Innovative Stormwater Management: From Monitoring to Solutions

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A thesis submitted to Newcastle University in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science, Agriculture and Engineering

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In memory of my beloved dad.

Abstract

Urban runoff poses flooding risks and pollutes surface water. Extreme weather events exacerbate these problems; in a changing climate and increasing urbanisation, innovation in stormwater monitoring and management is urgently needed. We therefore validated a method for onsite assays with a miniature speaker-sized qPCR instrument and other portable equipment items to rapidly identify faecal pollution marker genes in drainage systems and rivers. We deployed the portable method in a mobile laboratory ('lab in a van') and quantified HF183 marker genes for human host associated *Bacteroides* in river water within 3 h of sampling. We used the mobile laboratory to investigate urban river water and effluents from two storm drains and a retention pond and found significantly higher HF183 gene levels in the older storm drain compared to the river water (6.03 ± 0.04 vs. 4.23 ± 0.03 $\log_{10}$ gene copies per 100 mL). Based-on such qPCR methods and next generation sequencing (NGS) with the memory-stick sized MinION, we investigated spatiotemporal variation in the bacteriology of the urban river Ouseburn for different weather conditions. We found that the river microbiome of the Ouseburn changes from mainly freshwater bacteria during dry weather to mainly faecal bacteria during storm events. For a storm event, we matched >70% of bacteria in the Ouseburn where it flows through a public park to those found in combined sewer overflow (CSO) discharge. Nature-based solutions are sustainable practices for stormwater management to reduce flood risks whilst minimizing pollution. We designed an innovative 'pollution munching' permeable pavement with 2% activated carbon (AC) amendment in the subbase, which showed significant total nitrogen and nitrate reduction from leachates compared with a conventional sand base permeable pavement. Hydraulic tests showed that the AC amended system still met the design criteria for permeable pavements, making it a promising proposition for stormwater management.

Declaration

I hereby certify that this work is my own, except otherwise acknowledged, and that it has not been submitted previously for fulfilment of a degree at this or any other university.

Rixia Zan

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List of Acronyms

AC = Activated carbon

AOB = Ammonia-oxidizing bacteria

CSOs = Combined sewer overflows

DIC = Dissolved inorganic carbon

DOC = Dissolved organic carbon

NGS = Next generation sequencing

NOB = Nitrite-oxidizing bacteria

ONT = Oxford nanopore technologies

PAHs = Polycyclic aromatic hydrocarbons

PCA = Principal component analysis

QMRA = Quantitative microbial risk assessment

SuDS = Sustainable Drainage Systems

TDC =Total dissolved carbon

TDS = Total dissolved solids

TN = Total nitrogen

TP = Total phosphorus

TSS = Total suspended solids

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Chapter 1

General introduction

Chapter 1. General Introduction

In 2015, The United Nations published the 17 Sustainable Development Goals (SDGs) including 169 targets to address these global challenges by 2030 (UN, 2015). In line with other national governments, the UK government is committed to delivery of the SDGs. SDG 6 aims to achieve 'Clean water and sanitation for all' and the SDG 11, target 11.5 aims to reduce the number of death and number of people affected by disasters such as flooding. Sustainable stormwater management relates to these goals and targets, as stormwater is a potential flood risk (Chan et al., 2018) and pollution source to the water environment (Werbowski et al., 2021, Schreiber et al., 2019, Ahmed et al., 2019a). Globally, the urban population will reach 68% of total population by 2030 (UN, 2018). This rapid urbanisation, population growth, and climate change exacerbate the challenges for stormwater monitoring and management (Jegatheesan et al., 2019).

Conventional urban drainage systems convey sewage and stormwater runoff from impermeable surfaces in the same pipes, and release stormwater via overflows when the flow in these combined sewers exceeds the drainage capacity to prevent stormwater back-ups in the drainage systems (Tibbetts, 2005). However, the high frequency of combined sewer overflow (CSO) spillages is nowadays raising public concerns and leading to parliamentary debate in the UK. There is a lack of understanding of CSO discharge characteristics and related impacts on river water quality. For example, the House of Commons Environmental Audit Committee reported recently on the river water quality in the UK and found 'the claim made by the chief executive of Severn Trent that its sewer overflow discharges were 'pretty much rainwater' to be disingenuous. As water companies do not routinely test the quality of the discharges from storm overflows, they are in no position to make this claim (EAC, 2022).' This debate led to the Environment Act 2021 which makes provisions about improved monitoring and reducing storm overflows (GOV.UK, 2021). Storm water management challenges also occur in modern, separated sewer systems which reduce the risk of releasing untreated sewage into the environment by conveying surface runoff and sewage in separated pipes. However, wastewater may still reach separated surface water drainage systems via misconnected appliances from households (Ellis and Butler, 2015), and illicit dumping of waste into surface water drains, causing pollution of receiving water bodies (Panasiuk et al., 2015). Impacts on bathing water quality are a major uncertainty and concern for stormwater management (Ahmed et al., 2019a). In the UK, only two rivers are designated as bathing water, and consequently the microbial water quality of most rivers is not routinely monitored by the Environment Agency. However,

children will always play in rivers irrespective of what notices are put up next to them (Whitty et al., 2022). More broadly, according to the SDGs report 2022 (DESA, 2022), for 'at least 3 billion people the quality of water they rely upon is unknown due to lack of monitoring.' As part of this monitoring challenge, improved methods for the real-time and continuous monitoring of CSO impacts on river water quality are urgently needed to achieve the SDG 6 'clean water and sanitation for all.'

Molecular diagnostics for the monitoring of infectious disease have developed fast during the COVID-19 global pandemic (Anonymous, 2021). Nowadays, portable sequencing devices such as the MinION from Oxford Nanopore Technologies enable environmental DNA (eDNA) analysis on site and in near real-time (Acharya et al., 2020). However, limitations of nanopore sequencing technology such as lower accuracy compared with other platforms (i.e. Illumina), and time-consuming bioinformatic data processing of large sequencing data sets call for complementary water testing methods (Werner et al., 2022). Quantitative polymerase chain reaction (qPCR) is an ideal complement to sequencing, as it produces quantitative results quickly and can achieve high specificity for the targeted genetic region via the use of TaqMan probes (Acharya et al., 2019). Quantifying marker genes of special interest such as human pathogens (Capone et al., 2020) and human host associated *Bacteroides* (Ahmed et al., 2019b) by qPCR assays, enables faecal pollution source tracking. For all these reasons, qPCR methods should also be made portable to complement eDNA sequencing.

While monitoring is essential for understanding stormwater pollution sources and their impacts on river water quality, engineering interventions are ultimately needed to reduce and control these sources. To mitigate more frequent urban flooding and stormwater pollution, Sustainable Drainage Systems (SuDS) became a legal requirement for new developments in the UK (DEFRA, 2018). A good SuDS provides benefits for both, stormwater quantity and quality control, and in addition benefits biodiversity and amenity of blue-green city spaces (Woods Ballard, 2015). SuDS provide solutions for cost-effective management of stormwater, and permeable pavement is one of the SuDS widely applied in the UK. However, conventional permeable pavement reportedly results in nitrate and nitrogen leaching (Drake et al., 2014b, Brown and Borst, 2015, Razzaghmanesh and Borst, 2019) which poses a risk to groundwater resources. Better description of the microbial community characteristics in stormwater, permeable pavements and their leachates, is essential to understanding biotransformation processes such as nitrification and for improving permeable pavement designs and other stormwater management facilities. Activated carbon is produced from carbon rich materials

such as coal, coconut shells and wood chips (Chowdhury et al., 2013). It has been applied to water treatment (Chowdhury et al., 2013), sediment contamination remediation (Ghosh et al., 2011), and gas purification (Marsh and Rodríguez-Reinoso, 2006). Due to its large surface area, porous structure and diverse functional groups on the surface, AC is an excellent sorbent material of pollutant retention, and support material for microbial growth. Amending AC to stormwater management facilities such as stormwater bioretention systems (Sang et al., 2019) and stormwater biofilters (Aiello et al., 2018, Ulrich et al., 2015) showed promising removal of various pollutants. It could therefore be sensible to add a small amount of AC into the base layer of permeable pavements to enhance their pollutant removal efficiency. However, AC application in permeable pavements has not yet been investigated.

This thesis seeks to develop and demonstrate innovative stormwater management solutions from better monitoring methods to better understanding of related impacts on the water environment to better pollution control solutions.

1.1 Aim, objectives, and hypotheses

The overall aim of this thesis is to improve monitoring of stormwater pollution and CSOs impacts on river water quality at the catchment scale and evaluating an innovative permeable pavement design for improved stormwater quality control. Therefore, this thesis developed

1) on-site qPCR assays to enable faecal pollution source tracking in catchments with a mobile laboratory in a van; 2) eDNA based monitoring methods to investigate CSOs impacts on the bacteriology of an urban river in the northeast of England, and 3) an innovative permeable pavement using AC for improved stormwater management.

Objectives and hypothesis:

Objectives and hypotheses 1, 2, and 3 are associated with thesis Chapter 3, 4 and 5, respectively.

1. To develop and demonstrate a methodology for onsite qPCR assays that can rapidly quantify faecal pollution marker genes in urban drainage and river water samples using only portable equipment items.
   Hypothesis: qPCR assays can be performed on-site to quantify faecal pollution maker genes in drainage and river water samples within hours of sampling.

Author Contributions: Conceptualization, David Werner, Kishor Acharya and Rixia Zan; Methodology, Rixia Zan, Kishor Acharya and Adrian Blackburn; Software, David Werner;

Validation, Rixia Zan; Formal analysis, Rixia Zan and David Werner; Investigation, Rixia Zan, Kishor Acharya, Adrian Blackburn and David Werner; Resources, David Werner; Data curation, Rixia Zan and David Werner; Writing—original draft preparation, David Werner; Writing—review and editing, Rixia Zan, Kishor Acharya, Adrian Blackburn and Chris G. Kilsby; Visualization, David Werner; Supervision, David Werner; Project administration, David Werner; Funding acquisition, Chris G. Kilsby.

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2. To understand the short and longer-term impacts of CSO discharge on the bacteriology of an urban river, and implications for public health.
   Hypothesis: CSO discharge has high levels of faecal bacteria and substantially alters the bacteriology of a receiving river which poses a significant risk to public health.

Author contributions: Conceptualization, Rixia Zan and David Werner; Methodology, Rixia Zan and David Werner; Software, Rixia Zan and David Werner; Validation, Rixia Zan; Formal analysis, Rixia Zan and David Werner; Investigation, Rixia Zan, David Werner, Adrian Blackburn and Jidapa Plaimart; Resources, David Werner and Chris Kilsby; Data curation, Rixia Zan and David Werner; Writing—original draft preparation, Rixia Zan and David Werner; Writing—review and editing, Jidapa Plaimart, Chris Kilsby, Claire Walsh, and Ross Stirling; Visualization, Rixia Zan; Supervision, David Werner, Claire Walsh and Ross Stirling.

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3. To evaluate the performance of an innovative permeable pavement with an AC amended base material, in comparison with a conventional permeable pavement, in terms of stormwater infiltration and quality control.
   Hypothesis: AC amendment of the base material in permeable pavements can significantly improve the infiltrating stormwater quality.

Author Contributions: Conceptualization, Rixia Zan, David Werner, and Ross Stirling; Methodology, Rixia Zan, David Werner, and Ross Stirling; Software, Rixia Zan and David Werner; Validation, Rixia Zan; Formal analysis, Rixia Zan and David Werner; Investigation, Rixia Zan, David Werner and Ross Stirling; Resources, David Werner and Ross Stirling; Data curation, Rixia Zan and David Werner; Writing—original draft preparation, Rixia Zan; Writing—review and editing, Rixia Zan, David Werner and Ross Stirling; Visualization, Rixia Zan; Supervision, David Werner and Ross Stirling.

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Chapter 2

Literature Review

Chapter 2. Literature Review

2.1 Stormwater, urban drainage systems, and their impacts on the water environment

2.1.1 The stormwater management challenge

In 2015, the United Nations published 17 sustainable development goals (SDGs) which include 169 targets to address these global challenges by 2030 (UN, 2015). For example, SDG 6 aims to achieve 'clean water and sanitation for all'. The SDG target 6.3 seeks to reduce water pollution, eliminate dumping, and promote a safe water reuse, while the SDG target 6.6 seeks to protect and restore water-related ecosystems, such as aquifers and rivers (UN, 2015). Also, SDG 11 target 11.5 is to reduce number of death and number of people affected by water related disasters such as flooding (UN, 2015). The UK is committed to delivering the SDGs. With reference to SDG6 and its target 6.6, the European Water Framework Directive (WFD) (2000/60/EC) also seeks to achieve good chemical and ecological status for surface water and groundwater. It was adopted as The Water Environment (Water Framework Directive) (England and Wales) Regulations 2017 in UK law following Brexit (GOV.UK, 2017). To reach good chemical and ecological status of the environment, stormwater as a potential pollution source, should be monitored and managed properly.

With the world's population steadily growing, people living in cities will reach 68% of the global population by 2050 (UN, 2018). This rapid urbanisation and population growth brings challenges for stormwater management. Urban runoff transports various pollutants either from accumulation on impermeable surfaces during dry periods or precipitation from the atmosphere to receiving water bodies (Liu et al., 2018). These pollutants include suspended solids, nutrients (Aucour et al., 2013), heavy metals (Liu et al., 2018, Wicke et al., 2021), micropollutants, oil (Wicke et al., 2021), and human pathogens (Schreiber et al., 2019, Sidhu et al., 2012).

2.1.2 Conventional urban drainage systems

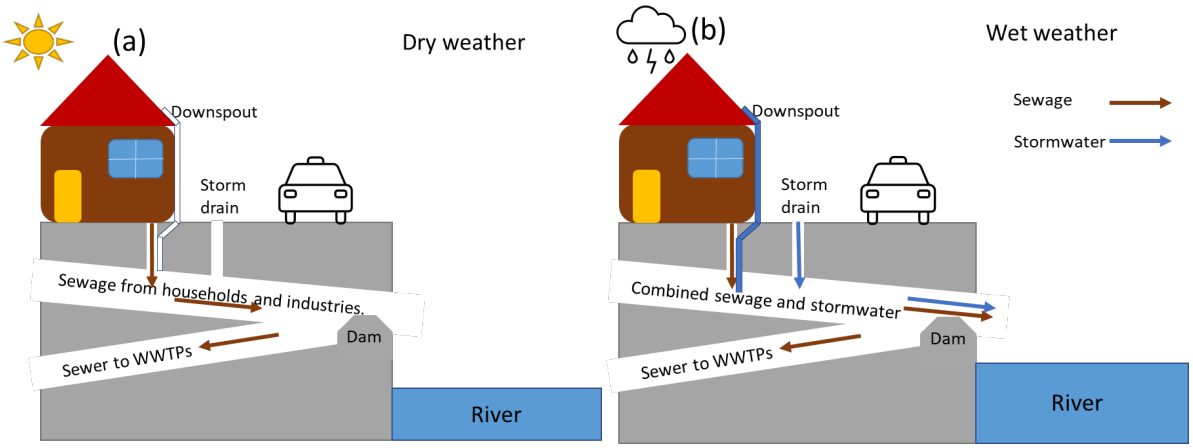

Figure 2.1. Combined sewer systems under dry (a) and wet (b) weather conditions.

In the middle of the 19th century, a lot of household sewerage was directly discharged into the river Thames in London creating the event called the "Great Stink" (Halliday, 1999). There were also several outbreaks of cholera in England in the 19th century, when most people believed that the disease was coming from the bad quality of air or 'Miasma.' Later, Dr John Snow proved that the cholera outbreaks were linked to the polluted water supply from shallow wells and the river Thames (Snow, 1849). Combined sewer systems such as the London sewer systems were subsequently introduced in the late 19th century when the government realised the urgency of improving Thames River water quality. Combined sewer systems were designed to collect municipal sewage, industrial wastewater and runoff from roads and roofs in the same pipes to carry waste and runoff out of cities, which was a great idea to reduce waterborne diseases in the late 19th century (Halliday, 1999). In London and elsewhere, combined sewer systems were designed many years ago for smaller cities with lower populations, but nowadays their capacity is more frequently exceeded due to population growth. For example, to increase the capacity of the combined sewer system in London, the Thames Tideway Scheme was started in 2016. This mega project will be finished by early 2025 and cost about £4.3 billion (Tideway, 2022). **Figure 2.1** illustrates how a combined sewer system works under different weather conditions. During dry weather, the sewage from households and industries is discharged into the combined sewers which typically rely on gravity flow to convey the sewage to wastewater treatment plants (WWTPs) (ASCE, 1992). When it rains, the urban run-off from roofs and streets also enters the combined sewer systems and is conveyed together with the sewage for treatment at WWTPs. During heavy rainfall events, the flow of municipal sewage and industrial wastewater in combination with the stormwater flow may exceed the capacity of sewers (Riechel et al., 2016). To prevent urban flooding and the risk of sewage backing up into houses during heavy rainfall or snowmelt, the combined sewer system contains outlets or

overflows (CSOs) that discharge directly into receiving water bodies without any treatment (Tibbetts, 2005, Riechel et al., 2016). Rapid urbanisation with increasing areas of impermeable surfaces (Tran et al., 2020) and climate change cause more frequent heavy rainfall events (Jiang et al., 2018) meaning that CSOs nowadays spill very frequently, causing concerns about related impacts on the water environment. The CSO discharges were also reported to correlate with gastrointestinal illness of recreational water users (Miller et al., 2022).

In the UK, CSOs were relabelled as storm overflows and are permitted to discharge when the flow in the sewer exceeds criteria for the minimum retained flow (GOV.UK, 2022b). There are around 15,000 CSOs on the network in England, of which 13,350 discharge to inland rivers (GOV.UK, 2021). The number of storm overflows spills recorded by water companies increased 27% in 2020 compared with the 2019 record (EAC, 2022). Indeed, the actual number of spills including unpermitted spills may be much larger than the water companies reported according to an artificial intelligence model developed by Professor Peter Hammond (EAC, 2022). The report of the Environmental Audit Committee also questioned claims made by Water Industry executives that storm overflows are 'pretty much already rainwater', as water companies are in no position to make this claim without routinely testing the storm overflows (EAC, 2022). The Hansard debate in parliament ultimately lead to The Environment Act 2021 which makes provisions about reporting, monitoring, and reducing storm overflows (GOV.UK, 2021). Following the Environment Act 2021, the Department of Environment Food & Rural Affairs (DEFRA) published the storm overflows discharge reduction plan in August 2022 (DEFRA, 2022). The plan aims to reduce overflows discharge from the 2020 levels by around 25% by 2025. Furthermore, 'the water companies will have: 1) to improve all overflows discharging into or near every designated bathing waters; and improved 75% of overflows discharging to high priority sites by 2035. 2) No storm overflows will be permitted to operate outside of unusually heavy rainfall or to cause any adverse ecological harm by 2050 (DEFRA, 2022).'

For better stormwater management, urban drainage systems are nowadays often designed with separated pipes for conveying sewage and urban runoff. Separated sewer systems is a legal requirement in UK for new developments (DEFRA, 2018). In separated sewer systems, only the sewage is conveyed to the local WWTPs, while the urban runoff is conveyed separately via stormwater pipes to be discharged into receiving water bodies, with or without treatment in stormwater management facilities. **Figure 2.2** illustrates how separated sewers operate under different weather conditions. Compared with the combined sewer systems, although separated

sewers minimize discharge from the foul sewers into receiving water via overflows, misconnections of household appliances into the surface water drainage pipes, illicit dumping of wastewater into gullies, and broken sewers may still cause wastewater discharge into receiving water bodies (Ellis and Butler, 2015). Households may unintentionally misconnect their dishwashers, toilets, washing machines and showers into stormwater drains, for example when building extensions, which results in regular discharges to surface water under both dry and wet weather conditions, and degradation of the receiving water quality.

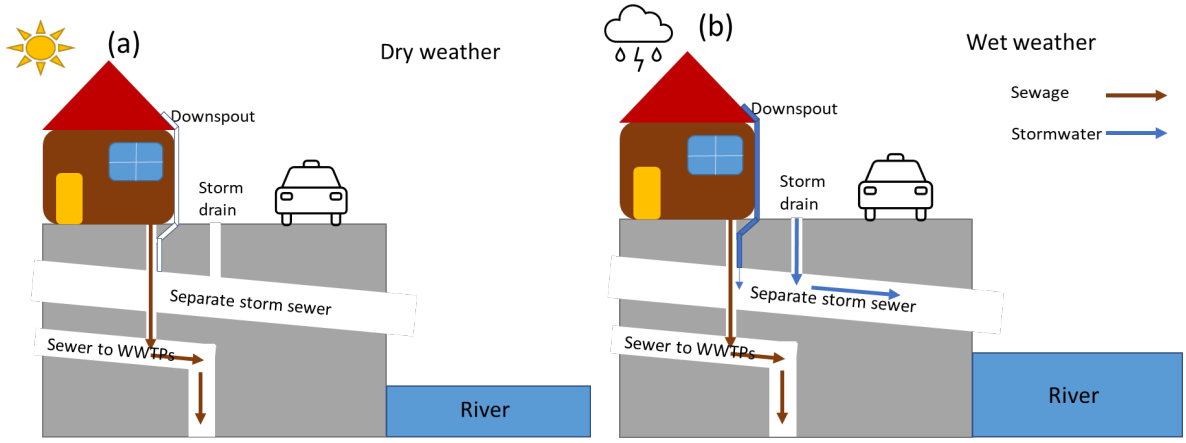

Figure 2.2. Separated sewer systems under dry (a) and wet (b) weather conditions.

2.1.3 Pollutants in urban stormwater and their impacts on surface water

Urban stormwater can be a significant source of nutrients (Yang and Toor, 2018), heavy metals (Ma et al., 2016), human pathogens (Schreiber et al., 2019, Ahmed et al., 2019a), and micropollutants (i.e. flame retardants, pesticides, polycyclic aromatic hydrocarbons) (Wicke et al., 2021) to aquatic systems (Werbowski et al., 2021). The chemical and microbial cocktail in stormwater may eventually result in the receiving water bodies failing to meet the standard for 'good chemical and ecological status (EAC, 2022).'

Discharging nutrients such as nitrogen or phosphorus with urban stormwater into the aquatic environment contributes to eutrophication (Yang and Toor, 2018), which is toxic to aquatic life, and affects urban aesthetics. In the UK, new developments need to carefully consider the nutrient impacts on internationally protected Habitats Sites, such as lakes and rivers, and nutrient neutrality has been imposed on sensitive catchments (GOV.UK, 2022c, Hughes, 2022). Nitrogen is an important nutrient widely present in the environment and exists in various forms including organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO), or inorganic nitrogen gas (N_2). The process of nitrogen cycling is biological and includes nitrogen fixation, ammonification, nitrification, and denitrification.

Figure 2.3 shows the typical nitrogen cycle in the natural environment. In aerobic environments, ammonium (NH_4^+) is converted to nitrite and nitrate by nitrifying organisms such as ammonia oxidizing bacteria (AOB. i.e. *Nitrosomonas europaea*, *Nitrosomonas oligotropha*, *Nitrosospira sp. 40K1*) and ammonia oxidizing archaea (AOA. i.e. *Nitrosopumilus maritimus, Nitrosopumilus piranensis, Nitrosopumilus adiactus)* (Martinez-Rabert et al., 2022) and nitrite oxidizing bacteria (NOB, i.e. *Nitrobacter winogradskyi*, *Nitrobacter hamburgensis*, *Nitrobacter vulgaris* and *Nitrobacter alkalicus)* (Koops and Pommerening-Röser, 2001). In anoxic environments, nitrate is converted to nitrogen gas by denitrification organisms such as denitrifying bacteria *Paracoccus denitrificans*, *Pseudomonads stutzeri,* and *Aeromonas spp.* (Karanasios et al., 2010). The nitrogen gas can be fixed from the atmosphere and converted to ammonium by nitrogen fixing bacteria such as *Azotobacter* spp. and *Azospirillum* spp. (Nassar et al., 2020). However, the nitrifying and denitrifying bacteria need a specific pH range and oxygen conditions for optimal growth. In conventional wastewater treatment, operational settings can be controlled to achieve nitrogen removal by nitrification followed by denitrification processes, but achieving the same in stormwater management facilities brings major challenges (Collins et al., 2010b). Ashoori et al. (2019) evaluated pilot-scale woodchip bioreactors that showed effective nitrate removal from the stormwater, which indicated a potential application of woodchips in stormwater management facilities for reducing nitrate under realistic scenarios.

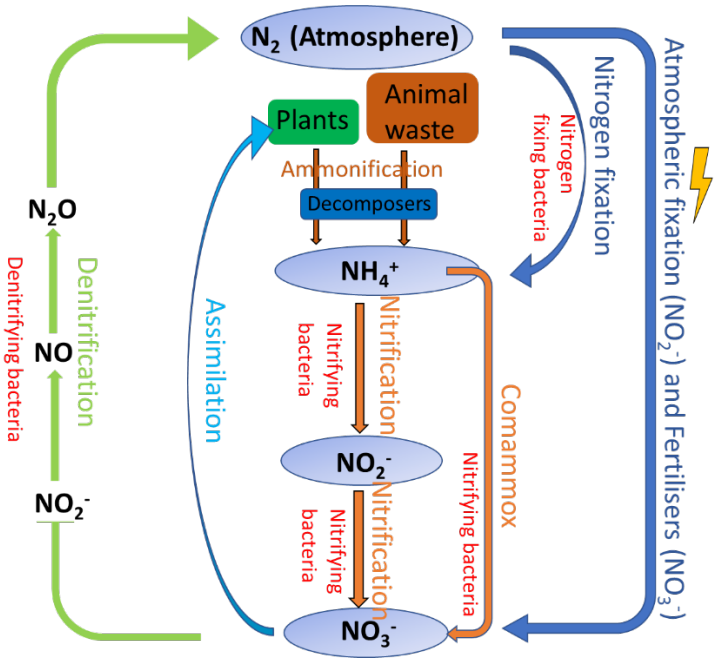

Figure 2.3. Nitrogen cycle in the natural environment.

Phosphorous is another important nutrient in the natural environment and largely used to produce chemical fertilizers for agriculture (Di Capua et al., 2022). Unlike nitrogen in the nitrogen cycle, phosphorous cannot convert into a gas that escapes from water or soil into the atmosphere (Paytan and McLaughlin, 2007). The major forms of phosphorous in water are particulate and dissolved (i.e. orthophosphate (PO_4^{3-})) (Paytan and McLaughlin, 2007). Dissolved phosphate can be converted into insoluble minerals that remove phosphorous from water into the solid phase of soils and sediments. In conventional wastewater treatment, phosphorous is removed by adding calcium, magnesium, aluminium and iron ions to react with the phosphate and form minerals (Di Capua et al., 2022). New ideas for phosphate removal include polyphosphate accumulating organisms (PAOs) that remove phosphorus from water into the sludge (Di Capua et al., 2022). For stormwater management, phosphorous can be removed mainly by sedimentation, for example in wetlands or other best stormwater management practices (Sample et al., 2012).

Heavy metals are also commonly found in stormwater, and the main sources of heavy metals are traffic related such as vehicle exhaust, tyre wear, and brake wear (Jegatheesan et al., 2019, Liu et al., 2018, Ma et al., 2016). For example, car park runoff may contain high concentrations of heavy metals together with polycyclic aromatic hydrocarbons (PAHs) in oil as shown in **Figure 2.4**, posing a threat to surface water (Wicke et al., 2021) and groundwater (Pinasseau et al., 2020). Potential methods to treat these pollutants include stormwater bioretention cells (DiBlasi et al., 2009) and media filter drains (Thomas et al., 2015) which showed effective reductions under laboratory conditions.

Figure 2.4. Oil pollution on the permeable (a) and impermeable (b) pavement of car parks after rain.

Human pathogens can also be detected in stormwater, and they normally get into the stormwater via mixing with sewage in combined sewer systems, and misconnected household

appliances in separated sewer systems (Ellis and Butler, 2015, Miller et al., 2022, Schreiber et al., 2019). Contamination of bathing waters by CSOs is a public health concern (Andersen et al., 2013, Ahmed et al., 2019a). In the UK, the Bathing Water Regulations 2013 assesses bathing water quality using *E. coli* and *Intestinal Enterococci* as indicators. However, there are only two rivers that include designated bathing water sites in the UK (GOV.UK, 2022a). The microbial water quality of UK rivers is therefore not regularly monitored by the Environment Agency. However, as stated in a joint opinion from Whitty et al. (2022) 'children have always played in waterways and always will, irrespective of what notices are put up next to them.' The absence of frequent monitoring data bring challenges for stormwater management and risk assessment (Andersen et al., 2013). Potential treatment solutions for microbial hazards such as stormwater biofilters and constructed wetlands were summarized by Feng et al. (2022). In general, stormwater biofilters and constructed wetland showed effectively microbial removal but need further disinfection for stormwater harvesting application.

Other emerging pollutants such as organic micropollutants, pharmaceuticals (Wicke et al., 2021) and microplastics (Jakubowicz et al., 2022) were also reported to be present in stormwater. The complex pollutant mixtures in stormwater not only bring challenges for stormwater management but also for the monitoring of stormwater quality, which is essential for better understanding the stormwater pollution.

Table 2.1 lists selected priority stormwater pollutants and their concentrations from published work (Wicke et al., 2021, Zgheib et al., 2012, Gasperi et al., 2012, Eriksson et al., 2007, Jakubowicz et al., 2022). In general, BOD, all the metals and PAHs (apart from benzo(a)pyrene) in stormwater exceeded the Water Framework Directive (England and Wales) 2017 standards for 'good chemical and ecological status' of surface water.

Table 2.1. List of selected priority stormwater pollutants, mean concentrations from published work, and related surface water quality standards.

Parameters	Concentration [1]	Standards
Basic water chemistry		Water Framework Directive (England and Wales) 2017
BOD (mg/L)	86 [b,d]	4-5 (90th percentile) [2]
COD (mg/L)	41 [b,d]	Not applicable
Ammonium (mg/L)	0.51[b]	0.3-0.6 (90th percentile) [2]
Total Phosphorous (mg/L)	0.66 [b,c]	Not applicable
Reactive Phosphorous (mg/L)	0.078 [b]	0.027 [2,3]
TSS (mg/L)	89 [b,c,d]	Not applicable
Metals		
Zn (µg/L)	544 [a,b]	10.9[4]
Cu (µg/L)	119 [a,b,c]	1[4]
Pb (µg/L)	48 [a,b,c]	14[5]
Cr (µg/L)	8 [b,c]	4.7, 3.4[4]
Ni (µg/L)	7 [a,b]	Not applicable
Caffeine (µg/L)	1.2 [b]	Not applicable
Acesulfame (µg/L)	0.26 [b]	Not applicable
PAHs (Σ16) (µg/L)	1.50 [b,c]	Not applicable
Benzo(a)pyrene (µg/L)	0.077 [b,c]	0.27[5]
Benzo(b)fluoranthene (µg/L)	0.147 [b,c]	0.017[5]
Benzo(k) fluoranthene (µg/L)	0.057 [b,c]	0.017[5]
Benzo(g,h,i)-pyrene (µg/L)	0.081 [b,c]	8.2×10^{-3}[5]
Indeno(1,2,3-cd)-pyrene (µg/L)	0.113 [b,c]	Not applicable
Faecal indicator bacteria		Bathing Water Regulations 2013 'Sufficient bathing water'
E. coli (CFU/100mL)	61,587 [e]	900 (90th percentile)
Enterococci (CFU/100mL)	Not applicable	330 (90th percentile)

a. (Jakubowicz et al., 2022) b. (Wicke et al., 2021) c. (Zgheib et al., 2012) d. (Eriksson et al., 2007) e. (Schreiber et al., 2019)

[1]: The concentrations were calculated from published mean values.

[2]: Standards for classification as good status rivers.

[3]: Annual mean concentration of reactive phosphorous for the site under reference conditions was estimated as 7 µg/L.

[4]: Standards for fresh water.

[5]: Maximum allowable concentration for inland surface water.

2.1.4 Real-time monitoring of stormwater discharges, quality, and related impacts

The monitoring of CSOs spillages in the UK as reported by water companies to the Environment Agency (EA) to fulfil their permitted conditions of storm overflows discharge via event-duration monitoring (EDM) data is published by the EA every year (EA, 2022). The Rivers Trust put these data into the public domain by mapping them for the whole of the UK, which raised awareness by the public (RiversTrust, 2022). Greater awareness led to an investigation by the Environment Audit Committee (EAC) of the House of Commons, which was holding an enquiry into water quality in rivers. The report published by the House of Commons highlighted that 'establishment of a complete overview of the health of rivers in England and the pollution affecting them is hampered by outdated, underfunded and inadequate monitoring regimes' (EAC, 2022). In addition, the report pointed out that the current range of pollutants being monitored is too narrow (EAC, 2022). Based on these concerns from the public, more real-time and continuous monitoring to improve understanding of water quality and help with decision making is urgently needed. Many innovative monitoring techniques such as online monitoring with in-situ sensors (Jiang et al., 2020) and the use of environmental DNA (eDNA) methods (Edge et al., 2021) for faecal pollution source tracking (Ahmed et al., 2019a) are emerging. Also, risk based water quality thresholds for recreational water have been proposed based on eDNA methods such as the genetic marker for human host associated *Bacteroides* HF183 (Boehm and Soller, 2020). Using eDNA data generated from portable equipment can inform rapid quantitative microbial risk assessment (QMRA) near the sampling site (Halla et al., 2022). A true DNA sensor has not yet been developed for the testing of river water (Werner et al., 2022), but portable sequencing device such as the MinION from Oxford Nanopore Technologies (ONT) enabled onsite analysis of eDNA in near real time (Acharya et al., 2020). However, portable sequencing generates large data sets that require time for processing with bioinformatic methods, which is delaying the availability of results, and current technology limitations also mean that the taxonomic assignments derived from portable eDNA analysis are not yet 100% reliable (Werner et al., 2022). Therefore, quantitative polymerase chain reaction (PCR) is an ideal complement to sequencing and should be made portable (Acharya et al., 2019). Progress has been made with semi-quantitative methods using paper-based devices for detecting pathogens in water (Hui et al., 2020), but more flexible and quantitative PCR (qPCR) methods for onsite water quality testing would be desirable for eDNA based monitoring of water quality.

2.2 Sustainable Drainage Systems (SuDS)

To mitigate against the more frequent urban flooding events, and stormwater pollution, innovative stormwater management systems are being put forward by many countries. For example, 'sponge city' strategies were initiated by Chinese government in 2014, when 29 cities were selected to become pilot 'sponge cities' with stormwater management facilities that reduce urban flood risks, increase water retention capacity and re-use stormwater resources (Chan et al., 2018). Similar strategies are pursued in Australia, where they are called water sensitive urban design (WSUD) (Jegatheesan et al., 2019). Also, Sustainable Drainage Systems (SuDS) became a legal requirement for new developments in the UK (DEFRA, 2018), and low impact developments (LIDs) have been successfully applied in many places in the United States and Canada (Jegatheesan et al., 2019). These innovative stormwater management practices are normally nature-based, sustainable and environmental-friendly for stormwater quantity and quality control. Birkinshaw et al. (2021) evaluated the impact of newly developed stormwater detention ponds at the catchment scale, showing that with these detention ponds, new residential development had only minimal impacts on the peak flow of the urban river Ouseburn. There is a growing body of literature recognizing that these innovative stormwater management strategies delivered multiple benefits not just for surface runoff control, but also for water pollution control, natural habitats, increasing aesthetic value and public amenity (Jegatheesan et al., 2019, Chan et al., 2018, Woods Ballard, 2015). The main benefits of SuDS are 1) protecting people and property from flood risks, 2) protecting the quality of groundwater and recreational surface water; 3) protecting natural habitats and increasing biodiversity and habitats for highly urban area; 4) creating attractive places to encourage people walking, playing, and enjoying living; 5) saving water resources where they are scarce; 6) delivering low-cost, low-carbon footprints and nature-based solutions for stormwater management.

Better understanding of SuDS benefits and application challenges for each type of SuDS is essential for appropriate design. The main design criteria for SuDS are water quantity management, water quality management, amenity and biodiversity (Woods Ballard, 2015). To retain or infiltrate water close to where it falls during high intensity and short duration rainfall events without flooding properties is the key driver of SuDS design (Woods Ballard, 2015). To protect the receiving water bodies such as groundwater and urban rivers, water quality control should be considered simultaneously (Woods Ballard, 2015). The water quality should be monitored regularly, and design criteria should be based on supporting the management of water quality in receiving surface waters and groundwaters. Apart from the water quantity and

quality, amenity and biodiversity are normally considered together (Jegatheesan et al., 2019). A good SuDS should be multi-functional and easy to adapt for future development and climate change (Jegatheesan et al., 2019).

In the UK, SuDS became a legal requirement for new developments since 2015 (Jegatheesan et al., 2019). Woods Ballard (2015) published the CIRIA SuDS manual to provide guidance for SuDS design and construction. There are many kinds of SuDS, which means they can be adapted flexibly to the local setting. **Table 2.2** lists various types of SuDS currently under development in the UK, and their benefits and challenges. Overall, the SuDS may deliver several benefits, however lack of guidelines for design, construction and maintenance are highlighted in several SuDS review papers. Also, due to SuDS being insufficiently advertised, few stakeholders install SuDS on their own volition (Muttuvelu et al., 2022). Current design manuals, long-term performance data and maintenance data for SuDS are insufficient. Based on experiences gathered by Muttuvelu et al. (2022), there is a need for standardization of design, construction, monitoring and maintenance of SuDS.

Table 2.2. List of application area and challenges for current SuDS practices.

Types of SuDS	Application area	Benefits	Challenges
Rainwater harvesting (Pala et al., 2021)	Rooftop, garden	Water saving and reuse	Unpredictable rainfall High installation and maintenance cost (Zang et al., 2022) Cannot aid large scale farming
Green/Blue roofs (Shafique et al., 2018)	Roofs	Temperature control, noise reduction, biodiversity enhancing, increasing biological habitats	High initial construction and maintenance cost Roof leakage problem
Filter strips & Swales (Gavrić et al., 2019)	Roadside, car parks, and public open spaces	Efficient for small rainfall events Efficient for nutrient removal	Limited efficiency during intense storm events Large spaces needed Less willingness from stakeholders to implement
Filter drains (Woods Ballard, 2015)	Public open spaces, adjacent to impermeable surfaces	Efficient for long-term metals removal (Thomas et al., 2015)	Less effective for heavy rainfall events
Bioretention basin/ Rain garden (Shafique, 2017)	Public open spaces, small area (less than 2 ha)	Enhance biodiversity and reduce temperature High efficiency for nutrient and organic pollutants removal	Difficult to retrofit Lack of guidelines for construction and maintenance Mosquito breeding reported
Permeable pavements (Muttuvelu et al., 2022)	Private driveways, car parks, side walks	Efficient for flood risk reduction, low-cost	Clogging Frequent maintenance required Higher nitrate leaching reported
Ponds and wetlands (Woods Ballard, 2015)	Open space	Multi-functional Permanent solution for water treatment Enhancing biodiversity	Large spaces needed

2.3 Permeable pavement systems

In response to the call for development of SuDS, permeable pavement systems (PPS) are nowadays more widely used to reduce runoff from hard-surfaced areas such as car parks, private driveways, open marketplaces, and footpaths (Eisenberg et al., 2015). A typical PPS can provide functions such as stormwater infiltration, attenuation, and groundwater recharge (Kuruppu et al., 2019, Woods Ballard, 2015). The benefits of PPS compared with conventional impermeable pavements are reducing runoff volumes and peak discharge rates, increasing infiltration and groundwater recharge, improving water quality, reducing stormwater temperature and heat island effects from pavements, and reducing drainage system infrastructure and costs (Eisenberg et al., 2015).

Figure 2.5. Base and sub-base of concrete block permeable pavement.

An example of permeable pavement is shown in **Figure 2.5**. **Figure 2.6** shows the typical structure for a permeable pavement system, base layer, sub-base layer, and surfaces. There are four types of PPS according to their surfaces, porous asphalt (PA), pervious concrete (PC), permeable interlocking concrete pavement (PICP) and grid pavement (GP) (Eisenberg et al., 2015, Kuruppu et al., 2019, Woods Ballard, 2015). Porous asphalt is similar to conventional asphalt but without the fines to increase the void space for water drainage (Eisenberg et al., 2015). For example, activated carbon amended porous asphalt has 20% increased void space compared with other porous asphalt and it showed good ability for pollutant removal (Huang and Liang, 2021). Pervious concrete is manufactured by adding aggregate into a cement mixture to maintain interconnected void space (Eisenberg et al., 2015). As a result, it has a coarser appearance than the conventional concrete, and additives can increase strength and improve binding. Permeable interlocking concrete pavement (PICP) or concrete block permeable pavement (CBPP) is made of impermeable concrete blocks paved with joint

materials that maintain drainage through stone/sand-filled gaps between the blocks (Interpave, 2018). The grid pavement systems are modular plastic or concrete elements filled with aggregates, and they can be vegetated (Woods Ballard, 2015).

Figure 2.6 shows the typical structure of PPS. According to the infiltration system of PPS, there are total infiltration systems which allow water to infiltrate to the subgrade without drainage pipes, partial infiltration and no infiltration systems with perforated drainage pipes to convey water away from the site, if required (Woods Ballard, 2015). The total infiltration system (**Figure 2.6. a)**) is simple, and no drainage pipes are needed which reduces the construction cost but requires high infiltrate water quality to avoid contamination of the subgrade soil. In a partial filtration system (**Figure 2.4 b)**), the proportion of the rainfall that exceeds the infiltration capacity of the subgrade soil flows to the receiving drainage system (Woods Ballard, 2015). This can occur by direct drainage through the sub-base or by conveyance via perforated pipes within or below it. If the subgrade soil has low permeability or strength, no infiltration system (**Figure 2.4. c)**) would be better (Woods Ballard, 2015). Under this system, the PPS is normally wrapped in an impermeable, flexible membrane placed above the subgrade. Once the filtrate reaches the sub-base, it is conveyed to the outfall via perforated pipes or drains. Partial and no infiltration systems would require pipe connections, thereby increasing the cost for construction and maintenance. Selection of the most appropriate system for PPS is depending on the application site, hydraulic demand, and cost. More technical details can be found in the CIRIA SuDS manual (Woods Ballard, 2015).

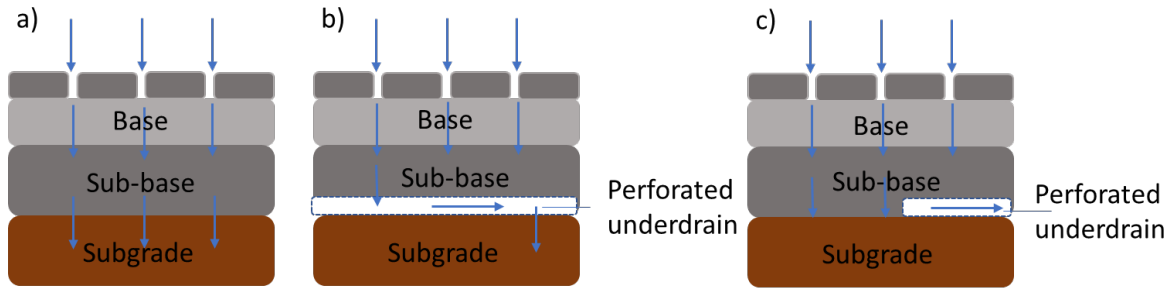

Figure 2.6. Typical structure of permeable pavement systems (PICP as example) for a) total infiltration system, b) partial infiltration system and c) no infiltration (Adapted from Woods Ballard (2015)).

A typical design consideration follows the order infiltration system selection, hydraulic and structural design (Woods Ballard, 2015, Eisenberg et al., 2015, Interpave, 2018). Selection of the PPS infiltration system is mainly depending on the subgrade soil permeability. **Table 2.3** lists the most suitable infiltration systems for different subgrade soil permeabilities. For the hydraulic design, volume control, peak flow mitigation and exceedance flow control are

compulsory requirements for the PPS design. The required capacity of PPS depends on characteristics of rainfall, return period, infiltration potential into the subgrade, and discharge constraints. For the structural design, the selection of surfacing, depth of base and sub-base layer is mainly based on the traffic loading and appearance that is required (Woods Ballard, 2015). The structural design for permeable pavement is based on conventional pavements, as surface materials used for PPS need to be strong enough to support the traffic loading (Woods Ballard, 2015, Eisenberg et al., 2015).

Table 2.3. Permeability of subgrade and suitable infiltration system for PPS (Interpave, 2018)

Permeability of subgrade (m/s)	Total infiltration	Partial infiltration	No infiltration
1×10^{-6} -1×10^{-3}	√	√	√
1×10^{-8} -1×10^{-6}	×	√	√
1×10^{-10} -1×10^{-8}	×	×	√

Table 2.4 lists the published performance evaluations of PPS for water quantity and quality control. According to Collins et al. (2008), three types of one-year old PPS showed good ability on surface runoff reduction and peak flow mitigation over 6 months monitoring. The performance of PPS on pollutants removal is inconsistent from the reviewed studies. Most of studies showed PPS had good ability for total suspended solid, ammonia, and metals removal (Collins et al., 2008, Tota-Maharaj and Scholz, 2010, Drake et al., 2014a, Drake et al., 2014b). However, higher levels of nitrate and total nitrogen leaching were reported and the potential reason is that the PPS cannot provide anoxic environments and carbon sources for denitrifying bacteria to complete the nitrification-denitrification cycle (Brown and Borst, 2015). Drake et al. (2014a) mentioned that PPS in winter had significant Na and Cl in effluent related to the use of road salt. Most studies showed that the pH of PPS effluent is higher than that of stormwater runoff (Drake et al., 2014a, Drake et al., 2014b, Tirpak et al., 2020). Although 7 years monitoring was conducted in one PPS study (Razzaghmanesh and Borst, 2019), the long-term monitoring of PPS performance in terms of hydraulic behaviour and water quality is scarce. Tota-Maharaj and Scholz (2010) evaluated faecal indicator bacteria (FIB) in PPS effluent, and although the PPS showed 98% FIB reduction, the monitoring was conducted only at the laboratory scale. The degree of stormwater treatment provided by a PPS is depending on the surrounding land use, pavement and drainage design, traffic loading and climatic conditions. There needs to be a more coordinated collection of data on the long-term

performance of permeable pavements across a wide range of water quality parameters relevant for better design, construction, and maintenance in the future.

Table 2.4. Permeable pavement system performance from published work (PC: porous asphalt, PICP: permeable interlocking concrete pavement, PC: pervious concrete, CGP: concrete grid pavement).

Reference	PPS Type	Study term, scale and site	Hydraulic behaviour	Water quality control performance
Collins et al. (2008) and Collins et al. (2010a)	PC, PICP and CGP filled with sand All PPS constructed with perforated underdrain	6 months Pilot-scale California, USA	98-99% surface runoff reduction for all three PPS	• Higher pH of PPS effluent compared with atmospheric deposition. • Lower NH_4^+ -N and TKN concentration of PPS effluent than runoff • CGP effluent had higher NO_3^- -N • No difference among three PPS was found regarding of TP and TSS concentration in the PPS underdrain.
Tota-Maharaj and Scholz (2010)	PICP with geotextile	14 months Laboratory Edinburgh, UK	Not applicable	• 84.6% and 77.5% removal of NH_4^+-N and ortho-P, respectively • 98-99% *E. coli* and faecal *Streptococci* removal • 91%, 82% and 88% removal of TSS, turbidity and BOD, respectively
Drake et al. (2014a) and Drake et al. (2014b)	PC and PICP Partial infiltration with underdrain	24 months Pilot-scale Ontario, Canada	Not applicable	• Higher pH of effluent compared with runoff • Less NH_4^+, NO_2^- and Organic N in PPS effluent, but higher NO_3^--N in effluent was found. • Higher TN concentration in effluent in winter compared with spring, summer, and autumn. • More than 80% TSS reduction • Significant reduction of Al (PICP only), Cu, Fe, Mn, and Zn. • Significant oil and grease reduction.
Brown and Borst (2015)	PICP, PC and PA	12 months Pilot-scale New Jersey, USA	Not applicable	• PA effluent had higher TN, but lower TP compared with PICP and PC. • The dominant nitrogen species in all three PPS was NO_3^- -N.
Razzaghmanesh and Borst (2019)	PICP, PC and PA	82 months Pilot-scale New Jersey, USA	Not applicable	• PA had higher NH_4^+ -N, NO_2^- -N, and TN, but lower ortho-phosphorous effluent. • PC and PICP didn't change TN concentration. • No difference between PC and PICP.
Tirpak et al. (2020)	PICP	15 months Pilot-scale Ohio, USA	Not applicable	• Higher pH of effluent compared with runoff. • 60% and 53% reduction for TKN and organic N, respectively. • 79% TSS reduction. • Fe, Mn, and Cr were significantly reduced.

The costs of PPS depend on surface materials, base and sub-base layer depth and design. Operation and maintenance costs should also be considered. However, Kuruppu et al. (2019) discussed that due to the cost of substructure requirements for PPS and clogging issues, installing it in large scales may not be economical.

In terms of maintenance, a common finding from PPS studies is that clogging negatively affects the hydraulic performance of PPS (Muttuvelu et al., 2022, Eisenberg et al., 2015, Kuruppu et al., 2019). The presence of oil and grease speeds up the clogging for PPS (Aryal et al., 2015). Eisenberg et al. (2015) thus suggested at least twice a year a vacuum sweep for the PPS to avoid the clogging.

2.4 Application of activated carbon in stormwater management

Activated carbon (AC) is a low-cost material normally generated from carbonaceous materials like coal, coconut shell and wood (Chowdhury et al., 2013). Due to its large surface area, porous structure, and diverse functional groups on the surface, it has been widely applied as a sorbent in agriculture, industries, gut disease treatment and environmental engineering applications such as drinking water filtration (Chowdhury et al., 2013), sediment contamination remediation (Ghosh et al., 2011), air purification (Marsh and Rodríguez-Reinoso, 2006), and so on. Granular activated carbon (GAC) and powdered activated carbon (PAC) are the two common types of AC being used for water treatment and soil remediation (Hale et al., 2012). The main mechanism of pollutant removal by AC is adsorption and ion exchange, but includes microbial pollutant degradation in biological activated carbon filters (Bushnaf et al., 2017). When biomass accumulates on GAC's large surface area (Vignola et al., 2018), biotreatment can become the primary treatment mechanism instead of adsorption (Ulrich et al., 2015). This mode of AC treatment is often called biologically enhanced activated carbon (BAC). **Table 2.5** shows the physical properties of typical GAC and PAC adsorbents. The surface area, carbon content, and surface functional groups of AC are dependent on its feedstock, activation chemicals, activation time and so on (Chowdhury et al., 2013). Nitrogen, oxygen, hydrogen, and sulphur are normally present in AC as functional groups or atoms chemically bonded to the structure. Typically, AC has excellent organic pollutant removal capacity especially for potable and wastewater treatment applications. Also, AC can be modified to obtain specific functional groups on the surface for targeted pollutant removal (Bhatnagar et al., 2013). The modification can be physical, chemical, and biological. According to Bhatnagar et al. (2013)'s review, there are eight types of modifications for AC, acid treatment, base (alkaline) treatment,

impregnation, microwave treatment, ozone treatment, plasma treatment, and biological modification.

AC as amendments for biofiltration or bioretention systems in stormwater facilities were reported to increase the pollutant adsorption capacity, retention time, and therefore increasing the lifetime and treatment efficiency of stormwater facilities (Aiello et al., 2018, Sang et al., 2019). According to Mohamed et al. (2023), incorporation of sludge-based AC and commercial AC in biofilter can reduce the negative environmental impacts up to 29-40% over a 40-50 years lifespan. Previous studies investigating the application of activated carbon mainly focus on stormwater pollution control in such facilities. **Table 2.6** lists the published work on application of activated carbon in stormwater management facilities. In general, AC has a great potential to be applied in stormwater management facilities, but 75% of the reviewed studies (Genç-Fuhrman et al., 2016, Björklund and Li, 2017, Aiello et al., 2018, Yue et al., 2018) were conducted under laboratory conditions. Various pollutants like nutrients, metals, organic pollutants and micropollutants can be removed by AC, but the removal performance is not consistent. For example, Björklund and Li (2017) reported that 0.5% sludge-based AC amended rain garden soil can enhance by 5-8 times the adsorption capacity for organic pollutants such as PAHs. However, to ascertain these benefits, pilot-scale and long-term studies should be conducted under realistic outdoor conditions with real stormwater in the future. Besides the chemical pollutants, human pathogens presented in stormwater should also be considered in the future evaluations of AC benefits.

Table 2.5. Typical activated carbon physical characteristics.

Porosity	Surface area (m^2/g)	Particle density (kg/m^3)	Dry density (kg/m^3)		Average particle size (mm)	
			GAC	PAC	GAC	PAC
0.5-0.8	500-1,200	600-850	300-650	200-750	0.6-3.0	0.01-0.03

Adapted from Chowdhury et al. (2013)

Although there are many benefits of AC for environmental engineering applications, saturation of AC sorption sites is a potential risk for pollutant control in the long term, and this would cause less effective treatment. Therefore, the effluent water quality from SuDS with AC should be monitored systematically. According to León et al. (2020)'s estimation, the AC from nutshell would cost approximately USD 2.15/kg for production. For a large-scale stormwater

management facility, using AC with regular replacement would thus increase the installation and operational costs.

Table 2.6. List of published work on stormwater management facilities using AC.

Reference	SuDS type	Type of AC	Target pollutants/property	Pollutant removal performance	Scale
LeviRam et al. (2022)	Stormwater biofiltration systems	Bioaugmented GAC	Atrazine	100%	Laboratory
			TN	94-98%	
			TP	85-92%	
			TSS	79-86%	
Huang and Liang (2021)	Porous asphalt permeable pavements	PAC	Engineering properties	20% void space increased with amending PAC	Laboratory
Hu et al. (2019)	Porous asphalt permeable pavements	PAC	COD, Zinc and Lead	Not applicable	Laboratory
Sang et al. (2019)	Bioretention system	GAC	COD	75%	Pilot-scale
			TN	22%	
			TP	95%	
Yue et al. (2018)	Batch study Adsorption	Sludge-based AC	PO_4^{3-} - P	4.8-46.3%	Laboratory
			NO_3^- - N	17.3-83.3%	
Aiello et al. (2018)	GAC and zeolite amended stormwater biofilter	GAC	Phosphorous	8.9%	Pilot-scale
			Dissolved copper	90.0%	
Björklund and Li (2017)	Batch and column study	Sludge-based AC	Organic pollutants	Not applicable	Laboratory
Genç-Fuhrman et al. (2016)	Batch study	GAC	Heavy metals	50-75% for all metals Cd, Cr, Cu, Ni and Zn	Laboratory

2.5 Research gaps

In the UK, the Environment Act 2021 calls for a reduction of stormwater discharges and their negative impacts on the natural environment and public health (GOV.UK, 2021). According to The Water Environment (Water Framework Directive) (England and Wales) Regulations 2017 the current chemical and ecological status of surface waters in the UK is well established, but

since only two UK rivers contain designated bathing water sites, the microbial water quality of rivers in the UK is not regularly monitored. In the absence of data, the nature of CSO discharge and resulting impacts on river microbiomes and public health remains uncertain. To close this knowledge gap, and to establish a baseline for the assessment of future improvements, innovative methods for faecal pollution source tracking that can monitor the impact of CSO discharges on the microbiology of UK rivers are urgently needed.

SuDS provide a clear way forward for improved stormwater management in the urban environment, and permeable pavement systems can be retrofitted into existing urban spaces to facilitate stormwater infiltration from hard-surfaced areas like driveways, foot paths and parking lots. However, while permeable pavements deliver many benefits for stormwater quantity and quality control, there are reports of nitrate leaching which is a potential risk for groundwater quality (Drake et al., 2014b, Brown and Borst, 2015). Also, the long-term evaluation of PPS performance in terms of their hydraulic behaviour and pollutant removal across the wide range of relevant water quality metrics are insufficient. Especially, understanding of their microbial community characteristics and resulting impacts on pollutant biotransformation in PPS is lacking. There are several studies suggesting that using AC in a sustainable drainage system may deliver many benefits, however, to the best of our knowledge, there is no published work on using AC as amendments for base materials of permeable pavements. Generally, there are three deficiencies in many stormwater management research projects, i) use of artificial instead of real stormwater in experimental settings, ii) short-term monitoring, iii) lack of outdoor pilot-scale experiments.

Chapter 3

A mobile laboratory enables faecal pollution source tracking in catchments with onsite qPCR assays

Chapter 3. A mobile laboratory enables faecal pollution source tracking in catchments with onsite qPCR assays

3.1 Abstract

Onsite molecular diagnostics can revolutionize faecal pollution source tracking. We aimed to validate a method for onsite qPCR assays with a miniature speaker-sized Q qPCR instrument and other portable equipment items. We showed that marker genes for total bacteria (16S) and *E. coli* (rodA) in 100 mL of river water measured with this method agreed within ±0.3 $\log_{10}$ units with results obtained when using conventional laboratory equipment items. We then deployed the portable method in a mobile laboratory ('lab in a van') and quantified HF183 marker genes for human host associated *Bacteroides* in river water within 3 hours of sampling. We also used the mobile laboratory to investigate urban river water and effluents from two storm drains and a retention pond and collected comprehensive microbial and physicochemical water quality data. We found significantly higher HF183 gene levels in the older storm drain compared to the river water (6.03±0.04 versus 4.23±0.03 $\log_{10}$ gene copies per 100 mL), and a principal component analysis revealed that storm drain effluent retention in a pond beneficially altered water characteristics, making them more similar to those of the receiving river. In conclusion, onsite qPCR assays can be performed with portable equipment items to quickly test water.

3.2 Introduction

As rapid progress is being made with molecular diagnostics in the global response to the Covid-19 pandemic, opinion pieces in the scientific literature (Aarestrup and Woolhouse, 2020) and mainstream media (Anonymous, 2021) are calling for near real time monitoring of microbial hazards not only "in the prison sick bay or the rural health centre", but also "on the farm, or at the town sewage works". In line with these calls for onsite diagnostics, environmental scientists and engineers are adapting nucleic acid-based tests like paper-based analytical devices (Mao et al., 2020), miniaturized loop-mediated isothermal amplification (LAMP) polymerase chain reaction (PCR) assays (Fu et al., 2021, Gowda et al., 2022), and portable next generation

sequencing (NGS) devices (Acharya et al., 2019) to applications in sewage epidemiology (Hui et al., 2020), faecal pollution source tracking (Pantha et al., 2021), environmental pathogen monitoring (Bridle et al., 2014) and antimicrobial resistance surveying (Reddington et al., 2020). According to the WHO, there are globally nearly 1.7 billion cases of childhood diarrheal disease every year, the second leading cause of death in children under five years old, which mostly results from contaminated food and water sources (WHO, 2017). Hence, it is vital deploy the molecular diagnostic tools and scientific advances made during the Covid-19 pandemic also in the fight against the 'permanent pandemic' of waterborne disease by testing water for indicators for faecal pollution (Tran et al., 2015) which may transmit pathogens like *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba coli*, rotavirus, norovirus, *Campylobacter jejuni*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, or enterotoxigenic and enteroadherent *Escherichia coli* (Teunis et al., 1996).

Environmental samples will typically contain much lower amounts of the targeted nucleic acids than clinical specimens, and the extraction of sufficient amounts of genomic material, the removal of interferents and inhibitors, and preventing the clogging of miniaturized devices are method development challenges for molecular diagnostics in environmental applications (Bridle et al., 2014). Such challenges are frequently side-stepped in proof-of-concept work for portable diagnostics by using cultured samples of high bacterial concentration (Gowda et al., 2022) or genetic material already extracted and purified with conventional laboratory methods (Fu et al., 2021, Martzy et al., 2017). However, the main advantages of onsite diagnostics are near-real time availability of data for decision making and the avoidance of sample alterations during transport and storage (Acharya et al., 2020). To realize these advantages the entire workflow from sampling to nucleic acid extraction and purification followed by molecular analysis and data interpretation must be performed onsite (Martzy et al., 2019).

For conventional, culturing-based microbial water quality assessments, field deployable tools like the Oxfam DelAgua® Water Testing kit are commercially available (Uprety et al., 2020). For molecular diagnostics, we have recently developed and validated a suitcase laboratory for

microbial community characterization by NGS of 16S rRNA gene amplicons with the memory-stick sized MinION sequencer of Oxford Nanopore Technologies (Acharya et al., 2020, Acharya et al., 2019). This suitcase laboratory cost about £10,000 and included a powerful laptop computer and all the required equipment items for the concentration, extraction, purification, amplification, and sequencing of environmental DNA (eDNA) in water samples. With this suitcase laboratory we have successfully demonstrated onsite characterization of microbial communities at a sewage treatment plant in the United Kingdom, and faecal pollution source tracking in low resource settings across Africa and South Asia (Acharya et al., 2020, Pantha et al., 2021, Thongsamer et al., 2021, Ho et al., 2021, Hiruy et al., 2022). NGS is an excellent tool for the fingerprinting of microbial communities to identify signatures of faecal pollution in water using SourceTracker (O'Dea et al., 2019, Pantha et al., 2021) or alternative multivariate data analysis methods (Ho et al., 2021, Thongsamer et al., 2021, Hiruy et al., 2022). However, NGS based diagnostics have limitations when it comes to the identification of rare species in diverse communities, and when the absolute rather than relative abundance of environmental microorganisms is of interest (Acharya et al., 2019). In such instances, quantitative polymerase chain reaction (qPCR) is the method of choice to more specifically and sensitively target and quantify marker genes of special interest such as those identifying human-host associated *Bacteroides* (Ahmed et al., 2016) or enteric human pathogens (Capone et al., 2020).

3.2.1 Aim

Our study aim was to develop and demonstrate a methodology for onsite qPCR assays which can rapidly quantify faecal pollution marker genes in water samples using only portable equipment items.

3.2.2 Objectives and hypotheses

To this end, we 1) compared portable with conventional qPCR workflows in a well-controlled laboratory setting; 2) compared the DNeasy Power Water and SoilPro extraction kits for a stormwater sample with a high amount of total suspended solids; 3) quantified marker genes

for human host associated *Bacteroides* onsite within hours of taking a river water sample; and 4) demonstrated the comprehensive onsite characterization of urban river, storm drain, and stormwater retention pond samples with microbiological and physicochemical methods using a mobile laboratory in the back of a van. These work packages tested the hypotheses that i) the mean results of qPCR assays obtained with portable and conventional equipment items are comparable within the typical range of uncertainty for three sample replicates; ii) the DNeasy Power Water kit is an appropriate choice for DNA extraction, even for stormwater samples with high suspended solid content; iii) qPCR assays can be performed onsite to generated quantitative microbial water quality information within hours; iv) onsite qPCR assays and physicochemical water quality tests can identify human sewage pollution sources in an urban catchment. This study is to the best of our knowledge the first field demonstration of onsite qPCR assays for the quantification of faecal pollution marker genes in environmental water at the sampling site.

3.3 Materials and Methods

3.3.1 Equipment

We purchased a portable, light-weight Q qPCR instrument from Quantabio (Beverly, USA). This instrument is miniature speaker-sized and the most portable qPCR instrument currently on the market. This qPCR instrument can process up to 48 samples per run and was controlled via a laptop computer. For comparison and method validation, we used a conventional Bio-Rad CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Watford, UK). For the water filtration and DNA extraction from the filter membranes we used a Rocker 400 oil free vacuum pump (Severn Sales, Shrewsbury, UK), Nalgene Reusable Filter Unit 250 × 250 mL (Scientific Laboratory Supplies, Nottingham, UK), Vortex (Thermo Fisher Scientific, Loughborough, UK) with adaptor for six 5 mL tubes (Scientific Industries, Bohemia, USA) , High-Speed Mini-centrifuge (Thermo Fisher Scientific, Loughborough, UK), Qubit fluorometer for DNA quantification (Life Technologies, Paisley, UK), and other small equipment items, as described in our previous publication (Acharya et al., 2020). For

comparison and validation of the DNA extraction procedure, we used a conventional bench-top ribolyser (FastPrep-24™ 5G bead beating grinder and lysis system, Thermo Fisher Scientific, Loughborough, UK). Specifications and costs of conventional and portable equipment items for cell lysis and qPCR are compared in **Table 3.1**.

Table 3.1. Specifications and costs of conventional and portable equipment items for cell lysis and qPCR.

Methodology	**Equipment**	**Weight kg**	**Dimensions (W x L x H in cm)**	**Costs £ incl. VAT**
Conventional	FastPrep-24™ 5G ribolyser	23.6	47.2 x 38.5 x 49	7,344
	Bio-Rad CFX Connect qPCR instrument with software	21.0	33 x 46 x 36	14,887
Portable	Vortex-Genie 2 with adaptor	4	12.2 x 16.5 x 16.5	518
	Q qPCR instrument	2	15 x 15 x 13	9,980

For the fieldwork we rented a "van with a lab" from Newcastle University's Bio Engineering: Wastewater Innovation at Scale facility BEWISe. This van contains a small laboratory in the back (**Figure 3.1a**) giving mobile access to power from rechargeable batteries, and shelter for setting up the portable equipment (**Figure 3.1b**) on a bench (**Figure 3.1c**). We also generated conventional microbiology and water chemistry metadata with a DelAgua® Water Testing kit (DelAgua, Marlborough, UK) and with cuvette tests on a portable spectrophotometer DR1900 (Hach, Manchester, UK). An ExStik handheld probe (Extech Instruments, Nashua, USA), HQ40D Digital two channel multi meter (HACH, Manchester, UK), and 2100Q portable turbidity meter (HACH, Manchester, UK) were used to measure water temperature, pH, conductivity, dissolved oxygen, and turbidity during sampling. A Model AL-DT portable alkalinity test kit (HACH, Manchester, UK) was used to measure alkalinity by titration.

Figure 3.1. a) Mobile laboratory in the back of a van; b) the equipment items needed for onsite marker gene quantification inside a suitcase for transportation, and c) the equipment items set up on the bench of the mobile laboratory, from left to right: Vacuum pump, filtration unit, qPCR instrument, laptop computer, pipettes and tips, centrifuge, fluorometer and vortex with adapter.

3.3.2 Comparison of portable and conventional qPCR workflows

For the portable method validation, we used river water from the Ouseburn, an urban river in Newcastle upon Tyne in northeast England. Several liters of river water with 2.6±0.1 NTU turbidity were collected in a sterile 5 L PE bottle from the Environment Agency gauging station at Crag Hall (55.025255 N, -1.634742 E), and 9 x 300 mL aliquots of the well-mixed river water were filtered through 0.22 µm Gridded Sterile Cellulose Nitrate Membrane Filters (Sartorius, Göttingen, Germany) and stored at -20°C. We then compared three different DNA extraction procedures: i) using a vortex with lysis tubes from the DNeasy PowerWater Kit following the manufacturer protocol (Qiagen, Crawley, UK); ii) using the same protocol with the vortex, but with the addition of 40 µl of 50 mg/mL lysozyme (Merck, Gillingham, UK) to

the lysis tubes followed by incubation at 37 °C for one hour; and iii) using our standard laboratory procedure with the ribolyser and Lysing Matrix E tubes (MpBiomedicals, Irvine, USA), instead of those provided with the extraction kits, so that the tubes would fit into the ribolyser slots. Each procedure was conducted in triplicate using one of the nine filters. Extracted DNA was quantified with the Qubit ds DNA HS Assay kit (Life Technologies, Paisley, UK) on a Qubit fluorometer (Life Technologies, Paisley, UK). Following DNA quantification, all the DNA samples were diluted with nuclease free water (Thermo Fisher Scientific, Loughborough, UK) to 5 ng/µL to avoid inhibitor effects in the qPCR assays. We then quantified 16S rRNA and rodA marker genes for each extract in duplicates using both the conventional and portable qPCR instrument. The 16S rRNA and rodA qPCR assays were chosen for this work because the portable and conventional qPCR instruments both had channels for the detection of the SYBR® Green and FAM dye used in these assays. The 16S rRNA assay, originally developed for Denaturing Gradient Gel Electrophoresis (DGGE) (Muyzer et al., 1993), is nowadays a widely utilized qPCR primer set for the 16S rRNA gene found in all bacteria and archaea, which informs phylogenetics. The rodA assay targets *E. coli*, a commonly used faecal pollution indicator bacterium, and uses a probe to increase the specificity of the assay (Chern et al., 2011). Details of the primers and probes and temperature programs used in these assays are shown in **Table 3.2.**

Table 3.2. Primers and probes with literature references, sequence, temperature cycling program, and metrics of the calibration curves on the portable qPCR instrument (N/A: no amplification).

Marker gene	**Primers and Probe**	**Program**	**Slope (Cq/(genes/µL))** **Average**±Stdev	**R^2** **Average**±Stdev	**Efficiency (%)** **Average**±Stdev	**NTC Cq** **Average**±Stdev	**Detection limit Cq Std1 (10 genes/ µL)** **Average**±Stdev
16S rRNA (total bacteria) (Harms et al., 2003)	F: ATGGCTGTCGTCAGCT R: ACGGGCGGTGTGTAC	3 mins at 98 °C, 40 cycles of 15 s at 98 °C, 30s at 60 °C, melt from 72°C to 95°C	-3.30±0.13	0.990±0.019	101±6	28.44±0.51	27.63±0.65
rodA (*E. coli*) (Chern et al., 2011)	F: GCAAACCACCTTTGGTCG R: CTGTGGGTGTGGATTGACAT P: FAM-AACCCCTACAACCGGCAGAATACC	3 mins at 98 °C, 40 cycles of 15 s at 95 °C, 30s at 60 °C	-3.63±0.09	0.992±0.002	89±3	N/A	34.89±1.59
HF183 (human host associated *Bacteroides*) (Ahmed et al., 2019b)	F: ATC ATG AGT TCA CAT GTC CG R: CTT CCT CTC AGA ACC CCT ATCC P: HEX-CTA ATG GAA CGC ATC CC	3 mins at 98 °C, 40 cycles of 15 s at 95 °C, 30s at 60 °C	-3.32±0.17	0.992±0.001	100±7	N/A	33.35±0.98

For the 16S rRNA gene qPCR assay, the reaction mixtures were prepared as follows: 2 µL of the DNA samples, 7.5 µL of SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix, 4 µL of nuclease free water (Thermo Fisher Scientific, Loughborough, UK) and 0.75 µL of each forward and reverse primer solutions (@ 10 micromol·L^{-1}) were combined for a 15 µL final volume with 500 (nmol·L^{-1}) of each primer. For the rodA probe-based reactions, we used 2 µL of DNA samples, 5 µL of PerfeCTa® qPCR ToughMix® (Quantabio, Beverly, USA), 1.75 µL of nuclease free water (Thermo Fisher Scientific, Loughborough, UK), 0.25 µL of the probe solution (@ 10 micromol·L^{-1}) and 0.5 µL of each forward and reverse primer solutions (@ 10 micromol·L^{-1}) for 10 µL of final volume with 500 (nmol·L^{-1}) of each primer and 250 (nmol·L^{-1}) of the probe. All the standard, samples and no template control reactions were done in duplicate. Standard curves were created by 10fold dilutions of a known target gene concentration from 10^7 to 10 genes/µL as described in our previous work (Acharya et al., 2019).

3.3.3 Comparison of soil and water DNA extraction kits

Since stormwater may contain a high amount of suspended sediment, we were interested to see if a soil DNA extraction kit (DNeasy PowerSoil Pro Kit, Qiagen, Crawley, UK) would perform better than a water kit (DNeasy PowerWater Kit, Qiagen, Crawley, UK) when analyzing such samples. For this experiment we used a stormwater sample containing a high amount of suspended solids (TSS =748±248 mg/L) which we collected on paved surfaces on the Newcastle University campus into a sterile 1L polyethylene (PE) bottle. This TSS content is equivalent to about 402 NTU turbidity (Rügner et al., 2013). We filtered 4 x 40-50 mL of stormwater through 0.22 µm filter membranes for DNA extraction, as explained above. Due to the high solids content, these were the maximum volumes we could readily filter. In these experiments, we used the portable qPCR instrument for the gene quantification.

3.3.4 Proof-of-concept: Onsite quantification of human sewage marker genes in river water

We first trialed the portable qPCR methodology onsite on Sept 15th, 2021. The analysis was conducted at the outlet of the river Ouseburn catchment, Newcastle upon Tyne, in northeast England, where the Ouseburn reaches the Tyne estuary at Foundry Ln (54.975582 N, -1.590909 E). Weather conditions were dry with baseflow in the river, and turbidity was 2.5±0.2 NTU.

We parked the van with the mobile laboratory opposite Ouseburn farm (**Figure 3.1a**) and collected 1 L of river water into an autoclaved PE bottle. In the van, we filtered 2 x 350 mL of the well-mixed river water and extracted DNA from each filter separately using the vortex method without enzyme and the DNeasy PowerWater Kit in accordance with the standard manufacturer protocol. We then processed this DNA for on-site qPCR using the portable equipment items illustrated in **Figure 3.1c**. For this trial we used a probe-based qPCR assay to specifically target human-host associated *Bacteroides* bacteria, instead of the rodA assay which targets *E. coli* bacteria also found in warm-blooded animal hosts like cattle and dogs. In the fieldwork, we were specifically interested in evidence for human sewage pollution of the river Ouseburn. This HF183 assay (details in **Table 3.1**) has been used successfully in many microbial source tracking studies (Ahmed et al., 2016). We have previously used this assay to analyze eDNA preserved from our fieldwork in Africa and have demonstrated strong association of *Vibrio cholerae* hazards in an Ethiopian catchment with this human sewage marker gene (Hiruy et al., 2022). The entire workflow from sampling to the data analysis was completed onsite by two skilled workers within 3 hours.

3.3.5 Faecal pollution source tracking with a mobile laboratory

Finally, a more comprehensive field demonstration of the portable qPCR method in combination with onsite physicochemical water quality testing was conducted on Oct 6th, 2021, further upstream in the catchment of the Ouseburn, near recently established stormwater retention ponds at Great Park (55.025255 N, -1.634742 E). Weather conditions were dry following a day of intensive rainfall which had filled the ponds to their capacity. Water samples were taken from the Ouseburn just upstream of two major storm drains (RiverUp @ 55.024800 N, -1.652820 E); the Kingston Park storm drain which is a drainage system from residential development in 1978 that discharges directly into the Ouseburn (KPStDrain @ 55.024714 N, -1.652305 E); the Great Park storm drain (GPStDrain @ 55.025304 N, - 1.649730 E) which originates in a residential area developed since 2004 and discharges into a stormwater retention pond; and from an outlet of this retention pond into the Ouseburn (PondEff at 55.024542 N, -1.650996 E). The sampled storm drains were in theory separated from the local sanitary sewer system and designed to drain excess urban surface run-off and groundwater but may have been impacted by some misconnections. Indications for sewage discharge into the river like sanitary plastic waste became notable in the low hanging branches and roots of the Ouseburn embankment after the older drain KPStD (**Figure A1 in Appendix A**). There was low-level discharge from both storm drains and the pond at the times of sampling. Water samples were

collected with a pole-mounted stainless-steel vessel which was repeatedly rinsed with the local water before taking composite samples into sterile PE bottles (2 x 1L) over a period of 5-10 minutes, by sampling each location repeatedly. Additional composite samples were collected in 250 mL PE bottles for the chemical analysis. Hand-held probes were used to immediately measure water temperature, pH, conductivity, dissolved oxygen and turbidity. All sample bottles were stored in a cooling box and returned to the mobile laboratory. In the van, we filtered the water samples and extracted the DNA from the filters using the vortex method without enzyme and the DNeasy PowerWater Kit, and then processed this DNA for onsite quantification of marker genes. For this fieldwork we used the 16S qPCR assay for total bacteria and the HF183 assay targeting human-host associated *Bacteroides* in combination with chemical markers, as recommended in a review of methods for identification, evaluation and characterization of faecal contamination in receiving urban surface waters (Tran et al., 2015). The DNA from two filter extractions was pooled to address low DNA content in the pond effluent extracts, which then enabled DNA quantification. We used the pooled extraction without dilution for qPCR assays run in triplicates.

For conventional membrane filtration plate counts, we filtered 0.5 mL of each water sample, diluted with 10 mL sterile saline solution, through 0.45 µm Gridded Sterile Cellulose Nitrate Membrane Filters (Sartorius, Göttingen, Germany). We placed the membranes onto sterile 47 mm pads (Pall Corporation, Ann Arbor, USA) soaked with sterile m-FC broth from 2 mL ampules (Hach, Manchester, UK) and placed inside autoclaved stainless steel petri dishes (DelAgua, Marlborough, UK). The petri dishes were incubated at 44 °C in the portable DelAgua® Water Testing kit. The next day, after 21 h incubation, the colonies on the membranes were counted and multiplied with a factor 200 to give the faecal coliform (FC) count per 100 mL.

3.3.6 Statistical methods

For the statistical analysis we used Matlab© (Version R2019a, Mathworks, Portola Valley, California, United States). We used Matlab© function ttest2 for the comparison of the measurement means between the two DNA extraction kits with the two-sample t-test. We used Matlab© function anovan for one-way or two-way crossed analysis of variance (ANOVA) to test the effects of factors such as DNA extraction procedures and qPCR instrument on the measurement means across more than two sample groupings. This was then followed by pairwise comparison of means using Matlab© function multicompare that applies the Tukey's

honest significant difference criterion. We also wrote a Matlab© script to quickly visualize the zscore transformed, multivariate data obtained onsite with a biplot based on principal component analysis (PCA) using the Matlab© function pca, and a hierarchical binary cluster tree with the Matlab© functions linkage and dendrogram. For the logarithmical data, we used the IBM SPSS Statistics (Version 28) to conduct the normality tests (Kolmogorov–Smirnov test/Shapiro–Wilk test). There is no evidence against the data being normally distributed.

3.4 Results

3.4.1 Comparing portable with conventional workflows for the DNA extraction and marker gene quantification by qPCR

Figure 3.2 illustrates how the choice of portable versus conventional DNA extraction and PCR equipment applied to the analysis of river water affected the measurements of 16S rRNA, and rodA gene copies. In this comparison, the vortex and portable qPCR instrument were considered field-deployable due to their smaller size, lesser weight, and lower costs, if compared with a benchtop ribolyser and conventional qPCR instrument. Overall, there was 87% reduction in weight and 53% reduction in costs for the two equipment items (**Table 3.1**). We also tested if the use of lysozyme to assist the cell lysis would be a worthwhile addition to the vortex protocol. More detailed results and statistical test outcomes for this method comparison are summarized in the **Appendix A** in **Table A1**.

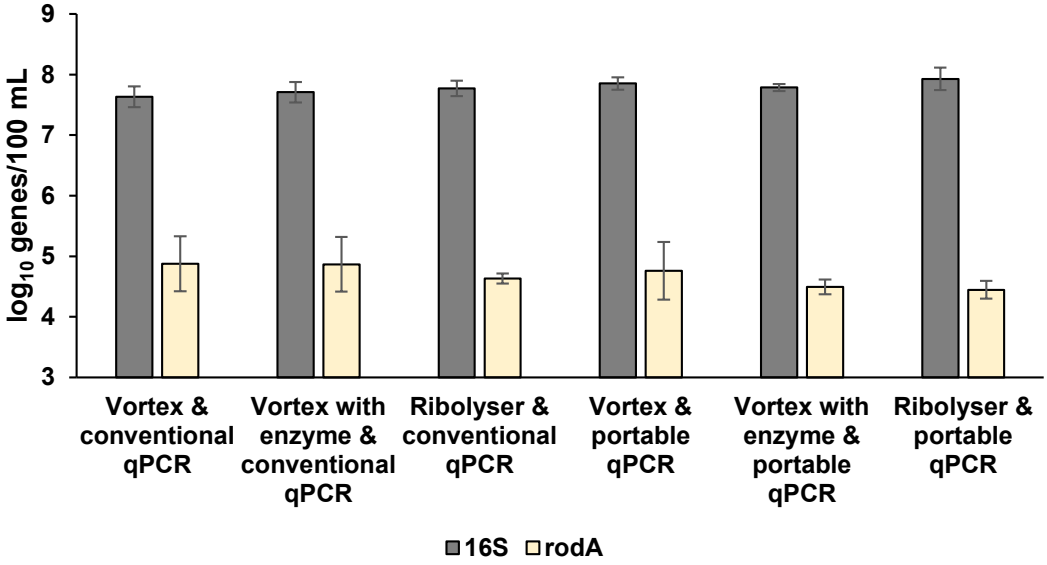

Figure 3.2. 16S rRNA and rodA marker gene copies per 100 mL of river water quantified with different DNA extraction methods and qPCR instruments (two-way across ANOVA, p=0.042, 16S only). Error bars indicate the standard deviation of triplicates.

Overall, there was no significant effect of the choice of the DNA extraction method on the mean DNA yield, 16S rRNA, and rodA gene copy numbers, while the choice of the qPCR instrument had a significant overall effect in the case of the 16S rRNA gene copy number (two-way crossed ANOVA, p=0.042). However, despite the significant difference, the mean 16S rRNA gene copy numbers quantified with the portable qPCR instrument differed by no more than 0.22 $\log_{10}$ units (they were higher) from those obtained with the conventional qPCR instrument. In the *post hoc* analysis with pairwise comparison of the six DNA extraction and qPCR quantification protocols, there were no significant differences between the protocols for any of the three metrics. There were also no significant interactions between the choice of the DNA extraction method and qPCR instrument for both qPCR assays. We concluded that the use of the vortex and portable qPCR instrument without the addition of lysozyme is the most appropriate methodology for qPCR work in the field, since it uses only portable equipment items and avoids the 1h enzyme incubation period, while the results were well aligned with the other protocols. The mean 16S rRNA and rodA gene copy numbers measured with this protocol agreed within ±0.3 $\log_{10}$ units (or a factor 2) with the results from five alternative protocols. The calibration curves metrics obtained with the portable and conventional PCR instrument (**Table A2** in **Appendix A**) both met qPCR quality requirements (Johnson et al., 2013).

3.4.2 DNA extraction kit comparison

Table 3.3 summarizes how the choice of the DNeasy PowerWater™ versus DNeasy PowerSoilPro™ extraction kits affected the DNA yields, 16S rRNA, and rodA gene copies

measured in stormwater with a very high suspended solid content. Overall, there were no significant differences between the mean values of the three types of measurements for the two DNA extraction kits (t-test, all $p > 0.05$). We concluded that the DNeasy PowerWater™ is an appropriate choice for our portable method, since there was no evidence for superior performance of the DNeasy PowerSoilPro™ kit, even for a sample with a very high total suspended solid content of 748±248 mg/L. We were also encouraged to see that high DNA yields were obtained when filtering relatively small volumes of this water (40-50 mL) with high content of suspended solids, which would have clogged the 0.2 μm membranes for larger filtration volumes.

Table 3.3. DNA yield, 16S rRNA, and rodA gene copies for different extraction kits, showing the data for each filter replicate. qPCR results were obtained with the ribolyser & portable qPCR method and are the average and standard deviation of duplicate analysis from each DNA extract.

Kit	Filter (#)	DNA yield (ng/100 mL)	Log_{10} 16S rRNA (genes/100 mL)	Log_{10} rodA (genes/100 mL)
DNeasy PowerWater ™	1	52,750	10.56±0.02	5.26±0.20
	2	32,200	10.31±0.00	5.22±0.26
DNeasy PowerSoil Pro ™	3	25,600	10.30±0.01	6.00±0.19
	4	22,800	10.23±0.01	5.61±0.43
Kit comparison, t-test		p=0.220	p=0.316	p=0.101

3.4.3 Pilot trial at the catchment outlet

On Sept 15th we successfully used our portable qPCR method in a "lab in a van" (Figure 1a) to quantify HF183 marker genes for human host associated *Bacteroides* at the Ouseburn catchment outlet within 3 hours of taking river water samples. The results of 4.25±0.02 log_{10} HF183 gene copies per 100 mL were repeatable within 0.12 log_{10} units for both filtration and qPCR replicates (**Table A3** in **Appendix A**).

3.4.4 Fieldwork to investigate the impact of storm drains on river water quality

Figure 3.3 shows the timeline of when different tasks were completed during our more comprehensive fieldwork to investigate storm drains and their impact on river water quality with the 'lab in a van'. We left the university around 8:00 AM and drove to the site at Kingston Park in half an hour. The sampling of four locations was completed within 1 hour and included the immediate measurement of water temperature, conductivity, pH, dissolved oxygen, and turbidity with portable probes. The water chemistry cuvette tests (ammonium, nitrate, nitrite, phosphate, fluoride) and alkalinity measurements were completed within 2.5 hours, which was

also the time required for filtering between 150 and 400 mL of water from the four locations in duplicate. The DNA extraction and cleanup took 1.5 hours and was completed by 1:30 PM. In the afternoon, each of two subsequent qPCR runs for 16S rRNA and HF183 gene quantifications took 1 hour to complete. The onsite qPCR results and physicochemical metadata were all available for interpretation by 4:30 PM, or within 8 hours from arrival at the site. Membranes for the plate counts were incubated onsite in the DelAgua® fieldkit at 12:30 PM, but because of the required incubation period, the colony counts only became available the next day at 10:00 AM.

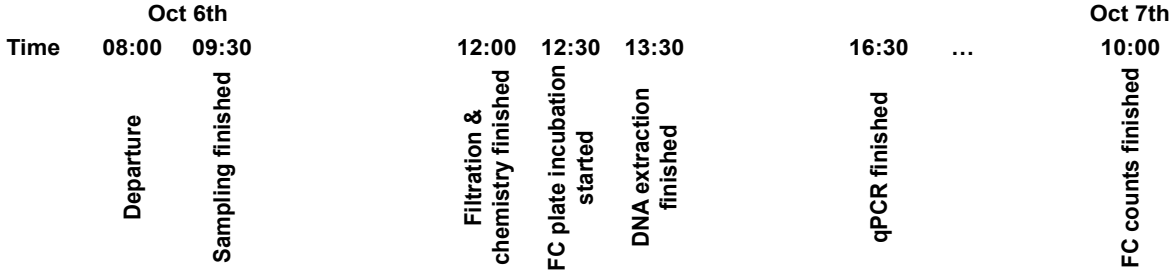

Figure 3.3. Timeline of the fieldwork at Kingston Park.

Figure 3.4 illustrates how 16S rRNA marker genes for total bacteria, HF183 marker genes for human-host associated *Bacteroides*, and faecal coliform counts varied between sampling sites. There was a significant overall effect of sampling locations on the mean value of the three microbiological metrics (one-way ANOVA, all $p < 4.18e\text{-}5$), and in the *post hoc* analysis there were significant differences in all the pairwise comparisons of sampling locations, except for 16S rRNA gene copies in the river upstream versus stormwater retention pond effluent ($p=0.61$), and for FC counts in the two storm drains ($p = 0.86$). More statistical details are provided in **Table B4** in **Appendix B**. The two storm drain effluents had higher numbers of total and FC bacteria than the river upstream of these two storm drains, while the storm water retention pond effluent had the lowest number of total and FC bacteria. The older storm drain from Kingston Park had the highest concentration of marker genes HF183 for human host associated *Bacteroides*, and was thus identified as a source augmenting these bacteria in the Ouseburn.

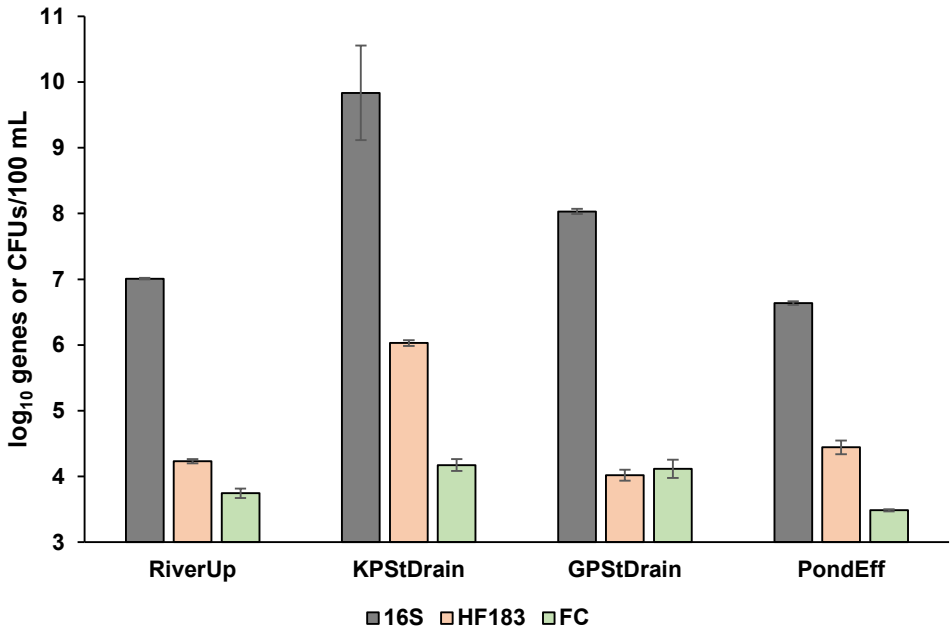

Figure 3.4. 16S rRNA and HF183 marker gene copies and FC per 100 mL of river (RiverUp), storm drain (KPStDrain and GPStDrain), and retention pond (PondEff) water quantified onsite, error bars indicate the standard deviation of triplicates (one-way ANOVA, all $p < 4.18e\text{-}5$).

Table 3.4. Physicochemical metadata collected with the mobile laboratory. For the hand-held probes, stabilized readings are reported. Errors are the average of duplicates for the other measurements.

Parameter	RiverUp	KPStDrain	GPStDrain	PondEff
Temperature (°C)	10.3	12.8	13.3	10.4
Conductivity (µS/cm)	610	746	769	358
pH	7.31	7.75	7.69	7.45
Dissolved oxygen (mg/L)	9.15	9.55	9.54	7.52
Alkalinity (mg/L $CaCO_3$)	96±16	145±8	113±4	64±1
Turbidity (NTU)	69.3±1.2	8.1±0.1	3.9±0.0	94.7±1.0
Ammonium-N (mg/L)	0.120±0.003	0.135±0.010	0.033±0.008	0.121±0.003
Nitrate-N (mg/L)	5.74±0.01	4.29±0.02	5.72±0.03	2.74±0.02
Nitrite-N (mg/L)	0.098±0.013	0.012±0.017	0.029±0.003	0.079±0.002
Phosphate-P (mg/L)	0.255±0.001	0.212±0.001	0.163±0.004	0.432±0.011
Fluoride (mg/L)	<0.1	<0.1	<0.1	<0.1

Table 3.4 summarizes the physicochemical water quality data measured on site. Storm drain effluent was characterized by higher temperature, higher alkalinity, and lower turbidity than the river upstream and pond effluent samples. Nutrient concentrations varied between the samples, and the highest ammonium concentration was measured in the Kingston Park storm drain, while the highest phosphate concentration was measured in the retention pond effluent.

Figure 3.5 visualizes the mean values or stabilized readings of the multivariate data that we had generated onsite by the end of the fieldwork day (i.e. excluding the FC plate count data) in a PCA biplot and hierarchical binary cluster tree. In the PCA biplot (**Figure 3.5a**), principal component 1 (PC1) accounted for 67.52% of the total sample variance and clearly separated the two storm drain samples from the river upstream and retention pond samples. In agreement with this, the cluster analysis (**Figure 3.5b**) also revealed greater similarity between the two storm drains versus the river and pond water samples. Principal component 2 (PC2) in the PCA biplot (Figure 5a) accounted for 21.71% of the total sample variation and was characterized by positive loadings of marker gene HF183 and ammonium, and negative loadings of nitrate, nitrite, and dissolved oxygen. PC2 was thus strongly shaped by the impact of sewage pollution indicators (HF183 and ammonium) versus indicators for more oxygenated conditions (i.e. higher dissolved oxygen concentration) which enable nitrification (formation of nitrate and nitrite from ammonium and organic nitrogen). PC2 separated the older storm drain sample

(KPStDrain) in a positive sign direction from the more modern storm drain sample (GPStDrain), indicating a stronger sewage signature in the older drain.

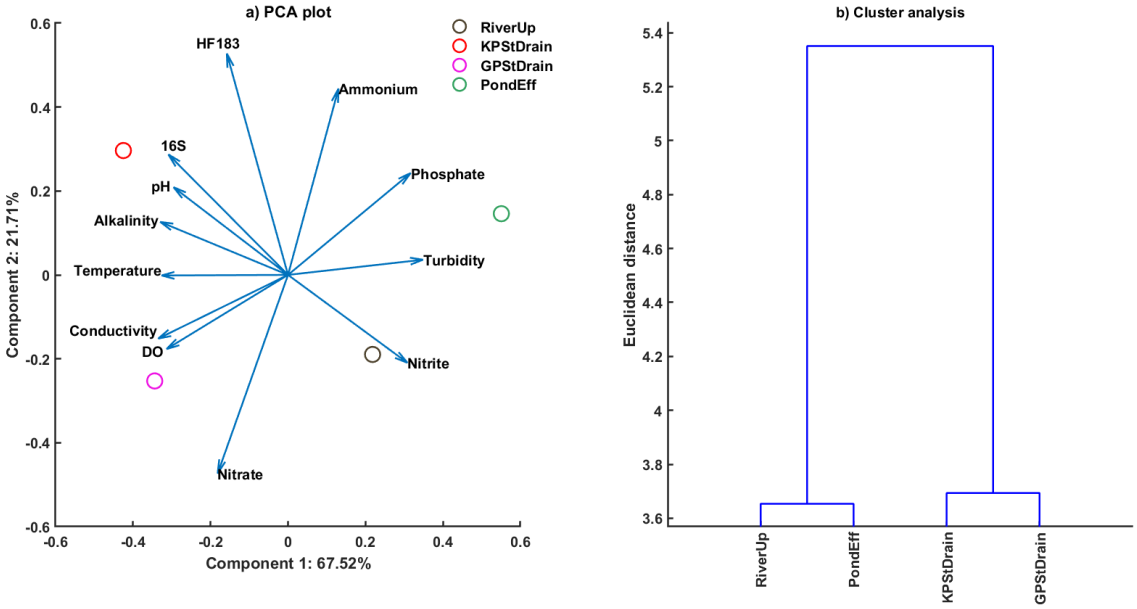

Figure 3.5. Multivariate analysis of the zscore transformed data collected on-site: (**a**) PCA biplot; (**b**) cluster analysis. Mean values of onsite qPCR assays and cuvette tests, and stabilized readings of the hand-held probes are shown in the plots.

3.5 Discussion

3.5.1 Validation of a portable qPCR method

We successfully validated our workflow for qPCR assays with portable equipment items by showing that the 16S rRNA and rodA marker gene results for river water samples obtained with the method agreed with those obtained when using our conventional laboratory equipment within the range of uncertainty for 3 filtration replicates. In previous studies we showed that 2-3 filtration replicates per site are sufficient to establish statistically significant differences between river sites and seasons, since spatiotemporal variation in metrics like 16S, rodA, and HF183 genes per 100 mL can be up to 6 $\log_{10}$ units across catchments (Hiruy et al., 2022). In comparison, the ±0.3 $\log_{10}$ unit variation between the final and alternative protocols in our study are very small indeed. Filtration of environmental water through 0.2 µm membranes takes ~30 min and is the most time-consuming sample preparation step (**Figure 3.3**). Numerous filtration replicates per sampling location would therefore be unrealistic in fieldwork, since the filtration should be done on the day of sampling to avoid sample alteration. From our fieldwork experience, 2-3 replicates are sufficient to establish significant differences between sampling locations (Ho et al., 2021, Hiruy et al., 2022, Thongsamer et al., 2021). Here we show that the differences between qPCR results obtained with portable and conventional cell lysis and qPCR

instruments fall within the range of measurement uncertainty when analyzing three filtration replicates. The addition of an enzymatic cell lysis step did not notably affect the results obtained. In principle, incubation of the enzyme could be done onsite by placing the amended lysis tubes inside the DelAgua® fieldkit after adjusting the temperature of its incubator to 37 °C. But an incubation step would add one hour to the sample processing time and work against our objective of rapidly generating results onsite.

3.5.2 Method suitability for the analysis of stormwater with high total suspended solids content

Previous studies of portable qPCR protocols have emphasized miniaturized devices for the sample preparation (Carvalho et al., 2021) or marker gene quantification (Gowda et al., 2022). However, samples with a high amount of suspended solids may clog microfluidic channels in miniaturized devices (Bridle et al., 2014), while the settling out of suspended solids from the water as pretreatment (Carvalho et al., 2021) causes sample alteration. Our onsite qPCR protocol with readily portable, but not fully miniaturized, equipment items enabled eDNA extraction and marker gene quantification from between 40 to 400 mL of unaltered environmental water samples. We successfully processed stormwater with a high content of 748±248 mg/L total suspended solids, which is at the upper limit of what is typically observed in surface water (Rügner et al., 2013). We found no advantage in using the DNeasy PowerSoilPro™ kit instead of the DNeasy PowerWater™ for the analysis of this sample.

3.5.3 Method suitability for rapid onsite water testing

The Unicef Office of Innovation has recently announced a rapid water quality testing challenge which calls for the detection of faecal indicator bacteria in water samples in under six hours (Unicef, 2022). Although formulated with drinking water testing in mind, the metrics of the Unicef challenge provide interesting points of reference for our onsite qPCR assays. Our portable qPCR protocol can easily meet the six hours challenge, as the analysis of a river water sample for the faecal pollution marker gene HF183 with duplicate filtration and triplicate qPCR assays was completed in the pilot test within 3 hours. How many water samples can be processed within a given time interval will depend on the number of workers and equipment items, as well as sample replication and metadata collection requirements. The water filtration step of ~30 min can be a time-consuming bottleneck which could be accelerated with the use of multiple vacuum pumps and filtration units. At Kingston Park, we processed 4 environmental samples and a blank in a working day which meets the Unicef challenge

requirement of 5-6 samples in a working day. However, consumables costs per qPCR assay were about £5, and total equipment costs were about £15,000, which gives a price tag of ~£20 per assay to test 1000 samples, versus a Unicef challenge requirement of $1-6 per test. We also used a mobile lab with rechargeable batteries instead of the portable power requirement specified in the Unicef challenge. Access to power and sheltered bench space is a requirement for our method, but this could also be provided from battery packs or in a building near the site of the fieldwork.

3.5.4 Method suitability for faecal pollution source tracking

The qPCR data and physicochemical water quality data we collected onsite provided useful and plausible information for faecal pollution source tracking. It revealed for example that the storm drain effluent from Kingston Park had significantly higher concentrations of marker genes for total bacteria and human host associated *Bacteroides* than the receiving Ouseburn sampled just upstream of this discharge. The same trend was observed, albeit only 17.5 h later, for the faecal coliform plate count results. The findings aligned with those of an MSc dissertation project at Newcastle University which previously identified the Kingston Park storm drain as a likely source of faecal coliform and chemical oxygen demand (COD) pollution of the Ouseburn (Rennie, 2012). The mean measured ammonium concentration were the highest in the Kingston Park storm drain samples, but ammonium levels were not particularly high in any of the analyzed waters. A previous study of storm water drains in the Ouseburn catchment already noted that ammonical nitrogen is variable and not a good indicator of sewerage inputs when used as a sole parameter (Baker et al., 2003). A literature review of faecal pollution source methods also recommended combining microbial parameters like HF183 marker gene copies with chemical sewage markers (Tran et al., 2015). In our PCA biplot the variables HF183 and ammonium were closely aligned, and their significant contributions to PC2 separated the 'fouler' Kingston Park storm drain discharge from the less polluted Great Park storm drain discharge. Kingston Park is a stormwater drainage system built in 1978 that discharges directly into the Ouseburn, whereas the residential development at Great Park has been built since 2004 and discharges stormwater into retention ponds before draining into the Ouseburn (Birkinshaw et al., 2021). In our PCA biplot and cluster analysis, the stormwater retention pond discharge had characteristics more comparable to those of the receiving river water than either one of the two storm drain effluents. These observations suggest that the retention pond at Great Park beneficially altered stormwater quality by making it more similar to that of the receiving Ouseburn. Previously, we demonstrated with onsite

sequencing methods that sewage treatment plant effluent microbiomes were beneficially altered by passage through constructed wetlands to produce microbial communities and water quality in the effluent more similar to that of the receiving river (Acharya et al., 2020).

3.5.5 Future work and potential for applications in low resource settings

Looking towards future applications, the portable qPCR methodology of this study is an ideal complement to the portable MinION nanopore sequencing method of our previous work (Acharya et al., 2020). 16S rRNA gene quantification by qPCR enables translation of the relative abundance data obtained for thousands of bacteria from 16S rRNA gene sequencing into absolute abundance estimates by multiplication. We have previously used this approach in faecal pollution source tracking applications in Nepal and Ethiopia by quantifying 16S rRNA genes in our UK laboratory using DNA preserved from the fieldwork overseas (Pantha et al., 2021, Hiruy et al., 2022). In future, as restrictions on international travel during the Covid-19 pandemic are being lifted, we will seek to demonstrate the combination of our portable NGS and qPCR methods with fieldwork in low resource settings. As shown in **Figure 3.1b**, all the equipment items used for onsite qPCR assays readily fit into a suitcase, which would not have been the case for the conventional ribolyser and qPCR instrument, given their much larger dimensions and weights (**Table 3.1**). The only additional items needed for the NGS are the memory-stick sized MinION device and a 13x7x9 cm mini-PCR thermocycler. With the further addition of the commercial DelAgua field kit for membrane filtration and plate counting we can in future fieldwork deploy a highly versatile laboratory for water microbiology in the field, which combines the complementary methods of culturing, sequencing, and qPCR, as envisioned in Acharya et al. (2019). While culturing based methods are necessary to test the viability of bacteria, sequencing comprehensively characterizes the microbiome of water samples, and qPCR assays can be used to validate sequencing results and to quantify genes of special interest such as the 16S rRNA gene for total bacteria, the rodA gene for *E. coli*, or the HF183 gene of human host associated *Bacteroides*.

3.6 Conclusions

We validated a methodology for qPCR assays with portable equipment items that produces results in close agreement with those obtained with conventional laboratory equipment items.

Our method can analyze turbid water samples without prefiltration, and we found no advantage in using the DNeasy PowerSoilProTM kit instead of the DNeasy PowerWaterTM for the analysis of a stormwater sample with a high amount of total suspended solids.

We demonstrated rapid water quality testing with an onsite qPCR assay in a mobile laboratory ('lab in a van') by quantifying HF183 marker genes for human host associated *Bacteroides* in river water within 3 hours of taking the sample.

We then deployed the mobile laboratory for faecal pollution source tracking in an urban catchment. Within 8 hours of sampling, we collected onsite comprehensive microbial and physicochemical water quality data which indicated human sewage pollution of the river via an old storm drain. The data also showed the benefits of storm drain discharge retention in a pond, with pond effluent water having characteristics more similar to the receiving river as compared to the discharges from the two storm drains.

The portable equipment items enabled a 87% reduction in weight and 53% reduction in costs in comparison with the conventional laboratory equivalents. All the equipment items used for the onsite qPCR assays readily fitted into a suitcase, making the method deployable for fieldwork overseas.

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Chapter 4

Environmental DNA clarifies impacts of combined sewer overflows on the bacteriology of an urban river and resulting risks to public health

Chapter 4. Environmental DNA clarifies impacts of combined sewer overflows on the bacteriology of an urban river and resulting risks to public health

4.1 Abstract

There is no reference of microbiological water quality in the European Union's Water Framework Directive, adapted into English law, and consequently microbial water quality is not routinely monitored in English rivers, except for two recently designated bathing water sites. To address this knowledge gap, we developed an innovative monitoring approach for quantitative assessment of combined sewer overflow (CSO) impacts on the bacteriology of receiving rivers. Our approach combines conventional and environmental DNA (eDNA) based methods to generate multiple lines of evidence for assessing risks to public health. We demonstrated this approach by investigating spatiotemporal variation in the bacteriology of the Ouseburn in northeast England for different weather conditions in the summer and early autumn of the year 2021 across eight sampling locations that comprised rural, urban, and recreational land use settings. We characterized pollution source attributes by collecting sewage from treatment works and CSO discharge at the peak of a storm event. CSO discharge was characterized by $\log_{10}$ values per 100 mL (average±stdev) of 5.12±0.03 and 4.90±0.03 for *faecal coliforms* and *faecal streptococci*, and 6.00±0.11 and 7.78±0.04 for *rodA* and HF183 genetic markers, for *E. coli* and human host associated *Bacteroides*, respectively, indicating about 5% sewage content. SourceTracker analysis of sequencing data attributed 72-77% of bacteria in the downstream section of the river during a storm event to CSO discharge sources, versus only 4-6% to rural upstream sources. Data from sixteen summer sampling events in a public park exceeded various guideline values for recreational water quality. Quantitative microbial risk assessment (QMRA) predicted a median and 95th percentile risk of 0.03 and 0.39, respectively, of contracting a bacterial gastrointestinal disease when wading and splashing around in the Ouseburn. We show clearly why microbial water quality should be monitored where rivers flow through public parks, irrespective of their bathing water designation.

4.2 Introduction

Legislation like The Water Environment (Water Framework Directive) (England and Wales) Regulations 2017 (GOV.UK, 2017), adapted from the European Union Water Framework Directive, stipulates criteria for the status of surface water according to physicochemical and

ecological parameters. However, there is no reference of microbiological water quality, and consequently, the bacteriology of rivers is not routinely monitored. Water quality standards for faecal indicator bacteria are set instead in the bathing water regulations (GOV.UK, 2013). However, in the United Kingdom (UK), only two rivers contain designated bathing water sites (GOV.UK, 2022a), and microbial river water quality remains mostly unmonitored. In the absence of monitoring, there is considerable public and parliamentary debate around the impacts on rivers of discharges from combined sewer overflows (CSOs), recently relabelled as storm overflows. Parliamentary debate resulted in the Environment Act 2021 (GOV.UK, 2021), and in response the government has formulated storm overflows discharge reduction plans that strengthen monitoring requirements and set new targets to better protect people and the environment.

In the UK, as in many other countries around the globe, CSOs function as safety valves for urban drainage systems in which sewers convey wastewater from households, commerce, and industry, together with run-off from impermeable surfaces (Owolabi et al., 2022). At critical junctures these networks have outlets to release stormwater into rivers when flow exceeds the sewer capacity. CSOs thus prevent stormwater backing up in the network and consequently urban flooding. Modern residential developments often convey runoff from impermeable surfaces in a separated surface water drainage system, but these drainage systems may still be polluted by wastewater, if households inadvertently misconnect appliances like showers, washing machines, or WC to the surface water drainage pipes (Zan et al., 2022, Baker et al., 2003).

In the UK, CSOs are permitted to discharge when the flow in the sewer exceeds criteria for the minimum retained flow (GOV.UK, 2022b), set by the so-called Formula A:

$$Minimum\ retained\ flow\ (liters\ per\ day) = DWF + 1360 \cdot P + 2 \cdot E \quad \text{eq. 1}$$

DWF (litres per day) is the dry weather flow calculated from

$$DWF = P \cdot G + I + E \quad \text{eq. 2}$$

P (capita) is the catchment population, G (litres per capita per day) is the per capita domestic flow, I (litres per day) is the infiltration, and E (litres per day) is the trade effluent flow.

Formula A sets criteria for flow conveyance in combined sewers largely based on the population served and, remarkably, no explicit reference is made to rainfall, catchment and receiving river characteristics. In contrast, concepts like “unusually heavy rainfall” and dilution

by “high flow in rivers” feature prominently in the discussion of CSO discharges and their impacts on the environment (EAC, 2022, DEFRA, 2022).

Without routine monitoring, the nature of CSO discharge and resulting impacts on the freshwater environment remain uncertain (Sojobi and Zayed, 2022). The House of Commons Committee report on water quality in rivers (EAC, 2022) concluded that “we therefore found the claim made by the chief executive of Severn Trent that its sewer overflow discharges were ‘pretty much already rainwater’ to be disingenuous. As water companies do not routinely test the quality of the discharges from storm overflows, they are in no position to make this claim.” Also, current bathing water quality indicators like *E. coli* and *Enterococci* target bacteria that are found in the gut of both, humans and warm-blooded animals. Accordingly, there is considerable debate around the relative contribution of human sewage versus alternative sources like wildlife, agriculture, or dog walking, on faecal pollution of the water environment (O'Keefe et al., 2005).

With molecular methods for the analysis of environmental DNA (eDNA), faecal pollution can increasingly be attributed to its sources (Ahmed et al., 2016). However, there remain challenges also for eDNA methods that relate to uncertainties around the quantitative attribution of genes to different sources, stability of DNA in the environment, differentiation between DNA from viable and nonviable cells, and lack of regulatory standards that are based on genetic methods (WHO, 2016, WHO, 2021). Recent scientific advances have started to address these challenges. They include a proposed framework for eDNA methods that integrate pollution source characterization with quantitative catchment survey data (Derx et al., 2023), the use of advanced bioinformatic tools like SourceTracker (Knights et al., 2011) and FEAST (Shenhav et al., 2019) for quantitative attribution of the bacteria in rivers to their various sources (Pantha et al., 2021, Liang et al., 2021), and quantitative microbial risk assessment (QMRA) methods that derive from eDNA data probability of illness via different exposure pathways (Halla et al., 2022), and improved standards for recreational water quality (Boehm and Soller, 2020).

In this study, we combined conventional and innovative eDNA methods with bioinformatic tools like SourceTracker and QMRA to quantitatively study the bacteriology of an urban river in Newcastle upon Tyne, UK, in relation to changing weather conditions and storm events that trigger CSO discharges, and for the assessment of risks to public health. We investigated the hypotheses that i) eDNA methods indicate human sewage pollution of rivers more sensitively than conventional methods; ii) eDNA methods can attribute the proportion of river bacteria

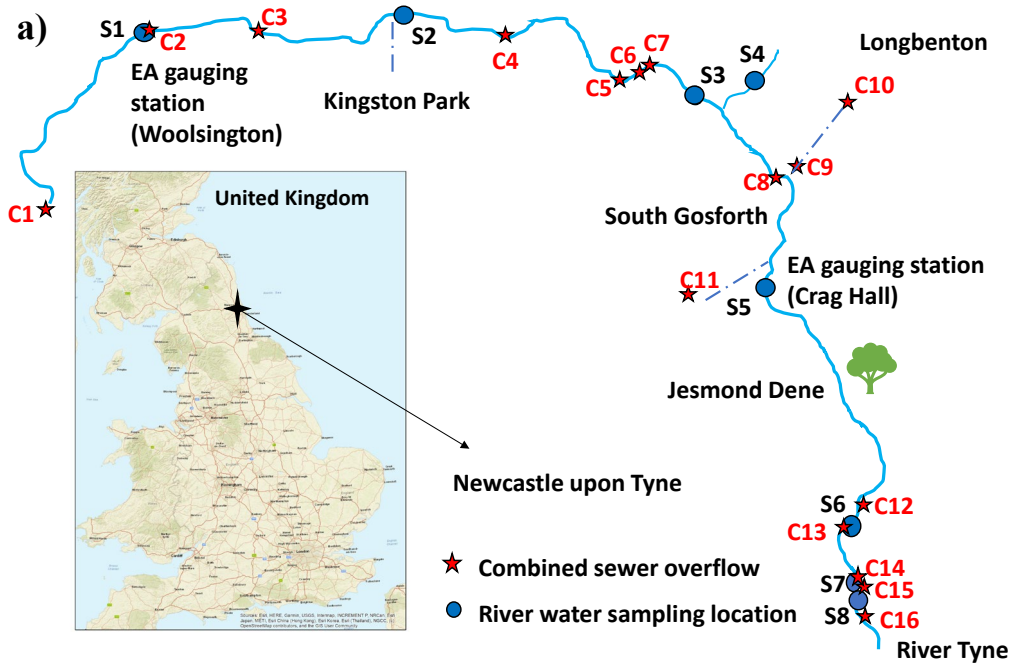
which originate from CSO discharge sources, and iii) conventional and eDNA methods predict substantial risks of contracting a gastrointestinal illness from wading and splashing around in an urban river affected by CSO discharges.

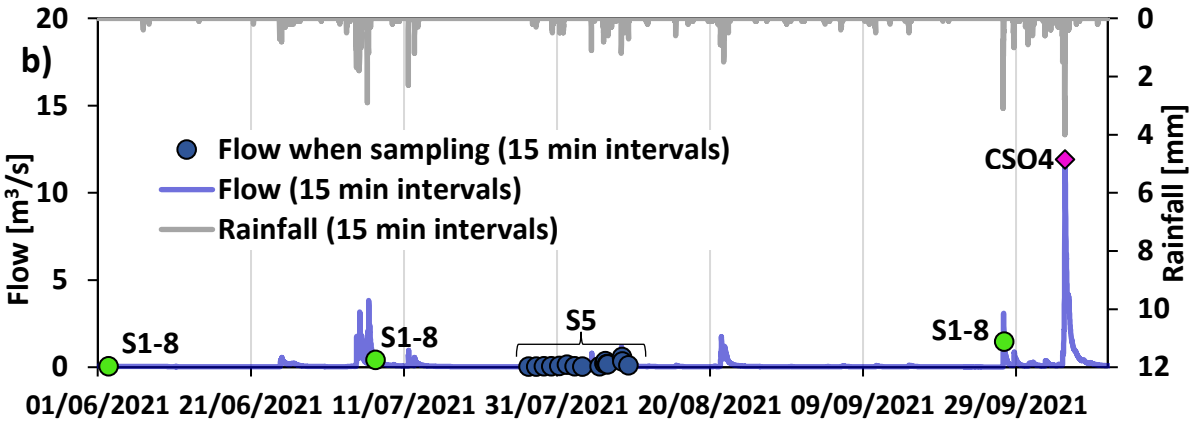
4.3 Method and sites description

4.3.1 Catchment characteristics and sampling locations

We studied the catchment of the Ouseburn, a small river in northeast England, that flows through Newcastle upon Tyne (**Figure 4.1a**). The catchment is drained by a mixture of combined and separated sewer systems (Birkinshaw et al., 2021), and the trunk sewer runs alongside the Ouseburn. During rainfall and snow melt sixteen CSOs are permitted to discharge into the Ouseburn (**Table B1 in Appendix B**). There is no wastewater treatment plant in the Ouseburn catchment and sewage was therefore obtained from the nearby wastewater treatment plant at Birtley.

The Ouseburn originates in the north-western rural part of the catchment. Sampling location S1 was in a public green space, where the Ouseburn enters the village of Woolsington, 100 meters downstream of an Environment Agency (EA) gauging station. The catchment to S1 is 9 square kilometres with predominantly arable land use (67.46%), some grassland (18.26%), and 10.8% urban area (NRFA, 2022). Proximity of the sampling point with the gauging station enabled calculation of rural pollution loads by multiplying concentration with flow measurements. From Kingston Park, the catchment becomes suburban, and the Ouseburn flows through an Accessible Water Environment within residential developments established during the 1970s (Birkinshaw et al., 2021). Sampling location S2 was at the boundary of these developments, after a major surface water drainage outfall. This outfall is nominally separated from the foul sewers, but a suspected source of faecal pollution from misconnections (Rennie, 2012, Zan et al., 2022). The Ouseburn then passes through residential areas with CSOs 4-7 and two golf clubs. Sampling location S3 was on land of the Gosforth Golf Club and reflected water quality at the end of this suburban reach of the catchment. For comparison, sampling location S4 was on a nearby tributary which passes through agricultural land and green space with a horse racecourse and nature reserve. In its upstream, this tributary is connected to surface water management schemes, but no CSO is discharging into it. The Ouseburn reaches an urbanized area with commerce and industry between South Gosforth and Longbenton. Here, a culverted





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Data collection	Public metadata	Data processing	Regulations
- Water chemistry (ammonium, nitrate, nitrite, phosphate, total N and P, COD, temp, pH, conductivity, DO, alkalinity, turbidity) **- Plate counts (Faecal Coliform and Streptococci)** **- Quantitative PCR (16S rRNA, rodA, and HF183 genetic markers for total bacteria, *E. coli*, and human host associated *Bacteroides*)** **- Sequencing (16S rRNA gene amplicons for bacterial taxonomy)**	- Environment Agency **rainfall** and **catchment data** - National **River Flow** Archive **- Water ingestion volumes** (different recreational activities) **- Indicator to pathogen ratios** **- Exposure dose response relationships**	- **Loads** of nutrients, coliform, and genes (concentration x flow) **- River status classification** (physico-chemical quality elements) **- Bathing water classification** **- Microbial Source Tracking** (SourceTracker) **- Quantitative Microbial Risk Assessment (QMRA)**	- The Water Environment **(Water Framework Directive)** (England and Wales) Regulations 2017 **- The Bathing Water Regulations** 2013 **- WHO guidelines** for recreational water quality

Figure 4.1 a) River sampling locations (S1-8) and combined sewer overflow locations (CSO1-16) in the Ouseburn catchment; b) average rainfall (grey line) from two monitoring stations in the catchment and flow at S5 (blue line) in relation to the sampling dates (shown by the markers); c) data collection and interpretation strategy.

Drainage system conveys surface water from the north-eastern catchment into the Ouseburn, and during storm events this culvert receives discharge from CSOs 9 and 10. Sampling location S5 was downstream of this merger of the north-western and north-eastern branches of the catchment and co-located with another EA gauging station at Crag Hall. The catchment to S5 is 55 km^2 with predominantly urban (41.14%) and arable land use (33.82%), and some grassland (16.34%) (NRFA, 2022). The Ouseburn then flows in Jesmond Dene, a wooded valley and public park. Jesmond Dene is considered "the jewel in the crown of Newcastle's parks and green spaces" (UrbanGreen, 2022), with decorative river features such as steppingstones and an artificial waterfall. After Jesmond Dene, the Ouseburn flows in a culvert below an infill constructed in the early 20th century. Sampling location S6 was before this culvert to reflect water quality at the end of the river stretch with mainly recreational land use (parks and allotments). Sampling location S7 was at the outlet of the culvert to understand impacts of the historic infill on water quality. Finally, the Ouseburn passes through Ouseburn Valley, an area with industrial legacy and brownfield regeneration where an urban farm is located next to the river on the former site of Northumberland Lead works (OuseburnTrust, 2022). Sampling location S8 was after Ouseburn Farm and in the tidal reach of the Tyne estuary to investigate the impacts on water quality of this complex setting.

4.3.2 Sampling schedule and procedures

Our sampling schedule in the year 2021 was targeted to reflect a range of weather conditions during the summer, when recreational activities in rivers are at their height. We collected water samples from locations S1-8, on June 2nd during dry weather, on July 7th after a day of heavy rainfall, and on Sept 27th during a storm event. We collected an additional sixteen samples in Jesmond Dene Park at S5 between July 27th and Aug 9th while the summer weather was changeable with scattered showers. On Oct 5th, we collected discharge directly from the outlet of CSO4 during a major storm event to establish discharge characteristics. For comparison, we collected settled sewage (STPInf) from the inlet of Birtley Sewage Treatment Plant in northeast England (Latitude 54°54'19.30" N, Longitude 1°35'57.8" W), on May 12th after 24 h with rainfall, and following 24 h of dry weather on July 21st and August 25th, 2021. **Figure 4.1b** illustrates the average rainfall recorded by the two EA rain gauges in the catchment, at Gosforth Race Course and Farne School, and flow in the Ouseburn at S5 in relation to the sampling events.

4.3.3 Sampling, analysis, and statistical methods

Our water quality assessment approach is outlined in **Figure 4.1c** and detailed in **supporting information.** In this study we integrated microbial source tracking (MST) and quantitative microbial risk assessment (QMRA) methods that we have developed with fieldwork in Asia and Africa (Pantha et al., 2021, Thongsamer et al., 2021, Hiruy et al., 2022, Acharya et al., 2020, Halla et al., 2022). In brief, composite river water samples were collected and a portion was immediately analysed for water temperature, pH, conductivity, and dissolved oxygen, using a calibrated ExStikII probe (Extech Instruments, Nashua, NH, USA) and HQ 40d meter with a LDO sensor (Hach, Manchester, UK). Samples were transported in cooling boxes to the laboratory and further processed on the day of sampling. Alkalinity was measured with a Model AL-DT alkalinity test kit and turbidity with a 2100Q turbidity meter (both from Hach, Manchester, UK). Water chemistry was characterized with cuvette tests (Hach, Manchester, UK) according to the manufacturer's instructions, comprising ammonium-N (LCK304), nitrate-N (LCK339), nitrite-N (LCK341), total-N (LCK138), total-P (LCK349), ortho-P (LCK349), and COD (LC1400). Bacterial analysis combined plate counting, qPCR, and MinION^{TM} nanopore sequencing methods to take advantage of complementarities between these approaches (Acharya et al., 2019). We quantified *Faecal Coliforms* (FC) and *Faecal Streptococci* (FS) by membrane filtration using m-FC and KF Streptococci ampules (Hach, Manchester, UK), respectively, as growth media for incubation in accordance with standard methods for the examination of water and wastewater (APHA, 2005). Biomass was concentrated onto gridded sterile cellulose nitrate membrane filters with 0.2 μm pore size (Sartorius, Göttingen, Germany) by filtering between 100 and 300 mL of river water, depending on the anticipated bacterial content. Membranes were preserved at -20 °C for subsequent DNA extraction and analysis. We extracted and cleaned-up DNA with the Dneasy PowerWater Kit (Qiagen, Crawley, UK) following the manufacturer's protocol. Using a portion of this DNA, we conducted qPCR with primer pairs targeting the 16S rRNA gene (F: ATGGCTGTCGTCAGCT, R: ACGGGCGGTGTGTAC) as a marker for total bacteria (Ferris et al., 1996), and two TaqMan qPCR assays targeting the *rodA* gene (F: GCAAACCACCTTTGGTCG, R: CTGTGGGTGTGGATTGACAT, P: FAM-AACCCCTACAACCGGCAGAATACC) as a marker for *E. coli* (Chern et al., 2011), and the HF183 genetic marker (F: ATCATGAGTTCACATGTCCG, R: CTTCCTCTCAGAACCCCTATCC, P: HEX-CTAATGGAACGCATCCC) for human-host associated *Bacteroides* (Ahmed et al., 2019b), as previously described (Zan et al., 2022). For qPCR assays, the average±stdev efficiency for 16S, *rodA*, and HF183 assays were 100±10%,

89.6±8.7%, and 81.9±8.4%, respectively. The Cq value for the no template control (NTC) of 16S assays was 30.8 on average, which was higher than any of the sample Cq values. No amplification for the NTC was found for the probe-based rodA and HF183 assays. Melt curve analysis was conducted for 16S rRNA assays to verify the specificity. Sequencing followed manufacturer protocols for PCR amplification of full-length 16S rRNA genes, followed by sequencing with the MinION^{TM} from Oxford Nanopore Technologies (ONT, Oxford, UK) and base-calling with Guppy version V5.0.11. We established the bacterial taxonomy at genus level with ONT's EPI2ME FASTQ 16S workflow using a quality score ≥7, as previously described (Halla et al., 2022). Sewage content estimation of CSO discharge were based on values of faecal coliforms, total nitrogen and total phosphorus that we measured in sewage from Birtley wastewater treatment works, and based on values reported in the literature for municipal sewage (Mihelcic and Zimmerman, 2014). We also estimated the sewage content of CSO discharge from Formula A which underpins the design of CSOs in the UK.

Our multivariate statistical methods comprised dendrograms, heatmaps, principal component analysis (PCA), and Pearson Correlation analysis using Matlab© (Mathworks, Natick, MA, USA), and analysis of similarity (ANOSIM) and BEST analysis using Primer7 (primer-e, Auckland, New Zealand). Stepwise regression using Matlab© was used to derive an appropriate linear model between HF183 and physicochemical water quality parameters that can potentially be monitored with sensors. We interpreted sequencing data at genus level, after rarefaction at 50,000 reads followed by square-root transformation, before calculating (dis)similarity as Euclidean distance. SourceTracker analysis (Knights et al., 2011) attributed river bacteria to either upstream or sewer sources (Pantha et al., 2021). Root-level taxonomy data derived from NGS of 16S rRNA genes was used to compare, for the June 2nd event (dry weather), the July 7th event (dry after a day of heavy rainfall), and the Sept 27th event (heavy rainfall), the composition of bacterial communities at sampling locations S2-S8 (sinks) with those in the rural upstream (S1), and CSO discharge that was sampled on Oct 5th, as potential source communities which are shaping these river water communities. Our quantitative microbial risk assessment (QMRA) approach followed WHO guidance (WHO, 2016) and previously described procedures for estimating pathogen to indicator ratios from eDNA data (Halla et al., 2022). The QMRA was conducted based on bacterial water quality data from 16 sampling events in Jesmond Dene Park between July 27th and Aug 9th, 2021. Dose response parameters values of the approximate Beta-Poisson model for *E. coli O157:H7* (Teunis et al., 2008), *Campylobacter jejuni* (Teunis and Havelaar, 2000), and non-typhoid *Salmonella* spp.

(WHO, 2002) were used to calculate the probability of contracting a bacterial gastrointestinal illness (*Shigellosis*, *Campylobacteriosis*, or *Salmonellosis*) by wading and splashing around in the Ouseburn. Details can be found in **Appendix B**.

4.3.4 Cost comparison

To enable a comparison of the relative costs of conventional and eDNA based methods, we compiled the directly incurred costs of one survey to sample and comprehensively characterize the water quality at 8 sites in the Ouseburn catchment (i.e., on June 2nd, 2021). These directly incurred costs included technical staff salary, transportation, equipment, and consumables expenditures needed for gathering the data. For the equipment items we assumed that their purchasing costs could be spread across 100 surveys. We did not include indirect costs such as university overheads.

4.4 Results

4.4.1 Distribution statistics of river water quality metrics

Table 4.1 summarizes statistical metrics for the data we have collected in the summer of 2021 by plate counting, qPCR, and physicochemical methods to characterize water quality of the Ouseburn. There was substantial variation for all parameters, but the highest coefficients of variation (CV) and percentile P90/P10 ratios were observed for sewage markers ammonium-N in the chemical data (CV = 1.78, P90/P10 = 52 for concentration values), and HF183 for human-host associated *Bacteroides* in the bacterial data (CV = 0.32 and P90/P10 = 2.47 for logarithmic concentration values). These observations hint at the importance of sewage inputs for water quality variation in the Ouseburn.

Table 4.1 Summary of microbial and physicochemical measurement statistics for the river Ouseburn (40 sampling events). For comparison, data for settled sewage (3 sampling events) and CSO discharge (2 sampling events, 15 min apart) were also included.

	Faecal coliforms **(FC)**	***Faecal streptococci*** **(FS)**	**16S rRNA (total bacteria)**	***rodA*** **(*E. coli*)**	**HF183 (human-host *Bacteroides*)**
	Log_{10} CFUs/100 mL	Log_{10} CFUs/100 mL	Log_{10} genes/100 mL	Log_{10} genes/100 mL	Log_{10} genes/100 mL
Ouseburn					
Average±Stdev	3.94±0.77	3.49±0.71	8.83±0.64	5.07±1.19	5.39±1.71
Median	3.80	3.50	8.61	4.98	5.55
10th percentile	2.97	2.47	8.24	3.67	3.29
90th percentile	5.24	4.50	9.83	7.10	8.12
Sewage					
Average±Stdev	6.49±0.43	Not available	10.59±0.34	7.88±0.30	8.16±0.36
CSO discharge					
Average±Stdev	5.12±0.03	4.90±0.03	11.16±0.00	6.00±0.11	7.78±0.04

	NH_4^+-N mg/L	**NO_3^--N** mg/L	**NO_2^--N** mg/L	**Total-N** mg/L	**PO_4^{3-}-P** mg/L	**Total-P** mg/L	**COD** mg/L
Ouseburn							
Average±Stdev	0.44±0.79	1.42±0.67	0.04±0.02	3.80±2.82	0.34±0.25	0.44±0.44	51.8±63.8
Median	0.12	1.28	0.04	2.73	0.30	0.30	23.5
10th percentile	0.03	1.00	0.01	1.87	0.11	0.14	17.9
90th percentile	1.38	1.90	0.07	7.97	0.65	1.06	140.8
Sewage							
Average±Stdev	47.8±27.1	1.95±1.38	0.19±0.15	80.7±19.1	5.87±2.71	6.89±2.96	636.8±362.3
CSO discharge							
Average±Stdev	0.28±0.01	1.86±0.03	0.03±0.00	4.08±0.00	0.24±0.01	0.41±0.02	134.8±11.7

	Temperature °C	**pH** -	**Conductivity** µS/cm	**DOSat** %	**Alkalinity** mg/L $CaCO_3$	**Turbidity** NTU
Ouseburn						
Average±Stdev	14.3±1.5	7.6±0.3	659±431	81.9±10.1	123±55	41.3±56.1
Median	14.1	7.6	617	84.6	120	17.6
10th percentile	12.5	7.2	247	71.0	51.3	1.7
90th percentile	16.5	8.0	981	90.2	187	132
Sewage						
Average±Stdev	17.1±2.8	8.4±0.1	1073±64	22.1±13.8	Not available	Not available
CSO discharge						
Average±Stdev	10.6±0.0	8.4±0.2	114±6	87.5±3.1	24.5±2.1	55.6±1.1

4.4.2 Characteristics of CSO discharge versus sewage

The oxygen saturation of CSO4 discharge was higher than in sewage, while nutrient concentrations and conductivity were reduced, as would be expected from the blending of oxygen deprived and nutrient rich sewage with well aerated and naturally soft rainwater. The CSO4 discharge collected on Oct 5th during very heavy rainfall contained high concentrations of faecal bacteria (i.e., FC, FS, *rodA* and HF183 genetic markers, **Table 4.1**). By noting that mean values of FC, total-N, and total-P in the CSO4 discharge were, respectively, a factor 23, 20, and 17, below those in sewage from Birtley STP, we estimate 4-6% volume sewage content in the CSO4 discharge. Across all metrics in **Table 4.1**, the CSO4 discharge characteristics were consistent with at least 0.6% volume sewage content. Based on reported concentrations of major constituents in average-strength wastewater of 6.48 $\log_{10}$ FC per 100 mL, 35 mg/L total-N, and 10 mg/L total-P (Mihelcic and Zimmerman, 2014), the estimated volume sewage content in the CSO4 discharge was 4.4%, 11.7% and 4.1%, respectively.

Substantial sewage content in CSO discharge agrees with a theoretical estimate derived from CSO permit regulations (GOV.UK, 2022b). Daily, our local water company provides 1.1 billion litres of water to its 4.4 million customers (NWL, 2022). If one attributes 22% to distribution losses (WaterUK, 2022), and 138 litres per capita per day to the domestic demand (EA, 2020), the average trade demand would be an estimated 57 litres per customer served per day. DWF at Birtley STP is about 255 litres per capita per day. Setting trade demand equal with trade effluent, the minimum conveyed flow in combined sewers according to Formula A becomes

$$Minimum\ retained\ flow\ (litres\ per\ day) = 255 \cdot P + 1360 \cdot P + 2 \cdot 57 \cdot P \quad \text{eq. 3}$$

The first summand is the DWF (i.e., "sewage"), and the second and third summands are the allowance for stormwater flow. It follows that the minimum conveyed flow is 1,729 litres per capita per day, or approximately seven times the DWF. Consequently, about 15% of the flow is "sewage" when a CSO starts to spill. From **Table 4.1**, sevenfold diluted sewage is still a very high concentration of faecal bacteria (~440,000 FC per 100 mL).

4.4.3 Spatiotemporal variation in river water quality

Figure 4.2 (a-e) illustrates the substantial spatiotemporal variation in concentrations of faecal bacteria across eight sampling locations along the Ouseburn, and three sampling dates with distinct weather conditions (dry on June 2nd, dry following a day of heavy rainfall on July 7th, and wet with heavy rainfall on Sept 27th). Despite dilution by storm flow in the river,

concentrations of faecal bacteria increased markedly during heavy rainfall, with peak concentrations in the suburban (S3) and urban (from S5) part of the catchment. The ratio HF183/16S rRNA shows an increased contribution of human gut derived *Bacteroides* (HF183) to total bacteria (16S rRNA) in the urbanized catchment during the Sept 27th storm event (**Figure 4.2f**). Two way-crossed ANOSIM of the plate count and qPCR data confirmed a significant effect of sampling dates ($R = 1$, $p = 0.001$) and locations ($R = 0.921$, $p = 0.001$) on the bacteriology of the river.

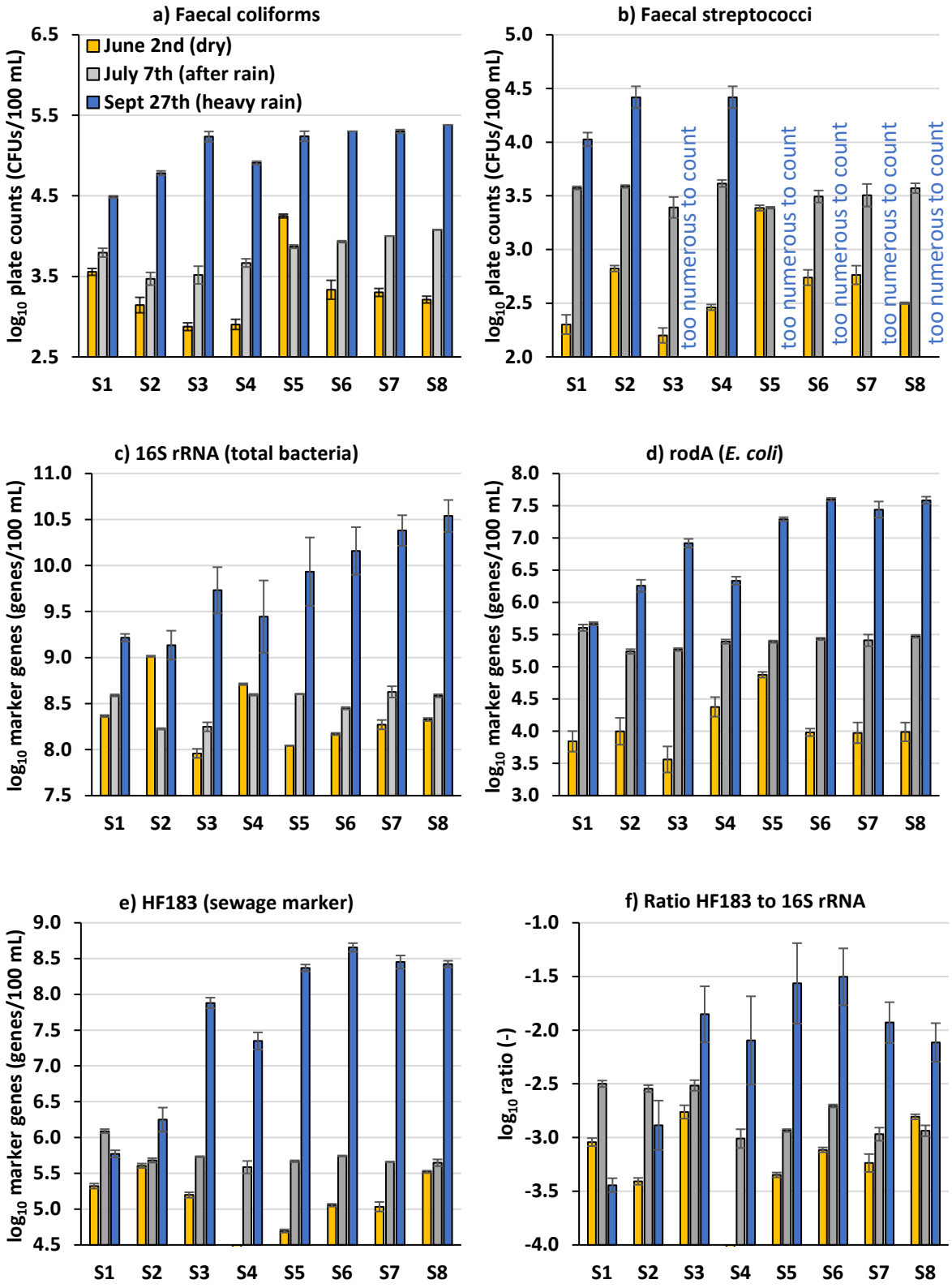

Figure 4.2 Bacterial water quality as indicated by plate counting and qPCR methods at sampling locations S1-8 for three sampling events with distinct weather conditions, average±stdev, a) Faecal Coliforms, b) Faecal Streptococci, c) 16S rRNA genes, d) rodA genes, e) HF183 genetic marker, f) HF183/16S rRNA ratio.

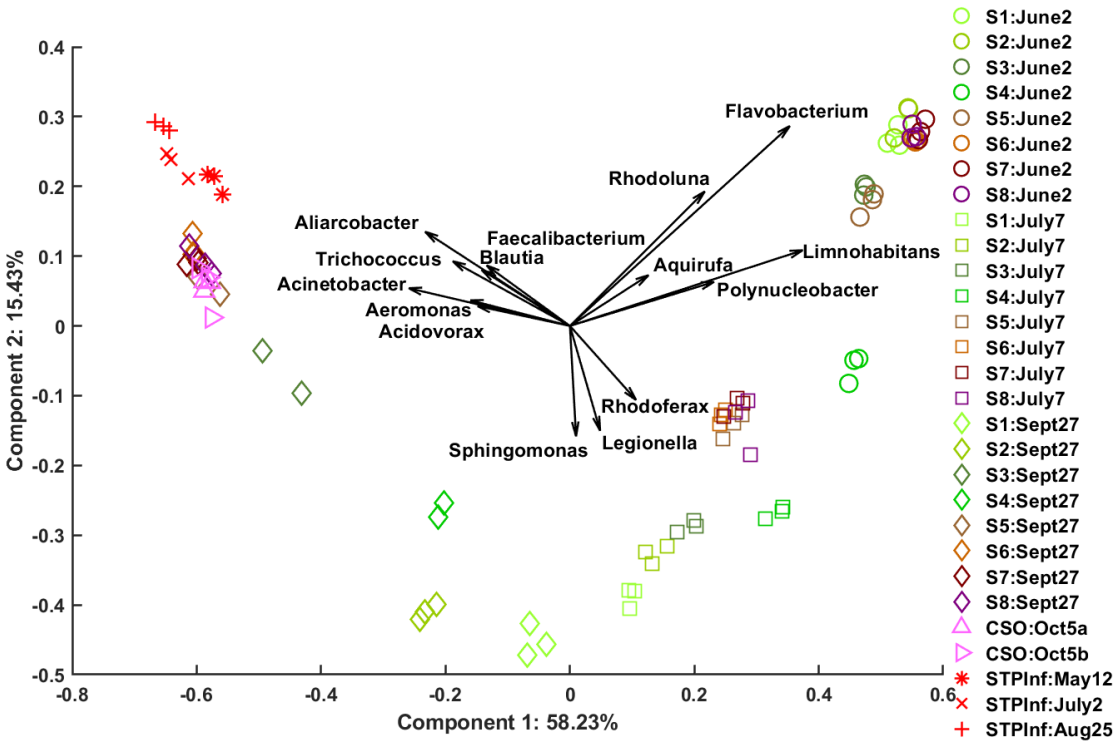

Figure 4.3 Principal component (PCA) analysis of 16S rRNA gene sequencing derived relative abundance data of the bacterial community characterized to genus level at sampling locations S1-8 for three sampling events, June 2nd (dry, circles), July 7th (after rainfall, squares), Sept 27th (heavy rain, diamonds). For comparison, data of combined sewer overflow discharge (CSO, triangles) and settled sewage from the inlet of a sewage treatment plant (STPInf, stars and crosses) have also been included in the plot. The percent variance explained by each component is shown in the axis label, and the arrows indicate which bacterial genera have a strong relationship (loading) with the principal components.

PCA of the relative abundance of different bacterial genera, derived from 16S rRNA gene sequencing data, clearly separated the river water samples according to sampling dates (**Figure 4.3**). Dry weather samples from June 2nd (circles, **Figure 4.3**) were characterized by high prevalence of freshwater bacteria like *Limnohabitans* (Kasalický et al., 2013), *Rhodoluna* (Hahn, 2016), *Polynucleobacter* (Hahn et al., 2017), and *Aquirufa* (Pitt et al., 2019). In contrast, Ouseburn samples collected during heavy rainfall on Sept 27th (diamonds, **Figure 4.3**) were characterized by high prevalence of bacteria that dominate microbial communities within urban sewer infrastructure, like *Acinetobacter*, *Trichococcus*, and *Aeromonas* (VandeWalle et al., 2012). Samples from July 7th, a day after heavy rainfall (squares, **Figure 4.3**), fell in between the dry and storm weather data. Two way-crossed ANOSIM confirmed a significant effect of sampling date ($R = 1$, $p = 0.001$) and location ($R = 0.865$, $p = 0.001$) on the river data illustrated in **Figure 4.3**.

During the Sept 27th storm (diamonds in **Figure 4.3**), the predominance of faecal bacteria was most notable in the urban part of the catchment (S3, and S5-8), in line with the plate count and qPCR observations (**Figure 4.2**). Indeed, bacterial communities at locations S5-8 on Sept 27th

had high similarity with those in CSO discharge, as indicated by the colocation of the respective samples in the PCA plot (respective diamonds and triangles in **Figure 4.3**). These samples furthermore resembled bacterial communities in sewage from Birtley STP (stars and crosses in **Figure 4.3**). The samples from June 2nd (dry weather) showed that the upstream bacterial community (S1) is the main source of the bacterial community in the river at locations S2-S8 (90%) while CSO discharge only contributed 1%. On July 7t, one day after heavy rainfall, the CSO discharge contribution increased to 8-10%, while the upstream was the main source of the bacteria at the location S2-S8. Source tracking analysis showed that the CSO discharge contribution has a significant impact on the bacterial community of the Ouseburn river location S3, S5-S8. SourceTracker analysis (Knights et al., 2011) attributed 72-77% of the river bacteria at S5-8 to CSO discharge, and only 4-6% to the bacterial community from the rural upstream (**Figure 4.4 c**).

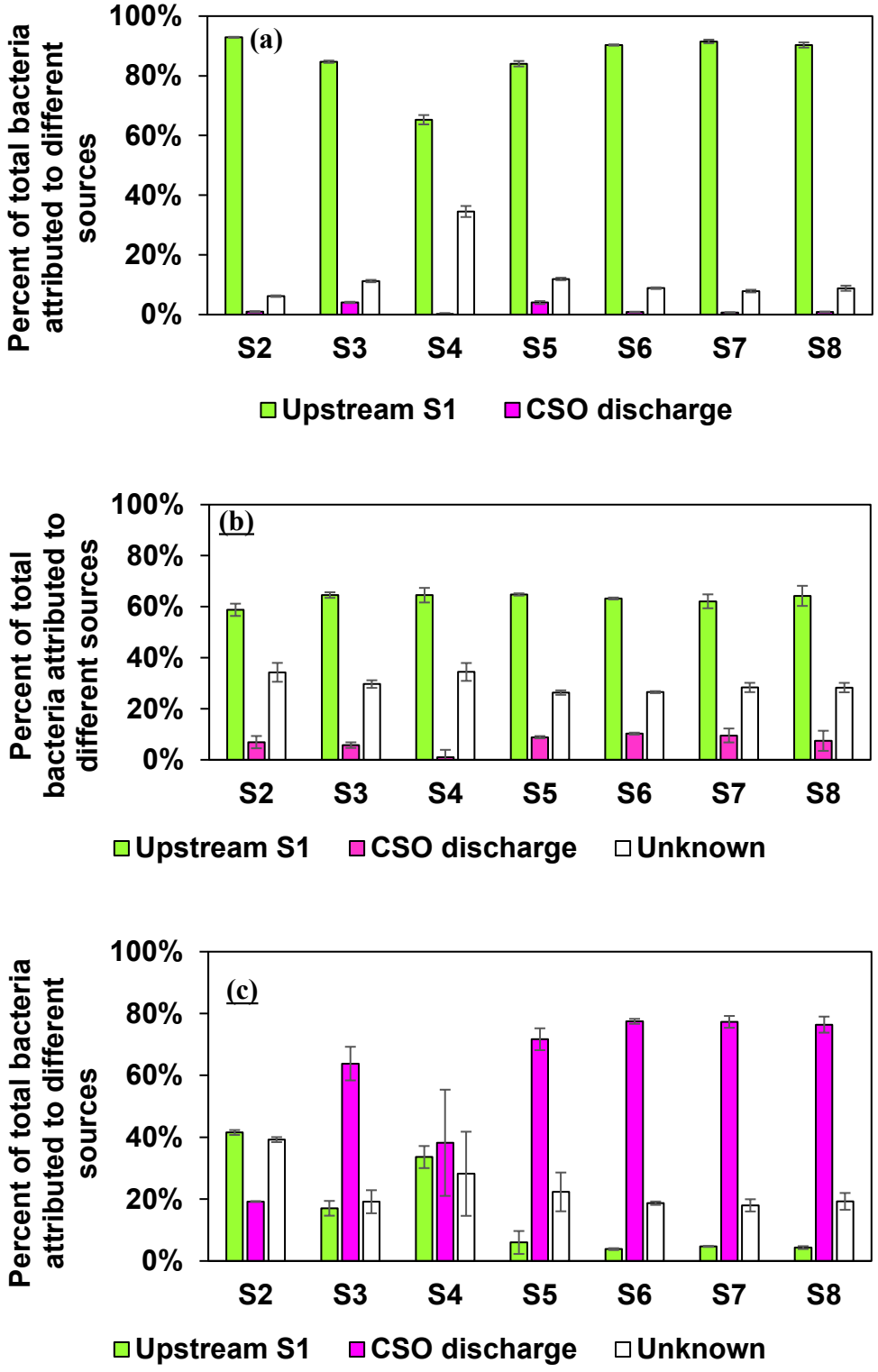

Figure 4.4 SourceTracker analysis using the 16S rRNA gene sequencing data from the June 2nd (dry weather, a) , the July 7th (dry weather after a day of heavy rainfall, b), and the Sept 27th (storm event, c) and CSO discharge samples from the Oct 5th storm event to attribute bacteria in the river at sampling locations S2-S8 to either rural upstream sources (based on the bacteriology at S1) or CSO sources (based on the bacteriology of CSO4 discharge).

A combined hierarchical clustering and heatmap of 16S rRNA genes attributed to 55 human gut associated bacterial genera (King et al., 2019) affirms the substantial spatiotemporal variation in faecal pollution signatures in the bacterial communities of the Ouseburn (**Figure B2 in Appendix B**). Concentrations of these genes varied by up to 6 orders of magnitude between samples collected during dry weather on June 2nd, and during the Sept 27th storm event. Two way-crossed ANOSIM confirmed a significant effect of sampling date ($R = 0.747$, $p = 0.001$) and location ($R = 0.651$, $p = 0.001$) for this data set.

Similar trends were observed in a corresponding PCA plot and combined hierarchical clustering/heatmap for sixteen additional samples collected at S5 in Jesmond Dene Park between July 27th to Aug 9th (**Figure B3-4 in Appendix B**). The bacterial community composition in the Ouseburn pendulated between predominantly freshwater bacteria during dry weather, and a high abundance of faecal bacteria following rainfall in the early afternoon of Aug 8th.

Apart from dissolved oxygen, which didn't correlate significantly with any other metric, significant and often high Pearson correlation coefficients were observed between logarithmic values of most measurements by physicochemical methods, plate counting and qPCR, showing that water quality in the Ouseburn varied in a systematic way (**Figure B5 in Appendix B**). For the HF183 genetic marker for human sewage, high Pearson correlations coefficients R with values > 0.90 were found with the *rodA* genetic marker for *E. coli*, ammonium-N, total-N, turbidity, and FC. We derived a relationship with statistics $R^2 = 0.94$ and $p < 0.001$ to predict HF183 from ammonium-N and turbidity:

$$log_{10}(HF183\ [genetic\ markers\ per\ 100\ mL]) = 1.74 \pm 0.27 \times log_{10}\left(ammonium - N\ \left[\frac{mg}{L}\right]\right) + 0.67 \pm 0.27 \times log_{10}(turbidity\ [NTU]) + 6.24 \pm 0.53 \quad \text{eq. 4}$$

BEST (or BioEnv) analysis of 16S rRNA gene sequencing data further showed that alkalinity together with nutrients (COD, ammonium-N, total-N, total-P) were environmental variables strongly associated with the observed differences in the bacterial community characteristics (**Table B4 in Appendix B**).

4.4.4 Spatiotemporal variation in loads of faecal bacteria transported by the river

While the assessment of local status is focused on pollutant concentrations, the downstream impacts on estuary and marine ecosystems and coastal bathing water quality will relate to pollution loads transported by rivers (Skogen et al., 2014, Neal et al., 1997). Dry weather flow

in the Ouseburn was very low, but flows spiked rapidly in response to rainfall, with hydrograph characteristics of an urbanized catchment (Birkinshaw et al., 2021). During heavy rainfall on Sept 27th, we observed a marked increase in pollutant loads (flow x concentration) transported by the Ouseburn as flow and concentrations of nutrients and faecal bacteria simultaneously peaked (**Table B5 in Appendix B**). Compared with the dry weather flow on June 2nd, the storm weather flow on Sept 27th was similarly elevated in the rural upstream (S1, 23-fold elevated), and after the urbanized part of the catchment (S5, 26-fold elevated). Loads of sewage pollution markers ammonium-N and HF183 increased beyond the flow, and most notably in the urban catchment. In the rural part of the catchment (S1), ammonium-N loads increased 232-fold, and loads of HF183 increased 65-fold, as compared to dry weather flow on June 2nd. In the urbanized part of the catchment (S5), ammonium-N loads increased 671-fold, and HF183 loads increased markedly by 124,158-fold. During the storm, flow at S5 was 9-fold higher than at S1, while loads of ammonium-N and HF183 increased 36-fold and 3,614-fold, respectively, between the two gauging stations.

Sixteen measurements at S5 between July 27th and August 9th, when the summer weather was changeable with scattered showers, confirmed increased loads of faecal bacteria transported by the Ouseburn after rainfall (**Figure 4.5**). While flow in this period at S5 varied by up to 21-fold, loads of total bacteria varied by up to 226-fold, and loads of HF183 varied markedly by up to 121,403-fold.

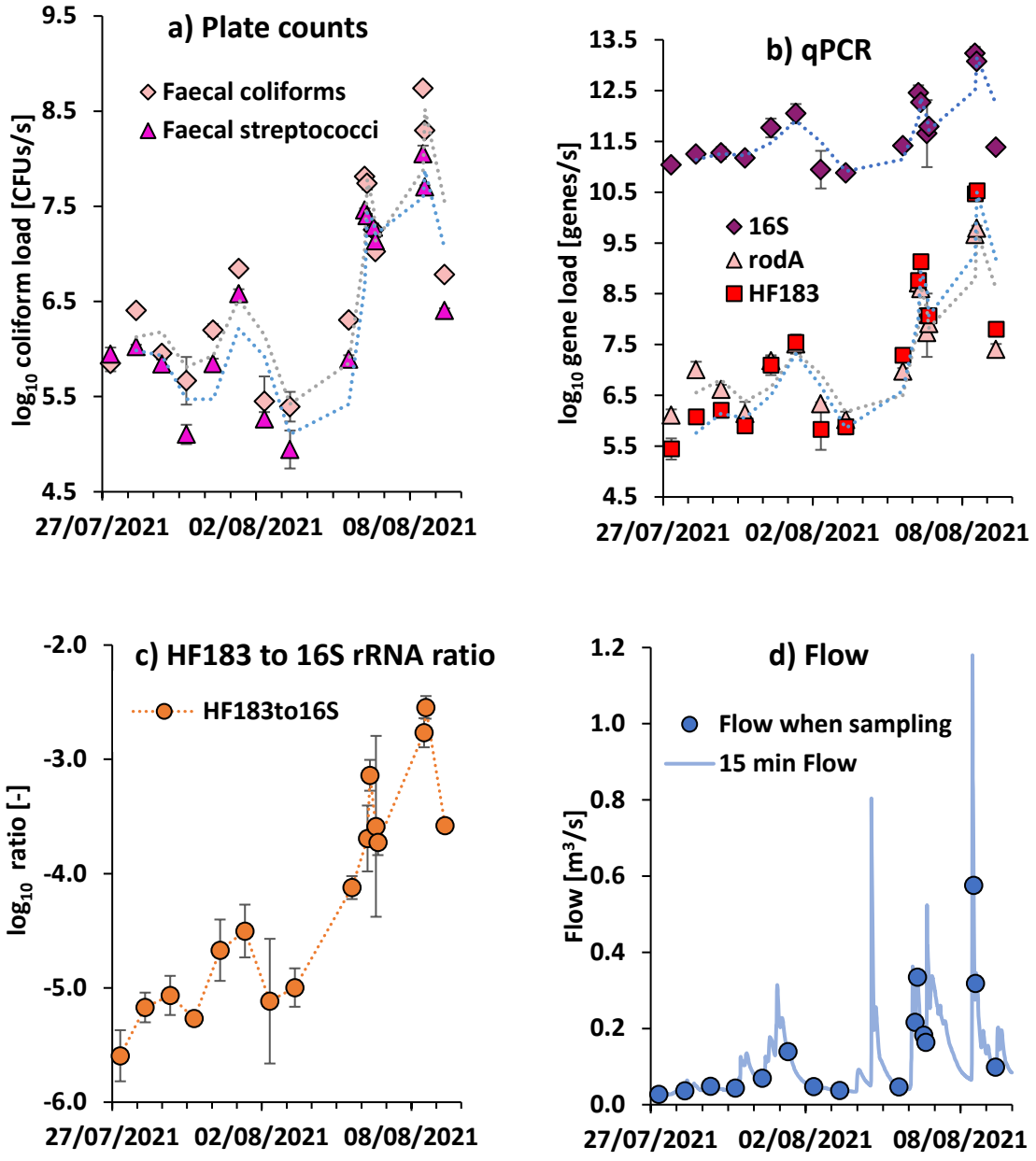

Figure 4.5 Observations at the Environment Agency gauging station at Crag Hall (S5) in the upper part of Jesmond Dene Park between July 27th and Aug 9th, 2021, average±stdev, a) loads of coliform bacteria, b) loads of genetic markers, c) ratio of genetic markers for sewage (HF183) to total bacteria (16S rRNA), d) Ouseburn flow at S5.

4.4.5 Quantitative microbial risk assessment (QMRA)

We based our evaluation of recreational water quality on the sixteen samples collected in Jesmond Dene Park at S5 between July 27th and Aug 9th in a period of changeable summer weather with scattered showers falling locally and resulting in moderately elevated river flows (**Figure 4.1b**). Most guidance values for recreational water quality are derived from risk

assessments for swimming to provide precautionary protection based on an activity with relatively high volumes of accidental water ingestion (WHO, 2021). However, wading and splashing around is a more likely recreational activity in smaller rivers like the Ouseburn flowing through urban green spaces. According to a swimming pool study, the estimated median and 95th percentile water volume ingested during recreation is 6 mL and 34.8 mL for swimming, versus 2.2 mL and 11.2 mL for wading and splashing around (Dorevitch et al., 2011). **Figure 4.6** illustrates the estimated risk of contracting a bacterial gastrointestinal illness (*Salmonella*, *Campylobacter*, *E. coli* O157:H7) when wading and splashing around in the Ouseburn on the sixteen sampling dates in Jesmond Dene Park between July 27th and Aug 9th. For this risk assessment, we used our eDNA observations and literature data for QMRA with assumptions detailed **in Appendix B**. By randomly combining one of the sixteen water quality assessments with a value drawn from a lognormal distribution of the ingestion volumes for wading and splashing around, we simulated 50,000 exposure events. From these simulations, our estimated median and 95th percentile risk of contracting a bacterial gastrointestinal disease was 0.03 and 0.39, respectively. The latter value is well above the 95th percentile risk threshold of >0.1 defining the lowest recreational water quality category D in the WHO classification (WHO, 2021).

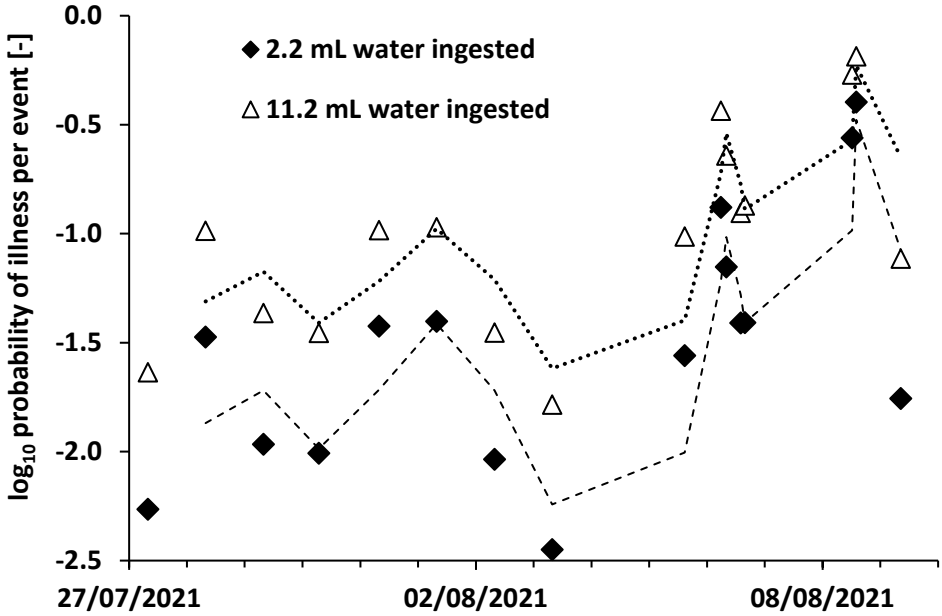

Figure 4.6 Probability of contracting a bacterial gastrointestinal illness per event for people wading and splashing around in the Ouseburn derived from quantitative microbial risk assessment (QMRA) using eDNA derived data, for the median (2.2 mL) and 95th percentile (11.2 mL) volumes of water ingestion.

4.4.6 Additional insights from event duration monitoring (EDM) data and sewage litter observations

In England and Wales, data on the total duration of CSO discharges and number of spills is published as aggregated annual data and summarized for the Ouseburn catchment and the year 2021 in **Table B1 in Appendix B**. These data reveal frequent spillages (41 and 52 events) for a total of 402-473 hours (or 4.6-5.4% of the time) from CSOs 9 and 10, in the north-eastern branch of the catchment. CSOs 9&10 discharge into a surface water drainage system that joins the Ouseburn just upstream of Jesmond Dene Park (**Figure 4.1a**). CSO4, from which we collected discharge samples on Oct 5th, had 14 annual spills for a total duration of 27 hours (or 0.3% of the time). While annual aggregate data does not allow matching CSO spillage to water quality on specific dates, the frequent spillages are consistent with poor water quality in the Ouseburn during rainfall events (**Figures 4.2-4.5**). Sewage litter observations substantiate frequent CSO discharges in the summer and early autumn of 2021, as summarized in **Appendix B with Figures B6-7**.

4.4.7 Surveying costs

The directly incurred costs at year 2021 prices of one of our surveys (i.e., June 2nd 2021) to sample and comprehensively characterize the water quality at 8 sites in the Ouseburn catchment are summarized in **Table B7 in appendix B**. Of the £4,705 survey costs, 6% were attributed to the sampling and onsite analysis, 19% to the water chemistry, 14% to the conventional microbiology, 11% to the eDNA extraction, 17% to the qPCR analysis, and 34% to next generation sequencing.

4.5 Discussion

4.5.1 Comparison of study data with official assessments of the water body

The Ouseburn is monitored by the Environment Agency (EA) in accordance with The Water Environment (Water Framework Directive) (England and Wales) Regulations 2017, and assessed to have overall moderate ecological status (Defra, 2019). In the official assessment, high status is indicated by temperature, pH, dissolved oxygen and ammonia, and moderate status by phosphate and biological observations. Our measurements (**Table 4.1**) would classify the Ouseburn as river type 5 (100-200 mg/L $CaCO_3$ alkalinity and elevation < 80 m), and in line with the EA assessment, all pH values, and the 10th percentile for dissolved oxygen saturation, were in the range for high status (6-9 and >70%, respectively). However, in our data set, the 90th percentile of 1.38 mg/L ammoniacal-N fell above the boundary defining moderate status (1.1 mg/L for river type 5), and the phosphate-P concentration (average±stdev) of

0.34±0.25 mg/L also exceeded the boundary defining moderate status (0.162 mg/L for 123 mg/L alkalinity and 68 m median elevation). The discrepancy with the official assessment resulted from the targeted inclusion of contrasting weather conditions in our sampling campaign (dry, day after rainfall, heavy rainfall), versus the random sampling underpinning the regulatory assessment. Stated reasons in the official EA assessment for why the Ouseburn is not achieving good status include "misconnections" contributing to moderate phosphate status, and "sewage discharge (intermittent)" contributing to poor status for fish (Defra, 2019). Despite these official acknowledgments of sewage inputs, the microbiological status of the Ouseburn is not monitored and reported by the EA, since like almost all rivers in England and Wales, the Ouseburn does not contain a designated bathing water site (GOV.UK, 2022a).

4.5.2 Value added by including eDNA in freshwater monitoring

Physicochemical water quality can be measured quickly, including with light scattering-based sensors for turbidity and ion-sensitive sensors for ammonium to potentially continuously monitor water quality in rivers (Winkler et al., 2004). Amongst the physicochemical metrics, ammoniacal nitrogen is most often used as a sewage pollution indicator, but previous reports from the Ouseburn concluded that this metric on its own is insufficient to reliably identify sewerage input into rivers (Baker et al., 2003). The previous work therefore combined ammonia with tryptophan fluorescence to attribute >10% of the rivers' discharge in this area to sewerage inputs. Our study found a strong correlation between ammonium-N, as well as turbidity, and the genetic marker for human sewage HF183. However, we also noted that the Ouseburn is officially assessed as having good status based on ammoniacal nitrogen concentrations and Water Framework Directive standards for physicochemical water quality (Defra, 2019), while our analysis of bacterial water quality indicated substantial risks of gastrointestinal illness for recreation in the Ouseburn. We deduce that continuous sensing of physicochemical water quality in rivers (Boënne et al., 2014) can complement, but not substitute, the monitoring of bacterial water quality in rivers. Catchment-specific, calibrated relationships between physicochemical and bacteriological water quality parameters, as exemplified by eq. 5, could, however, be used to infer from the continuous sensing data the water quality in between events when samples would be taken for more comprehensive assessment that includes microbiological methods.

Assessing bacterial water quality with conventional plate counting methods remains essential to demonstrate viability of faecal bacteria (Acharya et al., 2019) and for comparison with historic data and existing regulatory standards (WHO, 2016). However, the conventional

methods cannot distinguish between various sources of faecal pollution. We observed, for example, that at the most upstream, rural sampling location (S1 in **Figure 4.2**), FC and FS counts increased more in response to heavy rainfall than the human sewage marker HF183, perhaps due to runoff contaminated with animal manure from fields and farms.

Environmental DNA, especially qPCR methods, can help distinguish between human and animal sources of faecal pollution (Ahmed et al., 2019b, Boehm and Soller, 2020). In our study, the HF183 genetic marker most sensitively indicated the deteriorating bacteriology of the Ouseburn as it flows through the urbanized catchment during storm events (**Figures 4.2 & 4.5**). As expected, we measured high concentrations of HF183 genetic markers in both, sewage and CSO discharge (**Table 4.1**). But in contrast to our work in Africa (Hiruy et al., 2022), we detected HF183, albeit at much lower levels, also in rural settings (S1 & S4), and throughout the catchment for dry weather conditions when CSOs are unlikely to discharge (June 2nd in **Figure 4.2**). This background HF183 concentration could have resulted from effluents of septic tanks in rural areas, and sewage inputs via household appliances that are misconnected into surface water drainage systems (Zan et al., 2022). Although rarely, the HF183 genetic marker has been amplified at low concentration from some animal faecal samples (Boehm and Soller, 2020). A fraction of human-host associated *Bacteroides* frequently released into the environment via treated and untreated wastewater discharges may also survive and have become endemic in the UK freshwater environment. In short, although very useful, we advise against the sole use of the HF183 genetic marker for monitoring sewage derived hazards in UK freshwaters, and future work should establish the cause of the observed background levels.

Sequencing provides comprehensive characterization of microbial communities in a single analysis, generating data that can be used as an input for SourceTracker analysis to quantitatively attribute bacteria in a river to different sources (Pantha et al., 2021, Hiruy et al., 2022, Williams et al., 2022). Sequencing also simultaneously screens a wide range of potential hazards (Cui et al., 2019). As in other recent work (Urban et al., 2021, Acharya et al., 2020, Reddington et al., 2020, Kristensen et al., 2020), our sequencing data revealed, for example, a high abundance of *Aliarcobacter* bacteria in sewage, CSO discharge, and the Ouseburn during storm events (**Figure 4.3**). The genus *Aliarcobacter* contains several pathogens of emerging concern that may cause gastrointestinal disease (Chieffi et al., 2020).

4.5.3 Assessing risks to public health

In the absence of bathing water designation, microbial water quality in UK rivers remains unregulated despite a joint opinion piece from the Chief Medical Officer for England, the Water Services Regulation Authority (Ofwat) chair, and EA chair, stating that "children have always played in waterways and always will, irrespective of what notices are put up next to them"(Whitty et al., 2022). Indeed, people can be regularly observed wading and splashing around in the Ouseburn, especially in Jesmond Dene Park during summer months. Although not done for regulatory purposes, our findings indicate that the Ouseburn would struggle to achieve sufficient bathing water status in Jesmond Dene Park according to the Bathing Water Regulations 2013. The 90th percentile of sixteen $\log_{10}$ FC per 100 mL values measured at S5 between July 27th and Aug 9th was 4.64. From the literature, 68-93% of the FC count are *E. coli* (Hamilton et al., 2005). If we conservatively use 68% for the conversion, the estimated 90th percentile *E. coli* concentration is 29,398 CFU per 100 mL, well above the threshold for sufficient bathing water status (900 *E. coli* per 100 mL as 90th percentile). Our FS counts exceeded by a factor 7 to 611 the 32 per 100 mL threshold for which adverse health effects were identified in an exposure study with swimmers in UK coastal waters (Kay et al., 1994). Researchers in the United States used the HF183 genetic marker to derive sewage content and related pathogen concentrations (*Salmonella*, *Campylobacter*, *E. coli* O157:H7, *Cryptosporidium*, *Giardia*, norovirus, and adenovirus) in recreational water (Boehm and Soller, 2020). They proposed thresholds of 9,000 and 525 HF183 genetic marker copies per 100 mL, for recreational waters receiving fresh sewage or sewage of unknown age, respectively, for a median gastrointestinal illness probability that meets US EPA criteria of no more than 32 illnesses per 1000 swimming events. In Jesmond Dene Park, the proposed upper threshold for HF183 was exceeded for 62.5%, and the lower threshold was exceeded for 100% of the sixteen sampling events. We also derived risks of contracting a bacterial gastrointestinal disease from our 16S rRNA gene sequencing and qPCR data. The predicted 95th percentile risk is well above the threshold of >0.1 defining the lowest recreational water quality category D in the WHO classification (WHO, 2021). Since we did not monitor viruses and protozoa, we could not include them in our QMRA. Also, we could not include *Aliarcobacter* bacteria, since to the best of our knowledge, no dose-response curve has yet been established for *Aliarcobacter* species. Our predictions are therefore likely a floor rather than ceiling for the overall gastrointestinal illness risks. There is substantial uncertainty around various assumptions made in QMRA. For example, depending on the chosen dose-response study and fitting models, the predicted risks of illness may differ substantially for exposure doses far below the range of

experimental validation (WHO, 2016). In our QMRA, the 95th percentile gastrointestinal illness risk resulted mostly from exposure to pathogenic *E. coli* and *Salmonella* at doses within the range of their respective empirical study data (Teunis et al., 2008, WHO, 2002). For pathogenic *Campylobacter* the corresponding exposure dose was an order of magnitude below the range of the empirical study data (Teunis and Havelaar, 2000), but *Campylobacteriosis* made only a minor contribution to the overall 95th percentile gastrointestinal illness risk (0.35 without versus 0.39 with inclusion of *Campylobacter*). There is considerable debate about the most appropriate criteria for recreational water quality (Kay et al., 2004). To address these uncertainties, our analysis used a wide range of methods which, via multiple lines of evidence, consistently gave concern about the safety of people recreating in the Ouseburn.

4.5.4 Closing gaps in regulatory and monitoring frameworks

In our view, the lack of microbial water quality standards and resultant absence of official monitoring data is an elephant-sized hole in current regulation for protecting the freshwater environment in the UK, and beyond. It seems illogical that regulations are concerned with pollution risks to invertebrates and fish (Defra, 2019), but remain silent on the risks to people wading and splashing around in rivers. From our work, microbial water quality should be monitored where rivers flow through public parks, irrespective of their bathing water designation. Since parks are already managed and designated for public recreation, they provide a logical next step for expanding current monitoring programmes. Ultimately monitoring should cover all freshwater areas that are used for recreation purposes. To immediately improve the status quo, all event duration monitoring (EDM) data should be published in real-time, instead of as annual aggregated data (**Table B1 in Appendix B**). Digital applications could then be developed to alert the public to local CSOs discharges.

Analysis of eDNA from UK rivers as demonstrated in our study should in future work be expanded to the study of viruses (Rames and Macdonald, 2018) and antimicrobial resistance (Knapp et al., 2012), for example via metagenomics (Hu et al., 2018) or high throughput qPCR (Quintela-Baluja et al., 2019, Thongsamer et al., 2021). In response to the Covid-19 pandemic, the UK has built high throughput capabilities for wastewater monitoring with molecular methods via its Environmental Monitoring for Health Protection (EMHP) programme (Morvan et al., 2022). These assets and experiences could be built upon to better monitor the freshwater environment in the future. Our comparison of directly incurred surveying costs suggest the inclusion of eDNA methods would increase conventional monitoring costs (water chemistry and plate counts) by a factor 1.7 with inclusion of qPCR (to quantify 3 genetic markers) and a

factor 2.6 with inclusion of both qPCR and sequencing of 16S rRNA genes. Although eDNA based methods are more expensive than conventional methods, they help identify the microbial pollution source which is essential for risk assessment and decision making.

Ultimately, improving the bacteriology in urban rivers will need substantial financial investment by the UK water industry to address the root causes and implement well-known remedies such as sustainable urban drainage systems (SuDS) (Gimenez-Maranges et al., 2020). Government plans to substantially reduce the frequency spills from storm overflows (DEFRA, 2022) are welcome in this context.

4.6 Conclusions

CSO discharge had a high content of faecal bacteria and nutrients indicative of 4-6% sewage content which disproves the notion that CSO discharges are 'pretty much already rainwater.' A 2 $\log_{10}$ unit increase in concentrations of faecal bacteria in the river Ouseburn during storm events furthermore suggests that dilution of CSO discharge by the unpolluted catchment runoff can be insufficient to minimize hazards in a small river like the Ouseburn with an urbanized catchment area. Environmental DNA based monitoring, using genetic markers for human host associated *Bacteriodes* and SourceTracker analysis, clearly linked the faecal pollution of the river Ouseburn during storm events to CSO discharge. The faecal pollution of the Ouseburn posed a significant risk to people wading and splashing in the river, since data from sixteen sampling events in a public park and related QMRA exceeded guidance values for recreational water quality. Based on our findings, event duration monitoring data for CSO discharges should be shared in real-time to warn recreational water users of related health risks. And the authorities should monitor microbial water quality in rivers that flow through public parks, irrespective of their bathing water designation.

Data availability

Fastq files generated from 16S rRNA gene sequencing have been submitted to the NCBI Sequence Read Archive (SRA) with BioProject accession numbers PRJNA814853 (Ouseburn data) and PRJNA837409 (Birtley STP data). Additional data created during this research are openly available (https://doi.org/10.25405/data.ncl.22774688). Please contact Newcastle Research Data Service at rdm@ncl.ac.uk for access instruction.

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Chapter 5

Activated carbon amendment of sand in the base of a pilot-scale permeable pavement reduces total nitrogen and nitrate leaching

Chapter 5. Activated carbon amendment of sand in the base of a pilot-scale permeable pavement reduces total nitrogen and nitrate leaching.

5.1 Abstract:

Urban runoff from impermeable surface contains various pollutants. For one year, we collected monthly stormwater samples from car parks on the campus of Newcastle University located in northeast England to monitor seasonal variation in stormwater properties and study leachate quality following stormwater percolation through pilot-scale permeable pavements placed outdoors. The pilot study compared an innovative 'pollution munching' permeable pavement with 2% activated carbon (AC) amendment in the sand base with a conventional sand base permeable pavement. The measured physicochemical pollutant concentrations of stormwater were below drinking water quality standards, except for chloride and faecal bacteria. Faecal bacteria were detected at an average value of 3.75±0.79 $\log_{10}$ CFUs per 100 mL. The absence of genetic markers for human host associated *Bacteroides* (HF183) in eleven out of twelve stormwater samples showed that these faecal bacteria were mainly from animal sources. 16S rRNA gene sequencing results confirmed the presence of nitrifying bacteria from the genera *Nitrosomonas, Nitrobacter, Nitrosococcus, Nitrospira,* and *Nitrosospira* in stormwater. Nitrification and nitrate leaching was consequentially notable for the conventional permeable pavement and may pose a groundwater pollution risk. Two percent AC amendment of the sand base reduced nitrate and total nitrogen leaching significantly, by 57±15% and 40±20%, respectively, compared with the conventional permeable pavement. The AC amendment also resulted in significantly reduced Cu and TOC leaching, and lesser accumulation of PAHs by passive samplers embedded in the permeable pavement base. Hydraulic tests showed that the AC amended base layer still met the design specifications for permeable pavements, making it a promising proposition for pollution reduction in sustainable urban drainage systems.

5.2 Introduction

Due to rapid urbanisation, catchment areas covered by impermeable surfaces have dramatically increased in both developing (Tran et al., 2020) and developed countries (Strohbach et al., 2019). In addition, climate change may result in more frequent extreme weather events, and many countries have faced more frequent urban flooding, which threatens human lives, infrastructure, and environmental conditions (Jegatheesan et al., 2019, Jiang et al., 2018). Another impact of rapid urbanisation is water pollution caused by stormwater. Road surfaces are polluted by fuel and oil leaks, tyre and break wear and airborne dust. When rainfall flushes the road surface, this pollution will be transported with the runoff into stormwater drainage

systems and end up in the water environment (Müller et al., 2020). Combined sewer systems convey sewage and runoff in the same pipes. During a heavy storm event, rainfall enters these systems and may exceed their capacity, resulting in overflows (Tibbetts, 2005). Combined sewer overflows (CSOs) are increasingly recognized as a threat to aquatic ecosystems, given their high frequency of discharge, which can be as high as over 100 times a year (EAC, 2022). This situation has led to substantial public debate, including in the parliament of the United Kingdom (UK) (EAC, 2022). The parliamentary debate resulted in the UK's Environment Act (2021), which makes provision about improved stormwater reporting, monitoring and reduction of adverse impacts to improve the natural environment.

To reduce the urban runoff volume and stormwater pollution, many countries have applied nature-based strategies called Green Infrastructure (GI), Best Management Practices (BMPs), Low Impact Development (LID) and Sustainable Drainage Systems (SuDS) (Guan et al., 2021, Kuruppu et al., 2019). For example, the 'sponge city' programme in China aims to increase the capacity of water uptake in urban spaces and to reduce the impact of stormwater on the water environment (Guan et al., 2021). Permeable pavement is one of many ideas for better stormwater management, aiming to minimize urban run-off and maximize stormwater retention in urban catchments (Kuruppu et al., 2019). A typical permeable pavement includes a paving layer (blocks or porous asphalt), base layer under paving, and sub-base layer upon the subgrade (Woods Ballard, 2015). Instead of the conventional impermeable surface, permeable pavement reduces the surface runoff through water infiltration, treats this water by biofiltration in the base and sub-base layer, and then discharges into pipes connected to surface water drainage systems or infiltrates water into the subgrade. Various pollutants such as total suspended solids (TSS), nutrients like nitrogen and phosphate, metals, and hydrocarbons can be removed by permeable pavements (Tirpak et al., 2020, Razzaghmanesh and Borst, 2019, Kuruppu et al., 2019, Huang and Liang, 2018, Brown and Borst, 2015, Drake et al., 2014b, Drake et al., 2014a, Tota-Maharaj and Scholz, 2010, Collins et al., 2010a, Collins et al., 2008). Permeable pavements may thus be used for car parks, driveways, playgrounds, and light to heavy traffic roads. Although permeable pavements can reduce the runoff volume and remove pollutants, their removal performance depends on weather conditions, permeable pavement types, base materials, and pollution load (Kuruppu et al., 2019). In addition, Boving et al. (2008) pointed out nutrient pollution of groundwater may be caused by porous asphalt permeable pavements.

Activated carbon (AC) has been widely applied in water treatment (Chowdhury et al., 2013), for contaminated site remediation (Ghosh et al., 2011), and gas purification (Marsh and Rodríguez-Reinoso, 2006). AC has a porous structure, large surface area, and diverse functional groups giving it high adsorption ability for various pollutants (Marsh and Rodríguez-Reinoso, 2006). For example, Ulrich et al. (2015) proposed that AC amendment of bioretention systems such as rain gardens could enhance trace organic contaminant removal for up to 58 years. A few studies have evaluated AC amendment of porous asphalt permeable pavements, showing good removal of nutrients, metals, and pollutants from vehicle exhausts (Hu et al., 2019, Huang and Liang, 2018). Since base materials below permeable pavements cannot be readily replaced when the pollutant sorption capacity of AC becomes exhausted, AC amendment benefits should ideally result from synergistic interaction between pollutant adsorption and biodegradation processes (de Castro et al., 2018, Igun et al., 2019). For example, AC may initially capture pollutants by adsorption to increase their retention in a porous sand layer for subsequent biodegradation (Bushnaf et al., 2017). During storm events with high infiltration rates, AC may cleanse the percolating water by adsorption. Then, the AC bound pollutants will be slowly desorbed back into the stagnant porewater for biodegradation in the interval between two storm events. Many researchers reported nutrient transformation and removal via microbial processes in permeable pavements (Collins et al., 2010a, Tota-Maharaj and Scholz, 2010, Drake et al., 2014a, Drake et al., 2014b, Brown and Borst, 2015, Razzaghmanesh and Borst, 2019, Tirpak et al., 2020). But few studies have evaluated the microbial community characteristics that drive these bio-transformations under permeable pavements (Yu et al., 2019, Fan et al., 2014). To best of our knowledge, no previous study has evaluated amending AC into the sand base of a concrete block permeable pavement for improved pollution attenuation via biofiltration processes.

5.2.1 Aim

Our overall aim was to determine a cost-effective method that facilitates urban stormwater infiltration and at the same time minimizes groundwater pollution risks. We therefore evaluated the anticipated benefits of amending activated carbon into the sand base of concrete block permeable pavements for pollution attenuation.

5.2.2 Objectives and hypotheses

1. To evaluate the effect of amending sand in the base materials with various amounts of AC on the hydraulic properties of permeable pavements.

2. To evaluate the physicochemical and microbial characteristics of stormwater, including pH, conductivity, total suspended solids (TSS), various forms of nutrients, dissolved metals, and bacteria, over a period of one year.
3. To evaluate the effect of amending the sand in the base with 2% AC on the physicochemical characteristics of leachate from permeable pavements, including pH, conductivity, total suspended solids (TSS), various forms of nutrients, dissolved metals and bacteria, over a period of one year.
4. To establish AC amendment benefits and derive design recommendations for permeable pavements with optimized stormwater infiltration and cleansing properties.

Our hypotheses were: 1) An AC amended sand base layer will meet hydraulic requirements for permeable pavements; 2) Stormwater quality poses a potential groundwater pollution risk; 3) AC amendment improves the performance of permeable pavements in terms of removing various pollutants such as nutrients, dissolved metals, and faecal bacteria. 4) The experimental work can identify an optimal amount of AC amendment for effective pollutant removal whilst maintaining an acceptable hydraulic permeability.

5.3 Methodology

5.3.1 Permeable pavements set-up and base materials

The design of the pilot-scale permeable pavements was based on the CIRIA SuDS Manual (Woods Ballard, 2015) and the concrete block permeable pavements design manual (Interpave, 2018). A total infiltration permeable pavement was selected as it's a simple structure which does not require pipe connections. The two permeable pavement set-ups consisted of three layers, 4-6 mm size of gravels at the bottom, Leighton buzzard sand as base material in the middle, without (Control) or with AC amendment (AC), and 60(W)×120(L)×50(H) mm standard pavement blocks (Jewson, Gateshead, UK) on top. The thickness of each layer is shown in **Figure 5.1 (b)**. The gravel was collected from a farm located in Nefferton (UK). The AC was produced from coal by Calgon and obtained from Chemviron (Feluy, Belgium). The size of sand and granular activated carbon (GAC) ranged from 2-4 mm. The two pilot-scale permeable pavements were each assembled inside a 12 mm polyvinyl chloride (PVC) box with metal reinforcement 540(W)×1120(L)×350(H) mm (**Figure 5.1 (a)**). The PVC boxes were manufactured by the Mechanical Engineering Workshop of Newcastle University, School of Engineering, UK. The 25 mm tap was attached to the side of the permeable pavements for

collecting filtration effluents. A 1 permille gradient was applied when placing the boxes to promote flow towards the taps. Leak proof tests were conducted prior to filling the boxes.

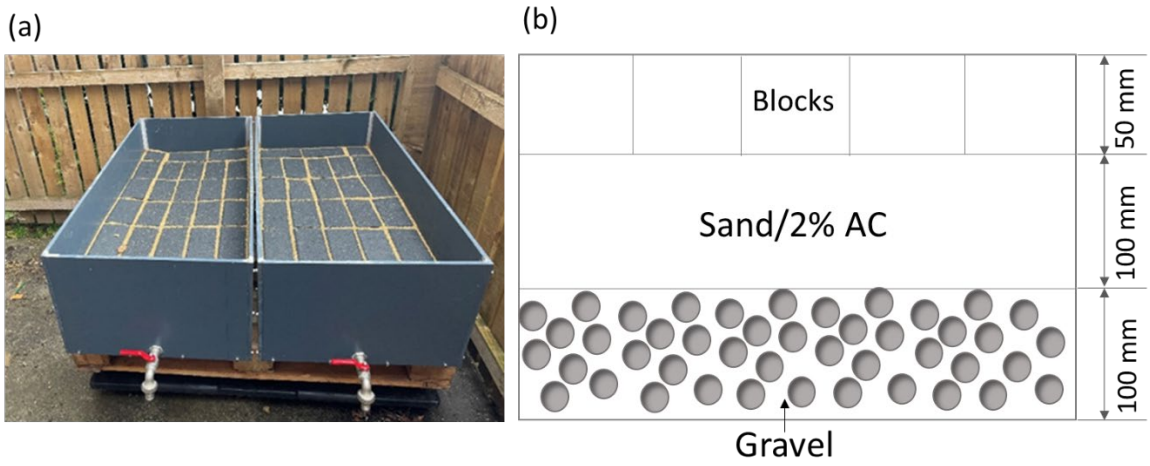

Figure 5.1 (a) Pilot-scale permeable pavements set-up with sand base on the left-hand side and 2% AC amended sand base on the right-hand side and (b) vertical view diagram.

5.3.2 Hydraulic tests

Hydraulic conductivity tests were conducted to determine the compatibility of an AC amended sand base with permeable pavement design criteria. Saturated hydraulic conductivity tests were conducted using KSAT from the METER Group AG (München, Germany) based on Darcy's Law. The saturated hydraulic conductivity (Ks) is calculated from the volumetric water flux V divided by the sample area A and the time t, the length of the soil sample L and the hydraulic gradient H along the flow direction as in equation 5.1.

$$Ks = \frac{L \bullet V}{H \bullet A \bullet T}$$ -(eq. 5.1)

All the measurements took place at room temperature (18-20 °C). The pure sand, and three AC amendment doses (1%, 2%, and 5% w/w) were tested. The Soil Water Retention (SWR) tests were conducted using a HYPROP instrument (METERO group, München, Germany).

5.3.3 Stormwater sample collection and infiltration tests

Composite stormwater samples were collected from puddles on impervious pavements and run-off alongside the curb stones of three car parks located on the campus of Newcastle University (Newcastle upon Tyne, NE1 7RU, United Kingdom) during heavy rainfall events. Twelve storm events were sampled over a one-year period at approximately monthly intervals between 12/07/2021 and 08/06/2022. **Figure 5.2**. shows the stormwater sampling locations. For each event, 20-30 L of stormwater were collected.

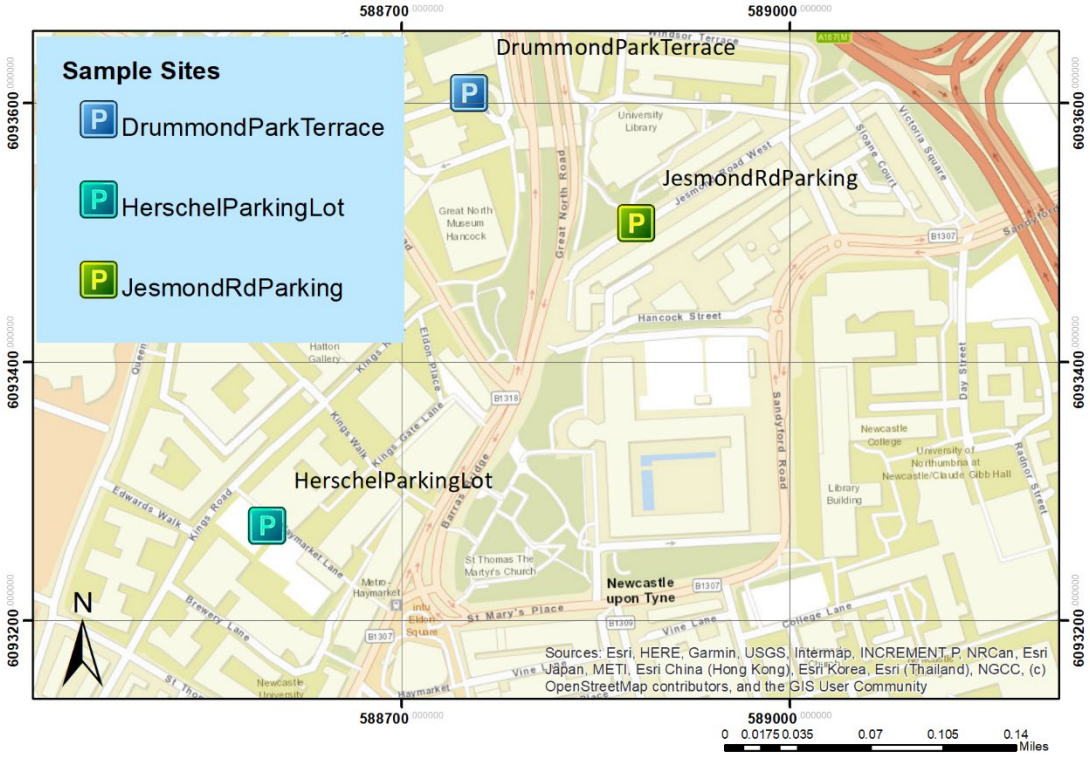

Figure 5.2 Stormwater sampling locations (Three car parks).

The infiltration tests were conducted within 2 hours after stormwater collection. Portions of the well-mixed stormwater samples were transferred into two 1L Neglen HDPE bottles (Thermo Fisher Scientific, Loughborough, UK) for later analysis, and ten litres were applied over each of the two pilot-scale permeable pavements to simulate an infiltration event. To compare the retention time and infiltration rate between the two permeable pavements, the timings were recorded for every 500 mL of effluent produced until 7L of effluent had been produced in total from each pavement. From each pavement, composite samples of the effluent were collected into two 1L Neglen HDPE bottles (Thermo Fisher Scientific, Loughborough, UK) for later analysis. In between the infiltration tests, the effluents from each pavement produced in response to rainfall were collected into 12L HDPE bottles, which were then subsampled about weekly, depending on the weather conditions, for a reduced schedule of analysis (total nitrogen, total phosphorus, and DOC only).

5.3.4 Physicochemical water quality analysis

Water samples were first filtered through Whatman® glass microfiber filters (1.6 µm pore size) (Merck, Gillingham, UK) for subsequent chemical analysis. The TSS were determined through the mass difference of the 100°C oven dried filters before and after the filtration. The filtrate was used for chemical water quality analysis using cuvette tests from HACH (Manchester,

UK). The water chemistry analysis comprised Ammonium-N (LCK 304), Nitrate-N (LCK 339), Nitrite-N (LCK341), Total-N (LCK138), Phosphate-P and total P (LCK349). We also measured the pH, conductivity, salinity, and total dissolved solids (TDS) with an ExStik handheld probe (Extech Instruments, Nashua, USA). A Model AL-DT portable alkalinity test kit (HACH, Manchester, UK) was used to measure alkalinity by titration.

We used filtrate from membrane filtration (0.2 μm, as explained below) to measure anions using a Dionex High Pressure Ion Chromatography instrument (Thermo Fisher Scientific, Loughborough, UK) to measure total dissolved carbon (TDC) and dissolved organic carbon (DOC) using a carbon analyser (Vario TOC cube, Elementar Analysen Systeme GmbH, Germany). The dissolved inorganic carbon (DIC) was calculated by difference from TDC and DOC results. Additionally, the filtrate was acidified by 1% v/v concentrated nitric acid to measure dissolved metals using a Varian Vista-MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) for higher concentrations, and Agilent ICP-MS 7700 Series instrument for trace level detection (Mayes et al., 2021). Certified 1000 ppm standards (accuracy of ≤ ± 1.0%; VWR Chemicals, VWR International, Leicestershire, UK) were diluted using 1% nitric acid solution for preparing calibration standards. Blanks and standards were run every 7 samples to check analytical accuracy and precision.

To analyse low concentrations of polycyclic aromatic hydrocarbons (PAHs) in the permeable pavement base layer, four passive samplers made of 1.200±0.005 g low density polyethylene (LDPE) (Merck Life Science UK Ltd, Gillingham, UK) were put into the sand/AC base layer on 1/02/2022 and removed on 28/06/2022. The passive samplers were then extracted twice by 10 mL of 50:50 (V:V) hexane: acetone as described by Meynet et al. (2012). A surrogate standard was added at the first extraction, which contains 2.5 μg/mL D_{10}-phenanthrene, 2.5 μg/mL D_{10}-fluoranthene, and 1.25 μg/mL D_{12}-perylene. The PAHs were analysed by GC-MS with an Agilent 6850 Gas Chromatograph (DB-XLB column length 30 m, i.d. 25 mm, and 1 μm film thickness) coupled to an Agilent 5973 mass spectrometer as described by Hale et al. (2012).

5.3.5 Microbial water quality

Conventional microbiology

For conventional membrane filtration plate counts, we filtered 1 mL of each water sample, diluted with 10 mL sterile saline solution, through 0.45 μm Gridded Sterile Cellulose Nitrate Membrane Filters (Sartorius, Göttingen, Germany). We used the Faecal Coliform ampules

from HACH (Manchester, UK) as growth medium. After 18-24 hours incubation at 44±0.5 °C, we counted the colonies visually.

Molecular microbiology

Bacterial biomass was concentrated by membrane filtration. Depending on turbidity, 50-300 mL water samples were filtered through 0.2 μm Gridded Sterile Cellulose Nitrate Membrane Filters (Sartorius, Göttingen, Germany). Filters were stored frozen at -20 °C for later use. The DNA was extracted using the DNeasy PowerWater Extraction kit from Qiagen (Crawley, UK). We followed the instructions provided by the supplier, but we used the lysing matrix E tube from MP Biomedicals (Eschwege, Germany) instead of the lysis tubes provided by the manufacturer. We also used the FastPrep-24 5G bead beating grinder and lysis system (MP Biomedicals, Eschwege, Germany) to strengthen the sample homogenization and cell lysis instead of the Vortex instrument mentioned in the manufacturer instructions. 50-100 μL of total volume containing cleaned-up DNA were obtained and stored at -20 °C for the downstream analysis. We qualified the DNA samples using the Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Loughborough, UK) and quantified the DNA concentration and yields using the Qubit ds DNA HS assay kits (Life Technologies, Paisley, UK) with the Invitrogen™ Qubit Fluorometer 2.0 (Thermo Fisher Scientific, Loughborough, UK). After quantification, the DNA was diluted with DEPEC-treated nuclease free water (Thermo Fisher Scientific, Loughborough, UK) to 5 ng/μL concentration for qPCR assays and the 16S rRNA gene amplicon sequencing library preparation.

The qPCR assays were conducted with a portable 2-channel Quantabio (Beverly, MA, USA) Q-qPCR thermocycler. The temperature programs, primers, and probes used for the qPCR assays are listed in **Appendix C Table C1.** The average efficiency, slope, and R^2 for qPCR assays are also stated in **Table C1**. The qPCR assays were conducted with 15 μL or 10 μL reaction solution. For the 16S rRNA assay, we used Bio-Rad Universe Inhibitor Tolerant SYBR green master mix (Bio-Rad Laboratories, Watford, UK) followed by forward and reverse primers and DNA-free water and 3 μL of DNA samples. For the human host associated *Bacteroides* (HF183) and *E. coli* (rodA), we used probe-based assays for enhanced selectivity as described in our previous work (Zan et al., 2022).

To characterize bacterial community characteristics in stormwater, permeable pavement base material, and effluents, we sequenced the DNA samples by MinION nanopore sequencing. For the base material, we collected samples on April 29th after temporarily removing pavement

blocks in four locations from each pavement, and we separately also collected material from in between the joints of the permeable pavement blocks to represent the surface material enriched with entrapped fine solids from the stormwater filtration.

We conducted full-length 16S rRNA gene sequencing with the MinION from Oxford Nanopore Technologies (ONT) (Oxford, UK) using the SQK16S024 multiplex barcoding kits from ONT (Oxford, UK). The sequencing library was prepared as per manufacturer's instructions. We used 20 ng of DNA for PCR amplification using specific 16S primers (27F and 1492R). The amplified PCR products were then purified with AMPure XP magnetic beads (Beckman Coulter, High Wycombe, UK) for a high-quality sequencing library. The sequencing libraries were loaded into the port of flow cells version R9.4.1 (ONT, Oxford, UK). The sequencing run was controlled with the MinKNOW software (v.21.02.1) developed by ONT (Oxford, UK), producing '.fast5' files, which were then base-called using Guppy (Version; v.5.0.11) from ONT (Oxford, UK) via the Windows Power Shell. Then the '.fastq' files were uploaded to the cloud-based analysis tool EPI2ME (v.5.0.4961252) (ONT, Oxford, UK) for taxonomic interpretation of the 16S rRNA gene sequences.

5.3.6 Multivariate data analysis

The CSV file including the taxonomic assignments (NCBI taxid) for each read were then downloaded from EPI2ME. The OTU tables were generated by Matlab© (Version R2019a, Mathworks, Portola Valley, CA, USA) using previously described scripts (Halla et al., 2022). We used the OTU tables generated at genus level to do cluster analysis, Principal Component Analysis (PCA) and analysis of similarity (ANOSIM) to compare the dissimilarity within and among sample groupings. To find out about nitrifying bacterial communities in stormwater and effluents, we extracted the relative abundance of OTUs matched to genera with ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) from the 16S rRNA gene sequencing results, then combined the data with the absolute abundance of 16S rRNA genes quantified by qPCR to compare between samples the absolute abundance of bacterial genera containing AOB and NOB. The list of AOB and NOB containing genera considered in this analysis is provided in **Appendix C Table C2**.

5.4 Results and discussions

5.4.1 Hydraulic tests

Tabe 5.1 compares the saturated hydraulic conductivity of sand without and with different AC amendment doses. AC amendments reduced the sand saturated hydraulic conductivity by 22%,

18%, 63% for a 1, 2 and 5% amendment dose, respectively. Nonetheless, the saturated hydraulic conductivity of the amended sand met the recommended range of permeable pavements for all AC amendment doses (Interpave, 2018). No significant difference in dry density among sand and different AC amended samples (1%, 2%, and 5%, t-test, $p>0.05$) was observed. This indicates consistency in sample preparation and the reliability of the saturated hydraulic conductivity test values as differences are attributable to sample structure and not the condition density under which tests were conducted.

Table 5.1 Saturated hydraulic conductivity and dry density of sand without and with AC amendments.

	Sand	1% AC	2% AC	5% AC	Literature values*
Saturated hydraulic conductivity (Ks, m/s)	2.69E-03 ± 6.50E-04	2.09E-03 ± 5.11E-04	2.20E-03 ± 3.86E-04	9.95E-04 ±2.42E- 04	1E-06 -1E-03[i]
Dry density (g/cm^3)	2.27 ± 0.00	2.31 ± 0.07	2.22 ± 0.01	2.27 ± 0.05	N.A

* Interpave (2018), i: Type A, total infiltration.

The soil water retention curves (SWRCs) for sand with and without AC amendment (sand, 1% AC, 2% AC and 5% AC) are shown in **Figure 5.3**. Water retention capacity is determined by the level of water content retained over a given range of pressure head (suction). The water retention capacity of 5% AC is the highest among the four types of media, while 1% AC amended sand has the lowest water retention capacity. 2% AC amendments had a similar water retention capacity compared with sand. According to the hydraulic conductivity and soil water retention tests results, 2% AC was selected as the treatment base material because it reduced hydraulic conductivity only moderately. Sand was chosen as the control base material under the permeable pavements. Additional infiltration rates for infiltration tests conducted between 12/07/2021 and 08/06/2022 are provided in **Appendix Figure C1**. The infiltration rates for Control permeable pavement were significantly higher than AC (t-test, $p<0.05$), which was in line with the saturated hydraulic conductivity results.

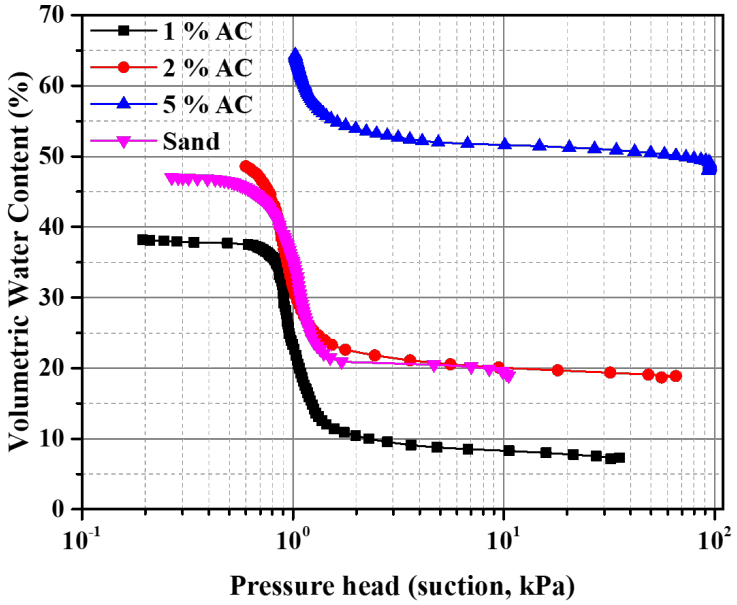

Figure 5.3 SWRCs for sand without and with 1%, 2% and 5% AC amendments.

5.4.2 Stormwater characteristics

We collected composite stormwater samples from the impermeable surfaces of car parks and along curb stones on the Newcastle University campus (Newcastle upon Tyne, UK). **Table 5.2** lists the physicochemical characteristics (TSS, chloride, conductivity, pH, and metals), and **Table 5.3** lists the nutrient (various forms of nitrogen and phosphorus, dissolved TDC, DIC and DOC) and microbial characteristics of twelve stormwater samples collected over one year. For comparison, water quality guidance values from the Groundwater (Water Frame Directive) (England) Direction 2016 and the Water Supply Regulations (2016) (England and Wales) were included, since stormwater infiltration may pollute groundwater resources that could be exploited for drinking water supply. In general, the stormwater samples collected from July 2021 to June 2022 had high total suspended solid (TSS) content above previously reported values of 9.8 mg/L (Collins et al., 2008). The pH of stormwater was in the neutral range (7.56±0.52), within the range stipulated in the drinking water standard, while the mean conductivity also met drinking water standards. The mean chloride was 10% higher than the drinking water standard, while the median chloride concentration was compliant. The Groundwater Direction 2016 doesn't regulate the threshold value for metals, but it lists As, Cd, Pb and Hg as pollution indicators. In general, the metals in stormwater, ranked from most to least abundant, were Al > Fe > Zn > Mn > Cu > Pb > As > Cd, while Hg was not detected in the stormwater samples. Compared with a previous study, the mean concentration of Al and Fe in stormwater were 0.8 and 0.86 times lower while the mean concentration of Cu was 1.6

times higher (Drake et al., 2014b). All metals concentrations in stormwater were below drinking water guidance concentrations. For nutrients, the mean concentrations of NO_x^--N and TN in stormwater were 23 and 3.1 times higher than reported in previous research, while TP was within the previously reported range (Tirpak et al., 2020). Compared with drinking water regulations, nitrate in stormwater was under the threshold value of 11.29 mg/L of Nitrate-N. Of the total dissolved carbon, stormwater samples contained > 80% in the form of DOC, which was 3 times higher than in a previous study (Brown and Borst, 2015). Microbial water quality is not defined in the Groundwater Direction 2016, but as a potential water supply source, the *faecal coliforms* count in stormwater should be considered. The mean *faecal coliforms* counts were 2 times less compared with the discharge we previously collected from separated sewer (Zan et al., 2022), and the median Faecal Coliforms counts were 10 times less than the discharge collected from separated sewers reported by Schreiber et al. (2019). The mean *faecal coliforms* counts were similar with those of driving lane surface runoff reported by Selvakumar and O'Connor (2018).

Spearman rank correlation analysis for stormwater between physicochemical and microbial parameters across the entire year are shown in **Table 5.4**. TP and ortho-phosphate had significant positive rank correlations with the mean counts of *faecal coliforms* ($p<0.05$) suggesting similar sources. The pH was significantly positively correlated with the mean absolute abundance of AOB/NOB and 16S rRNA genes ($p<0.05$). The 16S rRNA genes were positively correlated with TSS ($\rho=0.49$, $p=0.11$), suggesting dust deposits on roads are a source of total bacteria in the stormwater. The 16S rRNA genes were also significantly positively correlated with pH ($\rho=0.61$, $p<0.05$). To study seasonal trends, we compared April-September and October-March sample groupings, but there were no significant differences between these two data sets for all physical, chemical, and microbial parameters (t-test, $p>0.05$).

Table 5.2 Physicochemical characteristics (pH, conductivity, TSS, TDS, Cl^-, and metals) of stormwater influent, Control effluent and AC effluent samples in comparison with groundwater and drinking water standards.

Parameters		Stormwater	Control Effluent	AC Effluent	Standard [a, b]
pH	Mean±stdev	7.56±0.53	8.56±1.18	8.44±1.04	6.5-9.5[a]
	Median	7.49	7.93	7.89	
	10^{th} percentile	6.95	7.66	7.59	
	90^{th} percentile	8.27	9.83	9.67	
Conductivity (µS/cm)	Mean±stdev	931.25±1889.25	908.04±825.25	887.54±950.66	2500 µS/cm at 20°C[a, *]
	Median	242.50	565.00	470.50	
	10^{th} percentile	137.06	359.40	353.15	
	90^{th} percentile	1349.50	1796.95	1253.45	
TSS (mg/L)	Mean±stdev	545±396	24±19	32.3±26.4	N.A
	Median	442	17	24	
	10^{th} percentile	170	10	11	
	90^{th} percentile	1204	40	61	
Cl^- (mg/L)	Mean±stdev	281.64±618.40	175.02±276.24	195.17±366.89	250 mg/L[a]
	Median	36.62	51.95	57.65	
	10^{th} percentile	5.44	27.72	22.04	
	90^{th} percentile	417.98	501.39	326.17	
Al (µg/L)	Mean±stdev	94.04±121.47	103.58±148.99	103.19±133.19	200 µg Al/L[a]
	Median	51.14	49.84	46.84	
	10^{th} percentile	37.90	14.68	17.89	
	90^{th} percentile	134.63	241.31	351.70	
Fe (µg/L)	Mean±stdev	90.40±146.64	46.46±68.71	57.84±104.81	200 µg Fe/L[a]
	Median	28.09	22.40	9.17	
	10^{th} percentile	4.55	2.32	1.82	
	90^{th} percentile	195.96	82.04	105.93	
Cu (µg/L)	Mean±stdev	26.30 ±17.38	22.39±12.19	11.08±7.99	2000 µg Cu/L[a]
	Median	21.57	17.77	9.49	
	10^{th} percentile	13.29	13.78	5.13	
	90^{th} percentile	35.57	35.49	14.81	
Mn (µg/L)	Mean±stdev	30.34±57.61	2.98±2.19	3.10±2.05	N.A
	Median	8.18	2.42	2.90	
	10^{th} percentile	2.31	1.22	0.90	
	90^{th} percentile	55.48	4.80	6.19	
Zn (µg/L)	Mean±stdev	61.37±62.38	17.80±10.02	21.56±7.22	N.A
	Median	41.09	16.29	20.42	
	10^{th} percentile	19.27	7.46	14.04	
	90^{th} percentile	111.68	27.63	30.87	
As (µg/L)	Mean±stdev	0.71±0.32	13.42±16.23	11.29±14.72	10 µg As/L[a]
	Median	0.60	5.89	5.33	
	10^{th} percentile	0.48	2.44	3.33	
	90^{th} percentile	1.14	30.96	22.46	
Cd (µg/L)	Mean±stdev	0.05±0.06	0.02±0.03	0.02±0.03	5.0 µg Cd/L[a]
	Median	0.03	0.02	0.02	
	10^{th} percentile	0.01	0.01	0.01	
	90^{th} percentile	0.11	0.05	0.04	
Pb (µg/L)	Mean±stdev	2.65±4.00	0.67±0.83	0.88±1.08	10 µg Pb/L[a]
	Median	0.73	0.28	0.35	
	10^{th} percentile	0.37	0.10	0.12	
	90^{th} percentile	7.00	1.52	2.62	
Hg (µg/L)		N.A	N.A	N.A	1.0 µg Hg/L[a]

a: The Water Supply (Water Quality) Regulations 2016, b: The Groundwater (Water Framework Directive) (England and Wales) Direction 2016, *: Supply point

Table 5.3 Nutrients (NH_4^+-N, NO_3^--N, NO_2^- -N, TN, PO_4^{3-}-P, TP, TDC, DOC, and DIC) and microbial (Faecal Coliforms, 16S rRNA, and rodA) characteristics of stormwater influent, Control effluent and AC effluent samples in comparison with groundwater and drinking water standards.

Parameters		Stormwater	Sand Effluent	AC Effluent	Standard [a, b]
NH_4^+-N (mg/L)	Mean±stdev	0.40±1.25	0.11±0.16	0.09±0.13	0.39 mg/l (0.5mg/ L of ammonia) [a]
	Median	0.20	0.03	0.03	
	10^{th} percentile	0.06	0.01	0.09	
	90^{th} percentile	1.23	0.33	0.13	
NO_3^- -N (mg/L)	Mean±stdev	0.87±1.05	1.55±0.54	0.64±0.29	11.29 mg/L (50 mg of nitrate) [a,b]
	Median	0.51	1.49	0.58	
	10^{th} percentile	0.35	0.81	0.38	
	90^{th} percentile	1.25	2.19	0.86	
NO_2^- -N (mg/L)	Mean±stdev	0.05±0.06	0.17±0.39	0.06±0.11	0.15mg /L (0.5mg of nitrite) [a]
	Median	0.03	0.02	0.01	
	10^{th} percentile	0.02	0.01	0.00	
	90^{th} percentile	0.16	0.23	0.07	
TN (mg/L)	Mean±stdev	3.28±2.16	3.79±1.58	2.23±1.41	N.A
	Median	2.72	3.51	1.63	
	10^{th} percentile	1.65	2.38	1.44	
	90^{th} percentile	2.63	5.57	3.52	
PO_4^{3-}P (mg/L)	Mean±stdev,	0.29±0.33	0.28±0.18	0.22±0.10	N.A
	Median	0.14	0.22	0.18	
	10^{th} percentile	0.29	0.10	0.12	
	90^{th} percentile	0.33	0.47	0.38	
TP (mg/L)	Mean±stdev	0.35±0.33	0.30±0.18	0.26±0.13	N.A
	Median	0.21	0.26	0.21	
	10^{th} percentile	0.14	0.13	0.14	
	90^{th} percentile	0.60	0.48	0.43	
TDC (mg/L)	Mean±stdev	45.47±88.06	41.61±29.38	32.89±24.41	N.A
	Median	18.04	35.69	25.84	
	10^{th} percentile	13.13	24.35	20.34	
	90^{th} percentile	38.73	44.63	35.96	
DIC (mg/L)	Mean±stdev	8.37±2.57	23.18±5.79	21.25±5.36	N.A
	Median	7.71	22.53	19.70	
	10^{th} percentile	6.18	17.04	13.45	
	90^{th} percentile	11.41	30.56	26.25	
DOC (mg/L)	Mean±stdev	37.10±87.81	18.44±27.20	5.56±21.92	N.A
	Median	9.02	9.58	4.44	
	10^{th} percentile	6.29	7.29	3.13	
	90^{th} percentile	31.17	21.14	10.78	
Faecal Coliforms (log CFU /100 mL)	Mean±stdev	3.75±0.79	2.56±1.23	3.13±0.65	0 number/100 mL[a]
	Median	3.76	3.01	3.19	
	10^{th} percentile	2.72	0.53	2.22	
	90^{th} percentile	4.65	3.65	3.83	
16S rRNA (log genes/100 mL)	Mean±stdev	9.77±0.38	9.00±0.43	8.82±1.44	N.A
	Median	9.84	9.05	9.16	
	10^{th} percentile	9.22	8.34	8.68	
	90^{th} percentile	10.12	9.53	9.61	
AOB and NOB (log genes/100 mL)	Mean±stdev	6.63±0.54	6.27±0.73	6.02±1.64	N.A
	Median	6.64	6.46	6.46	
	10^{th} percentile	6.01	5.87	5.80	
	90^{th} percentile	7.20	6.79	6.80	

a: The Water Supply (Water Quality) Regulations 2016, b: The Groundwater (Water Framework Directive) (England and Wales) Direction 2016.

Table 5.4 Spearman rank correlation analysis for selected physicochemical and microbial parameters characterizing the stormwater samples.

Parameters	Faecal Coliforms		16S rRNA genes		AOB and NOB	
	Spearman rank coefficient (ρ)	p value	Spearman rank coefficient (ρ)	p value	Spearman rank coefficient (ρ)	p value
TDC	0.15	0.63	0.01	0.98	-0.06	0.83
DOC	0.10	0.32	-0.22	0.70	-0.38	0.23
TP	**0.83**	**0.001**	-0.11	0.73	-0.48	0.65
TN	0.06	0.84	-0.23	0.47	-0.14	0.11
TSS	0.27	0.40	0.49	0.11	0.45	0.14
pH	0.37	0.24	**0.61**	**0.03**	**0.61**	**0.03**
NH_4+	0.20	0.53	-0.17	0.60	-0.27	0.40
NO_3^-	0.39	0.21	-0.41	0.31	-0.50	0.10
NO_2^-	0.22	0.50	-0.32	0.19	-0.35	0.26
PO_4^{3-}-P	**0.70**	**0.01**	-0.22	0.50	-0.25	0.43

Bold denotes a statistically significant effect ($p < 0.05$)

5.4.3 Permeable pavement effluent characteristics, and impacts of AC amendment

The log removal efficiencies of key stormwater pollutants (TSS, PO_4^{3-}-P, TDC, DIC, DOC, Fe, Cu, Mn, Zn, As, Pb) from July 2021 to June 2022 are summarized in **Appendix C Table C3** and **Table C4**. Over the entire one-year sampling period TSS were removed effectively, by 1.3±0.4 and 1.2±0.5 log units, for the Control and AC permeable pavement, respectively, without significant difference between the two systems (t-test, $p>0.05$). The TSS removal efficiency in our study was higher than the range of 0.67 to 1.00 log units removal in previous reports (Drake et al., 2014a, Drake et al., 2014b, Tirpak et al., 2020).

For the total dissolved carbon (TDC), the log removal values for Control and AC effluents were not significantly greater than zero (z-test, $p>0.05$), which indicates for both pavements that only small amounts of carbon were lost to the atmosphere (i.e. as CO_2) or precipitated and retained as solids (i.e. as $CaCO_3$). For the dissolved inorganic carbon (DIC), the log removal values for Control and AC effluents were constantly negative, which indicates higher DIC in both effluents. No significant difference was found between Control and AC effluents for DIC (t-test, $p>0.05$). An increase in DIC during stormwater percolation through the permeable pavements results from DOC degradation to form DIC, or the dissolution of carbonate minerals. For the dissolved organic carbon (DOC), only AC significantly reduced the DOC with 0.35±0.13 log units removal (z-test, $p<0.001$) compared with stormwater, which was about 30% higher than a conventional permeable pavement reported by Brown and Borst (2015). The log removal values of DOC for AC effluent were significantly greater than that for Control effluent (t-test, $p<0.05$). The potential reason is that DOC adsorption may have

happened in addition to DOC biodegradation within the AC amended base layer. Similar findings were reported by Wang et al. (2023), when the leachates from soil amended with biochar, an AC-like material, had significantly lower DOC compared with the leachate from wheat straw pellets amended soil.

Figure 5.4 illustrates the log removal for different forms of nitrogen, total phosphorus and *faecal coliforms*, by the two permeable pavements. The AC pavement had significantly better total nitrogen removal than the Control pavement (t-test, $p<0.05$). On average, 0.7±0.4 and 0.7±0.3 log units of ammonium-N was removed by the AC and Control, respectively, without significant difference between the two permeable pavement types (t-test, $p>0.05$). On average, the log removal of nitrate-N for AC and Control were 0.02±0.24 and -0.37±0.30, respectively. Nitrate-N was initially removed in July by both permeable pavement types, but then the concentration of nitrate-N in the effluents eventually exceeded that of stormwater, with the Control releasing significantly more nitrate-N than the AC (t-test, $p<0.05$). From September 2021 onwards, both permeable pavement types showed removal of nitrite-N but there's no significant difference between AC and Control (t-test, $p>0.05$). Similar results were reported by Razzaghmanesh and Borst (2019), who found that permeable interlocking concrete pavement, porous asphalt, and pervious concrete, were able to remove ammonium-N and nitrite-N but not nitrate-N. Previous research also found much higher NO_X-N in permeable pavements compared with asphalt runoff (Collins et al., 2010a). This is due to the N cycling by microorganisms in permeable pavements. Aerobic conditions and the elevated pH range of the base materials in the permeable pavements provided optimal conditions for ammonium oxidizing bacteria (AOB) and nitrate oxidizing bacteria (NOB) and archaea species that convert reduced forms of nitrogen into nitrate (Tirpak et al., 2020). But subsequently, the denitrification process cannot proceed due to a lack of anoxic environments and carbon sources in conventional permeable pavements, which explains the high nitrate concentrations found in their effluent. Due to the highly porous nature of AC particles, AC amendment of the base layer of permeable pavements enhanced the water retention capacity as observed during the hydraulic conductivity tests and SWR tests. Therefore, it likely created more anoxic environments, including within AC macropores, where denitrification may occur. In addition, organic carbon remain in the AC pores may provide electrons to the denitrifying bacteria. Plaimart et al. (2021) found similar results when the addition of biochar, an AC-like material, to agricultural soil created more anaerobic niches for denitrification to occur, as compared to the unamended soil, resulting in reduced nitrate leaching from soil after digestate application.

For TP, the log removal efficiency of both permeable pavement types was not consistently positive and fluctuated, as shown in **Figure 5.4 (e)**. We observed similar trends for PO_4^{3-}-P removal. Differences between Control and AC pavement types were not significant for TP and PO_4^{3-}-P removal (t-test, $p>0.05$). This is contrary to previously reported TP and PO_4^{3-}-P reduction by permeable interlocking concrete pavement and pervious concrete (Drake et al., 2014a, Drake et al., 2014b, Brown and Borst, 2015, Tirpak et al., 2020). The fate of phosphorus in the permeable pavement depends on cycling between the pools of biomass phosphorus and inorganic phosphorus, and phosphorus mineral precipitation and dissolution (Brown and Borst, 2015). However, contrary to carbon as CO_2 gas, and nitrogen as N_2 gas, there is no gaseous release of phosphorus from a permeable pavement, and therefore all the phosphorus added will leach out once the phosphorus storage capacity of the system has been saturated.

For dissolved metals, in general both pavement types removed Fe, Zn, Mn, and Pb efficiently. However, their removal efficiency was not consistently positive. AC performed significantly better than the Control in terms of Cu removal (t-test, $p<0.001$). We found higher removal of Cu (AC only) and Pb (AC and Control) compared with previous research (Tirpak et al., 2020). The removal efficiency of arsenic was consistently negative across the 12 sampling events for both pavement types. Although no threshold value for As is stipulated in the Groundwater Directive (2006/118/EU), it is potentially of concern that the As effluent concentrations slightly exceeded standards for drinking water. Further optimization of the base materials to minimize release of arsenic should be considered in groundwater protection areas. The amount of TN, TP, and DOC leached out of the permeable pavements with the effluents in between infiltration tests is provided in **Appendix Figure C2**. In general, AC showed lower leaching of these nutrients in between the infiltration tests, but the difference to the Control was only significant for TP (t-test, $p<0.001$) and DOC (t-test, $p<0.05$).

For the microbial water quality, we measured *faecal coliforms* as indicator of faecal pollution. **Figure 5.4 (f)** illustrates the log removal of AC and Control from 12 sampling events. Overall, AC and Control had mean log removal values of 0.81±0.35 and 0.7±0.35, respectively, and they were significantly different from zero (z-test, $p<0.001$). The log removal values for Control and AC permeable pavements are much lower than a previous work, which reported 98% to 99% *E. coli* removal by the conventional concrete block permeable pavements (Tota-Maharaj and Scholz, 2010). The difference between Control and AC was not statistically significant (t-test, $p>0.05$). The removal efficiency of both permeable pavements fluctuated throughout the year. Although the Groundwater Direction (2016) doesn't regulate microbial

water quality standard, as a potential water supply source, extra treatment needs to consider for groundwater protection area.

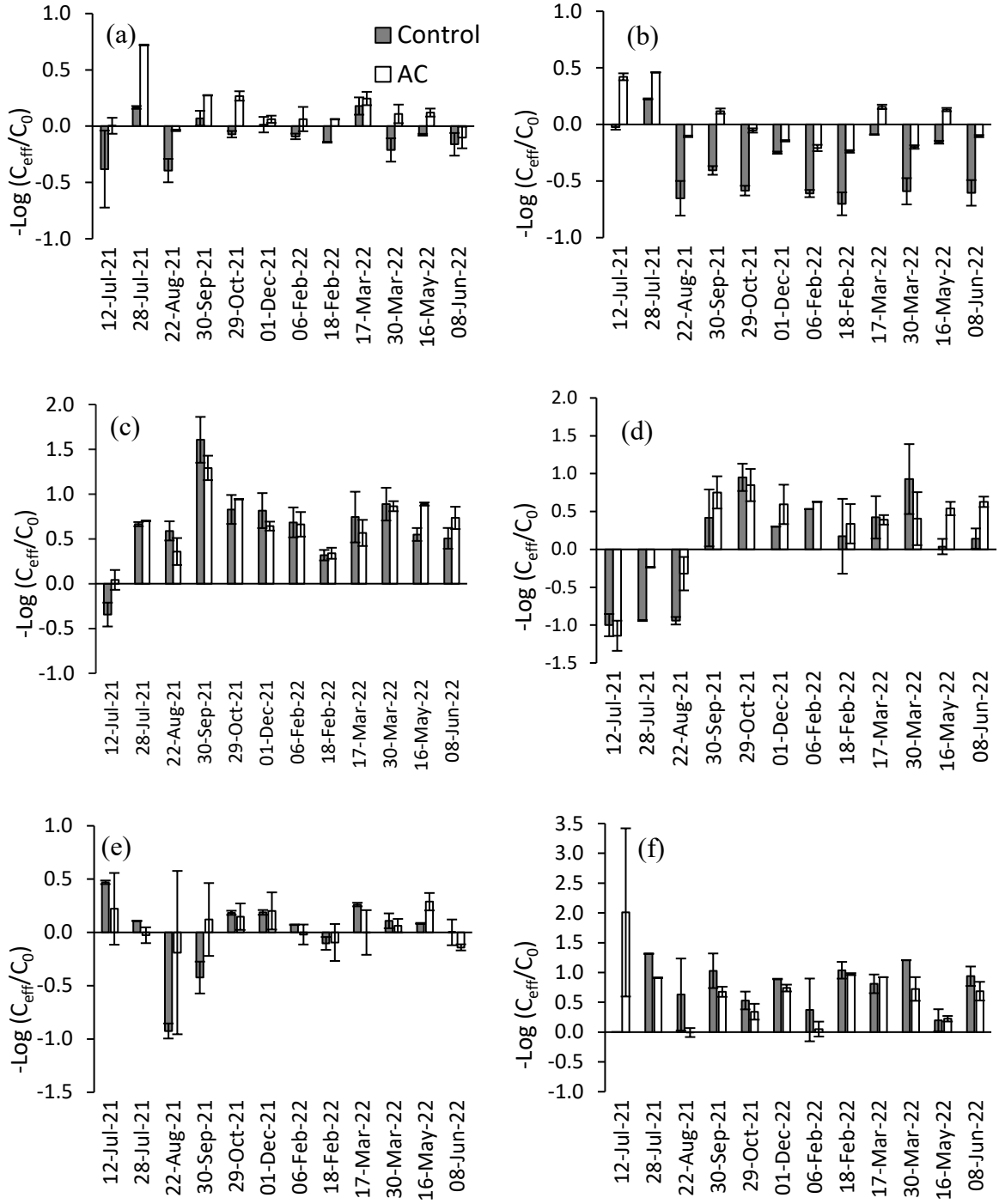

Figure 5.4 Total Nitrogen (a), Nitrate-N (b), Ammonium-N (c), Nitrite-N (d), Total Phosphorus (e), and Faecal Coliform (f); log removal for Control and AC amended permeable pavements (Log removal =-Log (C_{eff}/C_0); C_0: Concentration in influent, mg/L; C_{eff}: Concentration in effluent, mg/L. The results here are showed as the average value of duplicates, error bars represent the standard deviation. On 12/07/2021, the Faecal Coliforms concentration in Control effluent was 0 CFU/100 mL).

The PAHs concentrations accumulated by passive samplers in the base of the permeable pavements are summarised for compounds above the detection limit in **Table 5.5**. Overall, AC showed significantly lower accumulation of total PAHs than the Control (t-test, $p<0.05$). Similar results for sterile batch tests were reported by Meynet et al. (2012) who found that PAC and GAC amendment of an urban soil reduced PAHs uptake by passive samplers by 57% and 69% respectively, compared with unamended soil. And for the same urban soil, PAHs uptake by the passive samplers embedded in outdoor lysimeter drainage pipes was reduced by 93% for PAC amended soil and 56% for GAC amended soil, compared to the unamended soil (Hale et al., 2012). For individual PAH compounds, phenanthrene and fluoranthene were in our study not detected in AC, while pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene were significantly lower in AC as compared to the Control (t-test, $p<0.01$), while no significant difference was found for dibenz[a,h]anthracene. The lower concentration of PAHs in the AC amended pavement type is likely due to the very strong adsorption of PAHs and other petroleum hydrocarbons by AC particles (Hale and Werner, 2010).

Table 5.5 PAHs concentrations of passive samplers (µg/g) for compounds above the detection limit and statistical analysis. (A t-test was performed to evaluate differences between Control and AC permeable pavement. N.A indicates concentration below detection limit).

PAHs (µg/g)	Sand		AC		t-test
	Mean±Stdev	Median	Mean±Stdev	Median	
Phenanthrene	0.006±0.000	0.009	N.A	N.A	N.A
Fluoranthene	0.005±0.001	0.005	N.A	N.A	N.A
Pyrene	0.009±0.002	0.008	0.002±0.000	0.002	P<0.001
Indeno[1,2,3-cd]pyrene	0.014±0.002	0.014	0.011±0.001	0.011	P<0.05
Dibenz[a,h]anthracene	0.011±0.000	0.014	0.014±0.000	0.014	P=0.14
Benzo[ghi]perylene	0.019±0.000	0.019	0.013±0.000	0.018	P<0.001
Sum (µg/g)	0.074±0.008	0.072	0.050±0.004	0.050	P<0.05

5.4.4 Molecular microbiology results

To characterize the bacterial community of stormwater, and its alteration by percolation through the permeable pavements, we quantified the absolute abundance of different genetic markers (16S rRNA, rodA, and HF183) in stormwater and effluents samples. From **Figure 5.5 (a) & (b)**, on average over the year, the effluent from both types of permeable pavements showed significant reduction by log removal (z-test, $p<0.05$) of marker genes for total bacteria (16S rRNA) and *E. coli* (rodA) (Control only), relative to stormwater. The mean log removal values of 16S rRNA marker genes were 0.10±0.14 and 0.08±0.14 for Control and AC,

respectively. There were no significant differences between AC and the Control (t-test, $p>0.05$). For 16S and rodA marker genes, on July 12th, effluents in AC and Control showed significantly lower levels than the later 11 sampling events between 28/07/2021 and 8/06/2022 (t-test, $p<0.05$). The likely reason here is that over time bacteria from the stormwater colonized the base of the permeable pavement and eventually showed up in the effluents. The genetic marker for human host associated *Bacteroides* (HF183) was not detected in stormwater and effluents samples from either type of permeable pavements except for one sampling event in September. One of the possible reasons for the human faecal pollution maker occurrence in the September samples were freshers' week activities when outdoor venues such as tents and food stalls for the students were temporarily installed on the pavement of the sampling site. The absence of the HF183 genetic marker in all the other stormwater samples suggests that the faecal coliforms regularly detected by the plate counting method, and *E. coli* detected by the rodA qPCR method, were from animal rather than human hosts. In contrast, a very high abundance of HF183 marker genes were found in the outlets from separated stormwater drainage systems (Zan et al., 2022), which suggests that the human associated *Bacteroides* in these drainage systems were from misconnected household appliances rather than from road runoff.

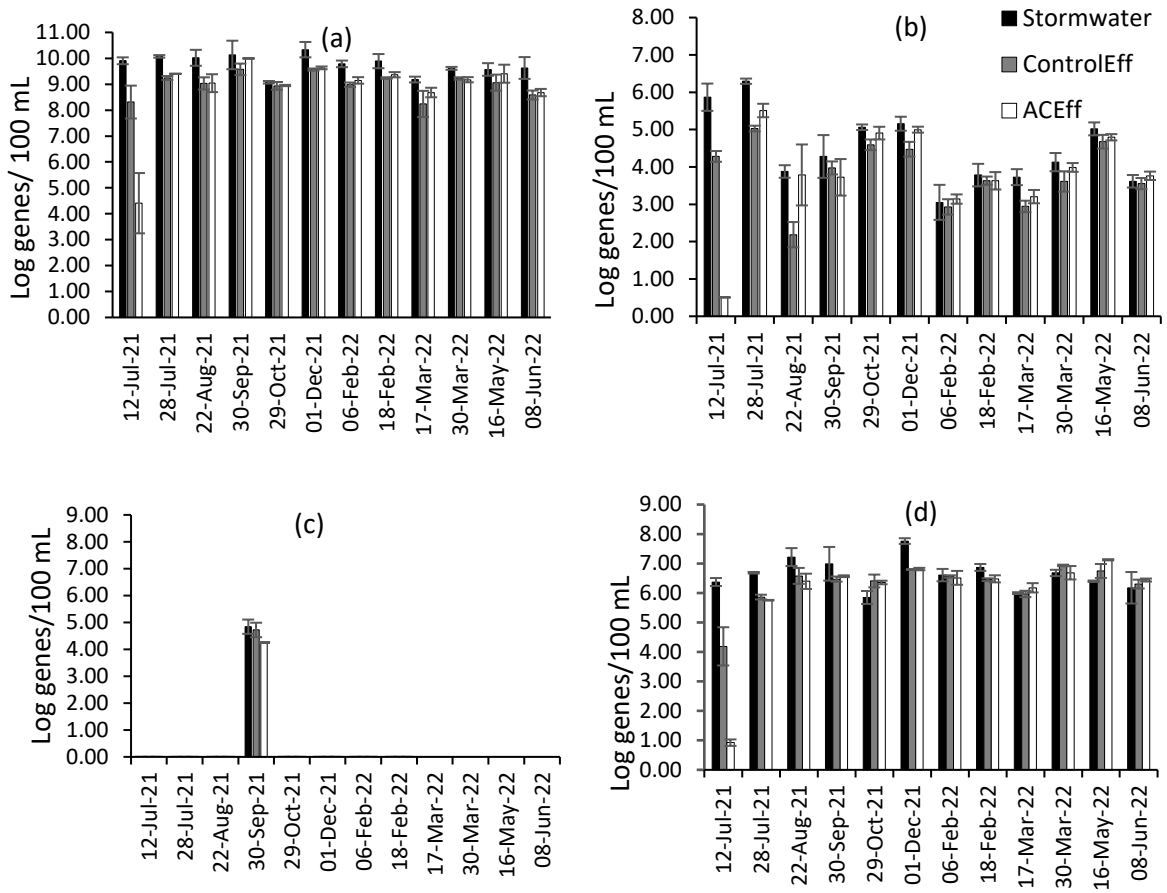

Figure 5.5 Absolute abundance of 16S rRNA genes (a), rodA genes (E. Coli) (b), HF183 genetic markers (human host associated Bacteroides) (c), and 16S rRNA genes attributed to genera containing AOB and NOB (d). The absolute abundance of 16S rRNA, rodA, and HF183 genetic markers were obtained from qPCR assays. Absolute abundance AOB and NOB was obtained from a combination of 16S rRNA sequencing and qPCR. Error bars indicate the standard deviation of duplicates.

Additional 16S rRNA gene sequencing results confirmed the faecal pollution of the September samples. A cluster analysis generated from sequencing data is provided in **Appendix C Figure C3**, while **Figure 5.6** shows a principal component analysis (PCA) for stormwater, Control effluents, and AC effluents collected from 12 sampling days between 12/07/2021 to 08/06/2022. The 3 samples located at the right bottom corner of the PCA plot are the September samples. Many of the 16S rRNA genes in those 3 samples were attributed to the genus *Lactococcus*, which is normally presented in wastewater (LaMartina et al., 2021). ANOSIM analysis showed that Control and AC effluents were significantly different from the stormwater ($R_{Control}$ = 0.4790, R_{AC}=0.3716, p=0.001), while no statistically significant dissimilarity was found between Control and AC effluent samples. The top ten median ranked genera in different sample types as attributed by 16S rRNA gene sequencing are provided in **Appendix C Table**

C5. Cyanobacterium genera such as *Aliterella* was found in stormwater samples, while the Control and AC effluents had bacterial genera typically found in aquatic, soil, and sediments environments such as *Flavobacterium* (Li et al., 2021) and *Hydrogenophaga (Willems and Gillis, 2015)*. **Figure 5.5 (d)** shows the absolute abundance of 16S rRNA genes attributed to genera containing AOB and NOB. There were no significant differences in the abundance of these genes between stormwater and both effluents, or between Control and AC effluents (t-test, $p>0.05$). In the first filtration, bacteria from genera containing AOB and NOB were present in both effluents at significantly lower abundance compared with the stormwater (t-test, $p<0.05$). But after the first flush, the abundance of these bacteria in both effluents increased dramatically and became more similar to the stormwater samples collected from 28/07/2021 to 8/06/2022. This aligns with the observed nitrification in the permeable pavements as discussed in section **5.4.3**, with significantly reduced nitrite and ammonium and increased nitrate in the effluents from permeable pavements compared to the stormwater. It also indicated that stormwater is a source of AOB and NOB. Additional 16S rRNA sequencing results showed that seven out of nine AOB and NOB genera were detected in the stormwater samples, and the dominant genera of AOB, and NOB were *Nitrosomonas, Nitrobacter, Nitrosococcus, Nitrospira,* and *Nitrosospira.*

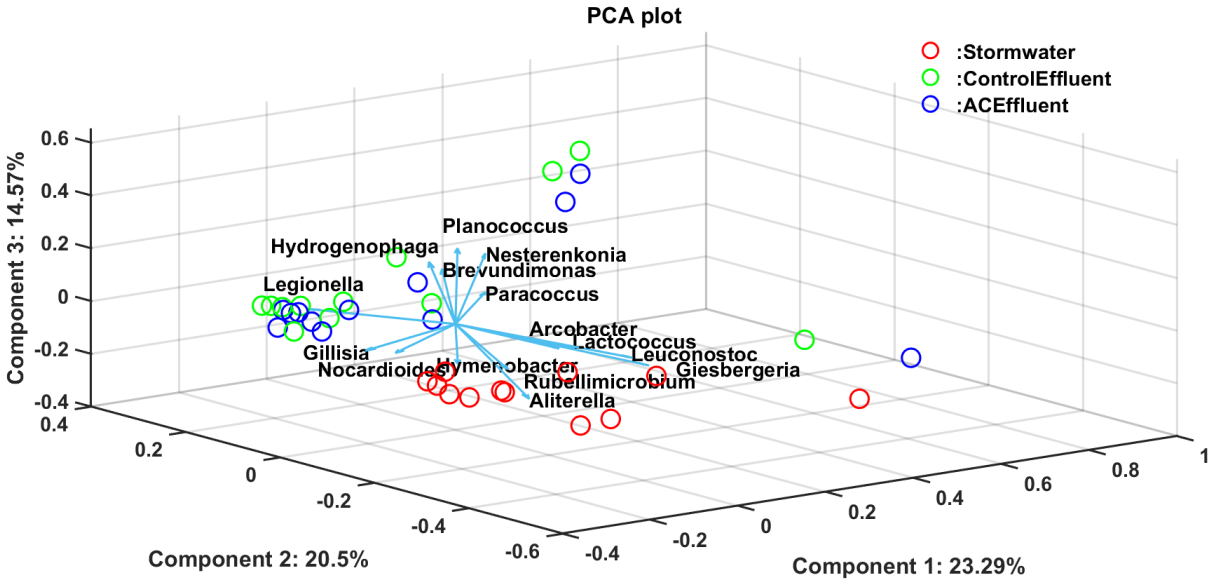

Figure 5.6 3D principal component analysis (PCA) for stormwater, Control effluents and AC effluents collected from 12 sampling events between 12/07/2021 and 8/06/2022. Each sample represents the average of four replicates.

Figure 5.7 (a) shows as Euclidean distance between samples in a cluster analysis the dissimilarity between bacterial communities associated with the base materials from the two permeable pavement types, materials from the joints in between the pavement blocks, which

included a high amount of entrapped suspended solids from the stormwater filtration experiments, and stormwater and effluent samples from the infiltration tests conducted on 30/03/2022 and 16/05/2022. The Control and AC base material samples were distinct from stormwater influent samples collected in March and May, but more similar to effluents from the infiltration tests for both pavement types. On the other hand, the pavement block joint samples were more similar to the stormwater samples. The cluster analysis showed a good separation of sample groupings, which indicated that the bacterial community of stormwater was altered when passing through the base material of the permeable pavements. **Figure 5.7 (b)** displays a PCA of the bacterial communities characterized at genus level. Cyanobacterium genus *Aliterella* was found in March and May stormwater samples. Denitrifying bacterial genera (*Denitratisoma)* (Fahrbach et al., 2006) and nitrogen fixing bacterial genera (*Azospirillum)* (Nassar et al., 2020) were enriched in Control and AC effluents. In addition, aromatic hydrocarbon degrading bacterial genera such as *Georgfuchsia* (Weelink et al., 2009) was also identified in effluents samples. The bacterial communities associated with materials from the permeable pavements fell in between stormwater and effluent samples, but the joint communities were closer to those in stormwater, and the base communities closer to those in effluents, as expected.

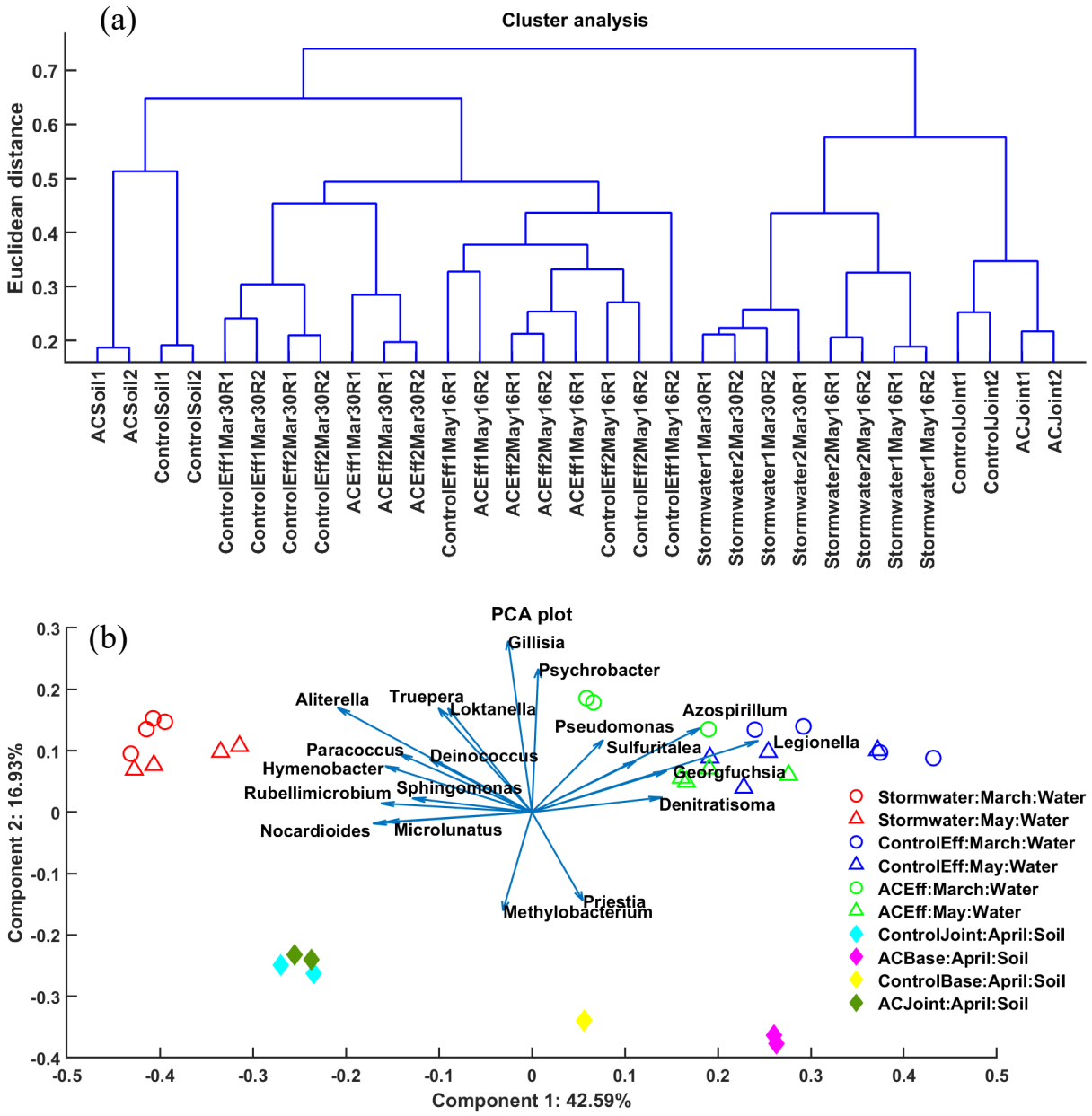

Figure 5.7 Cluster analysis (a) and principal component analysis (PCA) (b). Symbols show the scores of different samples, while arrows show the most notable variables (loadings) in the principal component (PC) 1&2 space. The percent variance explained by each PC is given in the axis legends). Joint indicates materials collected from in between the pavement blocks of the permeable pavements. Base indicates material collected from the base of the permeable pavements.

5.5 Conclusions

Using two pilot-scale concrete block permeable pavements with sand or AC amended sand as base materials, we compared their respective performance on stormwater pollution removal over a one-year period. We analysed the physicochemical and microbial water quality of stormwater collected from 12 storm events, and the effluents from either type of permeable pavement after applying for each event stormwater on top of the pilot-scale permeable pavements. We found that,

- 2% AC amendment of sand reduced its saturated hydraulic conductivity slightly, from 2.69E-03±6.50E-04 m/s to 2.20E-03±3.86E-04 m/s, but the AC amended sand still met the required specification for the base material of the permeable pavements.
- The characteristics of stormwater showed that measured pollutant concentrations were below drinking water quality the threshold values stipulated in The Water Supply (Water Quality) Regulations 2016 except for chloride and faecal bacteria.
- Spearman rank analysis showed that *faecal coliforms* in stormwater were significantly correlated with TP (t-test, $p<0.05$) and PO_4^{3-} -P (t-test, $p<0.05$), respectively.
- TSS were reduced by more than 1.2 log removal in the effluents of both types of permeable pavements compared with the stormwater samples. The effluents from both types of permeable pavements had higher pH than the stormwater, and removed Fe, Zn, Mn, and Pb. For As, the concentration in the effluent of both types of permeable pavements was higher than in stormwater, and slightly exceeded drinking water quality standards.
- The AC amended permeable pavement removed NH_4^+-N, NO_2^--N and TN efficiently, with a significant reduction of TN in AC effluents compared to stormwater (z-test, $p<0.05$). AC performs significantly better than the Control on TN, NO_3^--N and DOC removal (t-test, $p<0.05$), and Cu removal (t-test, $p<0.001$), and there was lesser PAHs accumulation by passive samplers in the AC base as compared with the Control (t-test, $p<0.05$).
- The conventional permeable pavement had significantly higher NO_3^--N in effluents as compared to stormwater (t-test, $p<0.05$), and insignificant TN removal (t-test, $p>0.05$). TN removal from permeable pavements may result from nitrification followed by denitrification, and our results indicate that the denitrification process was minimal in the conventional permeable pavement, but facilitated by the AC amendment, which reduces nitrate pollution risks from stormwater infiltration.

- Absolute abundance of 16S rRNA genes attributed to bacterial genera containing AOB and NOB showed the presence of *Nitrosomonas, Nitrobacter, Nitrosococcus, Nitrospira,* and *Nitrosospira*. Stormwater already contained these nitrifying bacteria and their activity during stormwater filtration can explain elevated nitrate concentrations in the effluents from both types of permeable pavements.
- The bacterial community of stormwater was changed when passing through the base material of the permeable pavements. The PCA analysis showed that the bacterial communities in the joints between pavement blocks on the top of the permeable pavements were more similar to those in stormwater, while bacterial communities in the base materials resembled those in the effluents from permeable pavements.

Chapter 6

Overall

Chapter 6. Overall

6.1 Overall conclusions

This thesis provides innovative stormwater management methods from monitoring to solutions. We first validated an innovative methodology for qPCR assays with portable equipment to rapidly quantify human host associated *Bacteroides* for indication of sewage pollution via genetic marker HF183. We demonstrated the qPCR workflow on-site in a mobile laboratory ('lab in a van') within 3 hours from sampling to results, and the results indicated human faecal pollution of the Ouseburn, an urban river flowing through Newcastle upon Tyne. We then deployed the mobile laboratory in the catchment of the river Ouseburn for faecal pollution source tracking in the stormwater management system of recent residential developments. The comprehensive physicochemical and microbial water quality data indicated faecal pollution from misconnections in the discharge of a surface water drain. The data also showed how stormwater retention in a pond produced effluent characteristics which were more similar to the receiving river water, as compared to the water quality of discharges from two storm drains. Compared with the conventional laboratory equipment, the portable equipment items had 87% reduced weight and 53% reduced costs. All the equipment readily fits into a suitcase, making the qPCR method transportable and applicable in the field. This would benefit water quality monitoring overseas especially, where there is no established laboratory, to address the United Nations Sustainable Development Goals (SDGs) such as SDG 6 'clean water and sanitation for all' (UN, 2015).

We then using the qPCR methodology combined with MinION nanopore sequencing to monitor the short-term and long-term impacts of combined sewer overflows (CSOs) on the bacteriology of the Ouseburn at the catchment scale. We found that CSO discharge had 4-6% of sewage content, as indicated by a high concentration of faecal coliforms and faecal *streptococci*. It also had high concentrations of *E. coli* genetic marker rodA and human host *Bacteroides* genetic marker HF183. The CSO discharges significantly altered the bacteriology of the Ouseburn, contributing 72%-77% of bacteria in the downstream of the river during a storm event according to SourceTracker analysis, while only 4-6% of bacteria were attributed to the rural upstream sources. Our findings disprove the claim made by the chief executive of Severn Trent that sewer overflow discharges were 'pretty much rainwater' (EAC, 2022). From our monitoring, the urbanized part of the catchment was the main source of high faecal pollution loads transported by the river during storm events. The faecal pollution poses a significant risk to people wading and splashing in the urban river, since the quantitative

microbial risk assessment (QMRA) based on sixteen sampling events in the summer with mostly dry weather and scattered showers exceeded guidance value for reactional water quality. Based on our findings, the impacts of CSOs discharge should be monitored systematically and data on CSO discharges should be shared in real-time to warn recreational water users of related health risks. To achieve the SDG 6.6 to 'protect and restore water-related ecosystem, including mountains, forests, wetlands, rivers, aquifers and lakes (UN, 2015)', the bacterial water quality of rivers should be monitored where they flow through public parks irrespective of bathing water designation.

To reduce storm overflows in accordance with the Environment Act 2021, water companies need to invest in more sustainable drainage systems (SuDS). Indeed, SuDS became a legal requirement for all new development in the UK in 2015 (DEFRA, 2018). In January 2023, DEFRA approved mandatory inclusion of SuDS in all new developments and removed developers' automatic right to connect surface water runoff to the public sewer network; this is to be implemented by 2024. However, the issue of existing developments remains; SuDS components should be retrofitted, and one of the options for reducing urban runoff are permeable pavements. We designed and evaluated an innovative permeable pavement as a solution to minimize the stormwater quantity and related pollution close to the source. Using two pilot-scale concrete block permeable pavements with sand or AC amended sand as base materials, we compared their respective performance on stormwater pollution removal over a one-year period. We analysed the physicochemical and microbial water quality of stormwater collected from 12 storm events, and the effluents from either type of permeable pavement after applying for each event stormwater collected on the Newcastle University campus on top of the pilot-scale permeable pavements. The stormwater characteristic showed that measured pollutant concentrations were below drinking water standard regulated in The Water Supply (Water Quality) Regulations 2016 except chloride and bacteria. 2% AC amendment of sand reduced permeability from 2.69E-03±6.50E-04 to 2.20E-03±3.86E-04, but the AC amended sand still meet the required specification for the base material of the permeable pavements. The 2% AC amended permeable pavement reduced nitrate pollution risks from stormwater infiltration, as it significantly reduced nitrate (NO_3^--N) and total nitrogen in leachate by 57±15% and 40±20%, respectively, compared to the conventional sand base permeable pavement. Absolute abundance of 16S rRNA genes attributed to bacterial genera containing AOB and NOB showed the presence of *Nitrosomonas, Nitrobacter, Nitrosococcus, Nitrospira,* and *Nitrosospira*. Stormwater already contained these nitrifying bacteria and their activity

during stormwater filtration can explain elevated nitrate concentrations in the effluents from both types of permeable pavements.

The permeable pavement showed efficiently TSS and metals removal except for As. The AC performance significantly better than Control on Cu removal. The AC amended permeable pavement also showed significant reduction of DOC in leachates and polycyclic aromatic hydrocarbons (PAHs) accumulation by passive samplers in the base compared with the conventional permeable pavement. The AC amended permeable pavement thus showed potential to contribute to SDG 6 target 6.3 to 'improve water quality by reducing pollution'(UN, 2015).

In summary, this thesis provides an innovative method for stormwater quality monitoring, closed the data gaps regarding bacterial water quality of urban rivers that are not regulated as bathing water, and provided a solution to reduce urban runoff -- an innovative pollution munching permeable pavement. This helps addressing the global sustainable development challenges identified by the United Nations.

6.2 Future work

Based on the work we did, we recommend that future work could focus on,

- Demonstrating the portable qPCR method in combination with MinION nanopore sequencing on-site, to make the most of these complementary methods, especially in low resource settings.
- Monitoring the impacts of CSO discharge more systematically to understand impacts on river water quality across the UK.
- Quantitative microbial risk assessment for recreational water quality should be improved based on real data for indicator to pathogen ratios, or even better using indicator to probability of illness data for people who have been wading and splashing around in rivers.
- Analysis of eDNA from UK rivers should be expanded to the study of viruses and antimicrobial resistance via metagenomics or high throughput qPCR.
- Keep monitoring the performance of permeable pavements for several years, to better understand the sustainability of the pollution removal process within the permeable pavements.

- Conduct more frequent characterization of the microbiome in the permeable pavements, incl. amoA marker gene qPCR assays, to better understand the nitrification process of microorganisms in the permeable pavements.
- Developing a fundamental understanding of biological processes in the base material of permeable pavements to optimize their long-term operation.

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Appendices:

Appendix A:

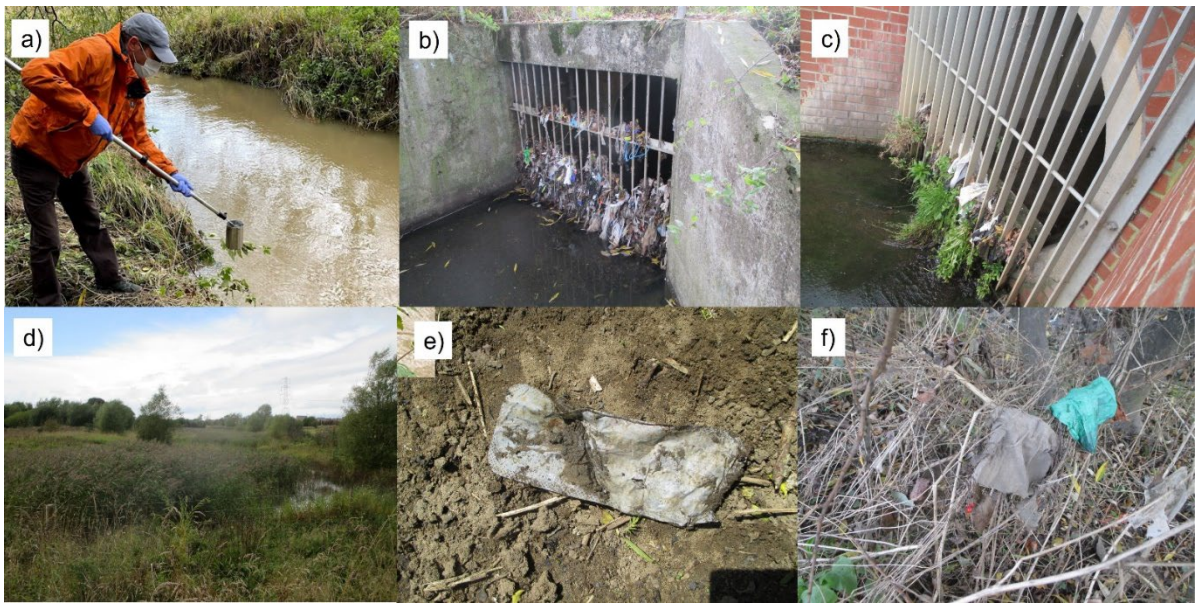

Figure A1. a) Sampling the Ouseburn upstream of the storm drains; b) Kingston Park storm drain; c) Great Park storm drain; d) stormwater retention ponds at Great Park; e & f) sewage litter and other plastic flotsam in the Ouseburn embankment downstream of the Kingston Park storm drain.

Table A1. DNA yield, 16S rRNA, and rodA gene copies for different extraction methods and qPCR instruments, showing the data for each filter replicate. qPCR results are the average and standard deviation of duplicate analysis from each DNA extract.

Method	Filter (#)	DNA yield (ng/100 mL)	Log_{10} 16S rRNA (genes/100 mL)	Log_{10} rodA (genes/100 mL)
Vortex & conventional qPCR	1	143	7.70±0.02	4.68±0.03
	2	157	7.76±0.10	4.68±0.16
	3	102	7.44±0.06	4.55±0.14
Vortex with enzyme & conventional qPCR	4	151	7.52±0.00	4.69±0.18
	5	184	7.84±0.11	4.54±0.04
	6	190	7.77±0.00	5.38±1.35
Ribolyser & conventional qPCR	7	280	7.92±0.10	4.68±0.03
	8	235	7.68±0.04	4.68±0.02
	9	132	7.72±0.20	4.54±0.03
Vortex & portable qPCR	1	See above	7.85±0.00	5.29±0.01
	2	See above	7.96±0.01	4.61±0.06
	3	See above	7.75±0.01	4.38±0.04
Vortex with enzyme & portable qPCR	4	See above	7.74±0.03	4.62±0.04
	5	See above	7.85±0.01	4.48±0.12
	6	See above	7.78±0.04	4.38±0.04
Ribolyser & portable qPCR	7	See above	8.14±0.00	4.54±0.27
	8	See above	7.80±0.51	4.52±0.11
	9	See above	7.85±0.01	4.28±0.07
Method comparison ANOVA with post hoc pairwise comparisons using Tukey's honest significant difference criterion		One-way Extraction method p=0.199 Pairwise comparison all p>0.177	Two-way crossed Extraction method p=0.358 qPCR instrument **p=0.042 *** Interaction p=0.692 Pairwise comparison all p>0.179	Two-way crossed Extraction method p=0.576 qPCR instrument p=0.295 Interaction p=0.338 Pairwise comparison all p>0.483

Table A2. Comparison of calibration curve metrics for the conventional and portable qPCR instruments.

PCR instrument	Gene	LOD[1] (genes/µL template)	Slope (Cq/(genes/µL))	Efficiency (%)	R^2
Conventional	16S	10	-3.376	98	0.993
	rodA	10	-3.440	95	1.000
Portable	16S	10	-3.383	98	0.996
	rodA	10	-3.595	90	0.992

[1] The limit of detection is stated as the lowest standard concentration, which is equal to 20 genes in the reaction mixture. All standards amplified, and all sample templates had marker gene concentrations above the lowest standard concentration.

Table A3. DNA yield and HF183 gene copies for 350 mL volumes of Ouseburn water collected at the catchment outlet at Foundry Ln and analyzed onsite, showing the data for each filter replicate. qPCR results are the average and standard deviation of triplicate analysis from each DNA extract.

Location	Filter (#)	DNA yield (ng/100 mL)	Log_{10} HF183 (genes/100 mL)
Catchment outlet at Foundry Ln	1	560	4.26±0.02
	2	523	4.23±0.06
Filtration replicate comparison, ttest			p=0.44

Table A4. DNA yield, 16S rRNA, HF183 gene copies, and faecal coliform (FC) counts for the water samples collected in the fieldwork and analyzed onsite. qPCR results are the average and standard deviation of triplicates prepared from each pooled DNA extract, while FC counts are for triplicates of 0.5 mL water filtrations.

Location	**Filter (#)**	**DNA yield (ng/100 mL)**	**Log_{10} 16S rRNA (genes/100 mL)**	**Log_{10} HF183 (genes/100 mL)**	**Log_{10} FC (genes/100 mL)**
RiverUp	1&2	112	7.01±0.01	4.23±0.03	3.72±0.07
KPStDrain	2&3	247	9.83±0.72	6.03±0.04	4.17±0.09
GPStDrain	3&4	61	8.03±0.04	4.02±0.08	4.12±0.14
PondEff	5&6	15	6.64±0.03	4.44±0.10	3.49±0.02
Blank[1]	7&8	n.d.	n.d.	n.d.	n.d.
Location comparison ANOVA with post hoc pairwise comparisons using Tukey's honest significant difference criterion			One-way location **p=1.95e-5***** Pairwise comparison all **p<0.05*** except RiverUp v.s PondEff p=0.61	One-way location **p=2.08e-9***** Pairwise comparison all **p<0.05***	One-way location **p=4.18e-5***** Pairwise comparison all **p<0.05*** except KPStDrain vs. GPStDrain p=0.86

[1] Method blanks did not give a quantifiable DNA yield. The 16S rRNA gene concentration in the qPCR tubes derived from the Cq values of no-template and blank sample control reactions was 17 to 26 genes, versus > 100,000 genes for all the water samples. No colonies were observed on filters incubated without the addition of water samples.

Appendix B:

1. Method details

1.1 Sampling and onsite analysis methods

River water samples were collected with a stainless-steel vessel mounted on a pole and composite samples were formed in sterilized 1 L polyethylene bottles by repeatedly sampling over a period of 5 minutes until 2 x 1 L had been collected. An additional sample was then collected in the stainless-steel vessel to immediately analyse water temperature, pH, and conductivity with a precalibrated ExStikII probe (Extech Instruments, Nashua, NH, USA) and dissolved oxygen with a HQ 40d meter and LDO sensor (Hach, Manchester, UK). Samples were transported in cooling boxes to the laboratory and processed on the same day by membrane filtration for plate counts, chemical analysis with cuvette tests on a DR6000 spectrophotometer (Hach, Manchester, UK), alkalinity by titration with a Model AL-DT alkalinity test kit (Hach, Manchester, UK), and turbidity with a 2100Q turbidity meter (Hach, Manchester, UK). Biomass was concentrated on the day of sampling onto Gridded Sterile Cellulose Nitrate Membrane Filters with 0.2 µm pore size (Sartorius, Göttingen, Germany) by filtering between 100 and 300 mL of river water, depending on the anticipated bacterial content. Membranes were preserved at -20 °C for subsequent DNA extraction and analysis.

1.2 Laboratory analysis protocols

Water chemistry of unfiltered samples was characterized with cuvette tests (Hach, Manchester, UK) according to the manufacturer's instructions comprising ammonium-N (LCK304), nitrate-N (LCK339), nitrite-N (LCK341), total-N (LCK138), total-P (LCK349), ortho-P (LCK349), and COD (LC1400).

Our microbial water quality analysis comprised plate counting, quantitative PCR (qPCR) and MinION^{TM} nanopore sequencing methods as described in Hiruy et al. (Hiruy et al., 2022),

taking advantage of the complementarities between these distinct approaches (Acharya et al., 2019). We quantified faecal coliforms (FC) by plate count using m-FC ampules for Faecal Coliform Bacteria (Hach, Manchester, UK) in accordance with standard methods for the examination of water and wastewater APHA, and USEPA approved 9222 D. We quantified Faecal Streptococci (FS, Group D and Q *Enterococci*) by plate count using KF *Streptococci* ampules (Hach, Manchester, UK) in accordance with standard methods for the examination of water and wastewater APHA, and EPA-600/8-8/017. These plate count methods have been used for many decades and enable comparison with historic data.

We extracted and cleaned-up DNA with the DNeasy PowerWater Kit (Qiagen, Crawley, UK) following the manufacturer's protocols. Using a portion of the extracted DNA, we conducted qPCR analysis with primer pairs targeting the 16S rRNA gene as a marker for total bacteria (Ferris et al., 1996), and two TaqMan qPCR assays targeting the *rodA* gene as a marker for *E. coli* bacteria (Chern et al., 2011) and HF183 gene as a marker for human-host associated *Bacteroides* (Ahmed et al., 2019b). The qPCR assays followed the procedures and quality assurance protocols detailed in Zan et al. (Zan et al., 2022). Initially, we also tested sewage treatment plant and some river water samples for the *ompW* gene as a marker of *Vibrio cholerae* as described in Hiruy et al. (Hiruy et al., 2022). We discontinued this work since no amplicons were obtained for this gene from any of the samples, incl. municipal sewage.

We used a portion of the extracted DNA for next generation sequencing of full-length 16S rRNA gene amplicons with the MinION platform of Oxford Nanopore Technologies (ONT, Oxford, UK) following procedures described previously (Halla et al., 2022). Samples were multiplexed with 24 barcodes and prepared for sequencing using the 16S rRNA sequencing kit SQK16S024 of ONT according to the manufacturer instructions. Amplicons were sequenced using flow cells version R9.4.1, base-called with Guppy version V5.0.11, and taxonomy was established with the EPI2ME FASTQ 16S workflow of ONT using a quality score ≥7.

1.3 Quality control measures

All cuvette tests were conducted in duplicate, while all microbial measurements were conducted in triplicates. In procedural blank samples, deionized water (for cuvette tests), autoclaved saline solution (for plate counts), or DNA free water (for molecular methods) replaced the river water. Cuvette tests with deionized water reported below detection limit values. For plate counting, no colonies were observed in procedural blanks. A DNA standard from Zymo Research (Irvine, CA, United States) consisting of genomic DNA from eight bacterial species and two yeasts was used as a positive control for the sequencing data. In line with previous reports (Halla et al., 2022) we found that the EPI2ME FASTQ 16S workflow of ONT attributed 79% of reads at genus level correctly to members of the mock community (**Table S2 in supplementary info**). Due to the limited nanopore read accuracy (~88%), 21% of reads were misclassified at genus level, although mostly to taxa closely related to the mock community members. For example, 5% of reads were misclassified as *Shigella*, a genus which is closely related to *Escherichia* (Pettengill et al., 2016) with up to 99% 16S rRNA gene sequence similarity (Acharya et al., 2019), resulting in a corresponding gap of 5.0% Escherichia detected, versus 10% expected (**Table S2**). In this study, we considered the 16S rRNA gene sequencing derived taxonomy as an indicative rather than definite account of the bacterial community composition. The MinION sequencing data were nonetheless useful for faecal pollution source tracking, as many previous studies have shown strong correlations between faecal pollution indicators derived from MinION nanopore sequencing data, and those obtained from culturing, qPCR, and alternative sequencing methods (Werner et al., 2022). Also, due to the PCR step, spurious amounts of DNA from filtration units and reagents can be amplified to detectable level in the 16S rRNA gene sequencing workflow (Thongsamer et al., 2021). In this study, a procedural blank sample generated 26,012 reads, of which >1000 reads, each, were assigned to *Geminicoccus roseus*, *Leptothrix discophora*, *Nakamurella flavida*,

Caulobacter mirabilis, and *Rhodobacter thermarum*. These species and their genera were not predominant taxa in the river water samples, which were all dissimilar from the blank and mock bacterial community samples (**Figure S1).** Hence, spurious DNA contamination of filtration units and reagents likely had only minor impacts on the bacterial community characterizations.

1.4 Data pre-treatment and multivariate data analysis

For one sample we didn't amplify a qPCR target (HF183 genes at S4 on June 2nd) and substituted 1 gene per 100 mL as detection limit to include these samples in the statistical analysis. For colonies that were too numerous to count (FS at S3 and S5-8 on Sept 27th), we substituted 4.5 for the $\log_{10}$ value of CFUs per 100 mL, based on the highest detected values. Our statistical analysis was largely based on percentiles and ranks (i.e. ANOSIM), which were not sensitive to the exact values substituted for above and below detection limit values, as long as they fell on either side of the boundaries of the quantification range.

Taxonomic data derived from 16S rRNA gene sequencing were interpreted at genus level and rarefied at 50,000 reads per sample to make samples more comparable. After exclusion of replicates with less than 50,000 reads (Aug 8th 2 pm S5a, Sept 27th S3c, S4a and S5c) at least two replicates remained for each sampling location and event. Square-root transformation was used as pretreatment for the analysis of taxonomic abundance data as recommended when using Euclidean distance as similarity metric (Legendre and Gallagher, 2001).

Cluster analysis used the Matlab© (Mathworks, Natick, MA, USA) function *dendrogram* and average Euclidean distance for the linkage tree. Principal component analysis (PCA) used Euclidean distance as similarity metric and other default Matlab© PCA settings. Pearson correlations were calculated with the function *corrplot* and a linear model for $\log_{10}$HF183 was derived from pH, $\log_{10}$Ammonium-N, $\log_{10}$Nitrate-N, $\log_{10}$Temperature, $\log_{10}$Conductivity, $\log_{10}$DissolvedOxygen, and $\log_{10}$Turbidity by stepwise regression with the function

stepwiselm. Absolute abundance estimates for 16S rRNA genes from 56 bacterial genera associated with the human gut microbiome (King et al., 2019) were generated by multiplying the total 16S rRNA gene copy numbers quantified by qPCR with the relative abundance derived from the 16S rRNA gene sequencing data. The estimated number of bacteria in each genus per 100 mL (with the addition of 1 to include below detection limit values in the analysis) was then $\log_{10}$ transformed before generating the heat maps with hierarchical clustering, using the Matlab function *clustergram* with default settings (except that the chosen color map was redblue and the scale not symmetric around zero). Primer7 software (primer-e, Auckland, New Zealand) was used for two-way crossed analysis of similarities (ANOSIM) and to investigate the linkage between environmental parameters and microbial communities with the *BEST* (or Bio-Env) procedure (Clarke et al., 2014). Since turbidity data was not available for June 2nd, and phosphate P was strongly correlated with total P (Pearson correlation coefficient r=0.89, with on average 83% contribution of phosphate-P to total P), we excluded these two environmental variables from the BEST analysis. We used square-root transformation for the relative abundance data of different bacterial genera and Euclidean distance as similarity metric in the ANOSIM and BEST analysis. SourceTracker analysis (Knights et al., 2011) followed procedures outline in Pantha et al. (Pantha et al., 2021).

1.5 Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) followed WHO guidance (WHO, 2016) and procedures outlined in our previous work to estimate pathogen abundance from genetic data by considering the number of gene copies per genome, and number of viable/culturable cells per molecular method derived genome (Halla et al., 2022). Transformation to viable/culturable cells was needed since most pathogen dose-response relationships were established using conventional culturing methods.

The dose of pathogen i per exposure event was estimated as:

$$D_i = \mathrm{IV} \times C_k \times f_{i,k} \qquad \text{eq. S1}$$

IV is the ingestion volume (mL), C_k is the concentration of faecal indicator k (colony forming units or genes/mL), and $f_{i,k}$ is the ratio of viable pathogens i (cells) per faecal indicator k (colony forming units or genes).

Published dose-response curves were then used to relate the ingested pathogen cell dose D_i to the probability of illness P^{ill}_i with pathogen i. The curves were modelled using an approximate beta-Poisson equation:

$$P_i^{ill} = R_{illtoinf,i} \cdot \left(1 - \left(1 + \frac{D_i}{\beta_i}\right)^{-\alpha_i}\right) \qquad \text{eq. S2a}$$

$$\beta = \frac{N_{50,i}}{\left(2^{\left(\frac{1}{\alpha_i}\right)} - 1\right)} \qquad \text{eq. S2b}$$

α_i and β_i (or $N_{50,i}$) are parameters obtained from the literature as detailed in **Table S3**. $N_{50,i}$ is the dose (cells) at which 50% of the exposed population is expected to become infected with pathogen i for a single exposure event. $R_{illtoinf,i}$ is the ratio of symptomatic (or ill) to infected persons for studies that relied on stool positive samples.

The total risk of illness per event P^{tot}_{ill} considering all pathogens i was then calculated according to

$$P_{tot}^{ill} = 1 - \prod_i \left(1 - P_i^{ill}\right) \qquad \text{eq. S3}$$

We considered the most common causes of bacterial gastrointestinal illness in our analysis. (Shigellosis, Campylobacteriosis, and Salmonellosis). Details of the parameter values and literature sources used are provided in **Table S3**.

WHO guidelines on recreational water quality classify recreational waters into categories A-D based on gastrointestinal illness risks at the upper 95th percentile value of anticipated exposures

(WHO, 2021). We therefore simulated 50,000 exposure events by randomly combining water quality metrics from one of the sixteen sampling events at S5 in Jesmond Dene Park between July 27th and Aug 9th with a water ingestion volume drawn from an assumed lognormal distribution with mean 0.3424 and standard deviation 0.4297 $\log_{10}$ mL. The ingestion volume distribution parameters were derived from the median and 95th percentile values reported for wading and splashing as recreational activity (Dorevitch et al., 2011).

2. Insights from sewage litter observations

As long-lasting legacy of sewage discharges, plastic wet wipes and sanitary products are noticeable throughout the Ouseburn catchment with higher density indicating the stormwater discharge locations (Zan et al., 2022). When we inspected CSO4 on Aug 17th, 2021, we noted freshly deposited sewage litter on its bar screen, and the green vegetation in the river, indicating recent spillage (**Figure S6**). We removed all deposits from the bar screen on Aug 19th and noted fresh accumulation on Aug 23rd (after a storm event on Aug 21st), Oct 3rd (after the Sept 27th storm event), and on Oct 9th (after the Oct 5th storm event). These observations substantiate frequent sewage discharges into the Ouseburn from CSO4 during summer and autumn storm events. Since CSOs 9&10 discharge into a culvert, we couldn't make the equivalent observations, but from the EDM data they spill more frequently than CSO4.

Following the autumn storm event on Oct 5th with the highest river flow in the observation period sewage litter was highly visible throughout the catchment (**Figure S7**). Sewage litter like wet wipes and sanitary products on footpaths, picnic lawns, and rocks either side of a picturesque waterfall showed that the river had left its embankment and flooded riparian areas frequented by the public in Jesmond Dene Park. A clump of wet wipes remained for at least eighteen days next to the park's most iconic sight, an artificial waterfall feature created by Lord Armstrong. These observations illustrate how CSO discharge related public health risks may

extend beyond those posed to people wading and splashing around in the river, to those walking, playing, sitting, or picnicking along the river embankment after major storm events.

Table B1: Event Duration Monitoring (EDM) annual return 2021 data for the 16 combined sewer overflows discharging into the Ouseburn.

CSO	Total spill duration (h)	Number of spills 12-24 h count
CSO1: Stamfordham Road Pumping Station	101.09	13
CSO2: Woolsington	0	0
CSO3: Woolsington Sewage System Bullocks Steads	8.5	3
CSO4: Acomb Crescent (Cathedral)	27	14
CSO5: Farne Avenue	6.25	9
CSO6: St Nicholas Hospital	3.25	2
CSO7: Three Mile Inn	4.75	3
CSO8: Turnberry Way	0	0
CSO9: Salters Lane	401.67	41
CSO10: Grassholm Place	472.5	52
CSO11: Moor Road South	3.75	3
CSO12: Blue Bell Inn	43.5	16
CSO13: Ouse Burn No8	0.1	1
CSO14: Foundry Lane No1	199.77	49
CSO15: Foundry Lane No3	212.97	44
CSO16: Lime Street/Out bank	210.79	52

Table B2 Agreement between the known composition of a mock community consisting of genomic DNA from eight bacterial species and classifications of reads on Epi2me at genus level after base-calling with the Guppy software of Oxford Nanopore Technologies. The percentage abundance of 16S rRNA genes from each species, as provided by the supplier of the MOCK community (Zymo Research), is considered as the true or actual abundance in this study.

Genus	**Theoretical composition** (% relative abundance)	**MinION (Guppy) results** (% relative abundance)
Bacillus	17.4	19.3±0.3
Enterococcus	9.9	7.65±0.02
Escherichia	10.1	4.97±0.20
Limosilactobacillus	18.4	11.0±0.2
Listeria	14.1	9.76±0.16
Pseudomonas	4.2	4.49±0.26
Salmonella	10.4	9.00±0.03
Staphylococcus	15.5	13.1±0.2
Others (*genus* >1%)		20.8±0.3 (*Shigella* 5.25±0.18, *Citrobacter* 1.82±0.11, *Kosakonia* 1.06±0.0.1)
Not classified to genus		<0.4%

Table B3: Parameters used in the quantitative microbial risk assessment based on sixteen river water samples collected in Jesmond Dene Park at S5 in the period from July 27th to Aug 9th, 2021.

Hazard indicator	Method	Dose-response model for illness & parameters	Indicator to pathogen ratio	Log_{10} ingestion volume (mL) Normal Distribution Mean±Stdev
E. coli (rodA)	qPCR	Beta Poisson* α: 0.248 β: 48.80 N_{50}: 750 $R_{illtoinf}$: 1	$0.08^{\beta} \cdot 1^{\gamma} \cdot 0.042^{\delta}$ = 0.00336	0.342±0.430$^{\$}$
*Campylobacter spp. (*16S rRNA)	qPCR & NGS	Beta Poisson† α: 0.145 β: 7.589 N_{50}: 897 $R_{illtoinf}$: 0.18	$0.33^{\eta} \cdot 0.40^{\varepsilon} \cdot 0.042^{\delta}$ = 0.00554	
Salmonella spp. (16S rRNA)	qPCR & NGS	Beta Poisson¥ α: 0.1324 β: 51.45 N_{50}: 9610 $R_{illtoinf}$: 1	$0.40^{\theta} \cdot 0.143^{\varepsilon} \cdot 0.042^{\delta}$ = 0.00240	

*Based on epidemiological data for outbreaks caused by faecally polluted soil, water, and food consumption (Teunis et al., 2008). Parameters are for the heterogeneous model (Butte et al., 2021). $R_{illtoinf}$ was set to 1 since the epidemiological data were for symptomatic cases. †Based on a dose-response study with *Campylobacter jejuni* A3249 and human volunteers (Black et al., 1988, Teunis and Havelaar, 2000). We used the median value of ill to stool positive volunteers to derive a $R_{illtoinf}$ value of 0.18 (Black et al. 1988). ¥Based on epidemiological data for outbreaks caused by water or food consumption (WHO, 2002). $R_{inftoill}$ was set to 1 since the predicted probability is already for illness. $^{\beta}$We used 0.08 *E. coli* O157:H7 per *E. coli,* according to Table C6 in (WHO, 2016). $^{\gamma}$We used 1 *rodA* gene copy per genome (Matsuzawa et al., 1989). $^{\delta}$We used 0.042 viable cells per eDNA derived genome. We derived this ratio by comparing the sum of genomes for *Escherichia*, *Shigella*, *Enterobacter*, *Citrobacter,* and *Klebsiella,* derived from 16S rRNA gene qPCR and sequencing data with the faecal coliform plate count. This ratio aligns with reported viable cells percentages for *E. coli* derived from Colilert to sfmD gene ratios of 3.4% ± 3.1% for river water samples (Sthapit et al., 2020). $^{\varepsilon}$We used the mean number of 16S rRNA genes per genome reported in the rrnDB (Stoddard et al.,

2015). [η]We used the median ratio of *Campylobacter* species associated with gastroenteritis (On, 2013) to total *Campylobacter* species, according to the Epi2Me 16S workflow taxonomic classification. Although species level classifications by this workflow are not always reliable due to limited MinION read accuracy (Acharya et al., 2019), these ratios were our best estimates from the available data. [θ]We used the median ratio of *Salmonella enterica subsp. enterica serovar Typhimurium* to total *Salmonella* species, according to the Epi2Me 16S workflow taxonomic classification. [$]Derived from the median and 95th percentile values reported by (Dorevitch et al., 2011) for wading/splashing in a swimming pool study.

Table B4 Combinations of up to 5 environmental variables, taken k variables at a time, yielding the best matches of 16S rRNA gene sequencing derived microbial community similarity matrices, and physico-chemical parameter similarity matrices for each k, as measured by weighted Spearman rank correlation ρ_s. The highest Spearman rank correlation is highlighted in bold. Global BEST test for a significant relationship between community and environmental variables, ρ_s= 0.777, p = 0.01.

k	Best variable combinations (ρ_s)		
1	COD (0.681)	Ammonium-N (0.665)	Total-N (0.628)
2	Total-N, Alkalinity (0.760)	Total-P, Alkalinity (0.749)	COD, Alkalinity (0.746)
3	Total-N, Total-P, Alkalinity (0.771)	Total-N, COD, Alkalinity (0.769)	Ammonium-N, Total-N, Alkalinity (0.767)
4	Ammonium-N, Total-N, Total-P, Alkalinity (0.775)	Total-N, Total-P, COD, Alkalinity (0.774)	Ammonium-N, Total-N, COD, Alkalinity (0.773)
5	**Ammonium-N, Total-N, Total-P, COD, Alkalinity (0.777)**	Total-N, Total-P, COD, Temp, Alkalinity (0.751)	Ammonium-N, Nitrate-N, Total-P, COD, Alkalinity (0.750)

Table B5 Flow, bacterial and chemical loads transported by the Ouseburn at the Environment Agency gauging stations of Woolsington (S1) and Crag Hall (S5) on June 2nd (dry weather), July 7th (after a day after heavy rainfall) and Sept 27th (during heavy rainfall). Note that the bacterial data is reported in logarithmic units.

Flow	***Faecal coliforms* (FC)**	***Faecal streptococci* (FS)**	**16S (total bacteria)**	**rodA (*E. coli*)**	**HF183 (human-host *Bacteroides*)**
	Log_{10} CFUs per second Avg±Stdev	Log_{10} CFUs per second Avg±Stdev	Log_{10} genes per second Avg±Stdev	Log_{10} genes per second Avg±Stdev	Log_{10} genes per second Avg±Stdev
			June 2nd		
S1 0.007 m^3/s flow	5.40±0.04	4.15±0.09	10.21±0.01	5.69±0.16	7.17±0.04
S5 0.056 m^3/s flow	7.00±0.02	6.14±0.03	10.79±0.00	7.62±0.04	7.44±0.02
			July 7th		
S1 0.031 m^3/s flow	6.29±0.05	6.06±0.02	11.08±0.01	8.10±0.05	8.58±0.03
S5 0.407 m^3/s flow	7.48±0.02	7.00±0.01	12.21±0.00	9.00±0.02	9.28±0.01
			Sept 27th		
S1 0.161 m^3/s flow	7.69±0.01	7.23±0.06	12.42±0.04	8.87±0.02	8.98±0.05
S5 1.470 m^3/s flow	9.41±0.06	n.a.	14.10±0.37	11.46±0.03	12.54±0.05

	NH_4^+-N grams per second Avg±Stdev	**NO_3^--N** grams per second Avg±Stdev	**NO_2^--N** grams per second Avg±Stdev	**Total-N** grams per second Avg±Stdev	**PO_4^{3-}-P** grams per second Avg±Stdev	**Total-P** grams per second Avg±Stdev	**COD** grams per second Avg±Stdev
				June 2nd			
S1	0.0003	0.011	0.0002	0.017±	0.0005	0.0006±	0.097±
0.007 m^3/s flow	±0.0000	±0.000	±0.000	0.001	±0.000	0.000	0.000
S5	0.0038	0.067	0.0016	0.102	0.0175	0.0188	1.011
0.056 m^3/s flow	±0.000	±0.000	±0.000	±0.028	±0.0001	±0.0001	±0.075
				July 7th			
S1	0.0041	0.151	0.0022	0.212	0.0036	0.0048	0.860
0.031 m^3/s flow	±0.0014	±0.000	±0.0000	±0.007	±0.0001	±0.0000	±0.029
S5	0.0499	0.790	0.0151	1.376	0.0484	0.0621	10.399
0.407 m^3/s flow	±0.0256	±0.012	±0.001	±0.012	±0.000	±0.0009	±0.720
				Sept 27th			
S1	0.0723	0.183	0.0124	0.663	0.0516	0.0760	18.354
0.161 m^3/s flow	±0.0000	±0.001	±0.0002	±0.050	±0.0017	±0.0009	±0.000
S5	2.5725	1.6538	0.0897	13.384	0.9687	2.5578	191.8350
1.470 m^3/s flow	±0.2703	±0.0104	±0.0104	±0.946	±0.0083	±0.0208	±7.276

Table B6 Risks per event of contracting gastrointestinal illness when wading/splashing around in the Ouseburn on various dates according to the quantitative microbial risk assessment (QMRA)

Disease	**Shigellosis**	**Campylo-bacteriosis**	**Salmonellosis**	**Overall risk bacterial gastroenteritis**
Model Pathogen	*E. coli* O157:H7	*C. jejuni*	Non-typhoid *Salmonella*	
Indicator	*rodA*	16S	16S	
Date	Risk of illness per event			
	Median (2.2 mL water ingested)/95th percentile (11.2 mL water ingested)			
July 27th	0.002/0.009	0.004/0.014	0.000/0.000	0.005/0.023
July 28th	0.010/0.047	0.021/0.047	0.003/0.013	0.034/0.103
July 29th	0.003/0.016	0.006/0.022	0.001/0.006	0.011/0.043
July 30th	0.001/0.006	0.008/0.025	0.001/0.004	0.010/0.035
July 31st	0.008/0.038	0.027/0.053	0.003/0.016	0.038/0.104
Aug 1st	0.009/0.040	0.028/0.055	0.003/0.016	0.040/0.107
Aug 2nd	0.002/0.009	0.007/0.022	0.001/0.005	0.009/0.035
Aug 3rd	0.001/0.005	0.002/0.009	0.000/0.002	0.004/0.016
Aug 5th	0.008/0.036	0.014/0.037	0.006/0.027	0.028/0.097
Aug 6th 05:40 AM	0.073/0.227	0.016/0.039	0.048/0.147	0.132/0.367
Aug 6th 07:55 AM	0.041/0.150	0.020/0.045	0.012/0.049	0.071/0.228
Aug 6th 01:55 PM	0.011/0.050	0.021/0.047	0.007/0.033	0.039/0.125
Aug 6th 03:45 PM	0.018/0.078	0.017/0.041	0.004/0.021	0.039/0.135
Aug 8th 12:20 PM	0.179/0.378	0.053/0.079	0.067/0.181	0.275/0.538
Aug 8th 02:00 PM	0.287/0.496	0.053/0.079	0.114/0.248	0.402/0.651
Aug 9th	0.010/0.044	0.004/0.016	0.004/0.018	0.018/0.077

Table B7 Directly incurred costs of one survey to sample and characterize water quality at 8 sites in the Ouseburn catchment, at year 2021 prices.

Item	**Costs (£)**
Sampling 8 sites with onsite analysis (T, pH, Cond, DO)	
Staff	170
Transport	27
Equipment*	29
Consumables	35
Subtotal (% of total costs)	261 (6%)
Sample chemistry (in duplicate, ammonium-N, nitrite-N, nitrate-N, TN, phosphate-P, TP, COD, alkalinity, turbidity), additional costs	
Staff	85
Equipment*	171
Consumables	653
Subtotal (% of total costs)	909 (19%)
Conventional microbiology (FC and FS, in triplicate), additional costs	
Staff	85
Equipment*	239
Consumables	323
Subtotal (% of total costs)	647 (14%)
DNA extraction and quantification (in triplicate), additional costs	
Staff	85
Equipment*	188
Consumables	226
Subtotal (% of total costs)	499 (11%)
qPCR (16S rRNA, rodA, HF183, in triplicate), additional costs	
Staff	85
Equipment*	125
Consumables	570
Subtotal (% of total costs)	780 (17%)
Sequencing (16S rRNA gene amplicons, in triplicate), additional costs	
Staff	510
Equipment*	53
Consumables	1,046
Subtotal (% of total costs)	1,609 (34%)
Total directly incurred costs	4,705

*For the equipment costing it was assumed that the equipment can be used for 100 surveys.

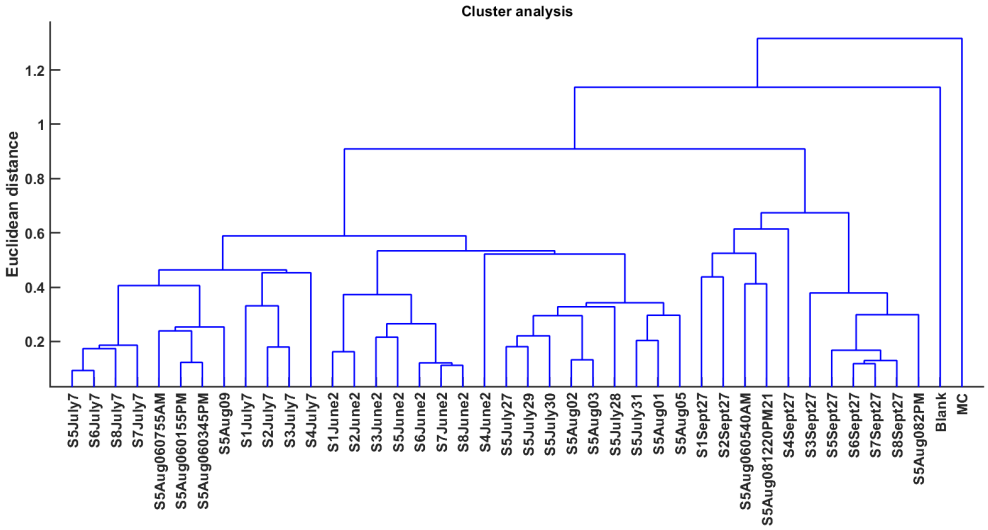

Figure B1 Dendrogram showing that the bacterial community composition derived from 16S rRNA gene sequencing data was for all the river water samples clearly distinct from the procedural blank and mock community samples.

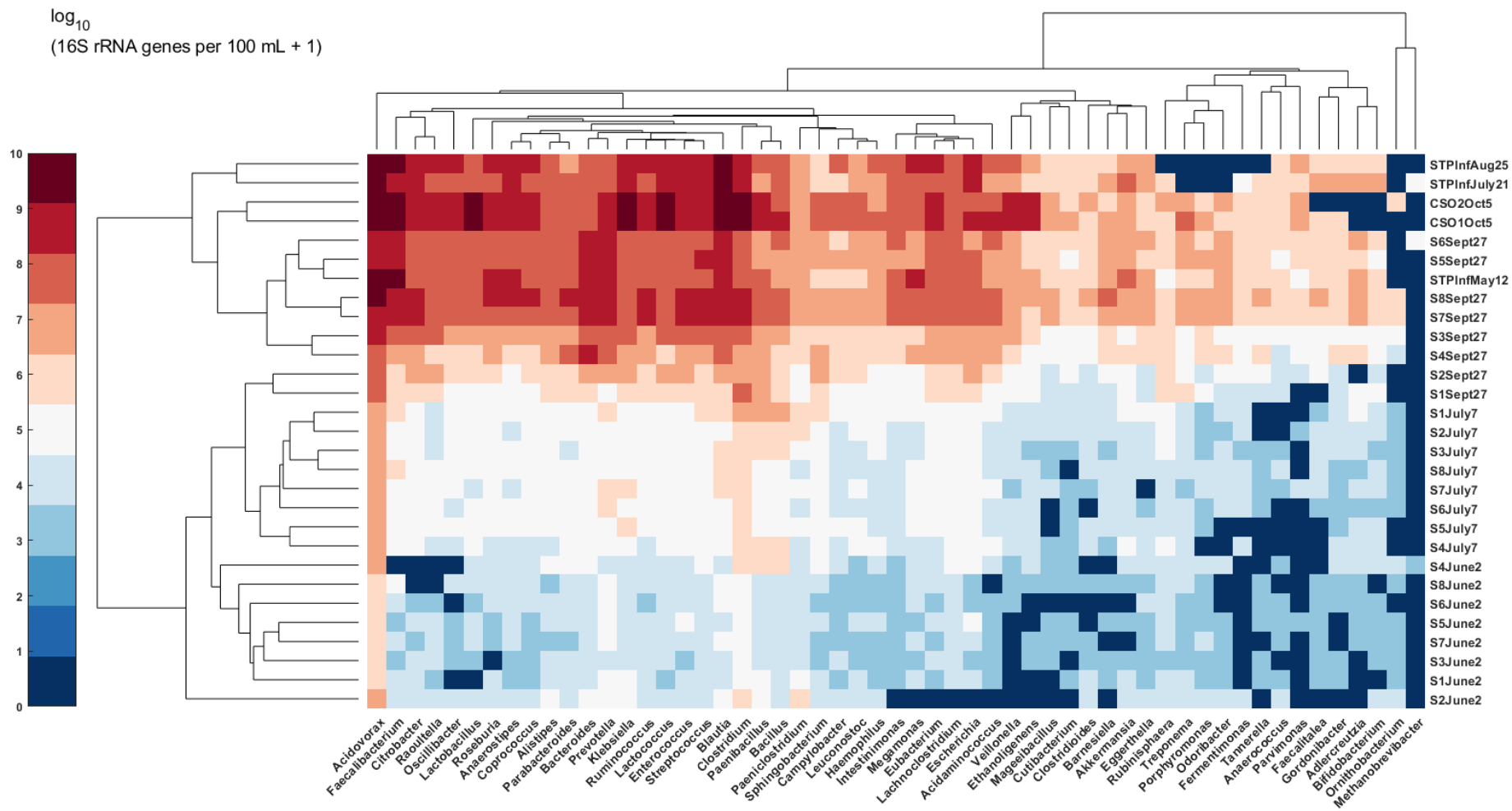

Figure B2 Combined hierarchical clustering and heatmap of 16S rRNA genes (mean values) attributed to 55 human gut associated bacterial genera at sampling locations S1-8 for sampling events, June 2nd (dry), July 7th (after rainfall), and Sept 27th (heavy rain). For comparison, data of combined sewer overflow discharge (CSO) and settled sewage from the inlet of a sewage treatment plant (STPInf) have been included in the plot.

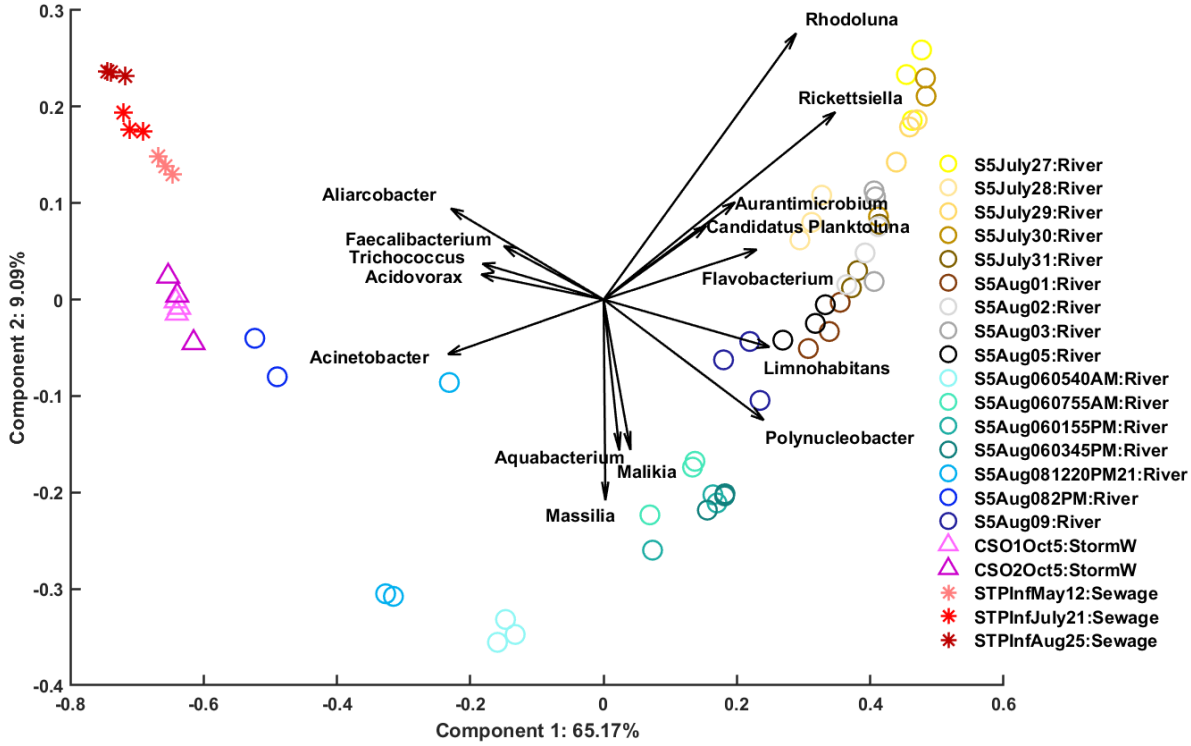

Figure B3 Principal component (PCA) analysis of 16S rRNA gene sequencing derived relative abundance data of the bacterial community characterized to genus level at sampling location S5 for 16 sampling events between July 27th and Aug 9th. For comparison, data of combined sewer overflow discharge (CSO, triangles) and settled sewage from the inlet of a sewage treatment plant (STPInf, stars and crosses) have also been included in the plot. The percent variance explained by each component is shown in the axis label, and the arrows indicate which bacterial genera have a strong relationship (loading) with the principal components.

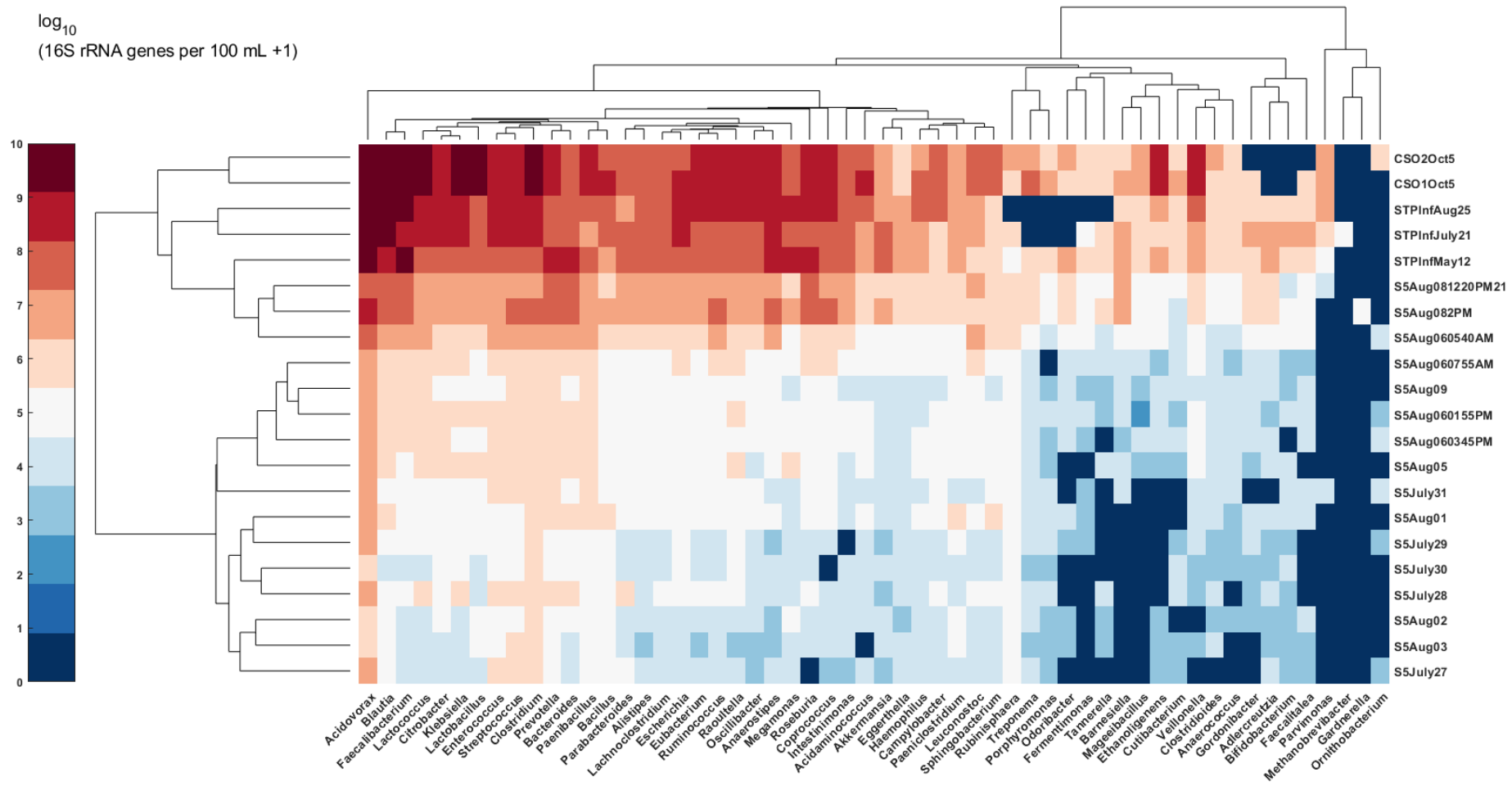

Figure B4 Combined hierarchical clustering and heatmap of 16S rRNA genes (mean values) attributed to 56 human gut associated bacterial genera at sampling locations S5 for 16 sampling events between July 27th and Aug 9th. For comparison, data of combined sewer overflow discharge (CSO) and settled sewage from the inlet of a sewage treatment plant (STPInf) have been included in the plot.

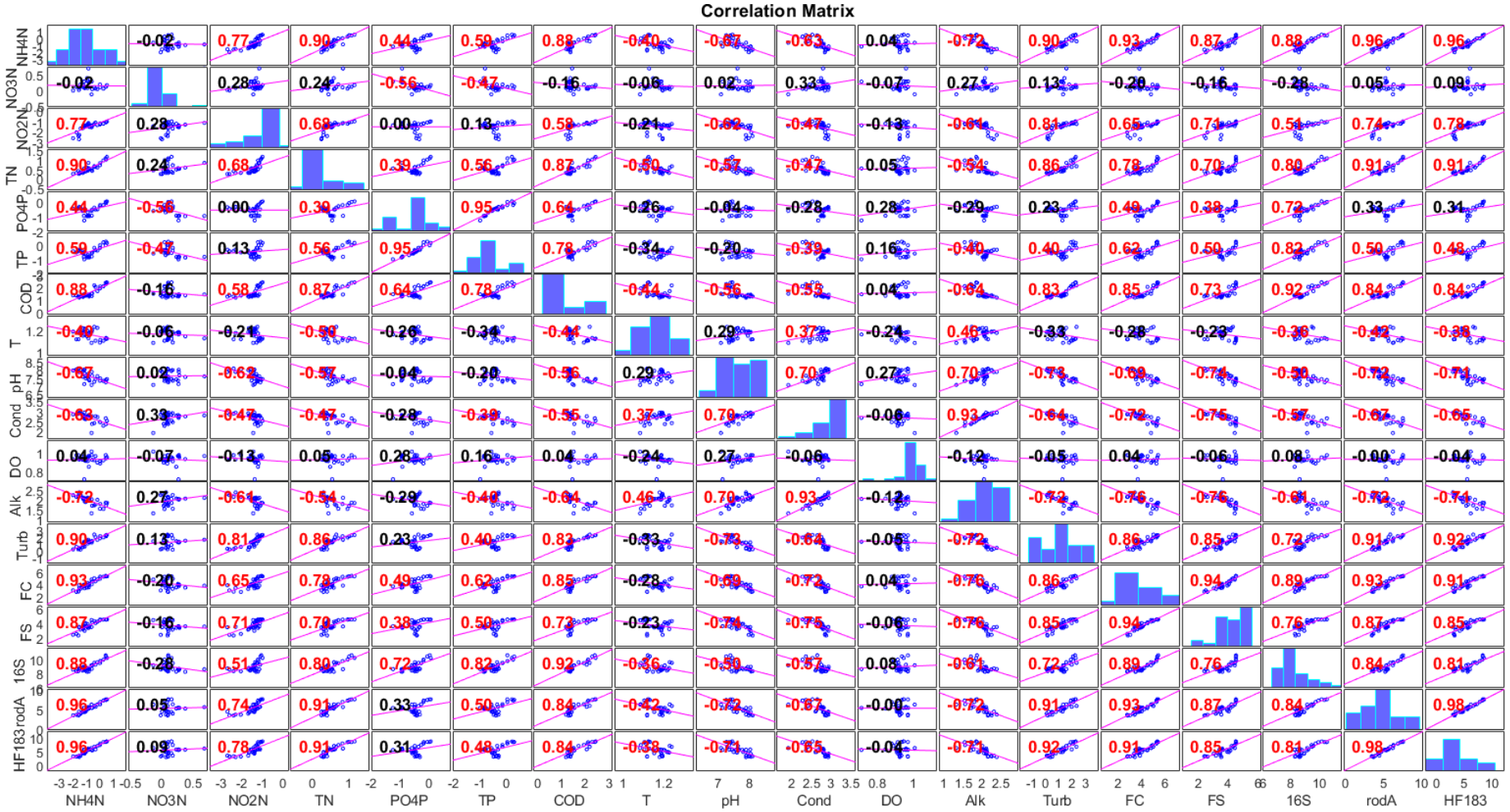

Figure B5 Pearson Correlation matrix for $\log_{10}$ transformed biogeochemical water quality parameters (pH was used without transformation). Significant Pearson Correlation Coefficients R (p = 0.05) are highlighted in red font. Units are shown in table 1 of the main manuscript.

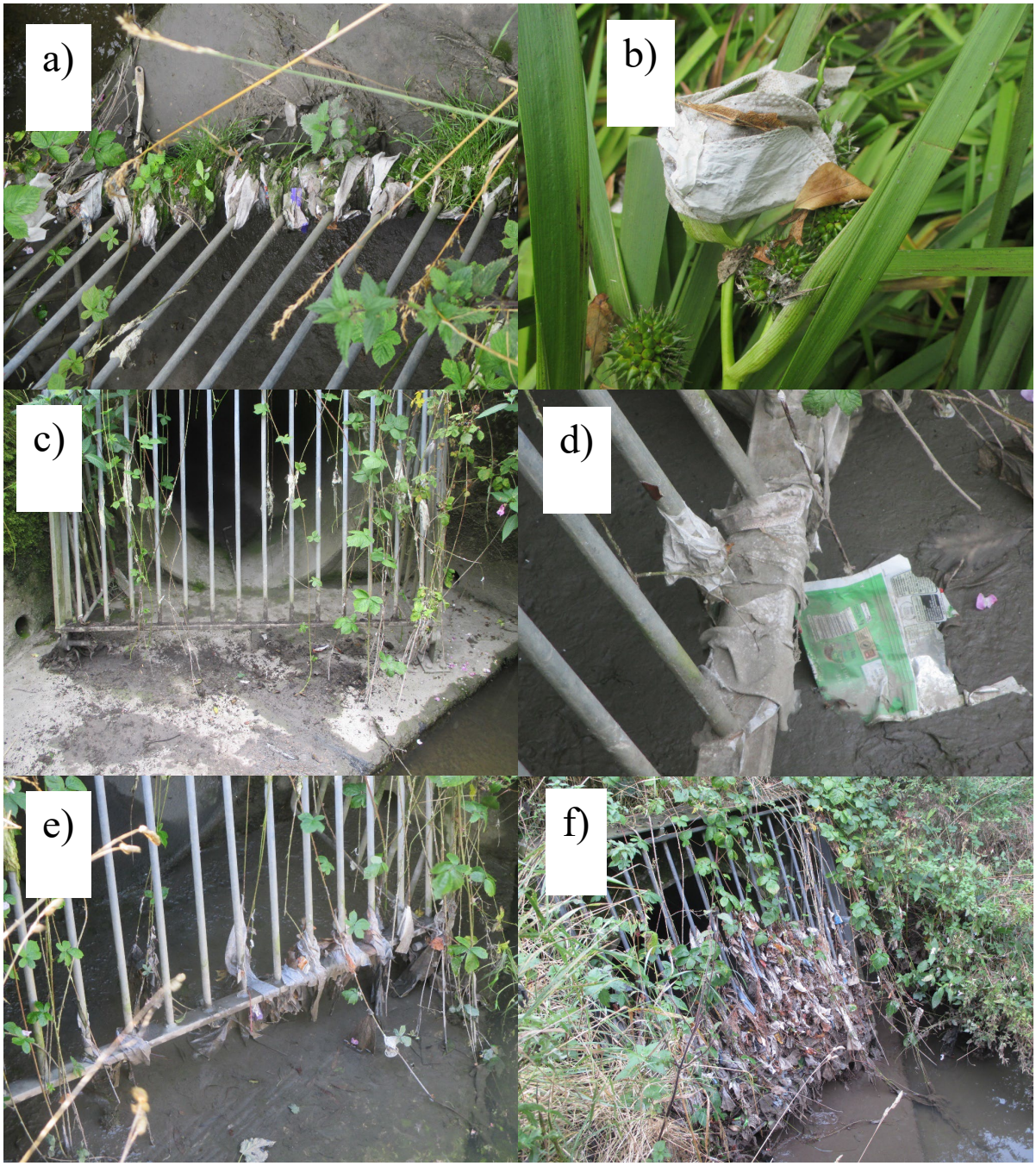

Figure B6 Sewage litter observations in the year 2021 a) on the CSO4 bar screen on Aug 17th; b) in the Ouseburn adjacent to CSO4 on Aug 17th; c) after manual removal of litter from the bar screen on Aug 19th; d) on Aug 23rd; e) on Oct 3rd; f) on Oct 5th. Sewage litter observed included wet wipes, sanitary products, and condoms.

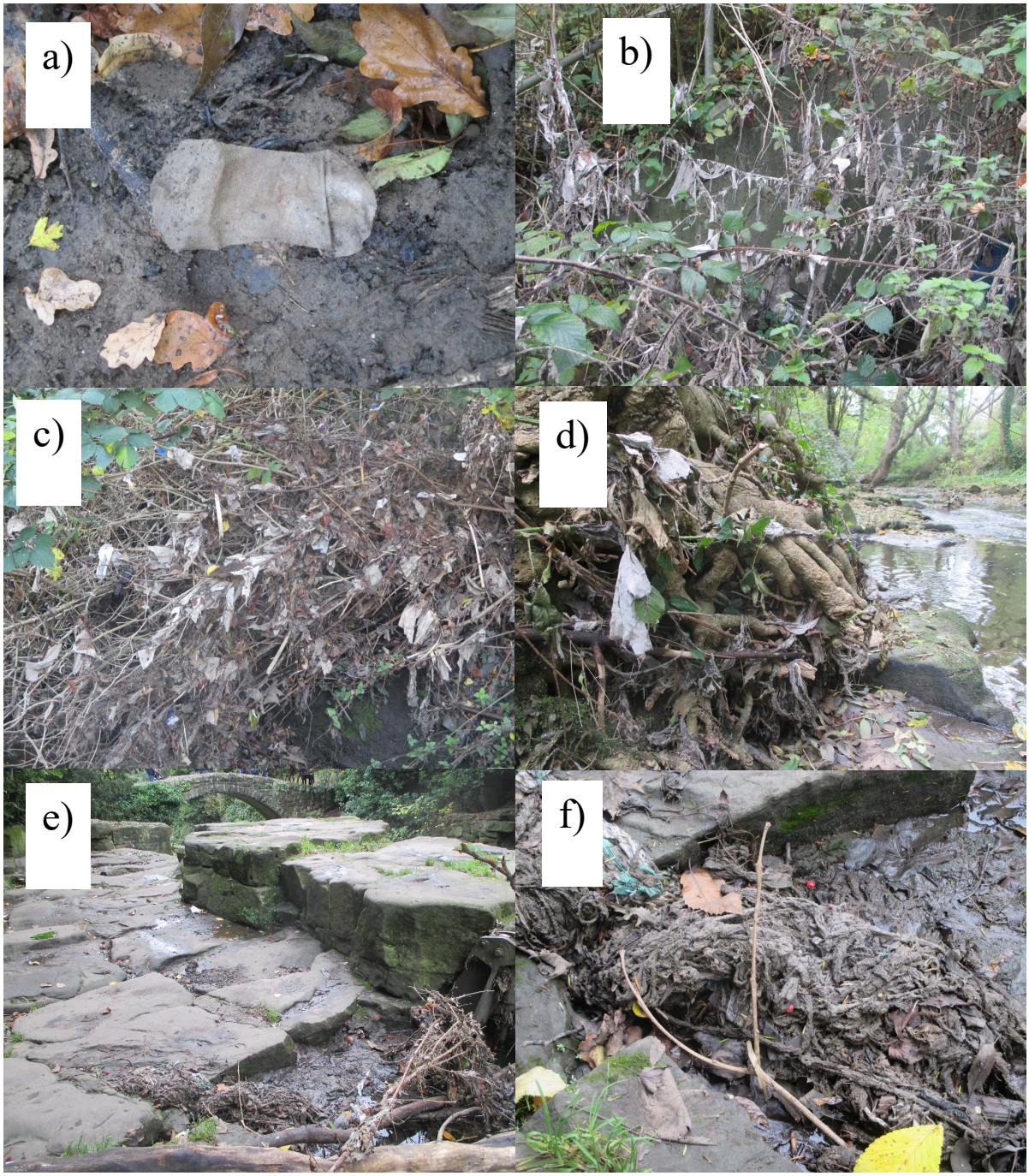

Figure B7 Observations after the major storm event on Oct 5th, 2021: a) a sanitary product deposited next to a surface water drain near S1; b) sewage litter in brambles covering the outlet of CSO7; c) sewage litter in riparian vegetation viewed from Gallalaw Terrace downstream of CSOs 9&10; d) sewage litter in riparian tree roots in Jesmond Dene Park, upstream of S5; e) wet wipe deposit on rocks next to the artificial waterfall in Jesmond Dene Park; f) detailed view of the wet wipe deposit on the rocks.

Appendix C

Table C1. Primers, probes, and programs for qPCR assays.

Target Gene	qPCR Cycle Program	Primers (3'-5') and probes	Efficiency	Slope	R^2
16S rRNA	3 mins at 98 °C, 40 cycles of 15 s at 98 °C, 30s at 60 °C, melt from 72°C to 95°C	F: ATGGCTGTCGTCAGCT	100±5	-3.32±0.11	0.988±0.008
		R: ACGGGCGGTGTGTAC			
rod A	3 mins at 98 °C, 40 cycles of 15 s at 95 °C, 30s at 60 °C	F: GCAAACCACCTTTGGTCG	93±4	-3.52±0.12	0.995±0.05
		R: CTGTGGGTGTGGATTGACAT			
		P: FAM-AACCCCTACAACCGGCAGAATACC			
HF 183	3 mins at 98 °C, 40 cycles of 15 s at 95 °C, 30s at 60 °C	F: ATC ATG AGT TCA CAT GTC CG	92±5	-3.53±0.14	0.995±0.05
		R: CTT CCT CTC AGA ACC CCT ATCC			
		P: HEX-CTA ATG GAA CGC ATC CC			

Table C2. List of bacterial genera containing nitrifying bacteria (Koops and Pommerening-Röser, 2001).

AOB	*Nitrosomonas*
	Nitrosolobus
	Nitrosococcus
	Nitrosospira
	Nitrosovibrio
NOB	*Nitrobacter*
	Nitrococcus
	Nitrospina
	Nitrospira

Table C3. Total suspended solids (TSS), total dissolved carbon (TDC, DIC and DOC), and dissolved metals (Fe, Cu, Mn, Zn, As, and Pb) average log removal values of 12 sampling days for Control and AC permeable pavements. The results are presented as mean±Stdev. The student t-test p value for log removal values between Control and AC permeable pavements are also stated.

	Control	AC	t-test
TSS	1.3±0.4	1.2±0.5	0.50
PO_4^{3-}P	-0.1±0.5	0.0±0.4	0.76
TDC	-0.19±0.25	-0.08±0.21	0.27
DIC	-0.45±0.15	-0.41±0.15	0.57
DOC	0.06±0.26	0.35±0.13	**0.003**
Fe	0.3±0.2	0.4±0.3	0.39
Cu	0.1±0.2	0.4±0.1	**0.0002**
Mn	0.6±0.6	0.6±0.5	0.94
Zn	0.5±0.3	0.4±0.3	0.38
As	-1.0±0.5	-1.0±0.4	0.74
Pb	0.5±0.3	0.5±0.2	0.53

Table C4. Total suspended solids (TSS), dissolved carbon (TDC, DIC and DOC), PO_4^{3-}-P, and dissolved metals (Fe, Cu, Mn, Zn, As, and Pb) log removal values of sand and AC permeable pavements for 12 sampling days during the one-year monitoring period. The results shown are presented as the mean log removal of duplicates ± Stdev.

	TSS	PO_4^{3-}-P	TC	TIC	TOC	Fe	Cu	Mn	Zn	As	Pb
July12 Sand	2.0±0.1	0.5±0.1	-0.63±0.04	-0.83±0.08	-0.47±0.17	-0.1±0.0	-0.4±0.2	1.2±0.2	0.5±0.4	-2.0±0.2	0.9±0.0
July12 AC	2.2±0.1	0.5±0.1	-0.38±0.13	-0.81±0.14	0.36±0.07	0.8±0.9	0.6±0.1	1.2±0.4	0.7±0.4	-2.0±0.2	0.9±0.0
July28 Sand	1.5±0.1	0.0±0.0	0.39±0.00	-0.50±0.00	0.48±0.00	0.6±0.0	0.1±0.0	1.9±0.0	1.2±0.0	-1.3±0.0	1.1±0.0
July28 AC	1.4±0.3	-0.1±0.0	0.48±0.00	-0.48±0.00	0.59±0.00	0.3±0.0	0.3±0.0	1.5±0.0	1.1±0.0	-1.2±0.0	0.7±0.0
Aug22 Sand	1.6±0.0	-1.5±0.1	-0.35±0.05	-0.43±0.04	-0.17±0.06	-0.1±0.2	-0.1±0.1	0.1±0.3	0.7±0.0	-1.8±0.1	0.6±0.1
Aug22 AC	1.2±0.0	-1.2±0.1	-0.21±0.02	-0.36±0.01	0.28±0.08	-0.3±0.1	0.3±0.1	0.2±0.1	0.4±0.3	-1.5±0.1	0.2±0.1
Sept30 Sand	1.9±0.0	-0.7±0.1	-0.14±0.01	-0.39±0.03	0.20±0.13	0.2±0.3	0.2±0.1	1.3±0.3	0.6±0.2	-1.4±0.1	0.5±0.3
Sept30 AC	1.5±0.0	-0.3±0.0	-0.08±0.01	-0.36±0.04	0.36±0.07	0.2±0.1	0.5±0.1	1.1±0.3	0.6±0.0	-1.2±0.1	0.4±0.0
Oct29 Sand	0.8±0.0	0.2±0.0	-0.17±0.03	-0.44±0.06	0.27±0.10	0.3±0.2	0.2±0.1	0.8±0.1	0.5±0.1	-1.0±0.1	0.5±0.1
Oct29 AC	0.7±0.0	0.2±0.0	-0.12±0.05	-0.40±0.07	0.43±0.08	0.2±0.1	0.4±0.1	0.7±0.1	0.2±0.0	-1.0±0.0	0.3±0.1
Dec01 Sand	1.0±0.0	0.2±0.0	-0.14±0.00	-0.18±0.00	-0.07±0.00	0.1±0.0	0.3±0.2	0.5±0.1	0.4±0.0	-0.9±0.0	0.0±0.0
Dec01 AC	0.9±0.1	0.3±0.1	-0.18±0.01	-0.12±0.00	0.37±0.05	0.3±0.0	0.4±0.0	0.6±0.2	0.3±0.0	-0.8±0.0	0.2±0.0
Feb06 Sand	0.9±0.0	0.0±0.0	-0.31±0.10	-0.51±0.15	0.15±0.11	0.1±0.2	0.2±0.0	0.3±0.4	0.5±0.1	-0.6±0.1	0.4±0.1
Feb06 AC	0.9±0.0	0.0±0.0	-0.23±0.04	-0.45±0.06	0.31±0.08	0.6±0.1	0.6±0.1	0.5±0.1	0.4±0.5	-0.7±0.1	0.4±0.0
Feb18 Sand	1.1±0.1	-0.2±0.1	-0.30±0.05	-0.40±0.09	-0.13±0.04	0.5±0.1	0.1±0.0	0.1±0.1	0.6±0.0	-0.8±0.0	0.3±0.1
Feb18 AC	0.9±0.1	-0.2±0.1	-0.19±0.04	-0.33±0.05	0.09±0.01	0.6±0.1	0.2±0.1	0.1±0.1	0.4±0.0	-0.8±0.0	0.5±0.1
Mar17 Sand	1.0±0.1	0.3±0.0	0.07±0.02	-0.44±0.14	0.38±0.14	0.5±0.2	0.1±0.0	0.9±0.2	0.3±0.3	-0.3±0.3	0.2±0.1
Mar17 AC	0.8±0.0	0.1±0.0	0.09±0.02	-0.47±0.02	0.47±0.00	0.5±0.0	0.2±0.1	0.9±0.1	0.0±0.2	-0.7±0.0	0.2±0.1
Mar30 Sand	1.4±0.1	0.1±0.1	-0.13±0.05	-0.31±0.07	0.09±0.03	0.6±0.1	0.4±0.1	-0.2±0.2	0.0±0.3	-0.7±0.0	0.8±0.0
Mar30 AC	1.3±0.0	0.1±0.0	-0.02±0.05	-0.29±0.07	0.36±0.03	0.9±0.2	0.3±0.1	0.2±0.0	0.1±0.1	-0.7±0.0	0.7±0.0
May16 Sand	1.2±0.3	0.1±0.0	-0.26±0.05	-0.45±0.16	-0.08±0.11	0.2±0.1	-0.3±0.0	0.3±0.0	0.0±0.1	-0.7±0.0	0.5±0.3
May16 AC	1.0±0.0	0.3±0.0	-0.06±0.02	-0.32±0.08	0.22±0.10	0.3±0.1	0.2±0.1	0.3±0.1	0.0±0.3	-0.7±0.0	0.3±0.1
June08 Sand	1.9±0.1	0.0±0.1	-0.25±0.03	-0.48±0.08	0.06±0.09	0.3±0.1	-0.1±0.0	0.3±0.0	0.3±0.1	-0.9±0.0	0.6±0.1
June08 AC	1.8±0.0	-0.2±0.0	-0.16±0.04	-0.44±0.05	0.31±0.02	0.3±0.1	0.3±0.0	0.2±0.2	0.1±0.3	-1.0±0.0	0.5±0.0

Table C5. Ten most prevalent bacterial genera in stormwater, Control effluents, and AC effluents, identified from 16S rRNA gene sequencing data according to their median relative abundance rank.

Stormwater	Control effluents	AC effluents	Control joint	AC joint	Control base	AC base
Aliterella	*Legionella*	*Legionella*	*Nocardioides*	*Nocardioides*	*Methylobacterium*	*Hydrogenophaga*
Sphingomonas	*Sphingomonas*	*Sphingomonas*	*Sphingomonas*	*Sphingomonas*	*Priestia*	*Methylobacterium*
Nocardioides	*Pseudomonas*	*Nocardioides*	*Microlunatus*	*Microlunatus*	*Nocardioides*	*Oxalicibacterium*
Rubellimicrobium	*Azospirillum*	*Pseudomonas*	*Ferruginibacter*	*Ilumatobacter*	*Robertmurraya*	*Thioalkalivibrio*
Microlunatus	*Nocardioides*	*Brevundimonas*	*Rubellimicrobium*	*Microbacterium*	*Sphingomonas*	*Legionella*
Truepera	*Hydrogenophaga*	*Flavobacterium*	*Spirosoma*	*Nakamurella*	*Rhabdothermincola*	*Acidiferrobacter*
Roseomonas	*Novosphingobium*	*Massilia*	*Aridibacter*	*Amaricoccus*	*Bacillus*	*Methylibium*
Deinococcus	*Brevundimonas*	*Psychrobacter*	*Flavisolibacter*	*Methylobacterium*	*Longimicrobium*	*Lysobacter*
Hymenobacter	*Devosia*	*Novosphingobium*	*Massilia*	*Marmoricola*	*Microlunatus*	*Mucilaginibacter*
Paracoccus	*Massilia*	*Devosia*	*Methylobacterium*	*Massilia*	*Lysobacter*	*Sulfurifustis*

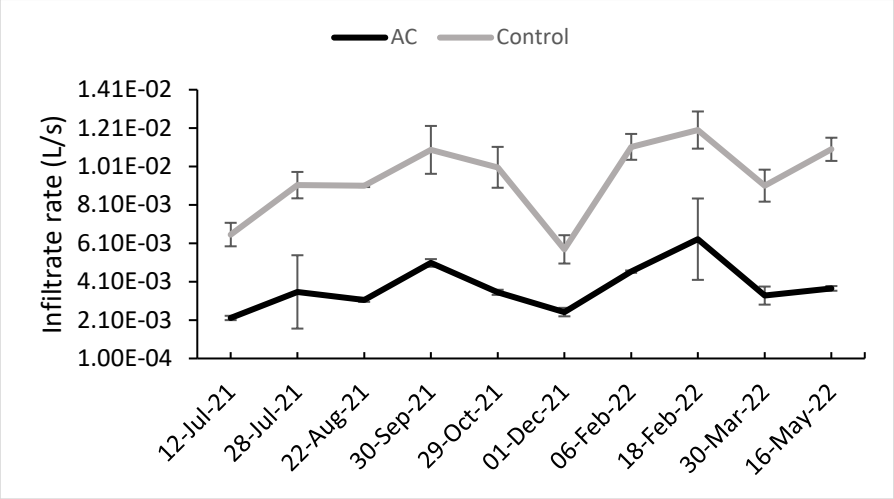

Figure C1. Infiltration rates for infiltration tests conducted between 12/07/2021 and 08/06/2022. Error bars represent the standard deviation of duplicate. The data for 17/03/2022 and 08/06/2022 were missing due to technical problems.

Figure C1 shows the infiltration rates for each sampling events conducted between 12/07/2021 and 08/06/2021. The infiltration rates were calculated as per Equation C1,

Infiltration rates = Effluent volume (L)/Retention time (s) (Eq C1)

Figure C2 shows the accumulative mass of TN, TP, and DOC released from the permeable pavements into the effluents collected in between the infiltration tests. The amounts released in between infiltration tests was calculated as per Equation C2,

Mass = Effluent volume (L) ×Concentration of chemical (mg/L) (Eq C2)

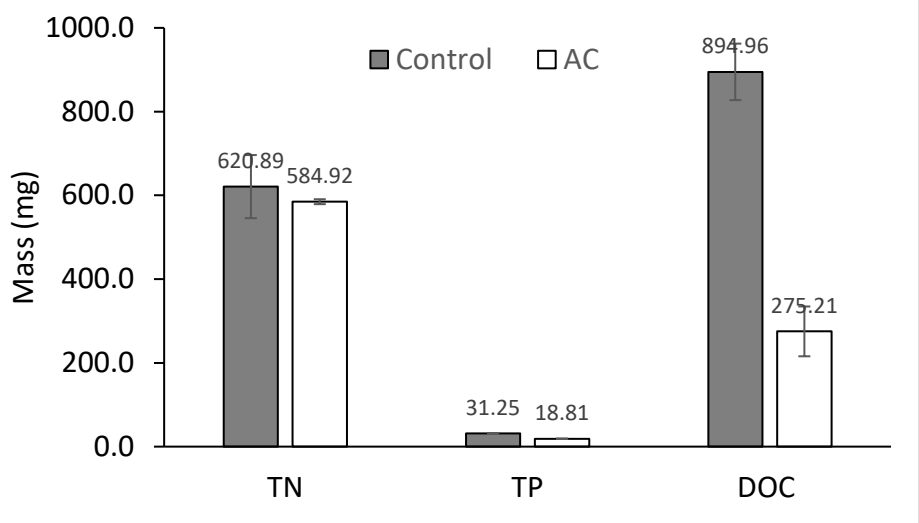

Figure C2. Total nitrogen (TN), Total Phosphorous (TP), and Dissolved Organic Carbon (DOC) mass released by the permeable pavements with effluents collected in between the infiltration tests (Error bars represent the standard deviation of duplicate).

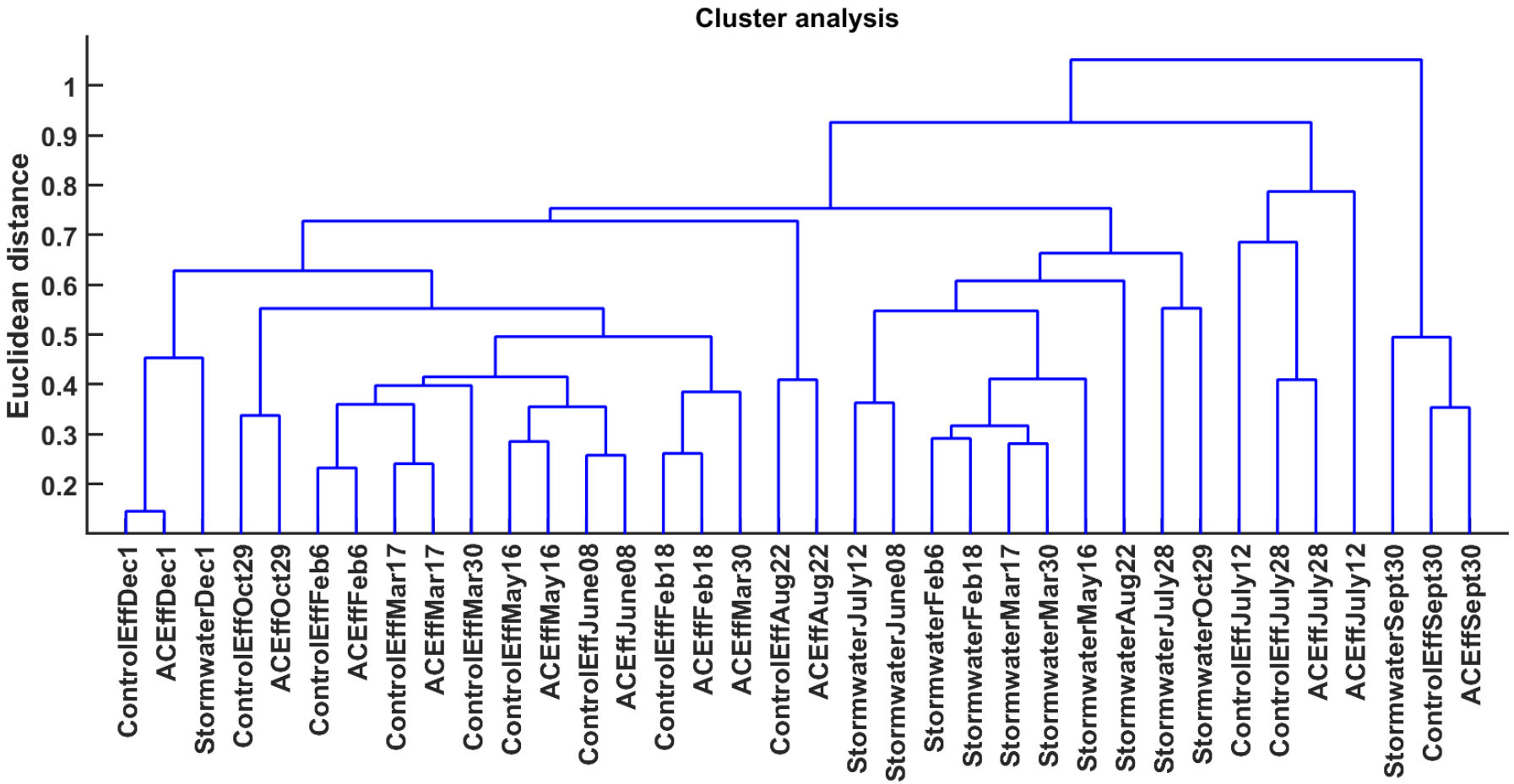

Figure C3. Cluster analysis for stormwater and effluents from Control and AC permeable pavement across over 12 sampling events from 12/07/2021 to 08/06/2022. Samples represent as average of four replicate.