the study sites (i.e. between the access site itself and upstream sites with no access), indicating an apparent lack of effect of direct cattle access to watercourses on sediment nutrient concentrations. Nevertheless, the GAM analysis suggested a potentially synergistic effect between cattle access (at the interface between land and watercourse) and estimated cattle density on total phosphorus concentrations in the silt+clay fraction. Additionally, and in contrast to sediment *E. coli* levels, there was no relationship between overall sediment nutrient levels at the study sites and the sites' RHI. These findings suggest that sediment nutrient reservoirs in agricultural streams are predominantly governed by catchment-scale activities. Nevertheless, unrestricted cattle access to watercourses potentially contributes to stream sediment reservoirs of phosphorus, which is of note given the role of P in eutrophication (Jennings et al., 2003) and deterioration of water quality and its potential to cause legacy issues (Sharpley et al., 2013).

The apparent contrasting nature of the mechanisms governing sediment faecal and nutrient contamination might be related to the distinct predominant pathways whereby nutrients and bacteria reach agricultural watercourses and their fate once in the aquatic system. Cattle faeces contain *E. coli* loads of 10^7 CFU (Avery et al., 2004; Davies-Colley et al., 2004; Weaver et al., 2005) and it is widely accepted that, once deposited, bacteria can become quickly incorporated in soils and sediment, where they can persist (Ishii et al., 2007; Oliver et al., 2007; Ishii and Sadowsky, 2008; Garzio-Hadzick et al., 2010; Badgley et al., 2011; Shelton et al., 2014; Hodgson et al., 2016) and multiply (Perchec-Merien and Lewis, 2012). In contrast, nutrient loads in cattle faeces may be relatively lower (Bond et al., 2012). In the aquatic system, nutrients also undergo biogeochemical reactions, including mineralisation, biotic uptake, and transport downstream in both particulate and dissolved forms, which may hinder localised nutrient accumulation in the sediment. The observation of a potential effect of cattle access on TP levels at the interface between land and watercourse, but not within the stream channel at the cattle access (CAS) might also indicate a rapid flush downstream of sediment TP within the stream channel with higher flows (Eder et al., 2014).

8.1.3. Cattle in-stream activity consistently results in increased concentrations of total suspended solids and *E. coli* bacteria in stream waters

Chapter 6 investigated the impacts of direct cattle access to watercourses and cattle in-stream activity on water quality at real-time. It was observed that cattle in-stream activity consistently resulted in increased concentrations of total suspended solids (TSS) and *E. coli* bacteria in stream waters, and that the increase in loads when cattle were present was significantly higher than when they were absent. Cattle in-stream activity was also significantly associated with increased loads of ammonium, while having no apparent effect on nitrate loads. Interestingly, it did not have a significant effect on stream loads of SRP or TP.

The increase in *E. coli* bacteria concentrations in stream waters during cattle in-stream activity occurred irrespective of the number of in-stream defecations. There was also an increase in TSS when cattle were in the water. These results indicate that the cattle disturbance of stream sediment, which can be enriched in faecal bacteria as observed in Chapter 4, can contribute through resuspension into the water column of high numbers of viable bacteria. Nevertheless the defecations which were recorded will result in increased numbers of *E. coli*, and faecal material, in the water. The findings indicate that cattle access to, and activity in, watercourses has an impact on water faecal contamination, and are line with the observations of Nagels et al. (2002), who suggested that disturbance of sediment enriched in faecal matter could be as important as diffuse contamination pathways in the overall bacterial yield in an agricultural stream in New Zealand.

The increase in TSS associated with cattle access is in line with the findings of Terry et al. (2014) and supports the evidence found by O'Sullivan et al. (2019), who reported significantly greater mass of deposited sediment downstream of cattle access sites than upstream of these sites for the sites used in this study. The lack of any immediate effect of cattle in-stream activity on phosphorus loads is less surprising for SRP, as P is a very reactive ion and exhibited high variation in concentrations in the study stream. Total phosphorus loads, however, would be expected to be influenced by bed sediment disturbance and direct defecation in waters. Generally, TP concentrations did increase in association with increases in TSS concentrations; however, due to the high background variability in TP loads at the study site, these increases were not significant.

8.1.4. Fencing of watercourses can have positive impacts on sediment nutrients and *E.coli* reservoirs

Chapter 7 described a small study assessing the effectiveness of fencing as a mitigation measure, including an assessment of potential improvements in sediment concentrations of nutrients and *E. coli* bacteria at cattle access sites after short-term fencing (~ one year) (in a *before-after* experiment), and an investigation of the effects of long-term fencing (nine years) on selected water quality parameters (nutrients and *E. coli*) (in a paired control-treatment sub-study). Although limited, the short-term experiment showed that fencing can lead to significant reductions on sediment nutrient concentrations (<2mm faction) at the stream-land interface in cattle access sites, an effect that can result from the physical recovery of bank and consequent elimination of these more impacted areas. Despite the statistically non-significant differences in sediment *E. coli* levels at the study sites before and after fencing, which might be related to time of sampling (i.e. post-grazing season, when, as seen in Chapter 4, *E. coli* sediment concentrations had been reduced), the findings of this short-term experiment also suggest that fencing can have positive effects on sediment *E. coli* reservoirs for individual sites. The longer-term sub-study did not reveal significant differences in water nutrient or *E. coli* bacteria concentrations. Overall, however, the results of this study add to the findings of the previous chapters in supporting the implementation of fencing as a mitigation measure.

8.2. Implications for management

This research has shown that unrestricted cattle access to watercourses can exacerbate contamination of stream bed sediments with *E. coli* and consequently of stream waters, increase suspended sediment levels, and potentially contribute to sediment phosphorus reservoirs. While the effects of direct cattle access on *E. coli* contamination of watercourses appear to be localised and thus strongly influenced by field-scale characteristics and practices, instream sediment nutrient levels seem to be mainly governed by catchment-scale activities. Thus, whilst there is evidence to support the implementation of measures aimed at excluding cattle from watercourses (e.g. fencing), such measures should be combined with larger scale, targeted mitigation measures to reduce diffuse losses of nutrients, sediments and faecal organisms, and effectively protect water quality.

Fencing off watercourses has been reported to have positive effects on water quality (see O'Callaghan et al., 2018). Under the COSAINT project, it was observed that short-term (~1 year) fencing of watercourses led to significant reductions in fine sediment deposits downstream of cattle access sites, with associated positive effects on macroinvertebrate communities in some cases (Ó hUallacháin et al., 2020). Additionally, an assessment of stream recovery following long-term fencing (~9 years) in paired fenced-unfenced streams in the Milltown Lake catchment, showed increased EPT (Ephemeroptera – Plecoptera – Trichoptera) richness in the fenced stream while the unfenced stream showed deterioration in water quality (Ó hUallacháin et al., 2020). In a study of faecal contamination in the same paired streams, Bragina et al. (2017) reported significantly lower streambed sediment concentrations of *E. coli* bacteria in the fenced stream in comparison to the unfenced stream during grazing season.

Also under the COSAINT project, Kilgarriff et al. (2020) determined the spatial distribution of cost-effectiveness of fencing watercourses in Ireland (using as metrics the reduction in faecal matter deposition within watercourses). They reported that the cost-effectiveness of fencing watercourses as a water quality protection measure is highest in regions with high agriculture intensity and low density of watercourses, which should therefore be prioritised (Kilgarriff et al., 2020). This is in line with the 4th NAP recommendation of fencing watercourses according to agricultural intensity (i.e. in derogation farms, which have stocking rates above 170 kg N.ha^{-1}), which will come into operation in Ireland in 2021.

Fencing watercourses eliminates the direct impacts of cattle access and cattle in-stream activity, but also promotes the establishment of a riparian buffer strip, which can further contribute to water quality protection by intercepting diffuse pathways of contamination. Conversely, the establishment of hedgerows in the riparian area can also be adopted as a mitigation measure, which would initially require fencing, but would later offset the costs of fencing maintenance (fences have a life spam of ~ 5 years) (Ó hUallacháin et al., 2020).

The need for supplementing cattle exclusion measures concurrently with diffuse pollution mitigation measures has been highlighted in Ireland (e.g. Bragina et al., 2017; Veerkamp, 2019) and elsewhere (e.g. Ranganath et al., 2009). Such measures often build on the concept of critical source area management, whereby pollution sources or activities are dissociated in space or time from hydrologically active areas (Easton et al., 2017). They can include nutrient management plans aimed at reducing excess nutrient runoff (i.e. reducing residual nutrient transfers), limiting the times of slurry spreading to periods of lower hydrological risk to reduce incidental nutrient transfers to watercourses, and, where possible, adopting rotational grazing, which has been shown to have beneficial effects in reducing agricultural pollution in comparison with continuous grazing systems (e.g. Sovell et al., 2010). In order to be effective, diffuse pollution mitigation measures must be implemented on a case by case basis, taking into account specific landscape and management conditions that can potentially combine to exacerbate diffuse losses to watercourses (Collins and McGonigle, 2008; Deakin et al., 2016).

8.3. Implications for policy

Ensuring the protection of water resources in Ireland is particularly relevant in the context of the recent and ongoing policies aimed at increasing the productivity of the agri-food sector, such as the Food Wise 2025 strategy. The second cycle of the current Irish River Basin Management Plan (RBMP) 2018 – 2021, maintains and strengthens key water quality protection measures. Under the current Green Low Carbon Agri-environment Scheme (GLAS), more than 21 000 farmers have fenced 16 000 km of watercourses. Furthermore, the 4th Nitrates Action Plan (NAP), which came into operation in 2017, requires derogation farmers to fence watercourses 1.5 m from the top of the banks from January 1st, 2021 (DoHPLG, 2018a). The 4th NAP also included strengthened measures to tackle diffuse sediment and nutrient losses from agriculture, such as a requirement for farmers to prevent direct runoff from farm roadways to watercourses and new conditions for slurry spreading derogation farmers (DoHPLG, 2018a).

The EU's legislative proposals for the post-2020 Common Agricultural Practice (CAP) (2021-2027) aim at delivering a higher level of climate and environmental ambition, while placing greater emphasis in the achievement of results at the regional and national scale. The policy comprises nine specific goals to be achieved by each Member State (MS). Three of these goals are directly related to protection and environment and climate, including to "foster sustainable development and efficient management of natural resources such as water, soil and air" (EC, 2019a). Each MS is responsible to delineate a CAP Strategic Plan, which will include specific targets and objectives for its territory and present actions to achieve them (EC, 2019a). The CAP 2021-2027 is interlinked with other EU policies for the protection of the environment and the agri-food sector such as the Water Framework Directive and the recent Farm to Fork Strategy and Biodiversity Strategy 2030, which are part of the climate and environment-focused European Green Deal (EC, 2019b). The Farm to Fork Strategy specifically requires the development of integrated nutrient management action plans in MS CAP Strategic Plans to tackle "nutrient pollution at source and increase the sustainability of the livestock sector" and the application of precise fertilisation techniques and sustainable agricultural practices particularly in areas of intensive livestock farming (EC, 2020b).

The current study provides information that is relevant for current policy as well as new policy developments. The findings of Chapter 4 and 6 relative to microbial contamination of waters show that unrestricted cattle access to watercourses can have implications in compliance with the Bathing Water Directive as well as the Drinking Water Directive. Additionally, the findings of Chapter 5 suggest cattle access can potentially contribute to sediment nutrient contamination which could impact on compliance with the WFD. Overall, the study supports the inclusion of fencing on-farm watercourses under agri-environment policy as part of the new 2021 – 2027 Rural Development Plan (RDP). Additionally, as seen in Chapter 5 where sediment nutrient reservoirs increased above cattle density rates of 1.2 (TP) to 1.6 (N and OC) animals per h, they favour the changes included in the 4th NAP regarding the targeting of intensive agriculture farms. This is further supported by Kilgarriff et al. (2020) who have shown that fencing would be a cost-effective water quality protection measure in intensive agriculture farms, including derogation farms, and recommended a vertical, targeted approach in agri-environment policy design as opposed to horizontal, one-size-fits-all regulations and incentives. Cullen et al. (2018) stated that the targeted approach adopted in GLAS resulted in higher farmer participation rates in regions with higher numbers of environmental features in need of protection or conservation, making this scheme more financially effective than its predecessors. This is because with the horizontal, flat-rate REPS and AEOS, farmers in extensive agriculture regions, who theoretically would be required to adopt fewer practical changes under the AES (but would receive the same payments as intensive farmers) would be more likely to adhere to the AES (Cullen et al., 2018). However, Madden et al. (2019) highlighted that in Ireland, high-status waterbody sites frequently coincide with designated Natura 2000 sites, which were also granted priority in entering GLAS. Farmers in Natura 2000 sites could select measures related to this feature, which do not necessarily include cattle exclusion measures, over water quality measures, which may be less financially rewarding (Madden et al., 2019). This highlights the need to ensure, in future AES, that incentives for adopting cattle exclusion measures are sufficient to cover the costs of the measure (i.e. fencing costs, provision of alternative drinking water sources, etc.), in order to make it more financially attractive for farmers and encourage its adoption where it is more beneficial. Where fencing of watercourses to completely exclude cattle is not a feasible or cost-effective measure, other measures such as the provision of alternative water sources (e.g. mains water, nose pumps) can be incentivised. Several studies have reported that providing cattle with alternative water sources resulted in cattle spending significantly less time in the watercourses (e.g. Clawson, 1993; Miner et al., 1992; Sheffield et al., 1997).

The current study highlights the need to combine cattle exclusion with diffuse pollution control measures where there are larger catchment-scale challenges. The need to adopt a more holistic approach rather than local reach scale measures to effectively mitigate agricultural impacts on water quality was emphasised in previous research (e.g. Weigel et al., 2000; Ranganath et al., 2009; Bragina et al., 2017). Research conducted under the recently concluded MARS Project launched by the EU also highlighted that restoring single, short stretches of rivers is insufficient to effectively address the environmental pressures currently faced by European rivers, which predominantly consist of diffuse pollution and hydromorphological degradation (Hering and Birk, 2018). Amongst the MARS Project recommendations is the implementation of riparian buffer strips along a substantial proportion of the European rivers network as a means to mitigate diffuse pollution impacts and allowing the regeneration of riparian and in-stream habitats (Hering and Birk, 2018).

Lastly, providing greater knowledge to farmers on the potential environmental and public health impacts of unrestricted cattle access to watercourses can help increasing awareness, understanding available measures and enhancing farmers' confidence in their ability to undertake water quality protection actions. Engaging farmers in water quality management through not only regulation but also knowledge transfer and participative approaches is crucial to more robust and effective management of water resources (Winter et al., 2011). This need was highlighted as part of the COSAINT project (Ó hUallacháin et al., 2020) and acknowledged in the Irish River Basin Management Plan (RBMP) 2018 - 2021.

8.4. Limitations of the study and future research recommendations

8.4.1. Uncertainty associated with the study

With empirical research such as the research reported in this study, there are uncertainties associated with the lack of control of numerous factors that can influence the variables studied, namely agricultural management practices as well as the effect of climatic conditions and site-specific factors. In this study, some attempts were made to conduct a more controlled field investigation of the impacts of cattle access on water quality parameters (i.e. by controlling number of animals in the watercourse, time spent in the watercourse, avoiding direct excretion to assess the impact of sediment disturbance only), however this proved unfeasible. Studies such as this are also dependent on the cooperation of farmers and landowners, which is not always guaranteed.

8.4.2. Influence of site-specific characteristics and management

This study did not specifically investigate several variables that can influence the extent to which unrestricted cattle access can impact freshwater systems, including site-specific management variables (stocking density, animal breed and age, density of cattle access point per length of watercourse). Future studies could further investigate the relationship between stocking density and impact of unrestricted cattle access, and investigate any differences in behaviour and impact caused by dairy and beef cattle.

8.4.3. Sediment faecal contamination and public health risk

Faecal indicator bacteria (FIB) such as *E. coli* have been widely used to detect recent environmental faecal contamination, under the assumption that the bacterium does not persist or multiply outside the host, and estimate the risk of exposure to faecal pathogens (Zhi et al., 2017). However, it is widely recognised that *E. coli* comprises environmental strains (Ishii et al., 2006; Ishii and Sadowsky, 2008; Perchec-Merien and Lewis, 2012; Jang et al., 2017) . Another limitation of the use of FIB to investigate faecal contamination is that, since *E. coli* is ubiquitous in warm-blooded organisms, it does not provide any information on the origin of such contamination, i.e. human origin, cattle origin, other animal origin, or indeed environmental *E. coli*. Bradshaw et al. (2016) have recommended a combination of FIB assessment and the use of faecal source trackers (FST) to determine the origin of contamination in order to better estimate the extent of environmental pollution. Incorporating such methods in further studies would allow for a better understanding of the extent of the impacts of cattle-based agriculture on faecal contamination of streams in rural areas, but also provide insight on the mechanisms that govern faecal freshwater pollution.

Some studies have also reported that *E. coli* bacteria levels may correlate poorly with the presence of certain pathogens and thus might provide inaccurate estimates of the public health risk (e.g. Abdelzaher et al., 2010; Abia et al., 2016; Bradshaw et al., 2016). Furthermore, the current study assessed sediment reservoirs of *E. coli* bacteria using plate count methods that depend on the culturability of bacteria in the samples. However, it has been shown that prolonged contact with surfaces including sediments may induce bacteria to enter a viable but non-culturable (VBNC) state, in which they will not grow in solid selective microbiological media, but may resuscitate under favourable conditions (Hassard et al., 2016). Studies have shown that *E. coli* bacteria, including *E. coli* O157:H7 (Poulsen et al., 1995; Wu et al., 2009), can leave the host already in a VBNC state, as bacteria in this state possess higher resistance to environmental stressors (Cheville et al., 1996; Saby et al., 2009). For these reasons, the current study might not provide an accurate estimate the true extent of faecal contamination and the public health risk in the study catchments. It would therefore be of interest to assess faecal contamination using methods not dependent on the culturability of microbial cells, e.g. quantitative PCR (qPCR). Targeting of specific pathogens in agricultural sediment reservoirs (e.g. VTEC, *Campylobacter jejuni*, *Crypstosporidium* sp., *Giardia* sp., and several viruses) could also provide a better estimate of the human and animal health risk posed by cattle-based agriculture and unrestricted access to watercourses.

8.4.4. Effectiveness of fencing as a mitigation measure

A further study could be undertaken on a more detailed investigation on the effectiveness of fencing in reducing nutrient and faecal contamination in streams. This would include sampling of a higher number of sites across a range of agricultural intensities and hydrological conditions, both in short-term and long-term, and at a higher temporal frequency. Where *before-after* studies are conducted, similarly to the study described Chapter 7, sampling at different points of the agricultural management cycle is recommended.

Such a study would also benefit of an assessment of the effectiveness of fencing on reducing nutrient sediment contents on the silt and clay fraction. Additionally, a study on the effects of cattle exclusion on the sediment nutrient reservoirs, particularly TP, similarly to the paired study on water quality described in Chapter 7, would provide a deeper insight of the effectiveness of the measure on such parameters, allowing a better understanding of the extent to which nutrient sediment reservoirs are controlled by diffuse pollution mechanisms.

8.4.5. Studies on phosphorus fractions at cattle access sites

It has been reported that, although stream banks and stream bed sediments might have large pools of TP, these do not necessarily represent an environmental concern. Of more concern will be the proportion of labile P at such sites (McDowell and Sharpley, 2001; Palmer-Felgate et al., 2009; Fox et al., 2016). Therefore, investigating the different fractions of P in bed sediments at cattle access sites would be of interest to better understand the extent to which these sites may affect water quality.

8.4.6. Studies on cattle behaviour

Although not described in detail in this study, the research provided some insight on the behaviour of cattle, in terms of watercourse usage, in Irish conditions. Cattle behaviour in terms of access to watercourses is influenced by factors such as temperature, provision of alternative water sources, provision of shade areas, and riparian vegetation (Belsky et al., 1999; Haan et al., 2010). Understanding how cattle behave (i.e. frequency of cattle access to the watercourse, duration of access, frequency of in-stream defecation and urination) in the Irish or western-European setting would provide relevant information to an understanding of the extent to which unrestricted access to watercourses might impact freshwater systems at the catchment scale , for example in modelling studies.

8.5. Overall conclusion

Unrestricted cattle access to watercourses has been recognised by policy makers as potentially detrimental for freshwater systems and water quality. However, although cattle exclusion measures have been included in Irish agri-environment policy for more than twenty years, few attempts at quantifying such impacts, and indeed at assessing the effectiveness of those measures, have been made to date. This study established that unrestricted cattle access to watercourses can negatively impact freshwater systems. Cattle access creates areas where nutrients, in particular phosphorus, can potentially accumulate. It is also apparent that systematic defecation within the stream channel at cattle access sites results in the accumulation of faecal organisms in stream sediments. The high density of cattle access sites found in several Irish agricultural streams (e.g *c*. 8 access sites per km) (Jordan and Ryan, 2011; Jordan and Smietanka, 2013) is likely to have an overall significant effect on water quality. The current study therefore favours the use of policy tools to incentivise farmers, particularly those in regions of high agricultural intensity, to implement measures aiming at eliminating or reducing cattle access to watercourses, while emphasising a need for a holistic approach in agricultural pollution mitigation plans.

Appendices

Appendix A1. Concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 2 (September 21, 2016).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	SRP (µg.L^{-1})	NH_4-N (mg.L^{-1})	TON (mg.L^{-1})	TSS (mg.L^{-1})
	27	no	-	-	166	0.01	2.23	0.5
	42	no	-	-	163	0.01	2.21	0.5
	57	no	-	-	168	0.01	2.33	0.3
	72	no	-	-	162	0.01	2.13	0.5
	87	no	-	-	165	0.01	2.10	0.6
	102	no	-	-	162	0.01	2.11	0.3
	117	no	-	-	161	0.01	2.12	0.4
Upstream	132	no	-	-	158	0.01	2.16	0.2
	147	no	-	-	159	0.01	2.16	0.4
	162	no	-	-	160	0.01	2.14	0.1
	177	yes	5	no	161	0.01	2.09	0.4
	192	yes	2	U+D	160	0.05	2.09	0.3
	207	yes	2	no	165	0.06	2.07	0.9
	222	yes	3	no	167	0.05	2.06	0.7
	237	no	-	-	162	0.04	2.04	0.4

Appendix A2. Concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 2 (September 21, 2016).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	SRP (µg.L^{-1})	NH_4-N (mg.L^{-1})	TON (mg.L^{-1})	TSS (mg.L^{-1})
	27	no	-	-	175	0.02	2.11	0.6
	42	no	-	-	171	0.02	2.09	1.0
	57	no	-	-	168	0.02	2.09	0.4
	72	no	-	-	166	0.02	2.14	0.2
	87	no	-	-	163	0.02	2.16	0.4
	102	no	-	-	165	0.02	2.14	0.3
	117	no	-	-	166	0.02	2.13	0.2
Downstream	132	no	-	-	165	0.02	2.09	0.3
	147	no	-	-	164	0.02	2.06	0.3
	162	no	-	-	163	0.02	2.04	0.1
	177	yes	5	no	162	0.06	1.97	0.4
	192	yes	2	U+D	162	0.14	1.97	5.5
	207	yes	2	no	192	0.08	2.03	1.8
	222	yes	3	no	169	0.07	1.99	5.3
	237	no	-	-	164	0.05	2.15	1.2

Appendix A3. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 7 (May 10, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	NH_4 - N ($mgN.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 – N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	-	-	0.02	73	83	4.43	20.65	2.8
	42	No	-	-	0.02	74	77	4.42	21.25	2.2
	57	No	-	-	0.02	74	76	4.47	21.69	4.4
	72	No	-	-	0.02	73	79	4.44	20.15	3.2
	87	Yes	5	No	0.02	73	73	4.48	21.01	2.4
	102	Yes	6	U+D	0.03	73	76	4.46	20.74	2.6
	117	Yes	1	U	0.02	73	84	4.42	20.61	3.4
	132	No	-	-	0.02	73	NA	4.41	22.01	4.2
	147	No	-	-	0.02	73	75	4.46	21.17	3.4
	162	No	-	-	0.04	76	NA	4.36	23.52	6.2
	177	No	-	-	0.04	79	96	4.12	23.45	9.2
	192	No	-	-	0.03	76	99	4.65	20.34	6.0
	207	No	-	-	0.03	76	85	4.45	21.87	4.2
	222	No	-	-	0.02	74	80	4.53	20.65	2.4

Appendix A4. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 7 (May 10, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	-	-	0.03	72	71	3.98	20.73	3.8
	42	No	-	-	0.03	71	72	4.03	20.70	2.6
	57	No	-	-	0.03	71	67	4.06	20.91	3.4
	72	No	-	-	0.03	72	68	4.07	20.77	2.8
	87	Yes	5	No	0.02	72	73	4.05	20.78	4.2
	102	Yes	6	U+D	0.04	79	130	4.03	21.81	14.8
	117	Yes	1	U	0.08	81	148	4.13	21.89	27.4
	132	No	-	-	0.03	73	79	4.05	21.09	5.4
	147	No	-	-	0.02	70	72	4.06	22.37	2.6
	162	No	-	-	0.02	71	77	4.00	21.02	3.4
	177	No	-	-	0.03	77	104	4.04	22.65	5.2
	192	No	-	-	0.04	78	93	4.07	20.55	5.8
	207	No	-	-	0.03	73	87	4.06	20.80	5.4
	222	No	-	-	0.03	72	76	4.01	21.24	2.4

Appendix A5. Water concentrations of .*E. coli* and Other Coliforms upstream of the cattle access site on Event 7 (May 10, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/Urination	E. coli Log_{10} CFU.100ml	OC Log_{10}CFU.100ml
Upstream	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	2.60	3.20
	87	Yes	5	No	2.69	3.06
	102	Yes	6	U+D	2.54	2.67
	117	Yes	1	U	2.62	2.46
	132	No	-	-	2.67	2.45
	147	No	-	-	NA	NA
	162	No	-	-	NA	NA
	177	No	-	-	NA	NA
	192	No	-	-	NA	NA
	207	No	-	-	NA	NA
	222	No	-	-	NA	NA

Appendix A6. Water concentrations of .*E. coli* and Other Coliforms downstream of the cattle access site on Event 7 (May 10, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/Urination	E. coli Log_{10} CFU.100ml	OC Log_{10}CFU.100ml
Downstream	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	2.61	3.56
	87	Yes	5	No	3.00	3.15
	102	Yes	6	U+D	4.24	3.23
	117	Yes	1	U	4.38	3.46
	132	No	-	-	3.61	2.76
	147	No	-	-	NA	NA
	162	No	-	-	NA	NA
	177	No	-	-	NA	NA
	192	No	-	-	NA	NA
	207	No	-	-	NA	NA
	222	No	-	-	NA	NA

Appendix A7. Water concentrations of nutrients, total suspended solids, *E coli* and Other Coliforms upstream of the cattle access site on Event 8 (May 24, 2017).

Location	**Minutes**	**Cattle activity**	**NH_4 - N ($mgN.L^{-1}$)**	**SRP ($\mu g.L^{-1}$)**	**TP ($\mu g.L^{-1}$)**	**NO_3 - N ($mgN.L^{-1}$)**	**Cl^{-1} ($mg.L^{-1}$)**	**TSS ($mg.L^{-1}$)**	***E.coli* log_{10}CFU.100ml**	**OC log_{10}CFU.100ml**
Upstream	27	No	0.05	131	194	4.21	23.65	4.8	4.11	3.72
	42	No	0.04	128	191	4.19	28.47	4.8	4.01	3.78
	57	No	0.03	127	180	4.16	22.53	3.4	3.67	3.36
	72	No	0.02	125	175	4.12	23.87	5.0	3.51	3.30
	87	No	< L.O.D	126	182	4.15	22.33	4.4	3.41	3.67
	102	No	< L.O.D	128	180	4.16	23.92	4.4	4.25	4.04
	117	No	0.02	125	187	4.15	25.32	8.5	NA	NA
	132	No	0.02	126	180	4.20	23.06	4.6	NA	NA
	147	No	0.02	128	174	4.18	29.98	7.5	NA	NA
	162	No	0.02	127	179	4.20	24.83	4.7	NA	NA
	177	No	0.02	127	174	4.20	23.14	8.6	NA	NA
	192	No	0.02	127	177	4.22	22.68	3.2	NA	NA
	207	No	< L.O.D	125	180	4.22	23.31	4.6	NA	NA
	222	No	0.02	128	169	4.24	23.12	4.2	NA	NA

Appendix A8. Water concentrations of nutrients, total suspended solids, *E coli* and Other Coliforms downstream of the cattle access site on Event 8 (May 24, 2017).

Location	Minutes	Cattle activity	NH_4 - N ($mgN.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mgN.L^{-1}$)	Cl^{-1} ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)	***E.coli*** log10CFU.100ml	OC log10CFU.100ml
Downstream	27	No	0.03	128	189	4.06	22.92	3.4	3.82	3.49
	42	No	0.03	128	191	4.14	22.45	4.6	4.10	3.89
	57	No	0.04	127	181	4.18	22.54	4.2	3.91	3.57
	72	No	0.03	129	182	4.16	22.34	4.6	3.72	3.51
	87	No	0.03	127	171	4.13	25.70	6.6	3.43	3.39
	102	No	< L.O.D	126	181	3.99	23.23	4.4	3.44	3.39
	117	No	< L.O.D	126	175	4.14	26.54	3.8	NA	NA
	132	No	< L.O.D	126	172	4.13	22.86	4.8	NA	NA
	147	No	< L.O.D	127	169	4.15	22.65	4.0	NA	NA
	162	No	0.02	126	168	4.12	30.13	2.6	NA	NA
	177	No	0.02	125	171	4.11	32.59	31.0	NA	NA
	192	No	0.02	124	169	4.10	23.62	4.0	NA	NA
	207	No	0.02	126	167	4.09	25.52	3.4	NA	NA
	222	No	0.02	126	166	4.15	23.19	5.2	NA	NA

Appendix A9. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 9 (June 14, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	NH_4 - N (mgN.L^{-1})	SRP (µg.L^{-1})	TP (µg.L^{-1})	NO_3 - N (mgN.L^{-1})	Cl^- (mg.L^{-1})	TSS (mg.L^{-1})
	27	No	-	-	0.02	100	243	3.05	23.34	5.2
	42	No	-	-	0.02	102	174	3.09	23.41	4.0
	57	No	-	-	< L.O.D.	102	154	3.22	23.31	5.4
	72	No	-	-	0.02	100	269	3.11	23.26	5.0
	87	No	-	-	< L.O.D.	100	217	3.30	24.56	4.8
	102	No	-	-	0.02	101	171	3.19	23.59	4.2
	117	No	-	-	0.02	100	348	3.17	23.10	5.0
	132	No	-	-	< L.O.D.	101	229	3.28	23.67	3.8
	147	Yes	2	No	< L.O.D.	101	217	3.15	24.07	3.8
Upstream	162	Yes	4	U	0.02	99	206	3.15	24.70	2.8
	177	Yes	4	No	< L.O.D.	101	155	2.04	23.97	4.4
	192	Yes	3	D	< L.O.D.	101	160	3.14	26.23	4.6
	207	Yes	1	No	0.02	101	208	3.09	24.88	5.2
	222	No	-	-	0.03	92	153	3.14	22.06	4.6
	237	No	-	-	0.04	101	225	3.11	22.76	3.4
	252	No	-	-	0.02	101	244	3.08	23.35	2.8
	267	No	-	-	0.03	105	232	3.14	22.76	5.2
	282	No	-	-	0.03	109	245	3.10	22.81	6.0
	297	No	-	-	0.02	105	266	3.13	23.06	5.6

Appendix A10. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 9 (June 14, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	NH_4 - N ($mgN.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mgN.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
	27	No	-	-	< L.O.D	97	205	2.72	31.08	12.6
	42	No	-	-	0.03	90	245	3.10	23.26	38.8
	57	No	-	-	0.02	102	234	2.95	24.16	7.6
	72	No	-	-	< L.O.D.	97	219	2.98	24.41	13.2
	87	No	-	-	< L.O.D.	100	226	2.91	24.54	3.0
	102	No	-	-	< L.O.D.	101	225	3.03	23.30	6.4
	117	No	-	-	< L.O.D.	97	414	3.00	22.80	2.6
	132	No	-	-	< L.O.D.	97	236	3.03	22.99	5.2
	147	Yes	2	No	< L.O.D.	96	199	2.70	24.03	6.8
Downstream	162	Yes	4	U	0.04	87	166	2.80	23.79	14.0
	177	Yes	4	No	0.05	89	309	3.11	23.90	18.4
	192	Yes	3	D	< L.O.D.	92	231	2.87	22.54	12.6
	207	Yes	1	No	< L.O.D.	94	259	3.07	23.02	12.2
	222	No	-	-	0.02	99	249	3.10	22.34	9.2
	237	No	-	-	0.05	98	248	3.13	22.15	7.6
	252	No	-	-	0.05	99	249	3.11	21.69	5.6
	267	No	-	-	0.05	98	243	3.10	22.58	4.8
	282	No	-	-	0.02	101	234	3.09	22.04	8.4
	297	No	-	-	< L.O.D.	102	279	3.026	21.45	6.0

Appendix A11. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 9 (June 14, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	NA	NA
	87	No	-	-	NA	NA
	102	No	-	-	NA	NA
	117	No	-	-	NA	NA
	132	No	-	-	2.90	2.99
	147	Yes	2	No	2.91	3.02
Upstream	162	Yes	4	U	2.98	2.99
	177	Yes	4	No	3.07	3.15
	192	Yes	3	D	3.02	3.17
	207	Yes	1	No	2.94	3.28
	222	No	-	-	2.95	3.11
	237	No	-	-	NA	NA
	252	No	-	-	NA	NA
	267	No	-	-	NA	NA
	282	No	-	-	NA	NA
	297	No	-	-	NA	NA

Appendix A12. Water concentrations of *E. coli* and Other Coliforms downstream of the cattle access site on Event 9 (June 14, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	***E. coli*** **Log_{10}CFU.100ml**	**OC Log_{10}CFU.100ml**
	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	NA	NA
	87	No	-	-	NA	NA
	102	No	-	-	NA	NA
	117	No	-	-	NA	NA
	132	No	-	-	3.03	3.08
	147	Yes	2	No	3.20	3.16
Downstream	162	Yes	4	U	3.88	3.69
	177	Yes	4	No	3.93	3.90
	192	Yes	3	D	3.68	3.74
	207	Yes	1	No	3.77	3.88
	222	No	-	-	3.57	3.56
	237	No	-	-	NA	NA
	252	No	-	-	NA	NA
	267	No	-	-	NA	NA
	282	No	-	-	NA	NA
	297	No	-	-	NA	NA

Appendix A13. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 10 (June 28, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	-	-	0.02	233	281	2.85	24.28	12.0
	42	No	-	-	0.02	243	289	2.79	25.85	6.0
	57	No	-	-	0.05	263	325	2.51	26.18	7.2
	72	No	-	-	0.08	272	340	2.78	25.33	11.2
	87	No	-	-	0.08	269	342	2.75	25.68	8.8
	102	No	-	-	0.07	261	320	2.69	25.08	9.2
	117	Yes	1	No	0.06	253	319	2.70	25.69	8.2
	132	No	-	-	0.06	249	299	2.69	24.75	6.2
	147	No	-	-	0.05	241	294	2.70	24.28	8.2
	162	No	-	-	0.04	239	295	2.71	24.74	5.6
	177	No	-	-	0.03	234	277	2.46	25.52	6.8
	192	No	-	-	0.02	233	262	2.70	26.66	6.0
	207	No	-	-	0.02	233	264	2.76	26.35	4.0
	222	No	-	-	0.02	232	271	2.81	24.47	5.6
	237	No	-	-	0.02	232	270	2.86	23.92	57.4
	252	No	-	-	0.02	231	262	2.79	23.62	5.0
	267	No	-	-	0.03	229	271	2.71	23.74	4.7
	282	No	-	-	0.02	236	214	2.74	31.35	6.0

Appendix A14. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 10 (June 28, 2017).

Location	Minutes	Cattle activity	Max cattle	Urination/ Defecation	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	-	-	0.07	233	291	2.75	30.39	12.2
	42	No	-		0.04	222	273	2.75	28.74	8.8
	57	No	-	-	0.06	247	314	2.70	29.23	9.0
	72	No	-	-	0.08	255	334	2.67	25.89	12.8
	87	No	-	-	0.10	266	336	2.62	47.74	9.4
	102	No	-	-	0.09	262	319	2.64	29.54	12.8
	117	Yes	1	No	0.07	250	312	2.62	25.76	10.6
	132	No	-	-	0.06	242	306	2.60	31.64	9.4
	147	No	-	-	0.04	236	283	2.58	31.15	9.0
	162	No	-	-	0.03	239	282	2.62	34.03	9.6
	177	No	-	-	0.03	234	298	2.67	31.22	9.8
	192	No	-	-	0.03	238	276	2.65	25.91	8.2
	207	No	-	-	0.03	229	267	2.64	30.63	9.0
	222	No	-	-	0.03	235	265	2.76	28.65	7.6
	237	No	-	-	0.03	226	263	2.73	26.24	6.6
	252	No	-	-	0.04	227	251	2.73	29.84	5.2
	267	No	-	-	0.03	226	266	2.67	27.98	5.8
	282	No	-	-	0.03	231	268	2.69	32.19	4.0

Appendix A15. Water conductivity upstream of the cattle access site on Event 10 (June 28, 2017)

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Upstream	27	603.5	14.7
	42	608.2	12.8
	57	594.2	12.2
	72	594.8	14.0
	87	596.5	14.7
	102	589.7	12.7
	117	593.4	11.8
	132	595.8	13.3
	147	594.9	12.3
	162	600.1	13.2
	177	605.1	12.2
	192	610.1	11.6
	207	602.8	12.5
	222	608.2	13.5
	237	604.7	14.1
	252	604.5	14.1
	267	604.5	13.1
	282	595.5	13.2

Appendix A16. Water conductivity downstream of the cattle access site on Event 10 (June 28, 2017).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Downstream	27	610.5	12.4
	42	599.7	12.0
	57	593.7	12.8
	72	598.6	11.8
	87	589.5	11.5
	102	588.3	14.6
	117	585.0	11.7
	132	587.8	12.3
	147	591.8	11.7
	162	595.9	11.0
	177	600.7	11.8
	192	595.4	11.4
	207	604.9	11.8
	222	600.9	12.2
	237	609.1	11.5
	252	604.7	12.9
	267	594.1	13.3
	282	604.8	12.6

Appendix A17. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 11 (August 8, 2017).

Location	**Minutes**	**Cattle activity**	**Max cattle**	**Defecation/ Urination**	**NH_4 - N ($mg.L^{-1}$)**	**SRP ($\mu g.L^{-1}$)**	**TP ($\mu g.L^{-1}$)**	**NO_3 - N ($mg.L^{-1}$)**	**Cl^- ($mg.L^{-1}$)**	**TSS ($mg.L^{-1}$)**
Upstream	27	No	-	-	0.15	241	265	2.81	23.03	3.4
	42	No	-	-	0.23	243	306	2.80	23.30	5.2
	57	Yes	3	No	0.13	240	279	2.80	22.58	4.0
	72	Yes	2	U	0.08	239	332	2.78	22.57	5.8
	87	Yes	1	No	0.12	242	268	2.79	22.71	5.0
	102	No	-	-	0.06	242	264	2.80	22.97	4.2
	117	No	-	-	0.13	238	260	2.78	22.64	5.0
	132	No	-	-	0.33	235	263	2.76	23.89	12.0
	147	No	-	-	0.16	227	277	2.65	22.44	16.0
	162	No	-	-	0.17	233	264	2.74	29.87	3.8
	177	Yes	2	No	0.24	225	279	2.63	23.63	13.0
	192	No	-	-	0.13	238	263	2.77	22.73	3.6
	207	Yes	1	No	0.18	238	278	2.69	23.05	4.0
	222	No	-	-	0.07	244	264	2.74	22.54	4.0

Appendix A18. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 11 (August 8, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3 - N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	0	-	0.15	241	263	2.80	23.26	8.2
	42	No	0	-	0.09	236	281	2.81	22.26	4.6
	57	Yes	3	No	0.18	245	308	2.83	23.75	15.0
	72	Yes	2	U	0.19	256	401	2.89	22.72	42.4
	87	Yes	1	No	0.16	247	367	2.84	22.93	32.2
	102	No	0	-	0.15	250	296	2.77	23.87	12.0
	117	No	0	-	0.13	244	276	2.77	22.54	6.6
	132	No	0	-	0.22	248	285	2.83	23.24	11.8
	147	No	0	-	0.12	234	261	2.84	23.68	6.4
	162	No	0	-	0.14	235	257	2.80	22.58	5.6
	177	Yes	2	No	0.10	238	274	2.84	23.47	7.2
	192	No	0	-	0.16	256	311	3.01	24.18	23.4
	207	Yes	1	No	0.08	243	320	2.80	22.51	17.0
	222	No	0	-	0.09	240	316	2.74	22.44	9.6

Appendix A19. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 11 (August 8, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/Urination	*E. coli* log_{10}CFU.100ml	OC log_{10} CFU.100ml
Upstream	27	No	-	-	NA	NA
	42	No	-	-	2.91	3.69
	57	Yes	3	No	2.86	NA
	72	Yes	2	U	3.00	3.10
	87	Yes	1	No	2.90	3.53
	102	No	-	-	2.94	3.28
	117	No	-	-	2.70	2.61
	132	No	-	-	2.91	NA
	147	No	-	-	2.81	3.35
	162	No	-	-	2.93	NA
	177	Yes	2	No	2.79	3.43
	192	No	-	-	2.90	3.66
	207	Yes	1	No	2.76	3.58
	222	No	-	-	2.76	3.53

Appendix A20. Water concentrations of *E. coli* and Other Coliforms downstream of the cattle access site on Event 11 (August 8, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/Urination	*E. coli* log_{10}CFU.100ml	OC log_{10} CFU.100ml
Downstream	27	No	-	-	NA	NA
	42	No	-	-	2.96	3.85
	57	Yes	3	No	3.36	2.70
	72	Yes	2	U	3.96	3.66
	87	Yes	1	No	3.88	3.53
	102	No	-	-	3.49	3.40
	117	No	-	-	3.33	3.30
	132	No	-	-	3.01	2.52
	147	No	-	-	2.76	1.78
	162	No	-	-	3.16	3.68
	177	Yes	2	No	3.27	3.26
	192	No	-	-	4.10	2.71
	207	Yes	1	No	3.62	2.73
	222	No	-	-	3.62	4.09

Appendix A21. Water conductivity upstream of the cattle access site on Event 11 (August 8, 2017).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Upstream	27	670.0	12.8
	42	671.0	13.2
	57	664.0	13.4
	72	681.0	13.5
	87	671.0	14.2
	102	675.0	13.9
	117	673.0	14.7
	132	665.0	14.9
	147	677.0	13.9
	162	675.0	15.3
	177	667.0	14.9
	192	671.0	15.9
	207	675.0	15.6
	222	672.0	15.5

Appendix A22.Water conductivity downstream of the cattle access site on Event 11 (August 8, 2017).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Downstream	27	667.0	13.7
	42	670.0	14.2
	57	672.0	14.3
	72	680.0	15.6
	87	676.0	14.8
	102	674.0	14.8
	117	678.0	15.0
	132	663.0	15.3
	147	664.0	15.3
	162	668.0	15.1
	177	673.0	15.8
	192	693.0	15.8
	207	667.0	15.9
	222	674.0	16.8

Appendix A23. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 12 (August 30, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	NH_4-N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	Cl ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	-	-	0.02	210	224	242	2.74	31.87	3.0
	42	No	-	-	< L.O.D.	208	225	234	2.72	35.31	3.4
	57	No	-	-	< L.O.D.	205	224	235	2.70	35.98	3.2
	72	No	-	-	0.09	213	231	245	2.72	33.83	2.4
	87	No	-	-	0.03	209	225	222	2.72	32.60	3.6
	102	No	-	-	< L.O.D.	205	223	224	2.68	31.88	1.8
	117	No	-	-	< L.O.D.	206	219	240	2.75	33.70	1.4
	132	Yes	1	No	< L.O.D.	208	221	226	2.79	34.24	1.4
	147	No	-	-	< L.O.D.	207	221	219	2.74	36.69	1.8
	162	Yes	3	No	0.02	211	222	218	2.76	34.31	3.4
	177	No	-	-	0.02	211	223	217	2.75	32.65	3.8
	192	Yes	3	No	0.02	208	224	217	2.70	36.19	1.6
	207	No	-	-	0.03	211	222	216	2.69	32.72	2.8
	222	No	-	-	0.22	216	220	218	2.72	35.13	2.2

Appendix A24. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 12 (August 30, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	NH_4-N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	-	-	0.03	223	254	279	2.55	45.13	11.0
	42	No	-	-	0.02	222	228	362	2.60	35.13	6.0
	57	No	-	-	0.02	207	214	234	2.60	33.63	6.2
	72	No	-	-	< L.O.D.	207	214	221	2.62	32.52	2.8
	87	No	-	-	< L.O.D.	211	221	213	2.63	34.23	2.8
	102	No	-	-	0.04	222	253	261	2.62	41.92	3.0
	117	No	-	-	< L.O.D.	204	219	198	2.60	35.69	3.2
	132	Yes	1	No	0.02	200	215	217	2.59	35.69	4.4
	147	No	-	-	0.05	204	214	238	2.62	37.93	9.2
	162	Yes	3	No	0.02	207	214	230	2.64	35.90	2.0
	177	No	-	-	0.10	210	219	349	2.60	35.79	11.0
	192	Yes	3	No	0.05	207	217	230	2.69	34.60	7.8
	207	No	-	-	0.07	207	208	236	2.68	35.35	9.2
	222	No	-	-	0.02	204	211	217	2.64	41.19	3.0

Appendix A25. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 12 (August 30, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
Upstream	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	NA	NA
	87	No	-	-	NA	NA
	102	No	-	-	3.13	3.01
	117	No	-	-	3.26	3.15
	132	Yes	1	No	3.29	2.67
	147	No	-	-	3.32	3.20
	162	Yes	3	No	3.32	3.17
	177	No	-	-	3.23	3.13
	192	Yes	3	No	3.45	2.95
	207	No	-	-	3.27	2.85
	222	No	-	-	3.23	2.89

Appendix A26. Water concentrations of *E. coli* and Other Coliforms downstream of the cattle access site on Event 12 (August 30, 2017).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
Downstream	27	No	-	-	NA	NA
	42	No	-	-	NA	NA
	57	No	-	-	NA	NA
	72	No	-	-	NA	NA
	87	No	-	-	NA	NA
	102	No	-	-	2.98	3.08
	117	No	-	-	3.08	3.01
	132	Yes	1	No	3.20	3.14
	147	No	-	-	3.70	2.93
	162	Yes	3	No	3.37	3.05
	177	No	-	-	3.71	3.34
	192	Yes	3	No	3.49	2.78
	207	No	-	-	3.62	2.83
	222	No	-	-	3.29	2.72

Appendix A27. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 13 (February 7, 2018)

Location	Minutes	Cattle activity	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	0.07	32	70	8.29	26.36	9.2
	42	No	0.07	31	64	8.27	25.93	4.0
	57	No	0.08	30	63	8.18	26.73	3.2
	72	No	0.06	32	64	8.30	26.79	4.6
	87	No	0.15	32	60	8.47	27.08	4.0
	102	No	0.08	32	62	8.41	26.66	3.2
	117	No	0.10	30	69	8.41	25.90	4.6
	132	No	0.09	31	72	8.25	26.65	3.4
	147	No	0.08	33	62	8.18	25.68	4.0
	162	No	0.08	32	64	8.27	25.45	4.8
	177	No	0.10	33	62	8.16	26.24	4.2
	192	No	0.10	33	62	8.25	26.00	4.0
	207	No	0.07	32	60	8.31	26.65	4.0
	222	No	0.10	33	60	8.53	26.69	3.2

Appendix A28. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 13 (February 7, 2018).

Location	Minutes	Cattle activity	NH_4 - N ($mg.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	0.08	30	70	8.08	25.72	3.6
	42	No	0.08	31	66	8.03	26.29	4.2
	57	No	0.12	29	67	8.07	26.04	3.0
	72	No	0.07	31	77	8.12	26.45	4.4
	87	No	0.06	31	74	8.46	26.36	4.0
	102	No	0.07	31	62	8.10	26.42	4.4
	117	No	0.09	29	68	8.20	26.32	3.2
	132	No	0.09	30	62	8.12	25.45	3.6
	147	No	0.09	31	65	8.13	26.35	3.6
	162	No	0.08	33	69	8.14	25.81	3.0
	177	No	0.09	32	63	8.23	25.68	2.6
	192	No	0.10	31	67	8.07	25.64	3.6
	207	No	0.07	33	103	8.08	25.66	15.8
	222	No	0.09	32	59	8.12	25.71	4.8

Appendix A29. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 13 (February 7, 2018).

Location	**Minutes**	**Cattle activity**	**E. coli Log10CFU.100ml**	**OC Log10CFU.100ml**
Upstream	27	No	2.61	2.34
	42	No	2.43	1.70
	57	No	2.56	2.32
	72	No	2.73	2.18
	87	No	2.81	2.45
	102	No	2.54	2.11
	117	No	2.43	2.36
	132	No	2.48	2.59
	147	No	2.48	2.86
	162	No	2.40	2.81
	177	No	2.54	2.86
	192	No	2.43	2.53
	207	No	NA	NA
	222	No	2.30	2.08

Appendix A30. Water concentrations of *E. coli* and Other Coliforms downstream of the cattle access site on Event 13 (February 7, 2018).

Location	Minutes	Cattle activity	***E. coli*** **Log_{10}CFU.100ml**	**OC** **Log_{10}CFU.100ml**
Upstream	27	No	2.40	2.51
	42	No	2.46	4.19
	57	No	2.40	2.30
	72	No	2.70	2.11
	87	No	NA	NA
	102	No	2.45	2.45
	117	No	2.43	2.23
	132	No	2.41	2.64
	147	No	2.43	2.72
	162	No	2.43	2.69
	177	No	2.36	2.69
	192	No	2.56	2.68
	207	No	2.56	2.54
	222	No	2.40	2.20

Appendix A31. Water conductivity upstream of the cattle access site on Event 13 (February 7, 2018).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Upstream	27	649.6	11.0
	42	636.3	10.5
	57	648.6	10.1
	72	640.0	10.1
	87	651.4	10.3
	102	653.3	10.6
	117	640.3	12.1
	132	646.1	12.2
	147	644.4	11.1
	162	NA	12.1
	177	653.6	10.3
	192	655.6	9.9
	207	640.7	10.3
	222	637.2	11.3

Appendix A32. Water conductivity downstream of the cattle access site on Event 13 (February 7, 2018).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Downstream	27	638.7	12.5
	42	645.1	11.5
	57	641.8	11.2
	72	655.3	11.2
	87	650.4	10.9
	102	653.4	10.5
	117	651.9	10.8
	132	648.3	10.8
	147	644.5	11.5
	162	671.5	10.8
	177	641.0	10.3
	192	648.9	10.1
	207	652.5	9.9
	222	638.5	10.3

Appendix A33. Water concentrations of nutrients and total suspended solids on Event 14 (March 21, 2018).

Location	Minutes	Cattle activity	NH_4-N ($mgN.L^{-1}$)	SRP ($\mu g.L^{-1}$)	TRP ($\mu g.L^{-1}$)	TP ($\mu g.L^{-1}$)	NO_3-N ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	0.08	49	53	80	7.58	27.88	9.8
	42	No	0.07	44	51	76	7.42	27.82	10.3
	57	No	0.07	44	51	63	7.64	27.73	7.5
	72	No	0.07	45	55	83	7.44	27.71	11.8
	87	No	0.07	49	56	82	7.46	27.60	8.3
	102	No	0.09	51	57	82	7.50	27.59	11.1
	117	No	0.08	53	59	76	7.52	27.80	7.4
	132	No	0.07	51	57	77	7.45	27.70	8.7
	147	No	0.06	50	60	78	7.46	27.82	7.7
Downstream	27	No	0.07	49	53	79	7.52	37.43	11.5
	42	No	0.05	44	48	64	7.68	32.97	11.0
	57	No	0.05	47	50	75	7.55	29.27	12.0
	72	No	0.04	47	53	66	7.54	28.13	12.0
	87	No	0.06	49	52	76	7.70	29.26	9.7
	102	No	0.06	53	59	78	7.54	27.66	9.5
	11	No	0.06	51	61	92	7.59	28.10	5.2
	132	No	0.06	49	59	93	7.50	27.84	9.0
	147	No	0.05	51	56	91	7.61	28.17	7.6

Appendix A34. Water concentrations of *E. coli* on Event 14 (March 21, 2018).

Location	Minutes	Cattle activity	*E coli* Log_{10}CFU.100ml
Upstream	27	No	3.65
	42	No	3.39
	57	No	3.27
	72	No	3.20
	87	No	3.15
	102	No	2.86
	117	No	3.05
	132	No	2.94
	147	No	2.90
Downstream	27	No	3.60
	42	No	3.41
	57	No	3.15
	72	No	3.22
	87	No	3.13
	102	No	3.10
	117	No	2.99
	132	No	3.01
	147	No	3.03

Appendix A35. Water conductivity on Event 14 (March 21, 2018)

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Upstream	27	691.4	10.2
	42	696.0	10.5
	57	694.0	11.3
	72	695.1	10.2
	87	689.4	9.6
	102	693.7	9.8
	117	693.6	9.7
	132	695.0	11.3
	147	704.0	11.3
Downstream	27	701.3	8.7
	42	692.2	9.4
	57	711.0	9.9
	72	695.8	9.8
	87	692.1	9.5
	102	694.4	9.0
	117	696.8	8.8
	132	700.0	10.6
	147	693.8	10.2

Appendix A36. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 15 (April 25, 2018).

Location	Minutes	Cattle activity	NH_4-N ($mgN.L^{-1}$)	SRP ($\mu gP.L^{-1}$)	TRP ($\mu gP.L^{-1}$)	NO_3-N ($mgN.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	0.27	100	145	6.58	29.07	7.7
	42	No	0.28	106	161	6.68	29.23	9.7
	57	No	0.21	105	147	6.74	29.27	8.7
	72	No	0.29	101	160	6.80	30.46	8.7
	87	No	0.27	97	158	6.77	29.83	9.7
	102	No	0.31	101	165	6.82	30.07	9.3
	117	No	0.30	101	168	6.83	31.00	9.3
	132	No	0.31	104	174	6.72	34.58	12.3
	147	No	0.30	108	172	6.83	35.75	11.3
	162	No	0.38	104	178	6.85	34.16	16.7
	177	No	0.48	104	173	6.96	35.41	19.7
	192	No	0.37	100	118	6.85	32.55	94.0
	207	No	0.40	104	97	6.90	31.92	10.0
	222	No	0.40	111	106	6.74	31.21	13.3

Appendix A37. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 15 (April 25, 2018).

Location	Minutes	Cattle activity	NH_4-N (mgN.L^{-1})	SRP (µg.L^{-1})	TRP (µgP.L^{-1})	NO_3-N (mgN.L^{-1})	Cl^- (mg.L^{-1})	TSS (mg.L^{-1})
Downstream	27	No	0.24	106	89	6.80	29.67	NA
	42	No	0.23	106	74	6.53	28.74	21.0
	57	No	0.24	115	101	6.61	28.86	9.0
	72	No	0.25	111	101	6.64	29.61	6.3
	87	No	0.24	110	93	6.63	29.53	6.3
	102	No	0.25	108	93	6.62	29.95	9.5
	117	No	0.25	109	85	6.59	30.14	6.0
	132	No	0.29	112	97	6.57	33.70	7.7
	147	No	0.29	103	87	6.50	34.84	7.7
	162	No	0.34	113	109	6.60	33.58	8.0
	177	No	0.31	107	124	6.63	31.14	9.7
	192	No	0.41	110	109	6.86	32.10	10.0
	207	No	0.33	106	117	6.86	31.79	11.0
	222	No	0.40	109	115	6.66	30.89	10.0

Appendix A38. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 15 (April 25, 2018).

Location	Minutes	Cattle activity	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
Upstream	27	No	3.38	3.34
	42	No	3.60	3.56
	57	No	3.48	3.72
	72	No	3.54	3.83
	87	No	3.30	3.90
	102	No	3.36	3.82
	117	No	3.30	3.79
	132	No	3.40	3.88
	147	No	3.34	4.08
	162	No	3.56	4.00
	177	No	3.58	4.18
	192	No	3.41	4.15
	207	No	3.48	3.94
	222	No	3.40	3.84

Appendix A39. Water concentrations of *E. coli* and Other Coliforms downstream of the cattle access site on Event 15 (April 25, 2018).

Location	Minutes	Cattle activity	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
Downstream	27	No	3.36	2.90
	42	No	3.20	2.78
	57	No	3.54	2.70
	72	No	3.46	2.95
	87	No	3.51	3.30
	102	No	3.56	3.34
	117	No	3.32	3.34
	132	No	3.40	3.32
	147	No	3.46	3.40
	162	No	3.57	3.32
	177	No	3.18	3.20
	192	No	3.32	3.15
	207	No	3.52	3.38
	222	No	3.40	3.34

Appendix A40. Water conductivity upstream of the cattle access site on Event 15 (April 25, 2018).

Location	**Minutes**	**Conductivity ($\mu S.cm^{-1}$)**	**Temperature (˚C)**
Upstream	27	640.3	10.7
	42	646.6	10.4
	57	636.6	10.6
	72	631.1	11.6
	87	636.6	11.5
	102	633.5	12.0
	117	647.9	12.3
	132	653.2	11.4
	147	656.5	12.4
	162	654.2	11.5
	177	649.3	11.0
	192	656.5	10.6
	207	652.3	11.3
	222	655.8	12.3

Appendix A41. Water conductivity downstream of the cattle access site on Event 15 (April 25, 2018).

Location	**Minutes**	**Conductivity ($\mu S.cm^{-1}$)**	**Temperature (˚C)**
Downstream	27	NA	NA
	42	NA	NA
	57	636.3	12.9
	72	644.3	8.3
	87	648.6	8.5
	102	642.0	9.9
	117	644.4	10.0
	132	659.8	9.1
	147	664.2	9.5
	162	653.9	9.4
	177	648.8	9.8
	192	645.4	10.4
	207	657.7	9.9
	222	649.0	10.7

Appendix A42. Water concentrations of nutrients and total suspended solids upstream of the cattle access site on Event 16 (June 13, 2018).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	NH_4-N ($mgN.L^{-1}$)	SRP ($\mu gP.L^{-1}$)	NO_3-N ($mgN.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Upstream	27	No	-	-	0.06	137	5.42	27.82	7.0
	42	No	-	-	0.03	135	5.40	27.92	8.3
	57	No	-	-	0.03	137	5.44	28.51	3.3
	72	No	-	-	< L.O.D.	136	5.56	27.85	7.5
	87	Yes	5	No	0.02	135	5.41	28.14	5.3
	102	Yes	5	U+D	0.02	132	5.45	27.98	4.3
	117	Yes	4	No	0.03	133	5.42	28.18	3.0
	132	No	-	-	0.06	135	5.45	28.41	4.7
	147	No	-	-	0.12	138	5.51	28.63	6.0
	162	No	-	-	0.11	138	5.51	28.14	5.3
	177	Yes	4	No	0.07	137	5.55	28.25	5.3
	192	Yes	3	No	0.04	134	5.49	28.69	6.7
	207	No	-	-	0.06	137	5.43	28.20	4.0
	222	No	-	-	0.06	132	5.16	27.92	4.7

Appendix A43. Water concentrations of nutrients and total suspended solids downstream of the cattle access site on Event 16 (June 13, 2018).

Location	Minutes	Cattle activity	Max cattle	Defecation/ Urination	NH_4-N ($mgN.L^{-1}$)	SRP ($\mu gP.L^{-1}$)	NO_3-N ($mgN.L^{-1}$)	Cl^- ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Downstream	27	No	-	-	0.02	137	5.37	27.73	10.0
	42	No	-	-	< L.O.D.	128	5.37	27.32	1.7
	57	No	-	-	< L.O.D.	132	5.39	27.40	6.7
	72	No	-	-	< L.O.D.	131	5.39	27.65	4.7
	87	Yes	5	No	< L.O.D.	129	5.01	26.93	7.7
	102	Yes	5	U+D	0.21	143	5.55	28.42	34.2
	117	Yes	4	No	0.24	139	5.62	29.01	16.0
	132	No	-	-	0.07	132	5.11	27.91	8.7
	147	No	-	-	0.11	131	5.48	28.19	4.0
	162	No	-	-	0.12	132	5.51	28.23	7.7
	177	Yes	4	No	0.12	133	5.51	28.17	9.3
	192	Yes	3	No	0.09	131	6.20	31.13	9.7
	207	No	-	-	0.06	130	5.50	28.29	8.3
	222	No	-	-	0.04	129	5.45	27.65	6.7

Appendix A44. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 16 (June 13, 2018)

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	*E. coli* Log_{10}CFU.100ml	OC Log_{10}CFU.100ml
Upstream	27	No	-	-	2.99	3.70
	42	No	-	-	2.92	3.15
	57	No	-	-	2.91	3.48
	72	No	-	-	2.82	3.30
	87	Yes	5	No	2.89	3.53
	102	Yes	5	U+D	2.74	2.62
	117	Yes	4	No	3.05	2.60
	132	No	-	-	3.71	NA
	147	No	-	-	3.90	2.59
	162	No	-	-	3.69	NA
	177	Yes	4	No	3.73	2.58
	192	Yes	3	No	3.66	2.54
	207	No	-	-	3.30	NA
	222	No	-	-	3.51	2.82

Appendix A45. Water concentrations of *E. coli* and Other Coliforms upstream of the cattle access site on Event 16 (June 13, 2018)

Location	Minutes	Cattle activity	Max cattle	Urination/Defecation	***E. coli*** $\mathbf{Log_{10}CFU.100ml}$	**OC** $\mathbf{Log_{10}CFU.100ml}$
Downstream	27	No	-	-	3.61	4.45
	42	No	-	-	3.56	3.79
	57	No	-	-	3.41	3.94
	72	No	-	-	3.36	3.67
	87	Yes	5	No	3.13	3.85
	102	Yes	5	U+D	4.90	NA
	117	Yes	4	No	4.79	NA
	132	No	-	-	4.31	3.23
	147	No	-	-	3.99	3.00
	162	No	-	-	4.02	3.45
	177	Yes	4	No	4.40	3.95
	192	Yes	3	No	4.28	NA
	207	No	-	-	4.25	NA
	222	No	-	-	2.75	NA

Appendix A46. Water conductivity upstream of the cattle access site on Event 16 (June 13, 2018).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Upstream	27	633.6	10.1
	42	631.8	9.9
	57	620.3	9.8
	72	638.5	10.6
	87	623.6	11.0
	102	626.5	10.8
	117	631.9	11.7
	132	627.5	11.6
	147	634.3	11.6
	162	634.2	11.5
	177	621.0	11.2
	192	628.7	11.2
	207	NA	NA
	222	627.2	11.3

Appendix A47. Water conductivity downstream of the cattle access site on Event 16 (June 13, 2018).

Location	Minutes	Conductivity ($\mu S.cm^{-1}$)	Temperature (˚C)
Downstream	27	631.2	10.5
	42	624.9	10.7
	57	645.2	11.7
	72	630.6	10.8
	87	633.5	10.5
	102	634.8	10.8
	117	641.2	10.9
	132	632.4	10.9
	147	632.4	10.8
	162	638.1	10.8
	177	633.5	10.7
	192	631.1	11.7
	207	629.5	11.6
	222	627.6	10.8

Appendix B1. Summary of the information obtained in the survey carried out with farmers involved in the study.

Catchment	Site	Type and number of animals	Cattle introduced in field	Cattle removed from field	Period of grazing	Slurry spreading	Nutrient management plan
Brackan River (BK)	BK1A	NA					
	BK1B	Mixed livestock, 25 – 30 cows plus calves	Early April	End of October 2016	7 – 10 days in field and off for one month	Farmyard manure in autumn	Yes
	BK2A/BK3B	Beef, 30 cows plus 15 calves	April	September	Animals in field for summer months	No slurry spreading	No
	BK2B	Mixed livestock, 25 – 30 cows plus calves, also silage/hay	Early April	October 2016	7 – 10 days in field and off for one month	No slurry spreading	Yes
	BK3A	NA					
Commons River (CM)	CM1	Replacements for dairy herd, 1.5 LU/Ha	Mid-March	Mid-October		No slurry spreading	Yes
	CM2	NA					
	CM3	Dairy, 1.6 LU/Ha	April 1	December 1		One slurry spreading	Yes
Milltown Lake (MT)	MT1	50 dairy cows	April	Mid-November	2 days in field, 3 weeks off	Slurry spreading once every 2 – 3 years	No
	MT2	20 beef suckler cows	April	Mid-November	4 – 5 weeks out, same period off	No slurry spreading	No
	MT3	NA					

Appendix B1 (continued): Summary of the information obtained in the survey carried out with farmers involved in the study.

Catchment	Site	Type and number of animals	Cattle introduced in field	Cattle removed from field	Period of grazing	Slurry spreading	Nutrient management plan
Munster Blackwater (BW)	BWA	Mixed livestock, 10 cows	Mid-April	September/ October	2 weeks, 3 times per year	Slurry spreading once in end of summer	No
	BWB	NA					
	BWC	Beef, calves and weenlings, 20 animals	Mid-July	End of October	Rotations depending on grass growth	Slurry spreading once in July, not always	Yes
Douglas River (DG)	DGA	Mixed livestock, 20 – 25 animals	June	Mid-October/Mid-November	1 week in field, 1 week off	Slurry spreading once a year in July	No
	DGB	NA					
	DGC	NA					

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