Analysis of the Chemistry & Microbiology of Urban Stormwater & Its Treatment by Rain Gardens

A thesis submitted by

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Declaration

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Abstract

Sustainable urban drainage systems (SUDS) are designed to both lower the quantity and increase the quality of urban runoff that is released to surface water bodies, reducing flood risk and minimising environmental damage. Little is currently known about the microbial communities within SUDS, despite the important role that bacteria play in pollutant removal. Optimising the water treatment potential of SUDS will require a better understanding of how water treatment and microbial community composition are affected by factors such as design and local pollutant concentrations.

This thesis examined the chemical and microbial variation in stormwater from Glasgow, Scotland and Florianópolis, Brazil, as well as in influent, effluent, and soil from Glaswegian rain gardens. Concentrations of contaminants were generally higher in Glasgow than in Florianópolis, with several potentially toxic elements (PTEs) exceeding local freshwater standards. Taxonomic and functional microbial diversity were significantly impacted by country of origin, suggesting that geographical factors such as climate are affecting the stormwater community.

The Glaswegian rain gardens' design differed only in soil particle size distribution. All four gardens were able to lower pollutant concentrations, but no clear trend in removal efficiency based on soil type was observed. Influent microbial communities differed significantly from those of the effluent, while the four effluent communities resembled one another and varied based on sampling date, suggesting that seasonal factors or community maturation have a greater impact on water microbiology than soil particle size.

Some variation in hydrocarbon and PTE concentrations was seen in the rain garden soil, but the overall level of contamination was low. Taxonomy of the soil microbial communities was similar between gardens, with several common hydrocarbon degraders identified. Differences in taxonomic and functional diversity caused by soil chemistry and morphology were found, suggesting that the choice of soil should be taken into account to maximise community stability.

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List of Abbreviations

ASV	Amplicon Sequencing Variant
BDL	Below Detection Limits
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
COD	Chemical Oxygen Demand
CSO	Combined Sewer Overflow
DCM	Dichloromethane
DOC	Dissolved Organic Carbon
EC	Electrical Conductivity
EQS	Environmental Quality Standards
GC-FID	Gas Chromatography - Flame Ionisation Detection
HDPE	High Density Polyethylene
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectroscopy
LLE	Liquid-Liquid Extraction
MCE	Mixed Cellulose Esters
PAH	Polycyclic Aromatic Hydrocarbons
PCoA	Principal Coordinates Analysis
PP	Polypropylene
PTE	Potentially Toxic Elements
qPCR	Quantitative Polymerase Chain Reaction
SEPA	Scottish Environment Protection Agency
SGV	Soil Guideline Value
SUDS	Sustainable Urban Drainage System
TDS	Total Dissolved Solids
TPH	Total Petroleum Hydrocarbons
TS	Total Solids
TSS	Total Suspended Solids

Introduction

1.1 The Need for Change

1.1.1 Flood Risk

Urbanisation of an area affects its natural hydrological processes (Figure 1.1). Impermeable surfaces such as roads, pavements, and roofs prevent direct infiltration of rainwater into the ground, while the clearance of vegetation reduces water removal via root uptake and evapotranspiration [1]. Rainwater that is unable to infiltrate instead flows over the ground until it reaches either the drainage system or a nearby body of water such as a river or lake. This water can be referred to as “surface runoff”, “runoff”, or “stormwater”. Urbanisation can increase the proportion of rainfall that becomes runoff from 10% to 55%, when compared to permeable areas such as woodland [2]. The high volume and flow rate of surface runoff leads to flooding and soil erosion, while the lack of infiltration contributes to water scarcity due to reduced groundwater recharge [3] [4] [1].

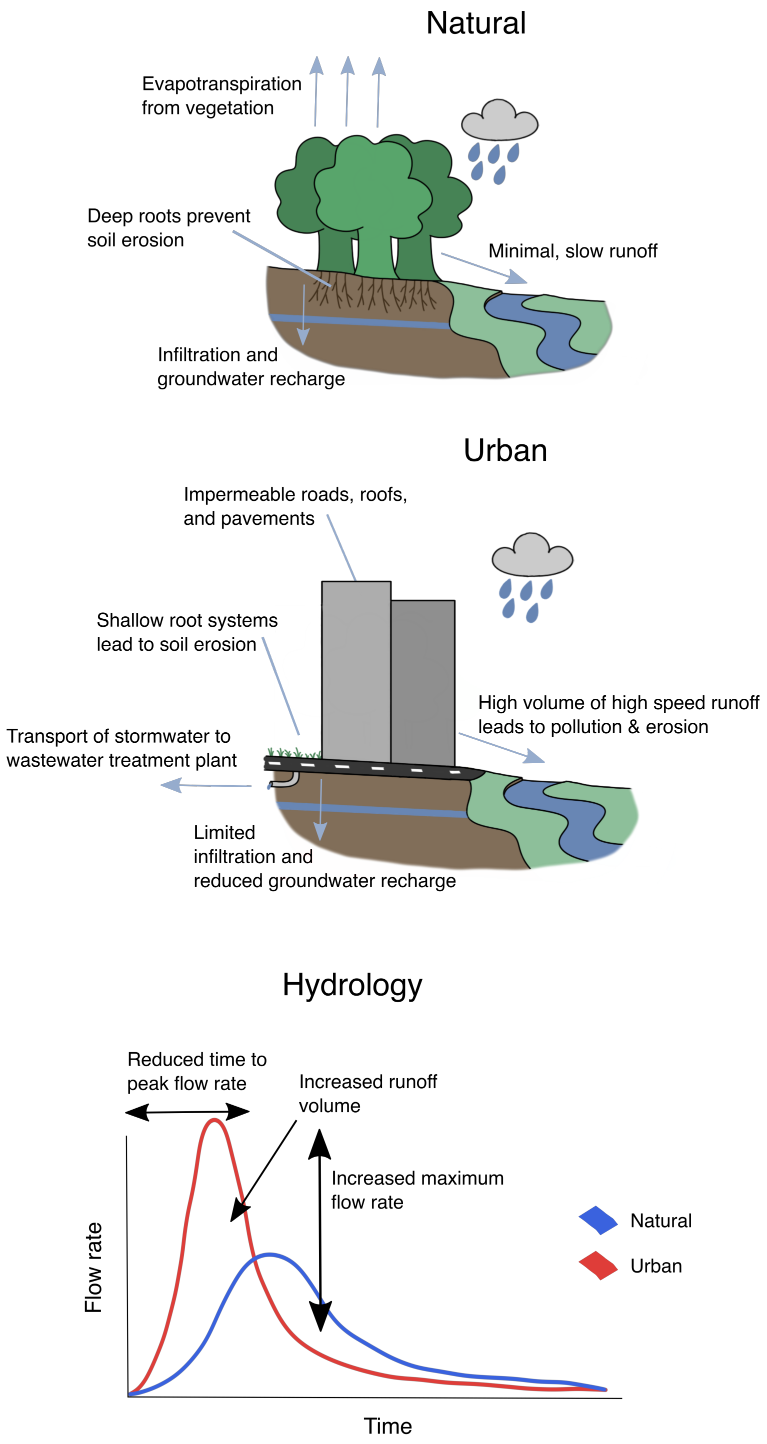

Figure 1.1: Urbanisation leads to changes in local hydrology, resulting in greater volumes of fast-flowing runoff.

Conventional urban drainage tends to control runoff in one of two ways – combined or separate sewers. Older systems rely on combined sewers – a single pipe system that transports both runoff and domestic sewage to a water treatment plant. After heavy rain or snowmelt, these systems are prone to "combined sewer overflows" (CSOs) as they are unable to handle the sudden influx of runoff. This results in the discharge of sewage-contaminated water to the environment [5] [6]. In 2012, the UK was called to the European Court of Justice due to repeated CSOs in Whitburn and London, England, breaching 1991 EU water quality regulations [7] [8]. As of 2021, these sewage discharges continue to be an issue [9]. In Scotland, the number of CSOs discharging to rivers and seas increased by 40% between 2016 and 2020. There were more than 12,000 sewage discharges in 2020 – and this is likely an underestimation of the true number, as Scottish Water monitors only 3% of CSO sites. The total volume of these discharges has also increased from 12.9 m^3 in 2016 to 48 m^3 in 2020 [10].

Separate sewer systems are now more common than combined systems [3]. These consist of one sewer system for transporting domestic sewage to treatment plants and another for transporting runoff, often discharging directly to the environment. While this minimises the risk of sewage overflow, flooding may still occur due to the discharge of high volumes of fast-flowing runoff to local bodies of water such as rivers. Flooding may also occur if the runoff cannot enter the drainage system quickly enough. Half of flooding incidents per year are a result of overload of the drainage system [11].

Climate change is set to worsen already present issues. Wetter winters with more frequent storms are predicted [12], with winter rainfall in Scotland expected to rise by 7% by the 2050s and up to 13% by the 2080s [13]. In the UK, 2020 was the wettest year since 2000, experiencing 1336 mm of rainfall – 116% of the 1981-2010 UK average. Widespread flooding occurred throughout the country during the summer and during what was the wettest February on record - with some areas experiencing 400% of the 1981-2010 average rainfall. A total of 474 mm of rain occurred during the winter months (144% of the 1981-2010 average) [14]. Winter rainfall is expected to rise further and, although summer rainfall is expected to fall slightly, year-round rainfall intensity in Scotland could rise by up to 25% [13], leading to significant pressure on drainage systems. Traditional drainage systems are not designed to cope with this increase in stormwater volume and adding additional capacity to the sewers would be both labour intensive and costly [5]. If a change in drainage strategy is not enacted, residential flood damage in the UK could increase by up to 160% by the 2080s [11].

1.1.2 Water Scarcity

The combination of population growth, population movement, and climate change is likely to lead to increased water scarcity in the coming decades. Between 1950 and 2000, the urban population rose from 737 million to 2.7 billion and, currently, half the world's population lives in cities [15]. By 2050, an estimated 6.3 billion out of 9.1 billion people will reside in urban areas [15], including 85.9% of the population of developing countries [2]. This high concentration of residents within comparatively small areas can lead to issues with water supply. Since the beginning of the last century, the global population has quadrupled but approximately 16 times more people are experiencing water scarcity, mainly due to changes in population distribution rather than per person increases in consumption [16].

The distribution of rainfall and thus water availability across a country does not necessarily match the urban distribution. For example, in the UK rainfall varies heavily depending on region, with the northern and western areas seeing the most. These areas are also the least densely populated, leading to water surplus in some areas while there are shortages in highly populated regions. In England and Wales, overall water availability per person is classed as "low" at 1400 m^3/year. London specifically has "very low" water availability at only 250 m^3/year per person [17]. To overcome this, surplus water must be stored during rainier seasons or else water must be transported from wetter areas to cover the deficit – both of which require significant space, energy, and money.

The effects of climate change will only exacerbate water shortages in urban areas. By the 2080s, global temperature could rise by up to 6.8° C [12], and an increase of only 2-3° C could negatively impact water availability for up to 3.2 billion people [18]. A decrease in rainfall during the warmer months is likely to result in droughts. In

Scotland, summer rainfall is predicted to fall by up to 7% by the 2050s and up to 16% by the 2080s [13]. Despite the higher than average overall rainfall in 2020, only 142 mm (60% of the 1981-2010 average) fell during spring [14]. By 2100, the UK could be in a water supply-demand deficit of between 1220 and 2900 megalitres per day, equivalent to the water use of 8.3 – 19.7 million people [13].

A more sustainable approach to water supply is required to minimise water scarcity. A decrease in water demand per person, coupled with the re-use of rainwater and stormwater may offer one solution to these issues.

1.1.3 Resource Costs

The average person in the UK uses 150 litres of water per day. Although all of this water is treated to drinking water standard, only 4% is actually used for drinking. The remainder is used for tasks such as washing clothes and dishes, watering plants, personal hygiene, and around a third is used for flushing the toilet [17].

Treating water to well beyond the standard required for most purposes results in enormous energy costs. The water industry emits 1% of the UK's greenhouse gas contribution and is the country's fourth most energy intensive industry [19]. Two thirds of the water industry's energy is used for treatment and pumping and the remaining one third is used in the construction and maintenance of infrastructure [19]. Although separate sewers reduce the risk of CSOs, the maintenance of two drainage systems comes with increased resource costs [3].

By decentralising water treatment and using local water resources, as well as harvesting rainwater and stormwater for non-potable use, resource costs in the water industry could be lowered significantly.

The indirect costs of continuing to rely on traditional drainage must also be considered. Attempting to adapt current drainage systems to deal with greater volumes of stormwater would cost the water industry £1 billion annually [20]. Even then, the increased capacity may not be sufficient and further expensive work could be required in the future to minimise the risk of flooding. Currently, it is estimated that flooding in Scotland causes £720-850 million worth of damage every year [21]. Widespread UK flooding in the summer of 2007 caused £3 billion worth of damage [20] and the deaths of 13 people [11].

1.1.4 Environmental Damage

Although the use of separate sewer systems for urban runoff reduces the likelihood of sewage discharge to the environment, they are not without their own harmful effects. Urban runoff is not treated by these systems before being discharged into rivers and lakes, taking with it various pollutants including metals, oil, and sediment [12]. As discussed further in Section 1.2, these contaminants can have a significant negative effect on the aquatic ecosystem and beyond. Even when as little as 10% of an area is urbanised, runoff is seen to have a negative impact on local water bodies [22]. Climate change is predicted to lead to drier summers in the UK, meaning there is a longer period of pollutant build-up on urban surfaces. When rain eventually happens and washes these surfaces, this can lead to bursts of high concentrations of pollutants entering the environment [1].

1.2 Urban Diffuse Pollution

1.2.1 Overview

Urban diffuse pollution is composed of the diverse range of contaminants that result from urbanisation - including oil, grit, pesticides, fertiliser, sediment, pathogens, and metals [1]. These pollutants do not originate from a single source but rather from a variety of sources and activities across a catchment area. While the impact of each source may be low on its own, together the pollutants can have a significant effect on the receiving water body. In some cases, the impact may be cumulative over time [23]. Unlike other sources of pollution such as industrial discharge, sewage, and agricultural waste, urban diffuse pollution is not generally monitored closely [23].

Urban diffuse pollution has a negative impact on the biodiversity of surface water, as well as on its suitability for anthropogenic uses including drinking water supply, swimming, and aesthetics [3].

1.2.2 Solids & Sediment

Solids and sediment pollution may arise from sources including vehicle corrosion, soil erosion, road salting during winter, washing and weathering of roofs and buildings, atmospheric deposition, and vehicle exhaust emissions [24] [1] [17] [25] [26]. High concentrations of suspended solids in rivers and lakes can clog fish gills and can limit the penetration of light into the water, which affects plant growth and in turn the rest of the aquatic ecosystem [27]. Additionally, solids and sediment act as carriers for other pollutants including nitrogen, phosphorus, metals, and hydrocarbons [23] [25] [28]. These pollutants may remain bound to the solids or be washed off during heavy rain. Fine particulates may be suspended in the air and if inhaled can cause health problems [29].

As well as being suspended solids themselves, winter road treatments like grit and salt increase vehicle abrasion, resulting in the release of higher concentrations of solids and metals. Chloride in road salt is not only toxic to wildlife and plants but can increase metal mobilisation, resulting in higher concentrations of dissolved or bioavailable metals [25] [30].

1.2.3 Hydrocarbons

Hydrocarbons are organic compounds that contain only hydrogen and carbon. Hydrocarbons may be aliphatic (straight, branched, or containing non-aromatic rings) or aromatic (containing rings with alternating double and single bonds). Compounds containing multiple aromatic rings are referred to as polycyclic aromatic hydrocarbons (PAHs). Common PAHs include benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX [28].

Hydrocarbon pollution is often a result of exhaust emissions, accidental oil spills, and incorrect disposal of chemicals [1] [24]. However, hydrocarbons may also result from natural sources such as leaves and decomposing organisms [28]. Pyrogenic hydrocarbons – formed by combustion of fuel – are often complex and not readily biodegradable, resulting in their accumulation in the environment [28]. PAHs can arise from exhaust emissions [26] and may also leach from pavements and road surfaces containing bitumen [25] [28].

Hydrocarbon contamination of surface water bodies can lead to decreased oxygen concentrations as the hydrocarbon degrades, which negatively impacts biodiversity and fish health [22]. Many hydrocarbons also exhibit acute toxicity to aquatic life when present in high concentrations [28]. Hydrocarbons can enter the food chain via bioaccumulation and can negatively impact growth, development, and reproduction [22]. PAHs such as BTEX are known carcinogens [31]

1.2.4 Potentially Toxic Elements

Both metals and arsenic can be described by the term ’potentially toxic elements’ or ’PTEs’. PTEs have wide-ranging sources and impacts. Traffic fumes and vehicle corrosion, for example, release several different metals [1]. Zinc is released from wheels, tyre filler, brakes, and engine oil [25] [32]. Cadmium, copper, lead, and iron are also released by wheels and tyres, while brake wear releases copper, nickel, antimony, lead, and cadmium [25]. Catalytic converters are a source of precious metals such as rhodium, palladium, and platinum; fuel additives are a source of manganese; and engines may leach carcinogenic chromium [25] [33].

Aside from traffic, buildings and other infrastructure also contribute to PTE pollution. Concrete pavement can leach chromium as well as increase the pH of stormwater, which affects the mobility of other metals [25]. Roofs, including flashing, guttering, and other trims, are a source of both zinc and copper [25] [32]. Zinc may also arise from brick, concrete, and galvanised steel infrastructure such as lampposts [25] [32]. Mercury, which is highly toxic even at low concentrations, can arise from the wear of roads and paving, as well as from vehicles [34]. While the typical concentrations of lead in the urban environment have decreased since the banning of leaded fuel, lead still enters stormwater by leaching from lead pipes, lead paint, lead decorations, brick, and slate roofs [25] [32] [33]. Lead is toxic to fish, and impairs plant growth and children’s cognitive development [33].

Like hydrocarbons, metals can enter the food chain by accumulating in biofilms that are then consumed by fish and other aquatic creatures [22]. Many metals are toxic and can cause damage to the kidneys and nervous system [31]. The most common hazardous metals in stormwater are cadmium, chromium, copper, nickel, lead, and zinc [27], with cadmium being the most toxic despite often being low in concentration [35] [36].

Although it is a metalloid rather than a true metal, arsenic is often considered alongside metals when considering stormwater contamination. Arsenic can damage the skin and circulatory system and may be a carcinogen. It typically enters stormwater from treated wood, pesticides, and herbicides [37].

1.2.5 Nutrients

Nitrogen and phosphorus can have significant impacts on the aquatic ecosystem. Nitrogen may cause acidification, and both nitrogen and phosphorus can promote the formation of algal blooms – a process known as eutrophication [24]. Not only do these blooms reduce light and oxygen levels in the water [22], but they may also produce toxins that damage the liver, kidneys, and brain [38]. The impact of eutrophication costs the USA $2.2 billion dollars per year [39].

Nutrient pollution may arise from fertiliser use in green spaces, traffic fumes, leaching from soil, fallen leaves, atmospheric deposition, or animal urine and faeces [1] [24] [25] [40].

1.2.6 Other Contaminants

The recent emergence of some organic contaminants means that their toxicology and environmental impact are not well-established. They are usually present in low concentrations and include hormones, antibiotics, and pesticides [38]. Although these micropollutants are not high in concentration and many are not regulated by water quality guidelines, they can still have a significant negative impact on human health and the wider ecosystem [41].

Microplastics are being increasingly found all across the world. They may be dispersed some way from their original source, and can arise from wear of tyres, road surface and road paint, litter, atmospheric deposition, plastic packaging, geotextiles, and drainage pipes [25] [42]. Plasticisers such as phthalates and the endocrine disruptor Bisphenol A (BPA) may leach from building coatings and litter [38] [25].

Thermal pollution is caused by the urban heat island effect, which is a result of dense traffic and population, a lack of vegetation, and buildings and roads retaining heat. The temperature difference between an urban area and the surrounding countryside is 1–2° C on average, though it can be as high as 12° C [43]. The increased temperature causes discomfort for the urban population, increased energy use for cooling, greater concentrations of ground level ozone, and increased evaporation of volatile organic compounds from engines [43]. This leads to risk of heat stroke and respiratory disease for the urban population [44]. When heated stormwater is discharged to rivers and lakes, it can raise their temperature and reduce their suitability for native species by increasing disease risk and altering migration patterns [22].

Microorganisms including pathogens may enter stormwater from litter, sewage leaks, vegetation, and animal faeces [1] [25]. There have been few studies on emerging pathogens in stormwater or the risk of antimicrobial resistance (which often co-occurs with metal resistance) arising in contaminated stormwater and sediment [41].

1.2.7 Typical Runoff Concentrations

The concentration of pollutants in runoff has been found to be highest near the start of rainfall events. The so-called “first flush” occurs early in the event, as it does not take a significant amount of rainwater to mobilise pollutants. The concentration of pollutants in the runoff typically begins to fall after the first flush, though it may increase after some time when contaminants flow in from further away sites. Concentration patterns vary depending on the type of pollutant (i.e. attached to sediment, dissolved) [1]. The exact nature and extent of the first flush is debated, with definitions disagreeing on factors such as the proportion of the total rainfall volume that should be considered as well as the minimum percentage of total pollutant concentrations that should be present in that rainfall [45]. The existence of a first flush may be dependent on total pollutant concentrations or rainfall intensity [46] and does not necessarily occur equally for all present contaminants [47]. Larger catchment areas may obfuscate the first flush effect, as runoff from across the catchment takes different lengths of time to reach the outflow, resulting in the mixing of water arising from different points in the rainfall event [45]. The potential for a first flush may have to be taken into account when planning the treatment or re-use of road runoff, as greater treatment capacity or even the discarding of initial runoff [48] may be required.

The concentration of pollutants in stormwater is dependent on many factors. These include the antecedent dry period (a longer dry period leads to greater pollutant build-up on roads and pavements), rainfall duration & intensity (light or short rainfall may be insufficient to mobilise pollutants), and the surrounding land use & design (e.g. traffic levels, percentage of impermeable cover, presence of residential vs industrial sites) [36] [49] [50].

Analysis of highway runoff across southern England between 1997 and 2002 consistently detected copper (average concentration 40.35 μg/l), zinc (139.19 μg/l), cadmium (0.47 μg/l), lead (24.58 μg/l), nickel (5.81 μg/l), and chromium (6.55 μg/l) across the six sites tested. Average total suspended solids (TSS) concentrations ranged from 53-318 mg/l and average chemical oxygen demand (COD, an indication of the concentration of organic matter e.g. oil or plant material in a sample) from 70-138 mg/l. The concentrations of pollutants were broadly similar across the six sites [51]. Antunes et al. (2016) found higher concentrations of copper (120 μg/l) and chromium (80 μg/l) and a similar zinc concentration (130 μg/l) in road runoff from Florianópolis, Brazil [52]. Analysis of German road runoff over two years by Helmreich et al. (2010) found higher average concentrations of copper (191 μg/l), zinc (847 μg/l), lead (56 μg/l), and nickel (55 μg/l) [36]. These high mean concentrations – particularly those of zinc and copper – were attributed to the impact of increased corrosion by weather and salt/grit during winter months, a factor that must not be overlooked in countries that experience sub-zero temperatures during winter.

Roof runoff is generally less contaminated than road runoff but can still be polluted with hydrocarbons and metals from air pollution [1] [32]. Hong et al. (2018) compared the quality of roof runoff and car park runoff and found that TSS, COD, oil, dissolved organic carbon, and metal concentrations were all higher in car park runoff. Biochemical oxygen demand, total nitrogen, and total phosphorus differed less between the two sources [53]. Gnecco et al. (2005) also found significantly higher concentrations of TSS, COD, copper, and lead in Italian road runoff compared to roof runoff. However, the average concentration of zinc was approximately five times higher in roof runoff [54].

In order to provide adequate treatment to runoff so that it may be released to the environment or re-used safely, close attention must be paid not only to the high-level source (e.g. roof or road) but to details such as surrounding land use, local climate, and building materials.

1.3 Sustainable Urban Drainage Systems

1.3.1 Overview

Sustainable urban drainage systems (SUDS) offer an alternative to conventional drainage. SUDS and the principles behind them may also be referred to as low impact development (LID), sustainable drainage systems (SuDS), best management practices (BMPs), stormwater control measures (SCMs), and water sensitive urban design (WSUD) [55].

SUDS provide de-centralised stormwater attenuation and treatment, avoiding the costs incurred through transporting runoff to dedicated treatment plants while also minimising pollutant release to the environment. The UK Department for Environment, Food, and Rural Affairs (Defra) analysed five sites including housing developments, a school, and a rail freight terminal to compare the costs of SUDS versus traditional drainage. At all sites, they found that using SUDS was the cheaper option [56]. Although SUDS require regular maintenance such removing blockages and caring for vegetation [57], maintenance costs remain lower than those of traditional drainage. Other cost savings include fewer fees to be paid to water/sewerage companies and reductions in residential and commercial flood damages [11].

SUDS come in a wide variety of styles, making them highly versatile and suitable for incorporation in almost any environment [11]. SUDS such as permeable pavement and green roofs take up minimal to no extra land compared to their impermeable counterparts, so they are well-suited to retrofitting into established urban spaces [12]. Larger SUDS such as retention ponds and artificial wetlands are better suited to installation in newly constructed sites, where the SUDS form a key part of the area's design. It is also possible (and in many cases recommended) to combine several SUDS to form a SUDS treatment train [1] [12].

Common types of SUDS include:

- Rain gardens/bioretention areas (discussed further in Section 1.3.2) - landscaped SUDS that contain plants and soil media. They can be easily incorporated into existing urban environments and come in a wide range of sizes [2] [1].
- Permeable pavements – provide a hard, load-bearing surface while also allowing infiltration of water through pores or joints (Figure 1.2). They may have additional underground water storage for re-use or to provide extra time for treatment [58] [59] [1] [60].
- Green roofs – vegetation planted on the roof of a building to provide both cooling and water treatment. As there is minimal soil available for infiltration, evapotranspiration is the main quantity control [58] [60] [12].
- Retention ponds & wetlands – SUDS that contain a permanent pool of water (Figure 1.3). Infiltration occurs slowly (allowing time for pollutant removal by sedimentation) and the growth of aquatic plants enhances local biodiversity [60] [1].

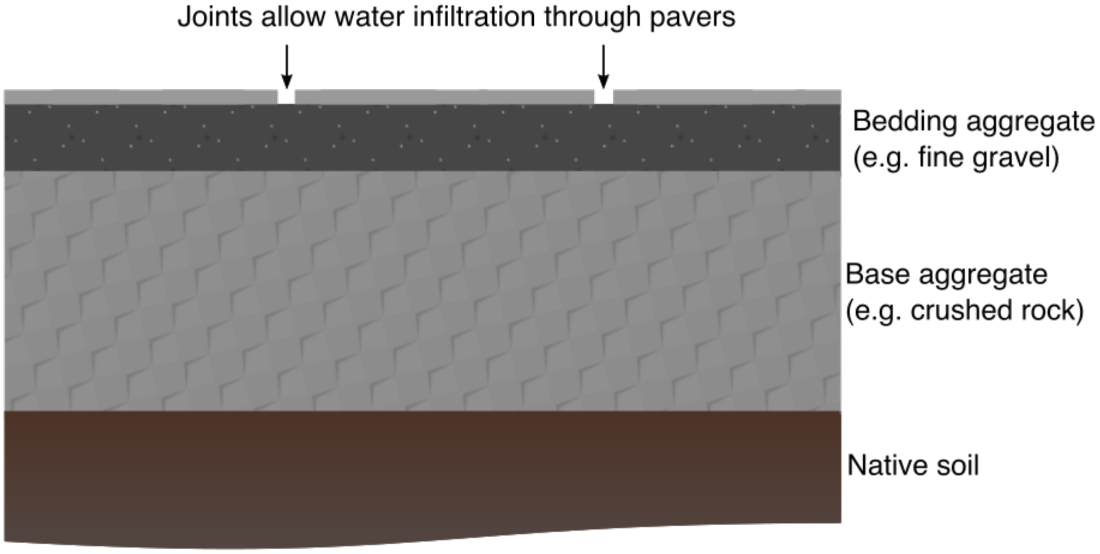

Figure 1.2: Schematic of a typical permeable pavement system.

Although SUDS vary significantly both visually and structurally, they are tied together by the "four pillars of SUDS design" - water quantity, water quality, amenity, and biodiversity [1].

Water Quantity

SUDS aim to reduce the impact of urbanisation by replicating natural hydrological processes to return the drainage of an area to as close to its pre-development state as possible [1]. They do so by providing opportunities for infiltration and evapotranspiration, as well as reducing the peak flow rate of discharge to local water bodies [60]. While conventional drainage systems are designed to remove runoff as quickly as possible, SUDS are designed to slow down stormwater flow via infiltration and detention, increasing the lag time of the water's discharge to the environment [12]. SUDS are generally more adaptable than traditional drainage, making them more resilient against climate change and increasing volumes of stormwater [5].

SUDS should be designed to reduce both volume and speed of stormwater, rather

Figure 1.3: Retention pond in Edinburgh, Scotland.

than focussing on one or the other. If the speed alone is controlled (e.g. by temporary detention), flood risk remains an issue as the volume of water being discharged to the environment remains greater than in pre-development conditions. This attenuation simply results in the peak flow of water being spread over a longer time, which can cause flooding further downstream [1]. If the speed of discharge is not lowered, the fast-flowing stormwater may cause erosion of stream banks [61]. Ideally, there should be no discharge to surface water bodies when rainfall is less than 5 mm [1].

Jefferies (2004) studied several SUDS in Scotland and found that all those tested reduced peak flow rate by at least 50%. Permeable pavement in particular was highly effective and increased discharge lag times by up to 3 hours compared to areas without SUDS [62]. Winston et al. (2020) saw a 27% reduction in runoff volume when studying a permeable pavement with an underground storage tank [63].

The volume discharged may also be reduced by harvesting the water and re-using it as “grey water” [1]. Grey water is water that has generally either already been used (e.g. in sinks and showers) or has been collected from rainwater harvesting systems or SUDS and has not been treated to drinking water standard. While not suitable for drinking, grey water can be used for flushing toilets, watering plants, or outdoor cleaning [17]. Modelling by Thives et al. (2018) suggested that up to 96.3% of the non-potable water demand in the city of Florianópolis, Brazil could be met by stormwater re-use [64]. Further modelling by Antunes et al. (2020) showed that potable water demand in a public building could be reduced by 69.6% if stormwater harvested from the building’s car park was re-used as a source of non-potable water [65]. In Cyprus, the use of grey water has reduced water consumption by 40% per person [18]. As the UK aims to reduce its individual water use from 150 litres per person per day to 130 litres by 2030 [17], the re-use of water from SUDS offers a useful opportunity.

Water Quality

SUDS are able to provide in situ treatment of stormwater, protecting local bodies of water from urban contaminants. Pollutant removal efficiency and mechanisms vary from system to system, depending on factors including oxygen availability, light availability, pollutant concentrations, and water retention time [66]. Using a treatment train containing multiple types of SUDS maximises the number of pollutants that can be targeted effectively [23].

Mechanisms of pollutant removal in SUDS include:

- Adsorption - the process through which dissolved pollutants such as metals become bound to soil or aggregate within SUDS through various chemical reactions [67]. The strength of binding is affected by the water's pH and salt content, therefore changes in water quality can result in the release of bound pollutants. Where pollutant concentrations are high, it is possible for the soil/aggregate to become saturated and unable to bind any more pollutants [1].

- Biodegradation - performed by the microbial community present within the SUDS. Microorganisms may degrade hydrocarbons, oxidise ammonium to nitrate, or convert nitrate to nitrogen. The efficiency of biodegradation depends on oxygen levels, nutrient availability, and temperature [1]. Storage tanks within SUDS may act as bioreactors to treat stormwater more effectively [60].

- Filtration - the trapping of sediment in the soil or aggregate, or on man-made filters such as geotextiles [6]. Filtration is very effective for particle removal, including smaller particles, and typically occurs in the upper layers of SUDS media [58].

- Photolysis - the breakdown of pollutants (particularly organic compounds) by UV light [1]. This is one benefit of SUDS being above ground or close to the surface, unlike traditional drainage systems.

- Phytoremediation – occurs in vegetated SUDS like rain gardens and green roofs where plants may take up pollutants including metals, phosphorus, and nitrogen [6]. Some plant processes convert pollutants to less toxic or bioavailable forms before releasing them, but others may result in the release of more bioavailable contaminants. There is also a risk of pollutant release when plants die or die back during colder months [60].

- Sedimentation - occurs when the flow rate of water through the system is low and generally works best for large particles [58]. Sediment particles settle out of the water, along with any pollutants that are bound to them. During heavy rain, there is a risk of sediment becoming re-suspended and pollutants being released to the environment, so sediment should be removed and disposed of safely as part of regular SUDS maintenance [1].

Winston et al. (2020) investigated pollutant removal in a stormwater treatment train consisting of permeable pavement over a concrete storage tank. They found that most pollutant removal occurred via filtration and sedimentation in the pavement, but some sedimentation occurred in the storage tank. The tank also provided anaerobic conditions in which denitrification could occur. Total phosphorus concentration was reduced by 78% while orthophosphate concentration increased by 76% from 0.005 mg/l to 0.01 mg/l, although this is still considered a low concentration. Aluminium, iron, and manganese saw at least a 70% reduction, but copper, lead, and zinc seemed to be retained for a while before leaching out of the system. There were also indications that calcium and magnesium were leaching from the pavement aggregate and concrete tank. TSS was reduced by 99.5% and total nitrogen concentration was reduced by 59% [63].

Pilon and Horner (2019) studied a similar system – pervious concrete over a limestone reservoir. They found that TSS fell by 97.3%, nitrite concentration fell by 98.8%, and chemical oxygen demand fell by 36.2%. Nitrate concentration did not change. The concentration of sulphate increased, potentially due to the oxidation of other forms of sulphur or from the degradation of hydrocarbons [61].

Chapman et al. (2010) studied a bioretention system in Washington, USA, and found the system effectively reduced concentrations of TSS (by 87-93%), total petroleum hydrocarbons (TPH, 92-96%), and dissolved copper and zinc (58-86%) [68]. Analysis of a Californian bioretention system by David et al. (2015) also found high mean removal of copper (83%) and zinc (93%) as well as cadmium (84%) and PAHs (90%). Although concentrations of nickel and lead were lower in the effluent than the influent, they were reduced to a lower extent than the other metals with percentage removal values of 20% and 51% respectively [69].

Nayeb Yazdi et al. (2021) investigated nutrient removal in a retention pond in Virginia, USA and found effective TSS removal year-round, ranging from 62% to 75%. Removal of phosphorus was low regardless of season (8%). Nitrogen removal varied seasonally, with a reduction of 47% in warm weather and an increase of 6% in cold weather, likely due to temperature-dependent biological activity [70]. Although the extent of water treatment varies, it is well-established that SUDS are able to remove a wide range of pollutants from stormwater. The installation of SUDS in urban areas can therefore be used to limit the contamination of local surface water bodies. Effective treatment by SUDS also enhances the potential of re-using stormwater as grey water in nearby buildings.

Amenity & Biodiversity

SUDS, especially those including vegetation or permanent water, have numerous amenity and biodiversity benefits. Not only does vegetation provide a habitat in and of itself, but it can also act as a link between larger green spaces to allow local wildlife to travel across areas that have been split by urbanisation [1]. SUDS are able to lower the effect of urban heat islands. Plants and trees can cool an area via evapotranspiration and by providing shade. Increasing the vegetative cover in an area from 15% to 50% can lower the local temperature by up to 7° C. Hard SUDS like permeable pavement systems can also cool an area via evaporation of surface or stored water, and large retention or detention ponds can act as a heat sink [12]. Lowering the temperature not only reduces discomfort, it also reduces the formation of ozone and decreases the amount of energy used for air conditioning [43]. Additionally, by allowing infiltration to soil rather than discharging to the environment or by storing water underground to cool down prior to discharge, the impact of thermal pollution on surface water can be minimised [40]. SUDS also have wider ranging effects on the neighbourhood and its residents. They may be used for recreation and education, and as a result can improve both the mental and physical health of local people. The presence of attractive SUDS in an area can also increase house prices [62] [1].

1.3.2 Rain Gardens

Rain gardens, also known as bioretention areas, are landscaped depressions containing soil, aggregates, and vegetation [2] [6] (Figure 1.4). They are generally not particularly large (about 2-4% of the area of their catchment), vary highly in shape and style, and are designed to cope with frequent, low intensity rain showers rather than prolonged heavy rainfall [1].

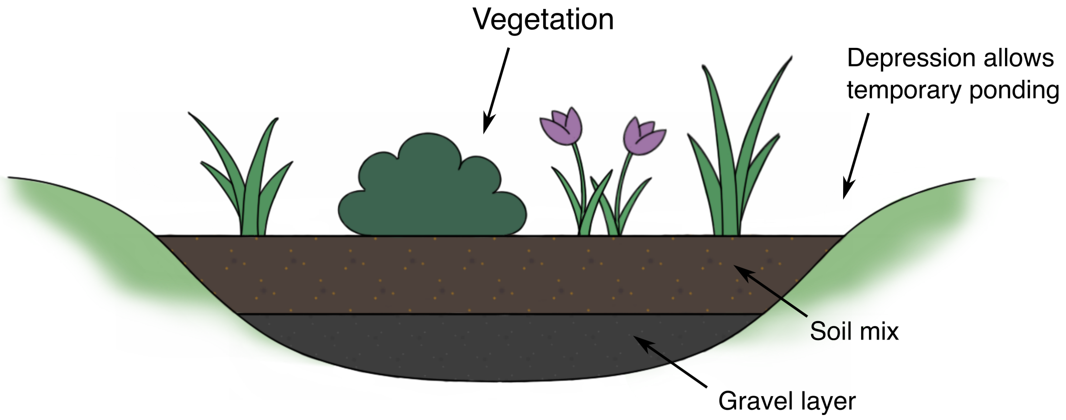

Figure 1.4: Schematic of a typical rain garden.

Rain gardens contain a number of components, some required and others optional. The inlet should be designed to spread input water evenly across the garden's surface. Some ponding is expected prior to infiltration, so the edges of the garden should be raised slightly. Any pooled water should drain within 48 hours of rainfall and be no more than 15cm deep [6]. Vegetation is a vital feature of rain gardens, contributing to water treatment, noise reduction, urban cooling, and aesthetic value [2] [1]. The species of plants chosen should be well-suited to water availability fluctuations, high concentrations of pollutants including road salt, and should release as low a level of biogenic volatile organic compounds as possible [1] [43]. Vegetation requires some maintenance in the form of pruning and weeding, but it also provides a habitat for wildlife [2] [6].

The filter medium is usually 70 cm to 100 cm deep and consists of a soil mix (which may contain different amounts of sand, gravel, and clay depending on local availability), and sometimes added nutrients in the form of organic matter/surface mulch [71]

[1]. Water treatment occurs primarily in the soil layer, so additional amendments can be added to improve pollutant removal or alter permeability [72]. A gravel drainage layer beneath the filter medium may be designed with perforated pipes to transport water away when infiltration is not desired, or it may provide temporary storage. Stored water can be used to supply water to the vegetation during droughts and can also encourage pollutant removal by providing an anaerobic environment for the microbial community [1] [72]. In some cases, a geotextile may be added beneath the soil media to minimise the loss of fines into the sub-base and effluent as well as to provide a surface for biofilm growth, enhancing biodegradation [71] [73].

Generally rain gardens are highly effective at removing metals and suspended solids, and slightly less adept at nutrient removal [71] [67] [6].

1.3.3 Impact of Design on Water Treatment

In many cases, SUDS are installed with a view to prevent flooding – i.e. focussing on the reduction of stormwater quantity rather than improving water quality [12] [53] [62]. Often the design of SUDS doesn't take into account surrounding land use, climate, or soil type, and tends to be standardised rather than optimised for specific hydrology and treatment goals [40]. Because of significant variation in surroundings and design, performance results from one system may not be applicable to others [58]. To overcome this, a deeper understanding of the various factors that affect SUDS function is required.

Jefferies and Napier (2008) performed lab-scale experiments to examine total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) removal in lysimeters and microcosms under various conditions. They found that only 30% of TPH was degraded in lysimeters containing sandy soil, compared to 70-80% degradation in other soils. Removal of TPH and PAHs was increased at 15° C compared to 5° C, due to increased evaporation and biodegradation. Water treatment in SUDS may be less effective during colder months, and this should be taken into account when designing the SUDS and deciding where and how water is discharged. Sterilisation of the soil prevented biodegradation, lowering the removal of low molecular weight PAHs from

60-80% within 3 hours to only 20%. This indicates that the microbial community of a system plays a key role in the removal of oil pollution. When moisture content of the soil was increased to 90%, removal of high molecular weight PAHs ceased [74]. When using SUDS to treat oil-contaminated water, it would therefore be more effective to use systems that aren't permanently wet i.e. using rain gardens and permeable paving rather than ponds and wetlands.

Davis et al. (2003) studied two bioretention systems in Maryland, USA. One system removed at least 95% of influent copper, lead, and zinc but the other showed much lower removal, including increases in zinc concentration in some effluent samples. This difference was attributed to factors including the soil media composition (higher soil content vs. sand resulting in better removal) and the age of the systems (the older system potentially having deeper and more widespread plant root systems for metal capture and uptake) [75].

Metals adsorb differently onto different media. Norris et al. (2013) studied water treatment by filter drains and found that microgabbro gravel was able to remove 10-70% more copper and zinc than dolomite and quartz gravels [76]. Additionally, the presence of a biofilm on the gravel improved metal removal by up to 29% [77]. The type of aggregate used in SUDS should be considered carefully to maximise pollutant removal. The use of concrete or limestone for storage tanks may increase stormwater pH [61] [63], and therefore also impact the mobility of metals.

Charbonnet et al. (2020) found that manganese oxide-coated sand was able to bind well to lead, copper, zinc, and cadmium, as well as being able to oxidise some organic contaminants. This coated sand is relatively inexpensive and, when saturated, can be regenerated with a mild acid wash [78]. Minamisawa et al. (2004) determined that lead and cadmium could be effectively adsorbed by biomaterials including tea, chitosan, aloe, and coffee beans. Chitosan and aloe were able to adsorb metals at a lower pH than tea and coffee were [79] so combinations of these materials could be used to provide better removal coverage. These inexpensive materials offer an alternative to more expensive aggregates such as activated carbon and zeolite – keeping the cost of the system low while also providing a use for waste products such as chitosan.

Soils containing higher percentages of clay are able to remove pollutants via cation exchange. Sandy and gravelly soil allow faster infiltration of stormwater, but this may be at the expense of water treatment [61]. The lower attenuation in sandy soil reduces the time available for sedimentation, biodegradation, sequestration, and cooling to occur. The high flow rate may also result in adsorbed pollutants being washed off and discharged [40]. A low hydraulic conductivity and therefore longer retention time is particularly beneficial for the removal of nutrients, salts, and pathogens [80]. The use of finer and deeper filter media as well as the presence of vegetation will increase retention time and can therefore improve water treatment.

1.3.4 Microbial Community

Bacteria exist as complex communities consisting of various bacterial species. All bacteria in a community interact with each other either directly through physical contact or indirectly through the release of metabolites that are used by other species [81]. In the natural environment, local mineralogy can impact microbial community structure. Hutchens et al. (2010) analysed microbial communities in three types of mineral environment – muscovite, feldspar, and quartz – and found significant differences in community composition [82]. Carson et al. (2009) studied the effect of minerals on soil microbial communities. Soil consists of a number of microhabitats that differ in mineral content, nutrient/water availability, and pore/particle size. They tested the impact of adding mica, basalt, or rock phosphate grains to a nutrient-limited quartzite soil, with planting of ryegrass, clover, or no planting, as plant roots are known to improve mineral dissolution and promote microbial activity in the rhizosphere. They found that both soil mineralogy and, to a lesser extent, plant type impacted community composition, as did the distance of the community from the minerals [83].

The diversity and composition of microbial communities can affect their function, which is particularly important in man-made systems that rely on microbial activity. For example, Haig et al. (2015) examined the microbial community in slow sand filters, which are used for water purification. They found that more even communities (i.e. those not dominated by any particular species) appeared to treat water more effectively, possibly due to being more robust against variation in input quality. Additionally, community evenness increased with the age of the slow sand filters, suggesting that systems like these may become more stable and more effective at pollutant removal with time [84]. Johnson et al. (2015) found that more diverse microbial communities in wastewater treatment plants were able to degrade micropollutants more quickly. Greater evenness and higher species richness (total number of species present) both contributed to increased rates of biodegradation [85]. When designing water treatment systems, including SUDS, the impact of design choices such as soil media must be considered to ensure that the community is able to function optimally.

Despite the importance of microbial activity in water treatment and the known associations between environment & community composition, and community composition & function, surprisingly little is known about the microbial communities in SUDS.

Hong et al. (2018) compared two rain gardens in Cheonan, South Korea – one of which took in roof runoff and one of which took in car park runoff [53]. The rain gardens also differed in plant species, filter media, surface area, and structural components. Microbial analysis was carried out on the rain gardens' soil, and it was found that the communities differed from each other as well as from control soil sampled from outwith the rain gardens. For example, the car park rain garden contained *Deferribacteres*, a known metal remover. The roof rain garden also contained some algae, due to greater light availability compared to the car park. By comparing the microbial data with chemical analysis performed on the input stormwater, Hong et al. concluded that pH, total nitrogen concentration, and organic matter content impact microbial community structure [53]. By also analysing output water quality in a similar experiment, the impact of microbial community composition on water treatment capability could be determined.

Liguori et al. (2021) analysed influent, effluent, and media from a pair of stormwater quality improvement devices (SQIDS) that contained different media but treated the same influent [86]. They found that the filter media communities were distinct from one another despite receiving the same water, suggesting that the design of the system impacts community composition and therefore potentially affects function. They also found differences between the influent and effluent communities [86]. Aquatic microbial communities typically bear little resemblance to urban communities, but the discharge of contaminated stormwater to nearby surface water bodies can result in changes to the natural community [87]. The exact impact of this influx of stormwater microbes is not yet fully understood. However, given that urban runoff is known to contain pathogens and antibiotic-resistant bacteria [87] [86], the potential for SUDS design to alter the effluent community should be considered more carefully as a route to limiting the spread of these microbes to the environment.

1.4 Thesis Outline

Sustainable urban drainage systems (SUDS) present a viable solution to increased flood risk and water shortages resulting from climate change as well as to the environmental damage caused by urban runoff. In order to ensure that SUDS are providing adequate water treatment and to both minimise environmental contamination and maximise the potential of water re-use, it is vital to understand the types of pollutants that are entering the SUDS and how SUDS design affects the removal of these pollutants. The SUDS microbial community plays a key role in pollutant removal, contributing to process such as adsorption and biodegradation. However, little is known about the composition of SUDS microbial communities, how their composition is affected by SUDS design, or how variation in community structure affects water treatment.

Chapters 2 and 3 of this thesis examine the chemical variation in stormwater within the cities of Glasgow, Scotland and Florianópolis, Brazil. This research aims to establish whether typical pollutants vary across urban areas to the extent that SUDS design should be targeted towards specific local contaminants.

Chapter 4 examines the chemical and microbial variation between stormwater from Glasgow and Florianópolis. The aim of this comparison is to identify how factors such as climate and population density impact pollutant concentrations and local microbial communities.

Chapter 5 and 6 examine the chemical and microbial variation in influent, effluent, and soil from four rain gardens in Glasgow, Scotland. These chapters aim to establish how soil composition impacts pollutant removal and microbial community structure within rain gardens, in order to inform future SUDS design and optimise water treatment.

Chapter 7 presents the overall conclusions of the work and puts forward recommendations for future research.

Variation in Stormwater Quality Across Glasgow, Scotland

Abstract

The city of Glasgow, Scotland, experiences regular rainfall leading to runoff from urban surfaces. This runoff may contain contaminants such as potentially toxic elements (PTEs), hydrocarbons, and nutrients. This study aimed to determine the chemical composition of runoff in Glasgow city centre, in order to understand how stormwater quality varies across an urban area. This is an important consideration as this variation could impact the efficacy of pollution removal by sustainable urban drainage systems (SUDS). Stormwater was sampled from five sites including high traffic, residential, and pedestrianised areas across Glasgow during March 2019. Samples were analysed for contaminants including PTEs, total petroleum hydrocarbons, anions, and suspended solids.

The results of the study show that pollutant concentration varies significantly within Glasgow stormwater. Concentrations of many PTES, including chromium, copper, and zinc, were greatest at the high traffic Hope Street site. Total suspended solids and chemical oxygen demand were highest at both Hope Street and the adjacent Sauchiehall Street. Stormwater from areas experiencing significant traffic volumes may therefore require more treatment than water from quieter areas. However, the highest concentrations of other PTEs including nickel and arsenic were observed in the pedestrianised

green space Kelvingrove Park and residential Montague Street, suggesting that traffic level alone is not a suitable predictor for stormwater quality. Therefore, widespread stormwater testing is recommended prior to the installation of SUDS. This will ensure that SUDS are designed and placed appropriately to deal with local contamination.

2.1 Introduction

Scotland is generally thought of as having high levels of precipitation and a plentiful supply of water, with annual average rainfall in the western highlands exceeding 3000 mm [88]. However, Scotland's rainfall shows significant spatial variation and the southern and eastern parts of the country experience as little as 500-800 mm of rain annually [88]. In recent years, periods of water scarcity have become more common, occurring in 2018 [89], 2020 [90], 2021 [91], and 2022 [92]. Climate change is predicted to cause a further reduction in summer rainfall, increasing risks of drought and water shortages. Conversely, winter rainfall volume and overall rainfall intensity are expected to rise over the next several decades, bringing greater chance of flooding. Between 1961 and 2004, winter rainfall across Scotland has already increased by 21.1% [88]. As of 2018, 284,000 residential properties in Scotland were at risk of flooding, with the city of Glasgow and the surrounding area containing one of the highest concentrations of communities affected by flood risk in the UK [13].

Glasgow is Scotland's largest city, with a population of over 635,000 as of June 2020, expected to rise to 644,274 by the year 2028 [93]. The city centre alone has a population of 28,000 and sees 75,000 vehicles every weekday [94]. The Glasgow City Council area currently covers 176.44 km^2, including 33.81 km^2 of parks & open spaces and 1859 km of road [95]. Although there are many green spaces in the city, the majority of Glasgow is covered by impermeable roads and buildings [10]. Increasing population is likely to lead to further urbanisation, resulting in less area for rainwater infiltration and therefore greater risk of flooding.

One method of combatting the issues arising from climate change and urbanisation is through the installation of sustainable urban drainage systems (SUDS), which lower flood risk and provide an opportunity for water harvesting and re-use (as discussed in more detail in Chapter 1). The combination of a wet climate (average annual rainfall 1370.2 mm [96]) and large urbanised areas make Glasgow an excellent candidate for the installation of SUDS such as permeable pavement and roadside rain gardens. Additionally, the use of SUDS could significantly reduce the volume of stormwater entering the local sewage system, enabling the potential regeneration of derelict sites that current wastewater infrastructure would otherwise be unable to support.

Modelling by Antunes et al. (2020) suggested that the installation of permeable pavement for rainwater harvesting in Glasgow could lower the environmental impact of water consumption by 98.6%, as well as lower the impact of PM2.5 and CO_2 emissions by 33.3% and 20.6% respectively [65]. Research by Meldrum & Smyth (2017) suggested that up to 100% of rainwater in Glasgow's George Square could be captured and harvested if SUDS including permeable pavement, rain gardens and a detention pond were installed in the area [21]. Work to improve Glasgow's relationship with water is already underway, including the Glasgow Avenues programme (discussed further in Chapter 5) and projects carried out by the Metropolitan Glasgow Strategic Drainage Partnership [97].

The impact of land use on stormwater quality has been studied in many urban areas including Singapore [98], Virginia Beach, USA [70], and Adelaide, Australia [59]. Overall land use and the proportion and distribution of impermeable areas are known to impact pollutant concentrations [99]. As the installation of SUDS and other drainage management strategies become necessary and more common, knowledge of stormwater composition is vital to ensure that these systems can be designed to treat water effectively [50]. However, despite the growing interest in SUDS in the city, little is currently known about Glasgow's urban stormwater composition.

By examining stormwater samples taken from across the city centre of Glasgow, this study aims to identify variation in quality caused by location. Such variation could affect the pollutant removal performance of SUDS and the suitability of stormwater for non-potable re-use, therefore impacting future SUDS design choices and installation areas.

2.2 Materials & Methods

2.2.1 Sampling

Stormwater samples were collected from five sites across Glasgow during the morning of the 12th of March 2019. The five sampling sites are shown in Figure 2.1 and their locations are shown in Figure 2.2. The sites are described below.

George Square (Site GS) is a pedestrianised, paved area in Glasgow city centre, surrounded on all four sides by roads. Road traffic surrounding the square is relatively heavy and includes cars, buses, and taxis. The square itself is widely used by pedestrians and for events, but no vehicular traffic crosses the square apart from for occasional maintenance. The animal population consists mainly of pigeons and there are several trees and grassy areas in the square. The stormwater sample was taken from the east side of the square, several feet away from the road. Latitude: 55.86092; Longitude: -4.24918.

Montague Street (Site MS) is a residential street in the west end of Glasgow. The road is a dead-end that is perpendicular to the busy Great Western Road but without vehicle access to or from it. There is a raised tree bed at the side of the road, as well as small gardens in front of the surrounding residences. The stormwater sample was taken from between a parked van and the raised tree bed. Latitude: 55.87338; Longitude: -4.27748.

Sauchiehall Street (Site SH) is one of the main shopping streets in Glasgow city centre. Part of the street is open to traffic and part is pedestrianised, although it is intersected in several places by roads that are used heavily by buses and taxis. Tree pits have recently been installed to provide drainage in some sections of the street, but not in the area that was sampled from. Water samples were collected from a pedestrianised area bordered by Renfield Street and Hope Street. The majority of the stormwater sample was taken from two puddles outside a pub, with the remainder being sampled from various smaller puddles in the same section of the street. Latitude: 55.86457; Longitude: -4.2557.

Kelvingrove Park (Site KG) is a large green space in the west end of Glasgow. The park is popular with joggers, cyclists, and dog walkers. The stormwater sample was taken from the northernmost part of the park, from a paved path bordered on both sides by grass. Latitude: 55.87277; Longitude: -4.28074.

Hope Street (Site HS) is a busy road next to Glasgow Central railway station. The street is consistently ranked as one of the most polluted in the country [100], due to being a main route for buses and taxis. There is a taxi rank in the street as well as several pubs and restaurants. The stormwater sample was taken from the rear of the taxi rank. Latitude: 55.85939; Longitude: -4.25903.

These five sites were chosen for sampling as they represent a variety of typical urban areas within Glasgow including pedestrianised areas, green space, residential areas, and high traffic roads.

Figure 2.1: Photographs showing the five sampling sites. Clockwise from top left – George Square (GS), Montague Street (MS), Hope Street (HS), Kelvingrove Park (KG), Sauchiehall Street (SH). Site MS, SH, and KG photographs were taken at the time of sampling. Site GS and HS photographs were taken at a later date.

Figure 2.2: Map showing the locations of the five sampling sites within Glasgow. Source: Google Maps.

Following heavy rain overnight, deep puddles suitable for water collection were present at four of the five sites. At site SH, no single puddle contained sufficient water, so samples were collected from several puddles both before and after a short rain shower. Approximately 2 l of water was collected from each site – 1x 1 l glass bottle, 1x 1 l plastic (high density polyethylene [HDPE]) bottle and 2x 50 ml centrifuge tubes (polypropylene [PP]). The pH of samples destined for organic (glass bottle) and elemental (PP tubes) analyses was lowered to pH 2 with HCl and HNO_3 respectively. Samples were stored at 4° C.

2.2.2 Chemical Analysis

Electrical conductivity (EC) and pH were measured in unfiltered samples at room temperature using a Mettler Toledo MPC277 pH/EC meter (Ohio, USA), immediately upon return to the laboratory after sample collection.

Total suspended solids were measured gravimetrically, according to the method described in Standard Methods for the Examination of Water and Wastewater [101]. Glass fibre filter paper (1.2 μm, Whatman, Maidstone, UK) was rinsed with deionised water and dried at 105° C overnight, then allowed to cool and weighed. Samples were filtered in duplicate using the weighed filter paper, which was then dried overnight at 105° C before re-weighing. TSS concentration was calculated using the equation $((Final\_weight(g) - Initial\_weight(g)) * 1,000,000)/Sample\_volume(ml) = TSS(mg/l)$.

Depending on the rate at which filter papers became clogged, different sample volumes were filtered (Table 2.1).

Sample	**Volume (ml)**
MS	100
GS	100
SH	11
KG	20
HS	10

Table 2.1: Sample volumes filtered for total suspended solids analysis.

Elemental analysis was performed using inductively coupled plasma – optical emission spectroscopy (ICP-OES). Duplicate samples were prepared and analysed for both dissolved and total elements measurement. For dissolved elements measurement, samples were filtered using 0.45 μm mixed cellulose ester (MCE) filters (Chromatography Direct, Runcorn, UK) and lowered to pH 2 with HNO_3 for preservation. Samples were stored at 4° C until analysis. Filtered ultrapure water (18.2 MΩ-cm) was prepared as a blank. Samples for total elements analysis were prepared by acid digestion of unfiltered, HNO_3-preserved samples. Acid digestion was carried out using reverse aqua regia (3:1 HNO_3:HCl) in a MARS 5 microwave digestion system (CEM, North Carolina, USA). Samples were allowed to "pre-digest" with the acid mix in the microwave vessels at room temperature for 1 hour before microwave digestion. Details of the digestion mixtures and MARS 5 settings are shown in Tables 2.2 and 2.3. Digested samples were each made up to 50 ml in ultrapure water before a 10 ml subsample was filtered through a 0.45 μm MCE syringe filter (Chromatography Direct, Runcorn, UK) for ICP-OES analysis. Digested samples were stored at 4° C until analysis.

ICP-OES was carried out using an iCAP 6000 Series ICP Emission Spectrometer (Thermo Fisher Scientific, Massachusetts, USA).

	Total Elements
Sample	6 ml unfiltered water sample 3 ml HNO_3 1 ml HCl
Blank	3 ml HNO_3 1 ml HCl

Table 2.2: Contents of sample and blank vessels used for acid digestion.

Step	**Pressure (psi)**	**Power (%)**	**Ramp (min)**	**Temperature (°C)**	**Hold (min)**
1	800	100	20	160	
2	800	100		160	20
3	800	100	20	180	
4	800	100		180	20

Table 2.3: Programme settings used for acid digestion in a CEM MARS 5 microwave.

Chemical oxygen demand (COD) was measured using COD cuvette tests (Hach, Iowa, USA), according to the manufacturer's instructions. Briefly, 2 ml of sample was added to each COD cuvette, mixed by inversion, and heated to 150° C for 2 hours. Cuvettes were allowed to cool before COD was measured colorimetrically using a Hach DR2010 spectrophotometer. COD was measured in unfiltered HCl-preserved samples using a 0 – 1000 mg/l test kit, and in filtered samples (0.45 μm MCE syringe filters, Chromatography Direct, Runcorn, UK) using a 0 – 150 mg/l test kit. Unfiltered deionised water was added to one cuvette as a blank. Filtered deionised water was tested as a control to check for contamination from the filters.

Total petroleum hydrocarbon (TPH) concentrations were measured by gas chromatography with flame ionisation detector (GC-FID). Samples were prepared via liquid-liquid extraction (LLE) into dichloromethane (DCM). Prior to LLE, 200 ml samples (including a deionised water blank) had been acidified to <pH2 with HCl and filtered through 1.2 μm glass fibre filter paper (Whatman, Maidstone, UK). Additionally, 10 g of NaCl and 1 ml 50 ppm 1-chloro-octadecane in methanol were added to each sample to aid extraction and act as a surrogate standard respectively. Samples were extracted

into approximately 30 ml of DCM (3x 10 ml extractions) and the extracts were then filtered through 1.2 μm glass fibre filter paper (Whatman, Maidstone, UK). Extracts were concentrated down to 1 ml using a Syncore evaporator (BUCHI, Flawil, Switzerland) followed by nitrogen blowdown evaporation. Tricosane (20μl, 20 ppm) was added to each 1 ml sample as an internal standard. GC-FID was carried out using a Finnigan Focus GC (Thermo Scientific, Massachusetts, USA). GC-FID settings are shown in Table 2.4.

Inlet temperature (°C)	250
Detector temperature (°C)	340
Split flow (ml/min)	25
Split ratio	10
Carrier (ml/min)	1
Initial hold temperature (°C)	40
Initial hold (min)	4
Ramp rate (°C/min)	10
Ramp to temperature (°C)	323
Hold (min)	15

Table 2.4: GC-FID settings used for TPH analysis of dichloromethane extracts.

Anion concentrations were determined using ion chromatography (Metrohm 850 Professional IC, Herisau, Switzerland), while ammonium concentrations were determined colorimetrically using a Konelab analyser (Thermo Scientific, Massachusetts, USA). Ammonium concentrations were measured by Carla Lopez, University of Strathclyde. Samples were filtered through 0.45 μm MCE syringe filters (Chromatography Direct, Runcorn, UK) prior to analysis.

2.2.3 Bioavailability

To better assess the impact of toxic metals on the ecosystem, the bioavailability of copper, manganese, nickel, and zinc was determined using the Metal Bioavailability Assessment Tool (M-BAT) [102]. The tool calculates bioavailability based on measured metal concentration, pH, dissolved organic carbon (DOC), and calcium concentration.

When DOC values are unavailable, the Scottish Environment Protection Agency (SEPA) assumes that the background DOC of the receiving water combined with the

DOC of the discharge results in a concentration of 2 mg/l in estuaries [103], such as the local segment of the River Clyde. Therefore, as DOC values were not determined for these stormwater samples, a value of 2 mg/l was used in the M-BAT calculator.

The measured concentration of calcium for site MS (2.67 mg/l) was below the minimum value required for bioavailable copper and zinc determination, and the measured calcium at site GS (3.06 mg/l) was below the requirement for copper determination. In these cases, the minimum value was therefore used in place of the measured concentrations – 3.1 mg/l for copper determination and 3 mg/l for zinc determination. The difference between the measured values and minimum required values was small and testing of the tool showed that such changes have negligible effects on the results given by the M-BAT calculator.

The measured nickel concentration was below the detection limit at sites MS, GS, and SH. The detection limit value of 1 μg/l was therefore used as the nickel concentration for these bioavailability calculations.

2.3 Results & Discussion

2.3.1 Water Appearance

Water samples from Montague Street (MS) and George Square (GS) were clear and contained very few visible solids, while samples from Kelvingrove Park (KG), Sauchiehall Street (SH), and Hope Street (HS) were significantly more turbid, with high levels of brown solids (Figure 2.3).

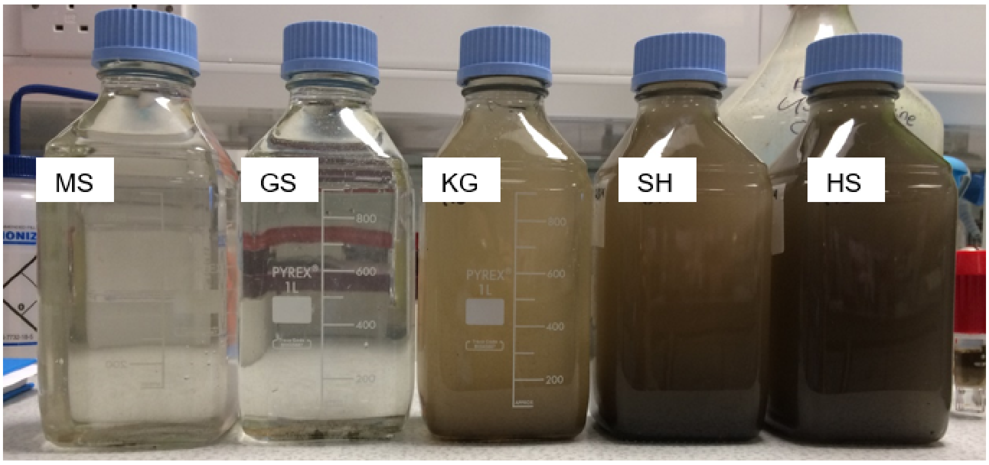

Figure 2.3: Glass sample bottles containing stormwater from the five sampling sites. Samples were shaken to prevent solids settling prior to the photograph being taken.

2.3.2 Solids

The lowest concentrations of total suspended solids were seen in samples MS and GS. The greatest concentration of suspended solids was found at site SH, and at 2,827 mg/l this was 2.8 times higher than site HS's 1,015 mg/l and 217 times higher than site MS's 13 mg/l. This large variation in suspended solids may be due to differing traffic levels at the sampling sites, as sites HS and SH see the greatest volumes of both vehicle and pedestrian traffic while site MS experiences only residential traffic. Common sources of suspended solids include vehicle exhaust emissions [25] [104], pedestrian debris [105], and dust kicked up by traffic [106]. Sand and grit from winter road treatment may be constituents of TSS as well as also increasing concentrations of other solids by causing vehicle abrasion [25]. Table 2.5 shows the average TSS of all samples.

Site	Total Suspended Solids (mg/l)	Standard Deviation
MS	13	0.011
GS	26	0.005
SH	2,827	0.617
KG	343	0.053
HS	1,015	0.092

Table 2.5: Mean total suspended solids measurements and standard deviations for each of the five sites.

2.3.3 pH & Electrical Conductivity

The pH of the samples ranged from 7.12 (KG) to 7.98 (HS) (Table 2.6). The pH of rainwater is typically around 5.6 [107]. Contact with concrete surfaces such as pavements increases the pH of runoff water [25], leading to higher pH in stormwater than rainwater, as seen in the Glasgow samples.

Stormwater from site KG had the lowest pH of the five sites. This is not unexpected, as the sampling site consisted of a paved path bordered by large grassy areas, so there is less impact from concrete compared to the other sites that were almost entirely paved.

Although the pH of the tested stormwater was greater than that of rainwater, it remained circumneutral. This fact, combined with the significant dilution of the water when it enters the River Clyde, indicates that the discharge of this stormwater is unlikely to have a significant effect on the river's pH.

Electrical conductivity (EC) of the stormwater ranged from 38.6 μS/cm to 636 μS/cm (Table 2.6).

Site	**EC (μS/cm)**	**pH**
MS	47.4	7.34
GS	223.0	7.29
SH	251.0	7.75
KG	38.6	7.12
HS	636.0	7.98

Table 2.6: Electrical conductivity (EC) and pH measurements for each of the five samples.

The EC of stormwater is affected by the concentration of dissolved solids (such as salts) in the sample. Within the Glasgow samples, there is a strong association between conductivity and chloride concentration, with a Pearson's correlation coefficient of 0.96 (Figure 2.4).

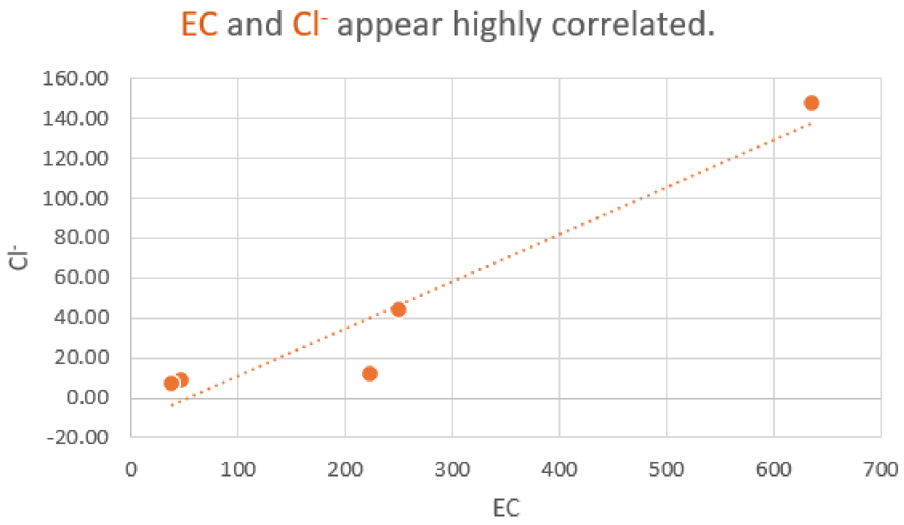

Figure 2.4: Correlation between electrical conductivity (EC) and chloride concentration (Cl^-) in the five Glasgow samples.

The highest conductivity of the five samples was seen at site HS (636 μS, 2.5x higher than the next highest EC), as was the highest concentration of chloride (147.3 mg/l, 3.3x higher than the next highest concentration).

Chloride pollution in stormwater is often a result of road salting. In Glasgow, roads and footways may be salted between the start of November and the end of March whenever the road surface temperature is predicted to fall below 1° C [108]. On Monday the 11th of March 2019 (one day prior to stormwater sampling), early morning temperatures fell to a low of 1° C [109]. Therefore, the high EC and chloride concentration seen at site HS may have been due to recent spreading of salt, as this is a main bus route and thus a priority for salting [108]. The next highest conductivities were found in samples from sites SH and GS, as were the next highest chloride concentrations. Both these areas are also prioritised for salting due to being busy, city centre footways. The lowest conductivities and chloride concentrations were seen at sites KG and MS, neither of which is a priority for council road salting [108].

2.3.4 Organics

The COD of unfiltered samples ranged from 32 mg/l at site MS to 1,169 mg/l at site SH. Common sources of COD are generally traffic-related – tyre wear, bituminous surfaces, and oil [25] [22], hence the greater COD at the high traffic sites HS and SH.

The COD in samples filtered through 0.45 μm MCE syringe filters was significantly lower – ranging from 12 mg/l at site GS to 150 mg/l at site SH. Filtering the samples resulted in a significant reduction (84-94%) in COD in four of the five samples. Conversely, the filtered MS sample had a COD 5 mg/l higher than when unfiltered. Filtered deionised water had a COD 7 mg/l higher than the unfiltered blank, suggesting that the increased MS result may be due to contamination from the filter. It may also be the case that the COD present at site MS is entirely dissolved, and the observed increase is within the bounds of expected error, which could be determined through future testing of extra replicates. Full COD results are shown in Table 2.7 alongside the percentage change in COD after filtering.

GC-FID chromatograms from TPH analysis showed no difference between the samples and the blank (deionised water extracted into DCM). Peaks representing 1-chloro-octadecane and tricosane spikes were identified in all samples, indicating that the lack of detected TPH was not due to issues in LLE or GC-FID performance.

The lower COD and lack of TPH present in filtered samples suggests that the majority of organic contaminants are in particulate form or are particle-bound. Previous studies have also found organic pollutants largely present in particle-bound form [110]. In particular, a high proportion of polycyclic aromatic hydrocarbons (PAHs) bind to particulates [28]. Many forms of SUDS including rain gardens and permeable pavement provide water treatment partially via filtering and sedimentation, so they are well suited to removing particle-bound contaminants.

Site	**COD (mg/l)**		**Percentage Difference**
	Unfiltered	Filtered (0.45 μm)	
MS	32	37	+15.6%
GS	77	12	-84.4%
SH	1169	150	-87.2%
KG	300	47	-84.3%
HS	884	56	-93.7%
Deionised water	-	7	-

Table 2.7: Chemical oxygen demand measurements for each of the five sites, and the percentage difference in COD resulting from filtering the samples through 0.45 μm MCE filters.

2.3.5 Anions & Ammonium

Full results for anion and ammonium concentration measurement are shown in Table 2.8.

The ammonium concentration at site SH was significantly higher than at the other four sites. One possible reason for this could be the use of cleaning products at the pub next to where the majority of the sample was taken, or possibly from animal or human urine. High traffic volume nearby may also be responsible, as vehicle emissions are a source of ammonium [111]. However, emissions are unlikely to be a major contributor in this case, as ammonium levels were much lower at the nearby site HS, which sees significantly greater volumes of traffic than site SH.

Phosphate concentration was below detection limits in all samples except GS, in which phosphate concentration was 0.08 mg/l. One potential source of phosphate in stormwater is animal faeces [25], so the high pigeon population at site GS could be responsible for the levels of phosphate found in the sample. There are also grassy and planted areas at the site, so some leaching of fertiliser could be taking place.

Nitrite concentration was below detection limits at sites GS and KG and was highest at site HS at 1.70 mg/l. Nitrate concentration was also below detection limits at site GS and again highest at site HS at 1.42 mg/l. Nitrogen oxides are released in vehicle exhaust fumes [104] and form acids when they dissolve in water, which then form nitrate or nitrite salts upon neutralisation [112]. This process is the likely cause of site HS having the highest concentration of these anions, as there is a significant amount of air pollution in the area due to traffic. Sulphate concentrations were also highest at site HS. Again, this is likely due to exhaust fumes, which contain sulphur dioxide [25].

Chloride concentration ranged from 7.08 mg/l at site KG to 147.30 mg/l at site HS. As discussed earlier, the chloride contamination at site HS is likely due to road salting. High concentrations of chloride can increase the mobility of toxic metals including lead, iron, chromium, zinc, and copper [104] [113]. The salt itself may contain contaminants such as sulphate, phosphate, and metals [25]. Chloride is difficult to remove from water as it is generally unreactive with sediment and other dissolved material, necessitating the use of techniques including ion exchange and distillation [114]. The impact of road salting should be taken into account when installing SUDS, as SUDS design may need to be altered to maximise chloride removal. This is particularly important where the SUDS drain directly into freshwater. The section of the River Clyde that would be impacted by the stormwater tested in this study is downstream of the Clyde Tidal Weir [115] and is a tidal estuary. Therefore, it already contains some saltwater and is likely to be less heavily impacted by chloride contamination.

Site	**Ion Concentration (mg/l)**							
	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	NH_4^+
MS	BDL	8.71	0.29	0.062	0.12	BDL	0.98	BDL
GS	0.011	11.62	BDL	0.088	BDL	0.087	2.03	BDL
SH	0.017	44.13	0.35	0.118	0.33	BDL	14.05	0.388
KG	BDL	7.08	BDL	0.058	0.02	BDL	0.99	BDL
HS	0.080	147.30	1.71	0.147	1.42	BDL	20.71	0.003

Table 2.8: Concentrations of anions in the five samples as measured by ion chromatography, and ammonium concentration as measured using a KONE analyser. Blank values have been subtracted from measured values. Concentrations below detection limits are labelled “BDL”.

2.3.6 Elements

Dissolved and total concentrations of all measured elements are shown in Tables 2.9 and 2.10 respectively. Of particular importance when considering stormwater quality are ’potentially toxic elements’ (PTEs). PTEs that are commonly found in stormwater or are regulated in surface water include aluminium, arsenic, cadmium, chromium, copper, iron, lead, mercury, manganese, nickel, and zinc. The dissolved concentration of iron was highest at site HS, as were the total and dissolved concentrations of aluminium, cadmium, chromium, copper, lead, titanium, and zinc. This is likely due to the street's high traffic levels, as these elements can all arise from vehicle parts and emissions. For example, tyre wear is a source of cadmium, copper, lead, and zinc pollution [25] [35]. Iron is a potential contaminant of road salt [25], so the high iron concentration at site HS is likely due to a combination of winter road salting and vehicle wear.

Dissolved concentrations of arsenic and mercury were highest at site MS, while total concentrations of these two elements were highest at site KG, along with total aluminium, iron, manganese, and nickel. Dissolved concentrations of manganese and nickel were also highest at site KG. Although site KG is a pedestrianised area and site MS experiences low levels of traffic, atmospheric deposition may result in PTE contamination from nearby areas such as the heavily trafficked Great Western Road or the Kelvinbridge subway station car park. Some PTEs may also be leaching from nearby soil at both sites.

Bioavailability results are shown in Table 2.11. As an estimated DOC was used when determining bioavailability, calculated values may differ from actual bioavailable concentrations [103]. Future stormwater analysis should include DOC measurement if bioavailability is to be calculated. Bioavailable concentrations of zinc, copper, and manganese were highest at site HS, while concentrations of nickel were similarly high at both site HS and site KG.

Element	**Concentration (mg/l)**				
	MS	GS	SH	KG	HS
Al	0.22	0.29	0.012	1.51	2.48
As	0.0015	0.0007	0.0005	0.0012	0.0001
Ca	2.67	3.07	11.41	10.07	40.91
Cd	BDL	0.0008	BDL	0.0009	0.0130
Co	BDL	BDL	BDL	0.008	0.0029
Cr	BDL	BDL	BDL	0.0008	0.0095
Cu	0.0043	0.0105	0.0041	0.0378	0.104
Fe	0.24	0.12	0.015	0.70	3.16
Hg	0.0041	0.0023	BDL	0.0024	BDL
K	BDL	BDL	1.89	0.55	3.85
Li	0.0003	0.0005	0.0042	0.0005	0.0064
Mg	0.49	0.83	1.33	1.66	10.58
Mn	0.024	0.040	0.023	0.45	0.25
Mo	BDL	BDL	BDL	BDL	BDL
Na	6.11	9.36	27.19	5.46	92.13
Ni	BDL	BDL	BDL	0.01	0.008
P	0.059	0.175	0.113	0.223	0.470
Pb	0.016	0.019	0.003	0.063	0.264
S	0.47	0.91	3.90	0.56	8.27
Sb	BDL	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL	BDL
Si	0.28	0.50	0.55	0.80	3.29
Sn	BDL	BDL	BDL	BDL	BDL
Sr	0.018	0.019	0.093	0.065	0.171
Ti	0.0033	0.0014	BDL	0.0026	0.026
Zn	0.075	0.128	0.040	0.247	0.617

Table 2.9: Table showing mean dissolved concentrations of elements in the five samples. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”. Standard deviations are shown in Appendix Table A.7.

Element	**Concentration (mg/l)**				
	MS	GS	SH	KG	HS
Al	1.51	3.08	7.82	25.58	22.47
As	BDL	BDL	BDL	0.022	BDL
Ca	2.01	4.37	25.57	13.46	52.21
Cd	BDL	0.0017	0.0017	0.0046	0.064
Co	BDL	BDL	BDL	0.028	0.021
Cr	BDL	BDL	0.018	0.065	0.083
Cu	BDL	0.013	0.057	0.109	0.203
Fe	3.01	5.53	12.71	47.14	46.01
Hg	BDL	BDL	BDL	0.0050	0.0033
K	BDL	BDL	BDL	BDL	2.25
Li	0.001	0.003	0.013	0.020	0.026
Mg	1.25	2.34	8.39	12.61	24.70
Mn	0.05	0.10	0.22	0.97	0.69
Mo	BDL	BDL	BDL	BDL	BDL
Na	6.43	10.08	34.21	6.40	98.58
Ni	0.12	0.01	0.05	0.17	0.05
P	0.17	0.67	2.38	3.45	1.74
Pb	0.031	0.088	0.435	0.349	0.489
S	0.25	1.11	7.82	2.88	10.36
Sb	BDL	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL	BDL
Si	2.90	6.62	14.58	34.38	36.82
Sn	BDL	BDL	BDL	BDL	BDL
Sr	0.025	0.035	0.176	0.107	0.245
Ti	0.09	0.23	0.49	1.50	2.11
Zn	0.11	0.25	0.55	0.60	1.04

Table 2.10: Table showing mean total concentration of elements in the five samples. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”. Standard deviations are shown in Appendix Table A.8.

Site	**Bioavailable concentration** (μg/l)			
	Cu	Mn	Ni	Zn
MS	0.52	14.21	0.52	75
GS	1.27	20.72	0.52	128
SH	0.57	14.43	0.71	30.52
KG	4.78	101.97	4.90	198.48
HS	19.27	250	4.92	388.84

Table 2.11: Bioavailable concentrations of elements as calculated using the M-BAT bioavailability calculator.

2.3.7 Comparisons to Standards

As there are no SEPA standards for urban stormwater, the results of this study were compared with SEPA environmental quality standards (EQS) for surface freshwater [103], which are shown in Table 2.12. This was done to provide a relative indication of how polluted the stormwater was, as well as to predict the possible impact that stormwater discharge could have on local water bodies.

EQS for chloride, fluoride and sulphate were not exceeded in any of the five samples - maximum measured values were 147,300 μg/l, 80 μg/l, and 20,710 μg/l respectively.

The concentration of arsenic in all samples was below the limit set by SEPA for freshwater. The detection limit of the technique used was higher than the EQS for mercury and cadmium, so although mercury concentration was below the detection limit at sites HS and SH, and cadmium concentration was below the detection limit at sites MS and SH, it cannot be determined whether these samples exceeded the freshwater EQS. Mercury and cadmium concentrations at the other remaining sites exceeded the EQS. Iron and manganese concentrations at site HS were greater than the recommended limits, as were copper concentrations at sites GS, KG, and HS. The concentration of nickel exceeded the EQS at sites KG and HS and the concentration of zinc exceeded the EQS at all five sites.

Although many of the stormwater samples exceeded SEPA standards, these standards are for surface freshwater such as rivers rather than for urban stormwater specifically. The stormwater will undergo significant dilution upon reaching the River Clyde, which will lessen its impact on the river's overall quality. However, given the potential for long-term impacts of chronic pollution such as accumulation in sediment and the food chain, the installation of SUDS for in situ treatment is still advisable.

Contaminant	**EQS (μg/l)**
Chloride	250,000
Fluoride	1,000
Sulphate	400,000
As	50
Cd	0.08 (dissolved)
Cu	1 (bioavailable, dissolved)
Fe	1,000 (dissolved)
Hg	0.07 (dissolved)
Mn	123 (bioavailable, dissolved)
Ni	4 (bioavailable, dissolved)
Zn	10.9 (bioavailable, dissolved)

Table 2.12: SEPA annual average quality standards for surface freshwaters. [103]

2.4 Conclusions

Although there is significant variation in stormwater quality across Glasgow, the pollution in all areas is enough that the city would benefit from stormwater treatment using SUDS. Concentrations of several contaminants such as zinc and copper exceeded environmental quality standards for freshwater, into which the stormwater may ultimately discharge. Although dilution will lessen the concentration of pollutants upon discharge, stormwater treatment is still advisable in order to prevent long-term damage to the local aquatic ecosystem.

Concentrations of many pollutants were highest at site HS, suggesting that traffic levels have a significant impact on water quality. However, concentrations of some contaminants were higher at other sampling locations (e.g. total concentrations of PTEs including arsenic and nickel at site KG), demonstrating that basing SUDS design, location, etc. on single factors such as traffic may not be sufficient. Testing of local stormwater should be carried out prior to the installation of SUDS to gain a full understanding of the type and extent of treatment required.

Future work should involve analysis of a wider range of sites over a longer time period, to better understand the impact of land use as well as seasonal differences. By using automated samplers to collect stormwater throughout rainfall events, variation in pollutant concentrations over time could also be determined.

Variation in Stormwater Quality Across Florianópolis, Brazil

Abstract

In order to avoid future water scarcity due to climate change, population increase, and urbanisation, a sustainable approach to water use is required. One possible method of preserving water is the harvesting of runoff from roads, pavements, and roofs. While rainwater harvesting from roofs is an established process, harvesting from roads and pavements is less common and its potential is less well understood. A key part of understanding this potential is establishing the chemical composition of the water. In this study, stormwater from the city of Florianópolis, Brazil, was sampled from three sites with varying traffic levels and analysed to determine how its quality varies across an urban area. Analyses included measurement of anions, potentially toxic metals, organics, and physicochemical parameters such as pH. Roof runoff that had passed through a rain garden was also analysed to demonstrate the potential of using rain gardens and related systems to treat water prior to non-potable re-use. Overall, the water tested was found to have low levels of contamination, with dissolved concentrations of total petroleum hydrocarbons and potentially toxic elements including arsenic, cobalt, chromium and lead below detection limits at all four sites. Comparisons to local water standards suggest that stormwater is a viable source of non-potable or (with some additional treatment) potable water. The results indicate that system design should be

considered carefully when harvesting runoff for re-use, as nearby infrastructure such as pipes and solar panels may contribute toxic metals to the water.

3.1 Introduction

By 2050, the global population is expected to reach 9.1 billion people, an estimated 6.3 billion of which will reside in urban areas [15]. This will lead to increased demand for water and significant stress on water treatment and distribution infrastructure. Thanks to increasing urbanisation coupled with changing weather patterns due to climate change as well as existing uneven water distribution, widespread water scarcity is a growing threat [16]. To overcome this, more sustainable approaches to water use and treatment are required.

Water is generally abundant in Brazil, with the country accounting for 11% of available water globally [116] and having an overall water availability of 33,000 m^3 per capita per year [52]. However, the Amazon Basin contains 69% of Brazil's water and only 8% of the population [116]. In contrast, the northeast and southeast regions of Brazil contain 28% and 43% of the population respectively [116] and water availability in these areas is classed as "low" by the United Nations Environment Programme (2000-5000 m^3 per capita per year) [117].

The city of Florianópolis lies on the southeast coast of Brazil. It is the capital of the state of Santa Catarina and the majority of the city is located on Santa Catarina Island. As of 2021, the population stood at 516,524 people [118]. Rainfall is higher during the summer months, with the greatest rainfall around February and the lowest around June [119]. The average annual rainfall in Florianópolis between 2000 and 2015 was 1607 mm [52]. Water availability in Santa Catarina is currently 10,000 m^3 per capita per year, though this could fall to as low as 2000 m^3 per capita per year by the year 2100 if the population increases as predicted [120]. Sustainable water use is therefore vital to avoid water scarcity.

Water conservation is written into Florianópolis law [121], ensuring that water consumption is controlled via methods such as the use of tap aerators, and that rainwater and wastewater are re-used for a variety of non-potable purposes. Rainwater harvesting already sees widespread use in several countries including Australia, Japan, Germany, and the USA, and is growing in popularity in Brazil. Many Brazilian states have introduced legislation and incentive programs related to rainwater harvesting, including Florianópolis [122]. While rainwater harvesting is often associated with roof runoff, other sources of non-potable water have recently been investigated in Brazil, including air conditioner condensate [123], washing machines [124], and road runoff [125] [64].

Florianópolis contains a high proportion of flats as opposed to houses, resulting in an average roof area of 60 m^2 per person – significantly lower than the average of 81.84 m^2 across Santa Catarina [120]. While this lowers the per capita volume of water available through rooftop rainwater harvesting, there is significant potential for stormwater harvesting from roads and paving to make up the difference. Modelling by Thives et al. (2018) suggested that between 52.65% and 96.3% of the non-potable water demand in Florianópolis could be met by stormwater re-use [64]. Additionally, modelling by Antunes et al. (2020) indicated that the use of permeable paving in the car park of a public building to supply non-potable water to the building could result in potable water savings of 69.6% [65].

Knowledge of stormwater quality is required to understand the scale of treatment systems necessary to allow for non-potable re-use. The aim of this study was to investigate how stormwater quality varies across Florianópolis, in order to determine the suitability of stormwater for re-use. Treated stormwater from a rooftop rain garden was also analysed to provide additional information about the potential of in situ stormwater treatment.

3.2 Materials & Methods

3.2.1 Sampling

Stormwater samples were collected by Professor Vernon Phoenix from four sites across Florianópolis during the week beginning 18th March 2019. Approximately 1 litre of water was collected from each site in plastic bottles (HDPE). Samples were stored at 4° C apart from during transport from Brazil to Scotland. The pH of sub-samples destined for organic and elemental analyses was lowered to 2 with HCl and HNO_3 respectively upon arrival at the laboratory in Glasgow.

The four sampling sites were as follows:

Rua João Pio Duarte Silva (Site Br1) is a busy main road in the Trindade district of central Florianópolis. The sampling site was a puddle close to the junction with Avenida Professor Henrique da Silva Fontes. Water was collected on the morning of the 20th of March, after rain the night before. Latitude: 27°36'0.52"S; Longitude: 48°30'58.31"W.

Casa Eficiente (Site Br2) is an energy efficient display house on Avenida César Seara in the Carvoeira district of central Florianópolis. Rainwater that lands on the roof of the house passes through a rain garden before being re-used in the home's washing machine and toilet. The water was collected on the 20th of March from a downpipe that collects water from the rooftop rain garden, after treatment and before re-use. Latitude: 27°36'19.74"S; Longitude: 48°31'16.66"W.

Rodovia Baldicero Filomeno (Site Br3) is a coastal road in the southwest of Florianópolis, in Ribeirão da Ilha village. Water was collected during heavy rain in the afternoon of the 22nd of March from a puddle outside a restaurant. Latitude: 27°43'13.22"S; Longitude: 48°33'50.63"W.

Rua Enginheiro Agronômico Andrei Cristian Ferreira (Site Br4) is a road within the campus of the Universidade Federal de Santa Catarina, in the Carvoeira district of Florianópolis. The road serves university traffic and has spaces for parking on both sides. Water was collected from a puddle on the morning of the 23rd of March, after rain the previous night. Latitude: 27°36'5.66"S; Longitude: 48°31'28.88"W.

These sampling locations were chosen to represent varying site uses and traffic volumes. Although sites Br1 and Br4 are located in the same region of the city, site Br1 experiences significantly higher traffic volumes. Site Br3 experiences moderate traffic but is located in a village rather than the city centre. Site Br2 provides insight into how runoff may be treated by rain gardens, although this system is based on roof runoff rather than road runoff. Images of the sites and their locations are shown in Figures

3.1 and 3.2.

Figure 3.1: Images of the four sampling sites in Florianópolis. Images of sites Br1, Br3 and Br4 are taken from Google Maps. The photograph of site Br2 was taken by Professor Vernon Phoenix.

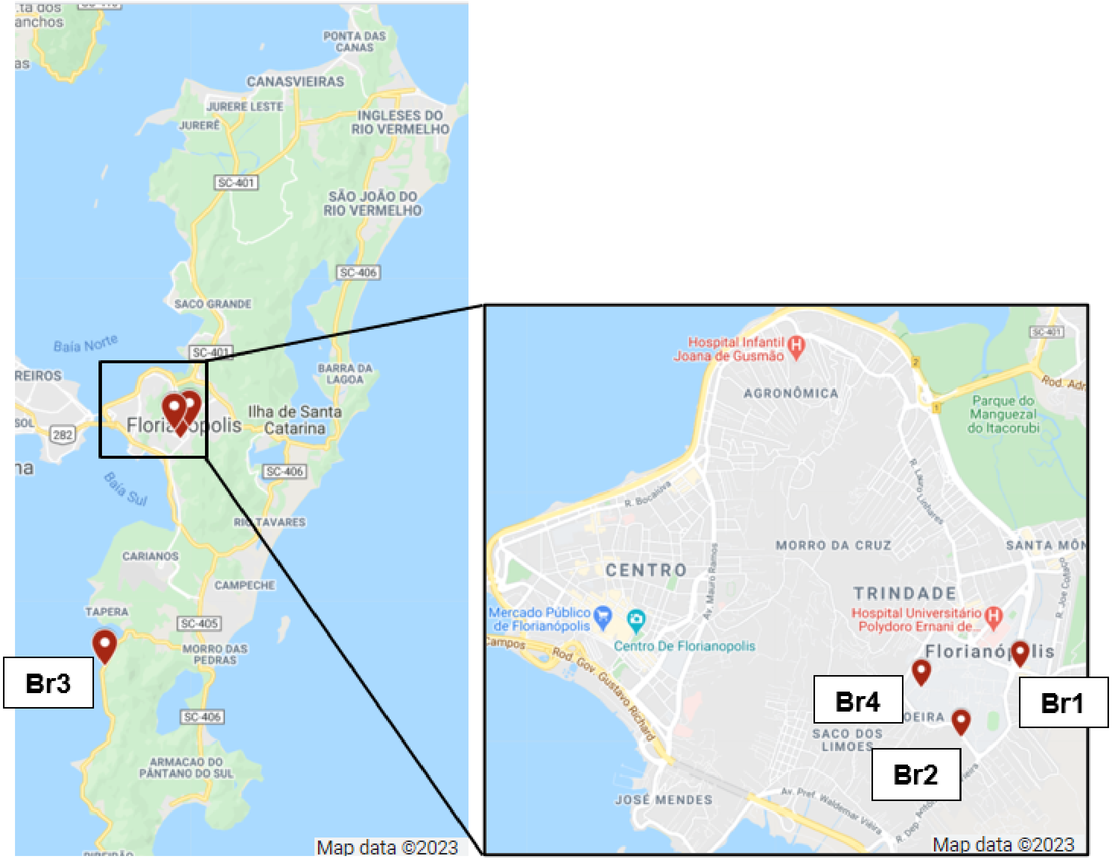

Figure 3.2: Maps of Florianópolis showing the locations of sampling sites Br1-Br4. Source: Google Maps.

3.2.2 Chemical Analysis

Analysis of total suspended solids (TSS), chemical oxygen demand (COD), total petroleum hydrocarbons (TPH), elements including potentially toxic elements (PTEs), and anions were performed as in Chapter 2.

The concentration of total solids (TS) was measured gravimetrically, according to the method described in Standard Methods for the Examination of Water and Wastewater [101]. Porcelain evaporating dishes were rinsed with deionised water and dried overnight at 105° C. Water samples were transferred to the cooled porcelain dishes and dried overnight at 105° C before re-weighing. TS concentration was calculated using the equation $((Final\_weight(g) - Initial\_weight(g)) * 1,000,000)/Sample\_volume(ml) =$

$TS(mg/l)$. Depending on the volume of the evaporating dish, different volumes of sample were used TS analysis. Duplicate samples were analysed from each site. Table 3.1 shows stormwater volumes used for TS and TSS analysis.

Sample	TS Sample Volume (ml)	TSS Sample Volume (ml)
Br1	10	70
Br2	10, 11	150
Br3	10, 11	100
Br4	10, 11	100

Table 3.1: Sample volumes for total solids (TS) and total suspended solids (TSS) analysis. Where two values are shown, different volumes were used for each duplicate.

3.3 Results & Discussion

3.3.1 Water Appearance

Sample Br3 had a slight brown tinge and Br1 & Br4 were a darker brown. Water was collected from sites Br1 and Br4 after rain the night before whereas sample Br3 was collected during heavy rain. This likely resulted in some dilution of contaminants and the weaker colouration in Br3. Samples Br1, Br3 and Br4 had some visible suspended solids. Sample Br2, which consisted of treated roof runoff, was clear and colourless with no obvious suspended solids. Samples are shown in Figure 3.3.

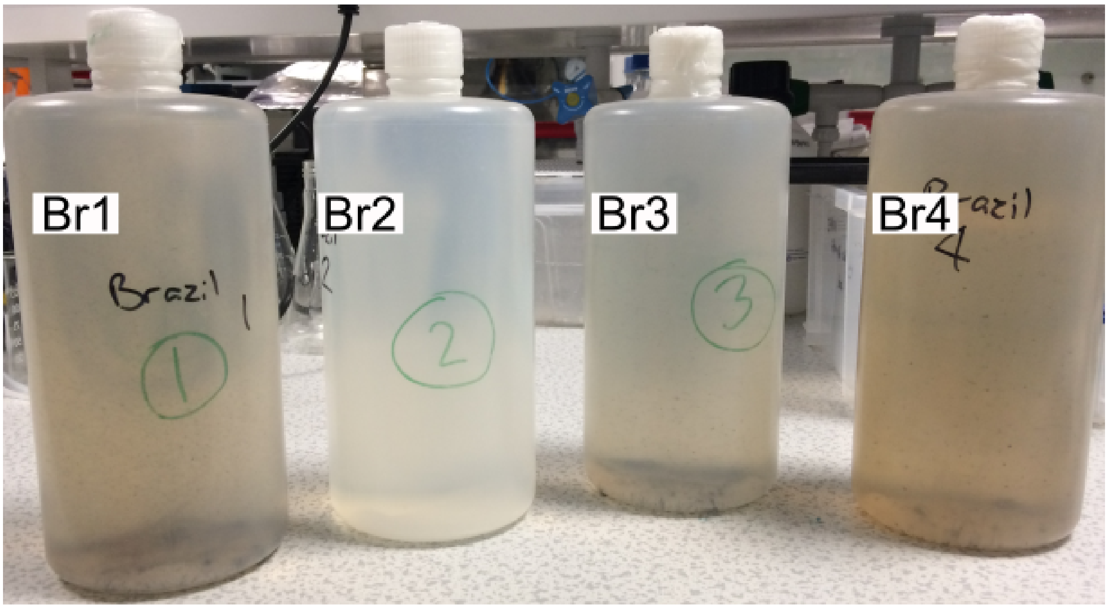

Figure 3.3: Appearance of the four samples upon return to the laboratory in Glasgow.

3.3.2 Solids

Concentrations of TSS and TS were highest at site Br1 and lowest at site Br2 (Table 3.2). Common sources of TS and TSS in the urban environment include dust stirred up by traffic [106], vehicle exhaust emissions [25] [104], and road, tyre, & brake wear [25], so it is unsurprising that the greatest concentrations of solids are seen at the site with the highest traffic. Site Br2 stormwater is roof runoff, which is typically significantly cleaner than road runoff although some contamination may still arise from precipitation, atmospheric deposition, and the roof surface itself [106]. Additionally, water at site Br2 has been treated by a rain garden, a form of sustainable urban drainage system (SUDS) that is effective at removing suspended solids [71].

Site	**TSS (mg/l)**	**SD**	**TS (mg/l)**	**SD**
Br1	97	0.0182	260	0.071
Br2	7	0.0014	19	0.140
Br3	54	0.0035	190	0.048
Br4	33	0.0064	215	0.021

Table 3.2: Mean total suspended solids (TSS) and total solids (TS) measurements for each of the four sites, alongside standard deviations (SD) for duplicates.

3.3.3 Organics

Full COD results are shown in Table 3.3 alongside the percentage change in COD after filtering. COD was highest at Site Br1 in both the filtered and unfiltered samples, at 36 mg/l and 115 mg/l respectively. Common sources of COD include oil and grease [22], bituminous road/pavement surfaces and tyre wear [25], all of which would be expected at the high traffic site Br1. Nearby trees and grass at the site may also contribute organic contamination, such as decaying plant matter [22].

Filtering resulted in a COD reduction of approximately 70% at sites Br1, Br3 and Br4. COD was lowest at site B2 and fell from 10 mg/l to 6 mg/l after filtering – a 40% reduction. The reduction in COD after filtering suggests that the majority of the organic contaminants in the water are particle-bound, which is common in urban stormwater [110]. The removal of solids by site Br2's rain garden would therefore have also removed bound pollutants, resulting in low COD measurements and a lower percentage reduction as a result of further filtering. Previous work has shown that adsorption prior to biodegradation is the predominant mechanism for removal of organic contaminants in rain gardens [126]. Low COD concentrations at site Br2 indicate that organic pollutants are strongly adsorbed to the soil and the rain garden is not releasing significant levels of oxidisable contaminants into the water. While this is beneficial for treatment of stormwater, strong adsorption can limit biodegradation of organic contaminants [126] and lead to the rain garden becoming saturated with potentially hazardous compounds. Regular analysis of rain garden soil should be carried out to ensure biodegradation is taking place at an acceptable rate.

GC-FID analysis detected no difference between the blank and the four water samples. Peaks representing the 1-chloro-octadecane spike and tricosane internal standard were found in all samples at the expected level, indicating that both the extraction and GC-FID analyses were successful. This result coupled with low COD measurements after filtering confirms that there were little to no dissolved hydrocarbons in the samples.

Site	COD (mg/l)		Percentage Difference
	Unfiltered	Filtered (0.45 μm)	
Br1	115	36	-68.7%
Br2	10	6	-40.0%
Br3	61	19	-68.9%
Br4	90	26	-71.1%

Table 3.3: Chemical oxygen demand (COD) measurements for each of the four sites, and the percentage difference in COD resulting from filtering the samples through 0.45 μm filters.

3.3.4 Anions

Nitrite and nitrate were below detection limits at all sites except Br3, where they were present at concentrations of 0.05 mg/l and 1.11 mg/l respectively. The concentration of phosphate was highest at site Br1, and phosphate was also detected at site Br3. Sites Br1 and Br3 were both near to green spaces, so fertiliser may be a source of nutrient contamination [114]. Fallen leaves can also leach phosphorus into stormwater [127]. In addition, phosphorus may arise from sea spray [125], and nitrogen oxides may arise from vehicle exhaust fumes [25].

Site Br1 had the highest concentrations of chloride, bromide, and sulphate. One source of chloride is sea spray and this has likely contributed to chloride contamination at site Br1. Site Br3 is considerably closer to the sea than site Br1, so higher chloride may be expected there. However, the heavy rainfall at site Br3 during sampling may have washed away some chloride. Other sources of chloride include animal waste, fertiliser, or cleaning products such as detergents and bleach that could be used by nearby businesses at site Br1 [114]. Bromide may also arise from sea spray [128]. Sulphate is another component of sea spray [129]as well as arising from sulphur dioxide in vehicle emissions [25].

The concentration of fluoride was highest at site Br2, at 0.65 mg/l. Fluoride concentrations are typically lower than 0.3 mg/l in surface waters, though they may range from 0.06 mg/l to 3 mg/l in natural waters [130].

Full results for anion concentration measurement are shown in Table 3.4.

Site	**Ion Concentration (mg/l)**						
	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Br1	0.34	36.69	BDL	0.08	BDL	0.44	18.06
Br2	0.65	6.43	BDL	0.07	BDL	BDL	8.96
Br3	0.14	14.04	0.05	0.07	1.11	0.12	9.89
Br4	0.19	6.47	BDL	BDL	BDL	BDL	2.86

Table 3.4: Anion concentrations in the four samples. Blank values have been subtracted. Concentrations below detection limits are labelled “BDL”.

3.3.5 Elements

Concentrations of arsenic, cobalt, mercury, molybdenum, antimony, selenium, and tin were below detection limits in both total elements and dissolved elements samples for all four sites. The dilution effect of the acid digestion process resulted in total lead concentration being below detection limits at all sites while the concentration of dissolved lead was above detection limits and highest at site Br2. Dissolved chromium was only detected at site Br3, while total chromium was below detection limits at all sites except site Br4. Cadmium, iron, and manganese concentrations were highest at site Br1, and aluminium, copper, nickel, and zinc concentrations were highest at site Br2. Full results for dissolved and total elements are shown in Tables 3.5 and 3.6.

The source of the higher concentration of cadmium at site Br1 is likely to be traffic, as this site is on a busy road and cadmium is a component of tyres and brakes [25] [36]. The iron and manganese at site Br1 also likely arose from the high volume of traffic in the area. Iron may originate from wheels [25], while manganese is a component of some fuel [25] and tyres [131].

It is somewhat surprising that the highest concentrations of aluminium, copper, nickel, lead, and zinc were found at site Br2, as this water is roof runoff rather than road and the water has been treated by a rain garden. It is possible that high concentrations of these metals entered the water through atmospheric deposition and treatment in the rain garden was not sufficient to remove them. However, atmospheric deposition of these pollutants is unlikely to be significantly higher here than at the other three sites, suggesting that the contamination has arisen from another source. Copper, zinc, and aluminium are commonly found in roof surfaces, guttering, pipes, and flashing

[106] [32] [25]. In addition, lead, zinc, and copper contamination may arise from brickwork [32]. The rooftop solar panels may be leaching aluminium, copper, lead, and zinc, among other metals including manganese [132]. It is therefore possible that high levels of metal contamination from the roof itself are not being completely reduced by the rain garden, or metals may be entering the water after treatment, e.g. while it flows through the downpipe. These possibilities should be considered when designing rainwater harvesting systems. For example, solar panels should be outwith the water harvesting zone and inert materials should be used for piping.

Element	**Concentration (mg/l)**			
	Br1	Br2	Br3	Br4
Al	0.345	0.953	0.474	0.288
As	BDL	BDL	BDL	BDL
Ca	29.08	5.69	6.42	3.95
Cd	0.0019	BDL	0.0003	0.0011
Co	BDL	BDL	BDL	BDL
Cr	BDL	BDL	0.0013	BDL
Cu	0.0062	0.073	0.0281	0.0089
Fe	0.594	0.507	0.465	0.312
Hg	BDL	BDL	BDL	BDL
K	6.85	BDL	5.99	1.66
Li	0.0039	0.0008	0.0020	0.0020
Mg	5.16	0.64	1.13	1.26
Mn	0.098	0.032	0.013	0.020
Mo	BDL	BDL	BDL	BDL
Na	27.96	2.33	11.86	3.85
Ni	0.0008	0.0009	BDL	BDL
P	0.295	0.017	0.078	0.057
Pb	0.0036	0.009	0.0041	0.0033
S	6.38	2.98	3.56	1.28
Sb	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL
Si	6.08	4.08	1.27	1.93
Sn	BDL	BDL	BDL	BDL
Sr	0.139	0.034	0.040	0.018
Zn	0.036	0.224	0.077	0.056

Table 3.5: Dissolved concentrations of elements at each of the four sites. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”. Standard deviations are shown in Appendix Table A.9

Element	**Concentration (mg/l)**			
	Br1	Br2	Br3	Br4
Al	1.54	0.93	1.97	1.28
As	BDL	BDL	BDL	BDL
Ca	27.97	5.42	6.22	4.00
Cd	0.0033	BDL	BDL	0.0017
Co	BDL	BDL	BDL	BDL
Cr	BDL	BDL	BDL	0.0058
Cu	0.007	0.071	0.023	0.005
Fe	1.50	0.55	1.33	0.85
Hg	BDL	BDL	BDL	BDL
K	0.44	BDL	BDL	BDL
Li	0.0067	0.0008	0.0025	0.0033
Mg	5.07	0.49	1.03	1.17
Mn	0.102	0.027	0.011	0.019
Mo	BDL	BDL	BDL	BDL
Na	29.35	3.06	11.81	4.76
Ni	0.008	0.024	BDL	0.005
P	0.371	BDL	0.057	0.082
Pb	BDL	BDL	BDL	BDL
S	7.83	15.77	3.46	1.73
Sb	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL
Si	7.90	3.96	3.46	3.51
Sn	BDL	BDL	BDL	BDL
Sr	0.139	0.035	0.040	0.018
Zn	0.108	0.293	0.089	0.088

Table 3.6: Total concentrations of elements at each of the four sites. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. The concentration of sulphur at site Br1 is based on a single value rather than an average of two, due to an issue with the duplicate reading. Concentrations below detection limits are labelled “BDL”. Standard deviations are shown in Appendix Table A.10

3.3.6 Comparisons to Standards

Concentration limits for discharge of effluent to surface waters in Brazil have been set for some PTEs by Resolution 430 of the National Environmental Council [133](Table 3.7). Concentrations at all four sites were well below these limits. This suggests that the discharge of stormwater in Florianópolis may not pose a significant threat to local aquatic environments. However, longer term analysis of a wider range of sites is recommended to account for variations in contaminant concentrations caused by factors such as land use, antecedent dry periods, or seasonal differences.

Element	**Maximum concentration (mg/l)**
As (total)	0.5
Cd (total)	0.2
Cu (dissolved)	1
Fe (dissolved)	15
Hg (total)	0.01
Mn (dissolved)	1
Ni (total)	2
Pb (total)	0.5
Zn (total)	5

Table 3.7: Maximum allowed concentrations of elements in effluent that is discharged to surface waters in Brazil [133].

Instead of being discharged, stormwater could be harvested and re-used as grey water or even drinking water. The Brazilian standards NBR 15527 and NBR 16783 (published in April 2019) provide limits for *Escherichia coli*, pH, turbidity, biochemical oxygen demand (BOD), and TSS for water being re-used for non-potable purposes [134]. TSS in this study was significantly below the 2000 mg/l limit.

Brazilian drinking water quality standards are regulated by Consolidation Ordinance No. 5/2017 [135]. Table 3.8 lists the maximum allowed concentrations of parameters that pose a threat to human health. The detection limit of the technique used in this study was greater than the maximum allowed concentrations of arsenic, antimony, and selenium, so it cannot be determined whether the measured concentrations exceeded the limit. Concentrations of the remaining parameters were all below the maximum allowed concentrations.

Table 3.9 lists parameters that are regulated for organoleptic quality i.e. acceptability in terms of taste and smell. The measured concentrations of aluminium and iron at all sites exceeded the maximum allowed values. The manganese concentration at site Br1 slightly exceed the maximum allowed concentration but was below the limit at the other sites. Concentrations of chloride, sodium, and zinc were below the maximum allowed value at all sites.

Given these results, stormwater appears to be a viable potential source for non-potable or potable water. However, further analysis is required to ensure that the water meets quality standards for parameters that were not tested in this study such as BOD or specific organic contaminants. Although the measured parameters met potable standards for health risk, the stormwater would require treatment to meet organoleptic standards. SUDS could be used to provide this treatment, providing they are designed appropriately to avoid contributing to contamination.

Parameter	**Maximum Allowed Value (Health) (mg/l)**
Antimony	0.006
Arsenic	0.01
Cadmium	0.003
Lead	0.01
Copper	2.0
Chromium	0.05
Fluoride	1.5
Total Mercury	0.001
Nickel	0.07
Nitrate (as N)	10
Nitrite (as N)	1
Selenium	0.04

Table 3.8: Maximum allowed values for parameters in Brazilian drinking water that pose a threat to health. Values refer to dissolved concentrations unless stated otherwise [135].

Parameter	**Maximum Allowed Value (Organoleptic) (mg/l)**
Aluminium	0.2
Chloride	250
Iron	0.3
Manganese	0.1
Sodium	200
Zinc	5

Table 3.9: Maximum allowed values for parameters in Brazilian drinking water that impact organoleptic quality. Values refer to dissolved concentrations [135].

3.4 Conclusions

As water scarcity in Brazil is expected to increase over the coming decades, finding alternative water sources is vital. This study demonstrated that stormwater in the city of Florianópolis is not highly polluted and could act as a source of non-potable or even potable water, with some treatment. However, future analysis of a wider range of parameters is required to ensure that the stormwater is treated effectively and meets all required quality standards before re-use. In addition, methods for collecting and treating recycled stormwater should be considered carefully to avoid unexpected contamination, such as that seen in roof runoff at site Br2.

Avenues for future research include the identification and installation of the most suitable treatment and collection systems, and subsequent analysis of the output water from these systems. High traffic levels may increase the concentrations of TSS, COD, and PTEs, so care should be taken in high traffic areas to ensure that stormwater receives adequate treatment prior to re-use.

Comparison of Stormwater Quality and Microbial Communities in Glasgow, Scotland and Florianópolis, Brazil

Abstract

Urban stormwater is a currently under-utilised resource that could alleviate the growing problem of global water scarcity. However, it contains an array of contaminants, which both limit its re-use potential and negatively impact natural water bodies that receive stormwater discharge. In order to appropriately treat stormwater for safe discharge or re-use, knowledge of its chemical and microbial quality is required. This study provides a comparison of stormwater sampled in Glasgow, Scotland and Florianópolis, Brazil with the aim of understanding how differences in urban environment affect stormwater composition. Water chemistry is compared between the cities and to prior studies, to provide an illustration of the impact of factors such as climate on contaminant concentrations. Stormwater microbiology from the cities is also discussed and contrasted, to determine how input communities in stormwater could affect both the communities within and the function of stormwater treatment systems. Maximum concentrations of pollutants including suspended solids, lead, nickel, and copper were

higher in Glasgow than Florianópolis but remain within the range seen in previous studies of urban stormwater. The lower contaminant concentrations in Florianópolis may be attributed to the city's lower population density and higher rainfall compared to Glasgow. Significant differences in microbial community composition were found between and within Glasgow and Florianópolis, including varying abundances of soil bacteria, and salt/metal-tolerant species. Key taxa found that could impact water treatment included *Rhodococcus*, *Mycobacterium*, and *Sphingomonas*, all known hydrocarbon degraders. Country of origin was identified as a significant contributor to taxonomic and functional diversity, suggesting that geographical differences including climate and urbanisation should be taken into account when considering the impact of stormwater microbiology on treatment.

4.1 Introduction

The need for sustainable water use is a pressing global issue. Climate change is predicted to result in both increased temperatures [12] as well as changes in rainfall patterns [13]. A temperature increase of 2-3° C is estimated to negatively impact water availability for up to 3.2 billion people [18]. One method of overcoming potential water scarcity is the harvesting and re-use of rainwater and stormwater for non-potable purposes. Rainwater harvesting is already popular in countries such as Japan and Australia [122] but stormwater harvesting is less common.

When stormwater is not harvested and re-used, it is often discharged directly to local water bodies. Stormwater collected from roads and pavements tends to contain more pollutants than rainwater either collected directly as it falls or from roof runoff [106]. Untreated stormwater contains a variety of pollutants such as oil and metals, which can do significant damage to the aquatic environment [12]. Because of this, stormwater should ideally receive some kind of treatment before it is either released or re-used. Sustainable urban drainage systems (SUDS) such as permeable pavements and rain gardens can be installed to provide in situ treatment to stormwater. The extent to which stormwater must be treated varies based on factors such as land use [99], so knowledge of local pollution levels is vital when planning SUDS installation.

Stormwater samples were collected in the cities of Glasgow, Scotland and Florianópolis, Brazil in March of 2019, as discussed in detail in Chapters 2 and 3. Both cities are actively working to improve their resilience against water scarcity and flooding, implementing measures such as legislation encouraging rainwater harvesting in Florianópolis [122] and plans to install SUDS across Glasgow city centre [136]. This study provides a comparison of stormwater in Glasgow and Florianópolis, with the aim of determining how stormwater chemistry varies between cities and whether water treatment must also take into account geographical factors beyond land use, such as climate.

Alongside chemical analysis, microbial community variation between the two cities was also considered. There have been few comprehensive studies on the stormwater microbiome, though the subject has gained some attention in recent years [137] [41] [86]. Microbial communities in stormwater can influence the community composition in receiving waters and stormwater treatment devices such as SUDS [138] [86]. Differences in SUDS communities could impact the efficacy of water treatment, so a deeper understanding of the stormwater microbiome is required to limit environmental damage and maximise the chances of stormwater re-use. This study aims to identify key features of microbial communities in Glasgow and Florianópolis, to provide some insight into how they may impact the functioning of future SUDS.

4.2 Materials & Methods

4.2.1 Sampling Locations

Glasgow has around 100,000 more residents than Florianópolis (635,000 vs. 516,000) and a significantly higher population density – 3,600 inhabitants per km^2 compared to 623.68 inhabitants per km^2 [118] [139] [93]. As of December 2018, there were 353,676 registered vehicles in Florianópolis [140] and 237,478 in Glasgow [141]. Florianópolis has a significantly larger area (675 km^2 [142] vs. 175 km^2 [143]) resulting in a greater traffic density in Glasgow – 524 vehicles/km^2 in Florianópolis compared to 1357 vehicles/km^2 in Glasgow.

The average minimum temperature in March is 3.5° C in Glasgow and 20.7° C in Florianópolis, while the average maximum is 10° C in Glasgow and 28.8° C in Florianópolis [144] [145]. While both cities have rainfall above the global average of 760 mm per year [116], Florianópolis's annual rainfall of 1607 mm [52] is greater than Glasgow's 1124.3 mm [96]. Average March rainfall is 173 mm in Florianópolis and 95 mm in Glasgow [144] [145].

Sampling sites in each city were chosen to represent a range of typical urban environments and are discussed in more detail in Chapters 2 and 3. A summary of sampling sites is shown in Table 4.1.

Site	**Abbreviation**	**Description**
Montague Street	MS	Glasgow, residential street with low traffic
George Square	GS	Glasgow, pedestrianised area bordered by intermediate traffic roads
Sauchiehall Street	SH	Glasgow, pedestrianised street intersected by high traffic roads
Kelvingrove Park	KG	Glasgow, pedestrianised green space
Hope Street	HS	Glasgow, high traffic road used by buses and taxis
Rua João Pio Duarte Silva	Br1	Florianópolis, high traffic road in city centre
Casa Eficiente	Br2	Florianópolis, energy efficient home, sampled water was roof runoff treated by a rain garden
Rodovia Baldicero Filomeno	Br3	Florianópolis, intermediate traffic road located in coastal village
Rua Enginheiro Agronômico Andrei Cristian Ferreira	Br4	Florianópolis, low traffic private road on university campus

Table 4.1: Summary of sampling sites.

4.2.2 Chemical Analysis

Procedures and results for the analysis of stormwater chemical and physical parameters are described in detail in Chapters 2 and 3. Briefly, total suspended solids (TSS) were measured gravimetrically [101], chloride was measured by ion chromatography, and total potentially toxic elements (PTEs) were measured on acid-digested samples using inductively coupled plasma - optical emission spectroscopy (ICP-OES).

4.2.3 DNA Extraction & Sequencing

Glasgow stormwater samples were stored at 4° C for approximately 48 hours prior to vacuum filtration through Nalgene Analytical Filter Test Funnels (0.2 μm, cellulose nitrate, Thermo Fisher Scientific, Massachusetts, USA). Florianópolis samples were filtered immediately upon arrival to the laboratory in Glasgow, having been stored at 4° C for 2-5 days between transport and collection. The volume of water filtered was based on how quickly the filter became clogged with solids (Table 4.2). Filters were stored at 4° C until DNA extraction.

Sample	Volume (ml)
MS	500
GS	350
SH	70
KG	150
HS	50
Br1	206
Br2	350
Br3	156
Br4	100

Table 4.2: Volumes of water filtered for DNA extraction.

DNA extraction was performed using the QIAGEN DNeasy PowerWater kit (Hilden, Germany), according to the manufacturer's instructions. Filters were cut into smaller pieces prior to DNA extraction using ethanol-sterilised scissors. DNA was eluted into 50 μl of elution buffer and stored at -80° C. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer (Winooski, Vermont). DNA concentrations are available in Appendix Table A.1.

Sequencing was carried out by Glasgow Polyomics (University of Glasgow, Garscube Campus) using the standard Illumina 16S protocol [146]. Libraries of the V3 and V4 regions of the 16S rRNA gene were created via two-step amplification using the 16SAmpliconPCRForwardPrimer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNG-

GCWGCAG) and the 16SAmpliconPCRReversePrimer (GTCTCGTGGGCTCGGAGATGT-GTATAAGAGACAGGACTACHVGGGTATCTAATCC). Sequencing was performed on the Illumina MiSeq using v3 chemistry to produce 300 bp paired end reads.

4.2.4 Bioinformatics

Paired-end 16S rRNA gene sequences (109,175 ASVs, 73 samples [combined dataset including 9 samples used for this study]) were demultiplexed, trimmed to remove adapter sequences (Trim F 17, Trim R 21), and truncated at the point median Phred quality score dropped below 30 (Trunc F 276, Trunc R 223). The open-source bioinformatics pipeline QIIME2 and the DADA2 denoising algorithm [147] were then used to construct Amplicon Sequencing Variants (ASVs) and generate abundance tables. Overall summary statistics for reads per sample were: Minimum: 12,650; 1st Quantile: 22,028; Median: 28,477; Mean: 69,613; 3rd Quantile: 104,365; Maximum: 269,669. ASVs were classified by aligning against the SILVA SSU Ref NR database (release v.138) [148]. QIIME2 was also used to generate a rooted phylogenetic tree and a BIOM file containing combined abundance and taxonomy data. The PICRUSt2 QIIME2 plugin was used to predict functional abundance and diversity in the form of KEGG enzymes (KEGG Orthologs [KOs]) and MetaCyc pathways. At this step, 1,712 of 109,011 ASVs were above the maximum Nearest Sequenced Taxon Index (NSTI) cut-off of 2.0 (a measure of closeness to the reference genome with values < 2.0 sufficient to obtain a reference hit) and were removed, leading to a very high alignment (98.42%). This high alignment combined with PICRUSt2's comprehensive database (approx. 20,000 genomes) and the strong correlation between the actual functions obtained through shotgun metagenomics and those predicted by PICRUSt2 on a test soil dataset ($R > 0.8$) in the original manuscript describing the software [149] allow high confidence in the predictions in this study. The full QIIME2 workflow is available here:
https://github.com/umerijaz/tutorials/blob/master/qiime2_tutorial.md.

Prior to statistical analysis, the output of the QIIME2 pipeline was further processed to remove ASVs that were unassigned or aligned with typical contaminants such as mitochondria and chloroplasts (as is suggested as a typical filtering step for QI-

IME2 analysis [150]). Additionally, as blanks were sequenced alongside samples and initial DNA concentrations were measured prior to sequencing, R's decontam package [151] was able to be used to remove any contaminants that may have arisen from reagents or library preparation [152]. For this purpose, after the initial filtering, method="frequency" in the isContaminant() function from the decontam package was used to remove 358 ASVs that met the criteria of contamination. The final reads per sample summary statistics for the 9 samples (19,847 ASVs) used in this study were [Minimum: 71,469; 1st Quantile: 88,258; Median: 117,939; Mean: 124,345; 3rd Quantile: 131,495; Maximum: 255,098].

4.2.5 Statistical Analysis

Statistical analyses were performed in R (version 4.1.2). Taxa bar plots of the overall top 20 most abundant taxa at appropriate taxonomic levels were generated to give an indication of how the abundance of the most common species changes between cities.

R's vegan package was used for estimating alpha and beta diversity indices [153]. Alpha diversity measurements used were as follows:

- Pielou's evenness – a measure of relative evenness that is constrained between 1 (complete evenness i.e. all species are present in equal abundances) and 0 (no evenness i.e. the community is dominated by a single species and other species are incredibly rare)
- Rarefied richness – the expected number of species in each sample (normalised to the minimum library size)
- Shannon entropy – takes into account both richness and evenness to provide a measure of overall community diversity, with diversity increasing as richness and/or evenness increases
- Simpson's index – an index similar to Shannon entropy but that is weighted towards more abundant/dominant species and so is more affected by changes in evenness than richness

For taxonomic beta diversity analysis, Principal Coordinates Analysis (PCoA) plots of ASVs were produced using three different distance measures:

- Bray-Curtis - considers not only whether ASVs are shared between samples but also takes into account variation in the abundance of those ASVs
- Unweighted UniFrac - considers the phylogenetic distance between two samples without considering ASV abundances i.e. closely related ASVs that share a high proportion of their phylogenetic tree will be treated as more similar than ASVs that have a greater proportion of unique branches
- Weighted UniFrac - a compromise between Bray-Curtis and Unweighted UniFrac which weights the phylogenetic difference by the abundance of ASVs, so is more heavily impacted by phylogenetic variation in abundant ASVs

For analysis of functional beta diversity, Hierarchical Meta-Storms [154] was used to generate a PCoA plot. While the calculation of taxonomic beta diversity assumes independence between features (treating each ASV as a distinct entity), this assumption does not hold when applied to functional abundance tables of KEGG Orthologs (KOs) such as those obtained from PICRUSt2. This is because of redundancy in KOs, with most functional pathways able to utilise one of several alternative KOs in enzymatic reactions. Therefore, taking into account only KOs rather than full pathways may result in erroneous detection of functional diversity, as different KOs may be performing the same function in different samples. Hierarchical Meta-Storms takes this redundancy into account by considering not only the relative abundance of individual KOs but also the relative abundances of functions at each level of the KEGG BRITE pathway hierarchy.

The phyloseq package [155] was used for calculating UniFrac distances. Vegan's ordiellipse() function was used to draw ellipses representing the standard errors of the weighted sample averages for each category.

Vegan's adonis() function was used to perform PERMANOVA. PERMANOVA is used to identify significant differences between the centroids/means of groups of samples (e.g. samples from Glasgow vs from Florianópolis). PERMANOVA also calculates the proportion of variance between groups that can be explained by individual covariates (e.g. water chemistry parameters) and gives the result as an R^2 value if significant. For example, an R^2 of 0.25 indicates that 25% of variance between groups is a result of changes to that parameter. PERMANOVA in this study was carried out on one environmental covariate at a time, due to the high number of covariates being tested in this study.

R's microbiome package was used to identify the core microbiome [156]. Although varying prevalence thresholds have been reported previously [157] the core microbiome in this study was considered to be ASVs that were present in at least 95% of samples. All figures in this study were generated using R's ggplot2 package [158]. The following annotations are used to denote significance: '***' ($p \leq 0.001$), '**' ($p \leq 0.01$), '*' ($p \leq 0.05$), and '.' ($p \leq 0.1$).

4.3 Results & Discussion

4.3.1 Chemical Analysis

Brazilian samples from sites Br1 and Br4 are most comparable in source to the Scottish samples, as these two samples were taken in the urban centre of Florianópolis, whereas site Br3 was more rural. As the water from site Br2 is treated roof runoff rather than untreated road runoff, it will not be discussed further in this section.

In general, the highest concentrations of contaminants in the Florianópolis samples were significantly lower than the highest concentrations in the Glasgow samples. For example, the TSS concentration in Florianópolis ranged from 33-97 mg/l, which is more in line with the lowest Glaswegian concentrations (13 mg/l at site MS and 26 mg/l at site GS) rather than the remaining concentrations in Glasgow, which range from 343 mg/l to 2,827 mg/l. Greater TSS concentrations in Glasgow could be due to higher population and traffic density, or due to the higher rainfall in Florianópolis resulting in less build-up of pollutants on the road surface. TSS concentrations measured previously in Scotland [62], India [159], and Singapore [98] ranged from 17.23 mg/l to 343 mg/l.

Only sites SH and HS in Glasgow had TSS concentrations outwith this range, likely due to a combination of high traffic and the impact of winter road salting/gritting. Stormwater analysis in Germany [36] identified abrasion from gravel/grit as a key factor in increased TSS concentrations during cold weather and measured a maximum TSS concentration of 3,165 mg/l. The maximum concentration of chloride was much greater in Glasgow (147.3 mg/l vs. 36.69 mg/l), again likely caused by winter road salting, which does not occur in Florianópolis.

Commonly analysed potentially toxic elements (PTEs) in stormwater include copper, nickel, lead, and zinc. Total concentrations of these metals in Glasgow and Florianópolis are shown in Table 4.3.

Sample	**Copper (mg/l)**	**Nickel (mg/l)**	**Lead (mg/l)**	**Zinc (mg/l)**
MS	BDL	0.12	0.031	0.11
GS	0.013	0.01	0.088	0.25
SH	0.057	0.05	0.435	0.55
KG	0.109	0.17	0.349	0.60
HS	0.203	0.05	0.489	1.04
Br1	0.007	0.008	BDL	0.108
Br3	0.023	BDL	BDL	0.089
Br4	0.005	0.005	BDL	0.088

Table 4.3: Mean total concentrations of copper, nickel, lead, and zinc in Glasgow and Florianópolis stormwater. Concentrations below detection limits are labelled "BDL". Standard deviations are shown in Appendix Table A.8.

Total copper concentrations in Florianópolis stormwater ranged from 0.005 mg/l to 0.023 mg/l whereas Glasgow concentrations ranged from below detection limits to 0.203 mg/l. The maximum Florianópolis concentration fell between the 3rd and 4th lowest Glasgow copper concentrations. A previous study of Florianópolis stormwater by Antunes et al. (2016) found total copper concentrations ranging from 0.02 mg/l to 0.27 mg/l [52]. This range is more in line with the Glasgow concentrations and illustrates the need for widespread sampling of a variety of stormwater under various weather conditions in order to gain a more accurate picture of urban stormwater quality within a city.

Total concentrations of nickel ranged from below detection limits to 0.008 mg/l in Florianópolis and from 0.01 mg/l and 0.17 mg/l in Glasgow. Concentrations measured previously in Scotland [62], India [159], and Singapore [98] ranged from below detection limits to 0.01 mg/l, similar in scale to the Florianópolis results from this study. A higher maximum total nickel concentration of 0.403 mg/l was found in Germany [36]. Nickel can arise from brake wear [25], as well as diesel exhaust fumes and oil [36], so variations in concentration may be due to differing traffic densities.

Lead was found at all five Glasgow sites at total concentrations ranging from 0.031 mg/l to 0.489 mg/l whereas the total lead concentration was below detection limits at all Florianópolis sites. This could be due to greater traffic density in Glasgow or the frequent historical use of lead in Glaswegian architecture such as roofing [160], which could then leach into runoff [32]. A review by Kayhanian et al. (2012) found global total lead concentrations in stormwater ranged from 0.00564 mg/l to 1.860 mg/l [161]. However, this maximum concentration was measured in a study prior to the banning of leaded petrol. More recent studies have found lower total lead concentrations, such as from <0.005 mg/l – 0.405mg/l in Germany [36] and from below detection limits – 0.28 mg/l in India [159].

Total concentrations of zinc were similar in the two cities, ranging from 0.11 mg/l to 1.04 mg/l in Glasgow and 0.088 mg/l to 0.108 mg/l in Florianópolis. Previous studies in Florianópolis [52] and Singapore [98] found total zinc concentrations ranging from 0 mg/l to 0.45 mg/l, somewhat lower than the concentrations identified in this study. However, analysis of stormwater in India [159] and Germany [36] found maximum concentrations greater than this study, up to 2.02 mg/l and 3.47 mg/l respectively.

It should be noted that this study considers grab samples taken after recent rainfall events, as opposed to flow-weighted or time-weighted samples taken throughout rainfall events and used to calculate event mean concentrations (EMCs). Grab sampling was used for this study due to equipment and time limitations. It is recommended that, where possible, future analysis of urban stormwater in these cities utilises automated sampling over longer timescales in order to gain a more detailed picture of contaminant fluctuations. This will allow the development of treatment regimes that are appropriate for the full range of pollutant concentrations seen at a particular site. Using EMCs rather than single time point values will also allow more direct comparison to previous studies.

Overall, concentrations of contaminants in Florianópolis stormwater appear more similar to the lower concentrations in Glasgow stormwater. This is likely due to differing population density and traffic levels at the sites sampled from, as well as the impact of winter road treatments in Glasgow. Higher rainfall in Florianópolis may also lead to less build-up of contaminants during dry spells as well as greater dilution of contaminants during wash-off. This comparison suggests that there may be a need to take into account climate factors such as rainfall frequency, rainfall intensity, and minimum temperatures as well as land use when designing systems for the treatment and re-use of stormwater.

4.3.2 Community Composition

Figure 4.1 shows the relative abundance of the top 20 phyla and genera in both cities. At phylum level, *Proteobacteria* was dominant in both Glasgow and Florianópolis. Florianópolis samples exhibited greater variation in phyla between sites than the Glasgow samples. Noticeable differences between Florianópolis samples included greater abundances of *Patescibacteria* and the sulphate-reducing [86] *Desulfobacteria* at site Br1, *Planctomycetota* and the metal-tolerant [86] *Acidobacteriota* at Br2, *Firmicutes* at Br3, and the ubiquitous soil phylum [162] *Verrucomicrobia* at Br4. The top 20 phyla accounted for 98-100% of all identified ASVs.

At genus level, taxonomic composition differed between all sites. The overall top 20 genera accounted for 25-53% of ASVs in the samples, with this wide range suggesting that some sites were dominated by genera that were not generally abundant throughout the two cities.

Some similarities in taxonomy were observed between Glasgow's sites GS and SH, with genera including *Haliangium* and *Nannocystis* seen here in greater proportions than at other sites. *Haliangium* and *Nannocystis* are myxobacteria, known for producing secondary metabolites and fruiting bodies [163] [164]. *Haliangium* is also halophilic [163], allowing it to tolerate the winter road salting that occurs at sites GS and SH. Both sites are pedestrianised and bordered by busy roads, so their similar taxonomy could be a result of shared environmental pressures. Samples from sites KG and MS also shared similar taxonomy, with high abundances of an uncultured *Planctomycetales* genus and *Rhizobacter*. Although these sites differ in their land use (green space vs. residential street), samples were taken close to soil in both cases. Given that both *Planctomycetales* and *Rhizobacter* are common members of soil microbial communities [165] [166], they have likely entered the stormwater as it passed over/through this nearby soil. Site HS contained greater abundances of *Flavobacterium*, *Alterythrobacter*, and *Sphingomonas* than any other site. Each of these genera is associated with oil contamination and degradation [41] [167] [168] [169] [170], which is likely why they are found in the heavy-traffic site HS.

Bradymonadales was the most abundant genus at site Br1. *Bradymonadales* was not identified in significant proportions at any other sites in Glasgow or Flórianopolis. *Bradymonadales* are predatory bacteria that are often found in saline environments, so may have arisen from sea spray [171]. Only 25% of ASVs at site Br2 were represented by the 20 overall most abundant genera, the lowest proportion of the 9 sites. This is not unexpected, given that Br2 samples were treated roof runoff rather than untreated road runoff and have therefore experienced different environmental conditions prior to sampling, resulting in a lower proportion of genera being shared with the other sites. Of the 25% of ASVs identified at Br2, the vast proportion was made up of an uncultured *Planctomycetales* genera, which likely entered the water via the rain garden soil, as in Glasgow's sites KG and MS. At site Br3, abundant genera included *Deinococcus, Exiguobacterium*, *Massilia*, and the hydrocarbon-degrading [41] *Pseudomonas*. *Massilia* is known to occur in the rhizosphere and promote plant growth [172] so may have entered the water from nearby planted areas. At site Br4, abundant genera included *Flavobacterium*, *Novosphingobium*, and *Luteolibacter*. Despite low COD measurements at Br4, both *Flavobacterium* and *Novosphingobium* [173] are associated with oil degradation. Site Br4 is a private university road with parking spaces on both sides, so it is possible that some oil is arising from leaking stationary vehicles. *Luteolibacter* occurs in the rhizosphere so may have arisen from green areas bordering the sampling site. *Luteolibacter* is also able to cope with metal stress, so can tolerate any soil contamination that may occur due to traffic [174].

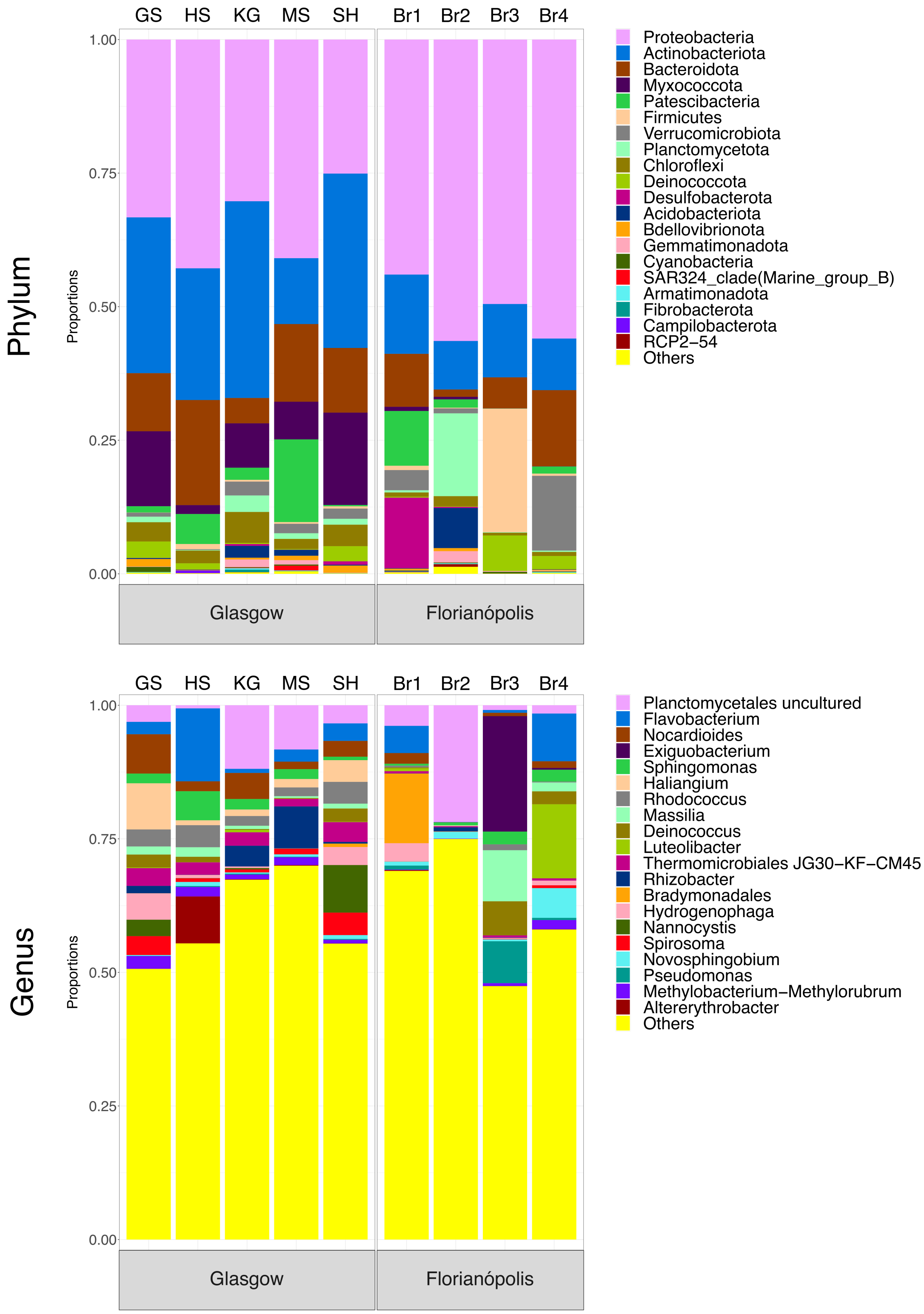

Figure 4.1: Bar plots representing the top 20 most abundant taxa at phylum and genus level. Taxa outside the top 20 are labelled “Others”.

The core microbiome of each site was defined as genera present in at least 95% of samples (Appendix Figures A.1 and A.2). Given the small number of samples in this study, this was equivalent to the core microbiome being genera present in all samples from each city. Glasgow's core microbiome contained many more taxa than that of Florianópolis. However, when site Br2 is removed from the analysis the two core microbiomes are similar in size. This demonstrates that the three Florianópolis road runoff communities share many more taxa with each other than they do with the treated roof runoff from site Br2.

Abundant core taxa found in both cities included *Nocardioides*, *Rhodococcus*, *Flavobacterium*, *Novosphingobium*, *Mycobacterium* and *Sphingomonas*, all of which are associated with hydrocarbon degradation [41] [167] [168] [175] [173]. Abundant genera unique to Glasgow included the salt-tolerant *Haliangium* (likely due to winter road salting, which is not carried out in Florianópolis) and an uncultured *Rhizobiales Incertae Sedis* genus. *Rhizobiales* species are often plant-associated and have been found to include methanotrophs, nitrogen-fixers, and hydrocarbon degraders [176] [177]. Abundant core taxa unique to Florianópolis (when Br2 was excluded from the analysis) included an uncultured member of the *Comamonadaceae*, a family common in polluted environments and capable of degrading complex organics [178], and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* from the family *Rhizobiaceae*, often found in soil and associated with plant roots [179].

4.3.3 Taxonomic & Functional Diversity

Taxonomic

No statistically significant difference was found in rarefied richness or Shannon entropy, indicating that communities in both cities were similar in their number of species and overall diversity. Significant differences were identified in Pielou's evenness and Simpson's index, which were lower in Florianópolis (Figure 4.2), suggesting that the Florianópolis sites were dominated by a smaller number of abundant bacteria compared to Glasgow. When site Br2 was removed from the analysis, no significant difference was found in Pielou's evenness but a significant difference in Simpson's index remained, indicating that overall diversity among abundant species in Florianópolis stormwater was lower than in Glasgow. However, in both Glasgow and Florianópolis (with and without Br2) Simpson's index was close to 1, suggesting that diversity was high in all samples despite the difference between the two cities.

PCoA using Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac distance measures yielded clustering based on country (Figure 4.3). This suggests that taxonomic similarity between sites from the same country was greater than any similarity seen between comparable sites in different countries (e.g. the low traffic sites MS and Br4). The Weighted UniFrac plot shows similarity between Glasgow's site HS and the Florianópolis stormwater samples, indicating that the abundant species at these sites were closely related. Site HS was significantly more polluted than the Florianópolis sites, so it is unclear what is driving this similarity.

Site Br2 was not closely clustered with the other 3 Florianópolis sites nor with the Glasgow sites. This is likely due to the Br2 sample both being roof runoff rather than road as well as having passed through a rain garden, resulting in a different microbial community. This suggests that although there is some variation between stormwater communities from different sites, road runoff communities remain distinct from those of stormwater treated by SUDS.

PERMANOVA identified country of origin as the only significant contributor to variation in beta diversity based on Bray-Curtis and Unweighted UniFrac distances (Appendix Table A.25). This suggests that factors beyond general land use and traffic levels (both of which affect pollutant concentrations) may be affecting the community. For example, climate factors unique to each country such as temperature and rainfall. Both country and phosphate concentration impacted beta diversity based on Weighted UniFrac.

Functional

No statistically significant difference in functional alpha diversity was identified by the metrics used (Figure 4.2). This indicates that the Glasgow and Florianópolis microbial communities were generally similar in the number and evenness of their functions. However, PCoA plots using Hierarchical Meta-Storms showed loose clusters based on country (Figure 4.3), indicating that, although the number of functions was similar between communities, the functions themselves differed somewhat.

Glasgow's site MS clustered more closely with the Florianópolis samples than the remaining Glasgow samples. Site MS somewhat resembled the Florianópolis sites as it consisted of a road adjacent to greenery and was minimally polluted, so this may have contributed to the similarity in functions between these sites. Site Br2 did not cluster closely with any of the other sites, again likely due to the sample being treated roof runoff resulting in a community distinct from that of the road runoff.

PERMANOVA identified country of origin ($R^2 = 0.29$, $p = 0.016$) and chemical oxygen demand ($R^2 = 0.24$, $p = 0.02$) as having a significant impact on variation in functional beta diversity (Appendix Table A.25). Differing COD concentrations may affect the functions present as high COD could enable the proliferation of bacteria capable of degrading and utilising organic material (such as hydrocarbons) for energy. Conversely, bacteria in low COD environments may require other functions such as the ability to use inorganic material for energy. The impact of country of origin on functional beta diversity suggests that local climate and geography affect the community. This could include factors such as proximity to the sea or to forest/rainforest, or the overall level of urbanisation in the area.

Disparity between functional and taxonomic beta diversity was also measured (Figure 4.4). The slope of the line can give an indication of community robustness by reflecting how changes in taxonomy affect the community's functions. The steeper gradient seen in Florianópolis (76.36°) indicates that its overall community is less robust than that of Glasgow (67.38°) – it takes less change in taxonomy to result in a change of function. This may mean that the functions of stormwater communities in Florianópolis are less resilient to changes in environmental pressures, and this potential loss of function could impact pollution removal in SUDS that receive the stormwater.

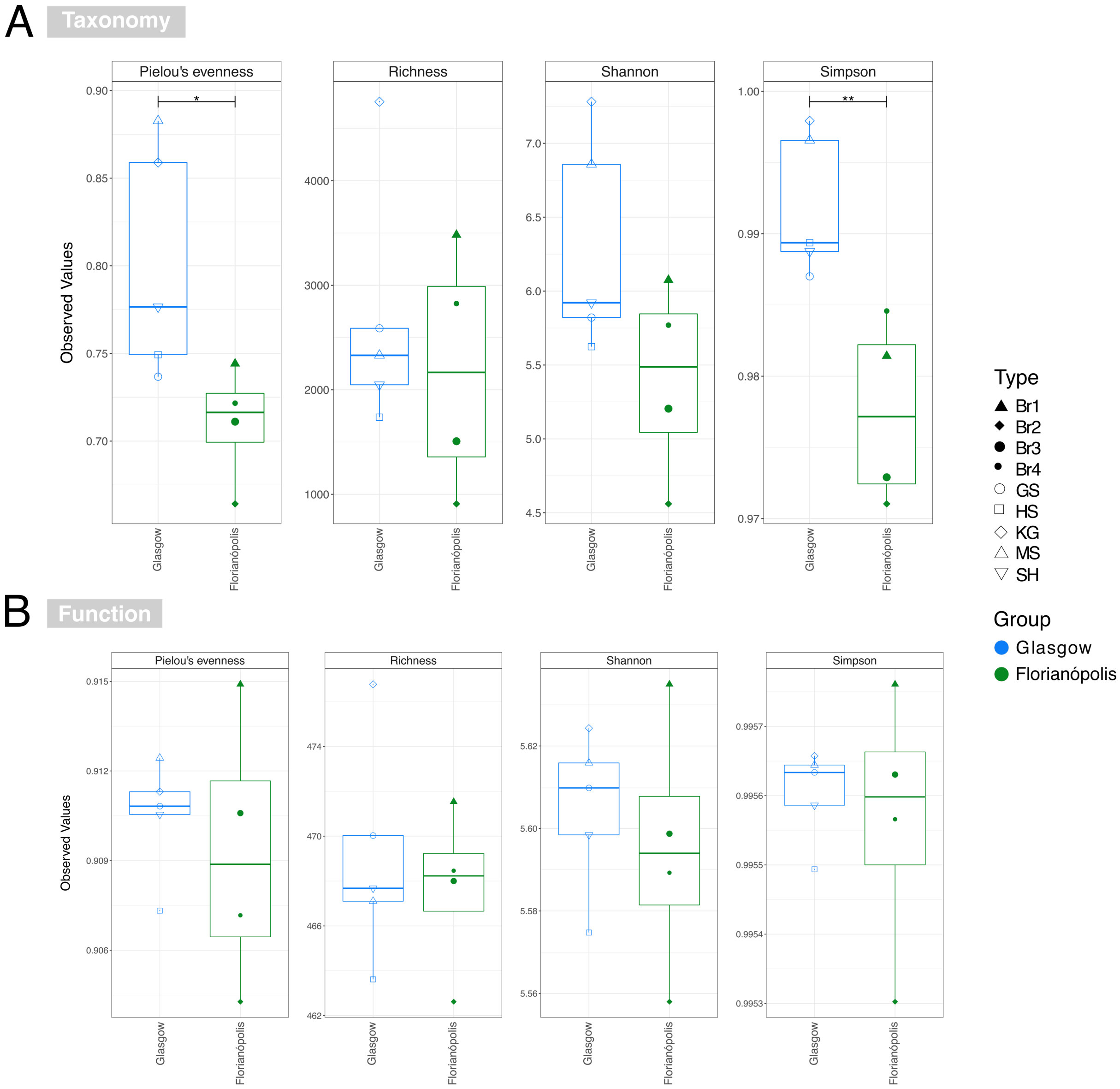

Figure 4.2: Alpha diversity estimates for (A) taxonomy and (B) function based on the PICRUSt2 prediction of the KEGG metagenome. Significant differences are represented by annotated lines connecting the two categories.

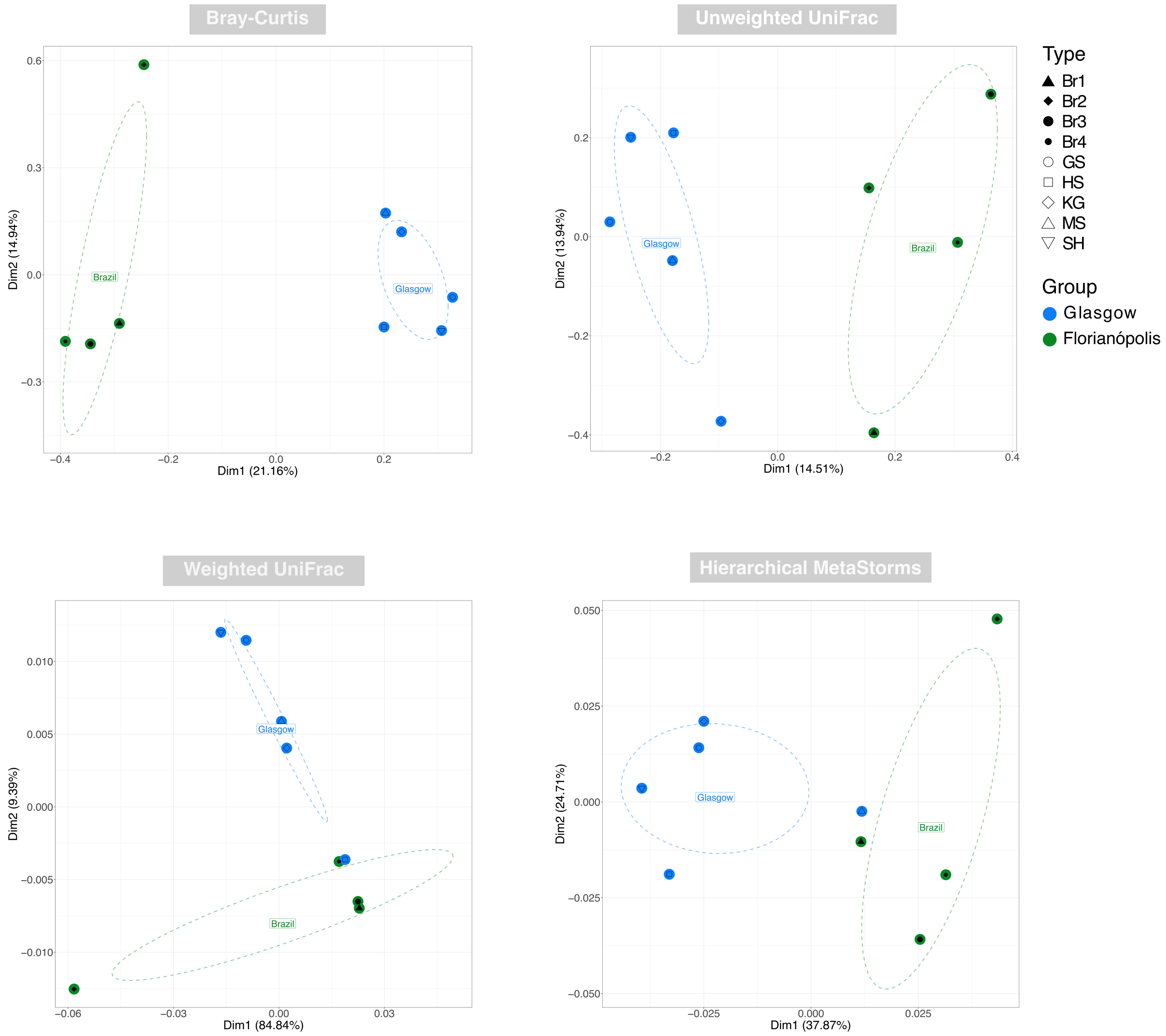

Figure 4.3: Principal co-ordinate analysis (PCoA) using Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac distances to represent taxonomic beta diversity and Hierarchical Meta-Storms to represent functional beta diversity. Ellipses for were drawn using 95% confidence intervals based on standard error of the ordination points for each category.

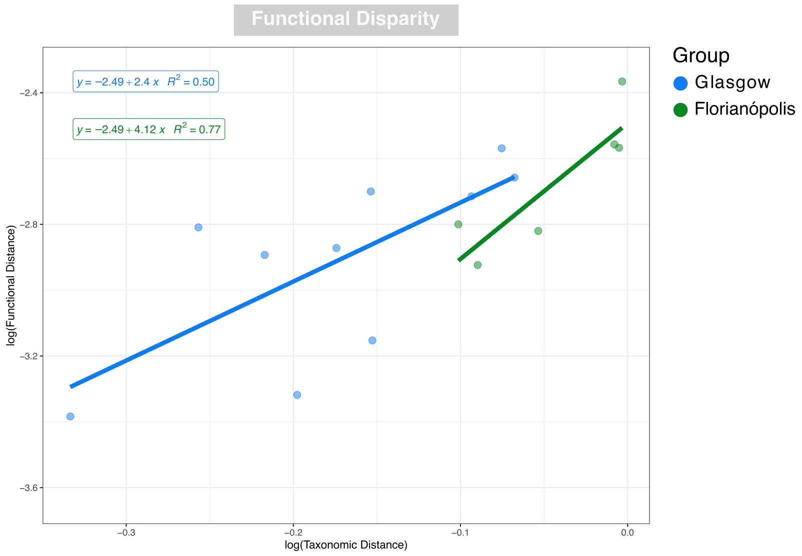

Figure 4.4: Functional disparity between taxonomic and functional distance is shown for each water source. Line gradient indicates the overall robustness of each community against perturbation.

4.4 Conclusions

Maximum concentrations of TSS, copper, nickel, and lead were greater in Glasgow than in Florianópolis. These differences can be attributed to a number of factors including traffic density, local building styles, and rainfall patterns. Overall, contamination in stormwater from both cities remained within the ranges seen in prior studies. In order to gain a more accurate picture of stormwater quality, longer-term flow-weighted sampling from a wider range of sampling points is recommended.

A number of known hydrocarbon degrading organisms including *Rhodococcus*, *Mycobacterium*, and *Sphingomonas* were identified in both Glasgow and Florianópolis, the presence of which could enhance the treatment of stormwater contaminated by oil and grease. Other common taxa included salt-tolerant and plant-associated genera, suggesting that factors such as winter road treatments and proximity to the coast or green space can impact community composition.

Country of origin was identified as having a significant impact on both taxonomic and functional diversity, emphasising the need to take into account geographical differences such as climate when considering stormwater microbiology and its potential impact on water treatment. Water from site Br2 differed from the stormwater samples in composition as well as taxonomic and functional diversity, suggesting that the treatment of runoff by rain gardens could have a significant impact on the water's microbiology.

Variation in Stormwater Quality & Microbial Community Composition Across Four Rain Gardens in Glasgow, Scotland

Abstract

Sustainable urban drainage systems (SUDS), such as rain gardens, are an alternative to traditional drainage, able to lower flood risk and reduce environmental contamination from stormwater. Rain garden soil composition has been shown to affect water treatment, so a better understanding of the impacts of soil mix on effluent quality is required in order to design rain gardens and other SUDS for optimal treatment ability. This study analysed influent and effluent from four rain gardens in Glasgow, Scotland, which were identical in design apart from variation in soil particle size distribution. The study aimed to determine the impact of soil type on both water chemistry and microbial community. Chemical analyses included measurement of pH, conductivity, solids, potentially toxic elements, and anions. Microbial analysis included alpha and beta diversity analysis of 16S sequencing data. All four rain gardens were able to remove metals and suspended solids from stormwater, although treatment efficacy varied based on influent quality. When influent contamination was low, less change

in quality was seen between the influent and effluent, suggesting that there may be a minimum level to which the rain gardens can reduce pollutants. Some variation in pollutant removal was seen between soil types, but a lack of clear pattern prevented the identification of the optimal particle size distribution. Effluent microbiology was noticeably different from that of the influent, and taxonomic evenness and functional richness were both greater in the effluent than influent, indicating that the rain garden soil had altered the microbial community in the water. However, effluent microbiology was more impacted by sampling date than soil type, which may be a result of the soil communities maturing and changing over time.

5.1 Introduction

Climate change is expected to have a significant impact on rainfall in Scotland, with summer rainfall predicted to fall by 6-7% and winter rainfall predicted to rise by 7% by the 2050s [13]. This change in rainfall patterns will cause increased flood risk during winter while some areas experience water scarcity during summer [13]. Traditional drainage systems such as the combined sewers found commonly in Glasgow, Scotland [180] are not resilient against the impacts of climate change. Incidences of combined sewer overflows (CSOs) are already on the rise [10], resulting in the discharge of human waste to the environment. Separate sewer systems are designed to discharge stormwater directly to local surface water, which reduces the pressure on sanitary sewers during heavy rainfall and lowers the risk of CSOs. However, this stormwater discharge is untreated and carries a variety of urban pollutants including metals and hydrocarbons [5]. An alternative drainage strategy is required to ensure that urban areas are equipped to deal with climate change.

The city of Glasgow is carrying out various projects in order to improve its resilience against climate change. This includes the Climate Ready Clyde initiative [181], work carried out by the Metropolitan Glasgow Strategic Drainage Partnership [97], and the Glasgow Avenues programme [136].

The Glasgow Avenues programme is the result of a £136 million investment in

Glasgow city centre, which aims to make the city more sustainable, more attractive, and more accessible for pedestrians and cyclists [136]. One aspect of the Avenues programme is the installation of green/blue infrastructure such as sustainable urban drainage systems (SUDS), including rain gardens.

SUDS are able to lower flood risk by slowing stormwater flow or allowing infiltration to soil, as well as providing in situ water treatment to reduce the impact of stormwater on the environment and provide an opportunity for stormwater recovery and re-use [1]. Rain gardens are a form of SUDS that typically consist of landscaped areas containing soil and plants. They have a high capacity for the removal of metals, suspended solids, and hydrocarbons [71]. Vegetation in rain gardens also provides aesthetic value and noise reduction. Their relatively low installation and maintenance costs, and their ability to be installed in established urban areas [6] make rain gardens particularly well-suited for a city centre environment. Rain gardens and SUDS are discussed in more detail in Chapter 1, Section 1.3.

Guidance for SUDS design is often based on the system's ability to control water quantity rather than water quality [53] [66], despite evidence that the ability of SUDS to remove pollutants varies depending on the materials used. For example, the composition of the soil mix used in rain gardens can impact the system's hydrology by controlling the speed of infiltration, which in turn affects pollutant removal – slower infiltration allows more time for sequestration and degradation of contaminants [40] [182] [72]. Previous studies have shown that changes in soil composition (such as variation in proportions of sand, soil and clay as well as the addition of modifiers including compost and wastewater treatment by-products) impact metal removal efficiency in rain gardens [39] [183].

In addition to directly impacting pollutant removal, soil composition may also affect the microbial community that resides within the rain gardens, which could cause changes in biodegradation capability. Changes in either the stormwater community or the soil community could alter the effluent community. There has so far been little research into the microbiology of stormwater and SUDS effluent. Recently, Liguori et al. (2021) investigated the microbial community in the influent, effluent, and media of two stormwater treatment devices on the same high traffic road. They found that the community composition differed between the influent and effluent, as well as between the effluent and media of both devices [86]. This demonstrates the need for further investigation into the impact of SUDS design on output microbial community.

In early 2019, a pilot set of rain gardens for the Avenues programme was installed in Washington Street, Glasgow. These rain gardens each received the same input stormwater and contained the same plant species but varied in soil type. This study presents the results of analysis of influent and effluent water from the rain gardens with the aim of determining the impact of soil composition on pollutant removal and microbial community composition, in order to inform future rain garden design.

5.2 Materials & Methods

5.2.1 Rain Garden Construction

A system consisting of four rain gardens with a shared input and individual outputs was installed in Washington Street, Glasgow by civil engineering company Civic Engineers and landscaping company idverde during the spring of 2019 (Figure 5.1). The rain garden system was designed to take in stormwater from a short section of Argyle Street, which is a busy major road that runs across Glasgow and connects to the M8 motorway close to the rain garden site. Stormwater drains from Argyle Street into a trough on Washington Street before baffles within the trough distribute the water evenly between the four rain gardens. Once the water has passed through the rain gardens, it is released into a shared pipe and directed into the pre-existing Washington Street drainage system (Figure 5.2).

Figure 5.1: Rain gardens on Washington Street, Glasgow prior to the addition of soil and plants (A), shortly after installation in 2019 (B), and in 2021 (C). Photos B and C by Civic Engineers.

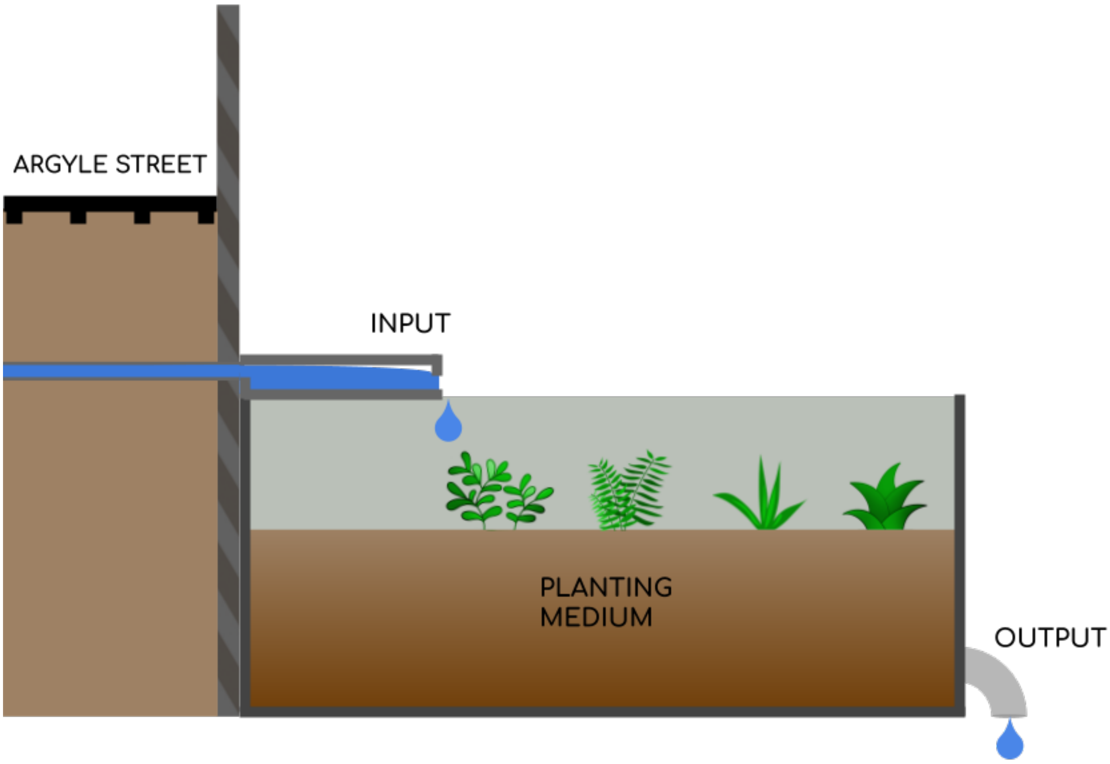

Figure 5.2: Schematic of the Washington Street rain garden system.

The four rain gardens (referred to as A, B, C & D) in the Washington Street system differed only in the particle size distribution of their soil mix. Particle size distribution was tested by European Turfgrass Laboratories prior to the construction of the rain gardens and this data was provided by idverde. All four soil samples tested were moist, of friable consistency and medium homogeneity. Soil particles in each soil mix were sub-angular and of medium sphericity. Soil chemistry data was not provided by the supplier. Table 5.1 shows a summary of the particle size distribution in each mix.

All four rain gardens contained the same plant species in the same layout (Table 5.2).

Particle Size Distribution (Detailed)	**Soil Mix**			
	A	**B**	**C**	**D**
% coarse gravel > 3.4 mm	-	0.3	0.1	0.7
% fine gravel 2 -3.4 mm	0.1	2.1	0.3	2.7
% very coarse sand 1 - 2 mm	1.3	9.6	1.5	10.7
% coarse sand 0.5 - 1 mm	15.7	21.1	15.9	18.7
% medium sand 0.25 - 0.5 mm	40.2	34.4	41.6	31.2
% fine sand 0.15 - 0.25 mm	35.6	23.4	35.0	24.4
% very fine sand 0.05 - 0.15 mm	5.0	6.5	3.1	7.0
% silt + clay <0.05 mm	2.1	2.6	2.5	4.6
Particle Size Distribution (Summary)				
% >1 mm	1.4	12.0	1.9	14.1
% coarse + medium sand	55.9	55.5	57.5	49.9
% fine sand	35.6	23.4	35.0	24.4
% fines < 0.15 mm	7.1	9.1	5.6	11.6
Saturated Hydraulic Conductivity				
At 35 mm tension (mm/hr)	258.2	105.4	152.1	47.1

Table 5.1: Soil mix particle size distribution and saturated hydraulic conductivity. Data provided by European Turfgrass Laboratories/idverde.

Scientific Name	**Common Name**	**Category**
Luzula nivea	Snow rush	Shrub
Calamintha nepeta	Lesser calamint	Shrub
Daboecia cantabrica ‘Rodeo’	Daboecia	Shrub
Geranium macrorrhizum	Geranium	Shrub
Vinca major ‘Variegata’	Greater periwinkle	Shrub
Carex oshimensis ‘Evergold’	Japanese sedge	Grass
Sesleria caerulea	Blue moor grass	Grass
Stipa arundinacea	Bent grass	Grass
Molinia caerulea ‘Heidebraut’	Purple moor grass	Grass
Molinia caerulea ‘Karl Foerster’	Purple moor grass	Grass
Primula bulleyana	Bulley’s primrose	Herbaceous perennial
Bergenia ‘Baby Doll’	Elephant’s ears	Herbaceous perennial
Geum rivale	Water avens	Herbaceous perennial
Achillea ‘Moonshine’	Yarrow moonshine	Herbaceous perennial
Phlomis russeliana	Turkish sage	Herbaceous perennial

Table 5.2: Plant species in each of the four rain gardens. Information provided by Glasgow City Council.

5.2.2 Sampling

Water samples were collected from the Washington Street rain gardens in May 2019, August 2019, and December 2019. Due to an issue with flow distribution, May samples were taken only from the input and outputs A and C. Rainfall measurements for each sampling date and the week preceding are shown in Figure 5.3. Measurements were taken from the Scottish Environment Protection Agency (SEPA) online data for the Dalmarnock rainfall gauge, as this is the closest gauge to Washington Street [184].

Approximately 2 litres of water was collected from the shared input and each of the rain garden outputs – 1x 1 l glass bottle, 1x 1 l plastic (HDPE) bottle and 2x 50 ml centrifuge tubes (PP).

In March 2021, approximately 2 litres of water was collected from the River Clyde in HDPE bottles. This was to allow comparison of the pollutant concentrations in the rain garden effluent with those of the receiving water body.

The pH of samples destined for organic and elemental analyses was lowered to 2 with HCl and HNO_3 respectively. Samples were stored at 4° C until analysis.

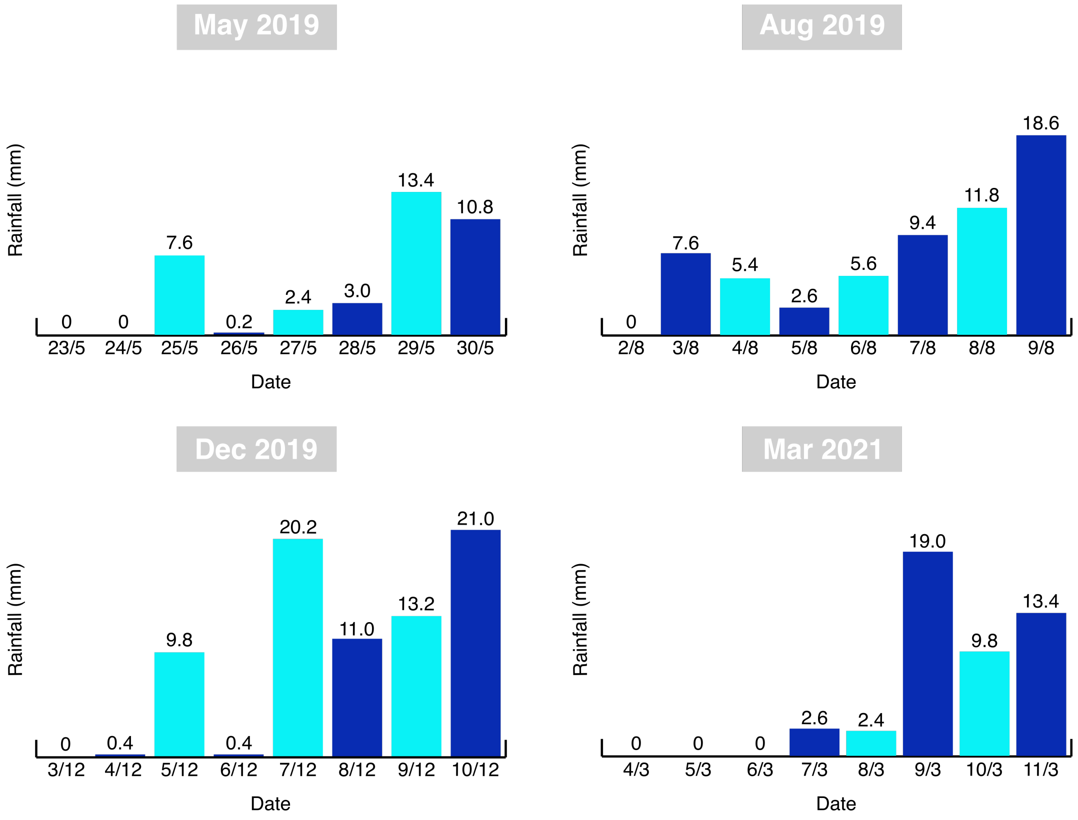

Figure 5.3: Rainfall measurements for each sampling date and the preceding week, taken from SEPA's online data for the Dalmarnock rainfall gauge [184]. Each value reflects the total rainfall in the 36 hours up to 9am on the stated date.

5.2.3 Chemical Analysis

In May 2019 and August 2019, pH and electrical conductivity (EC) were measured as in Chapter 2 Glasgow. For December 2019 and River Clyde samples, a Thermo Scientific Orion Star A211 (Massachusetts, USA) meter was used to test pH and EC. All pH and EC measurements were performed in the laboratory.

Total suspended solids (TSS) were measured as in Chapter 2. Total solids (TS) were measured as in Chapter 3. Total dissolved solids (TDS) were measured gravimetrically in May and August 2019 and using a Thermo Scientific Orion Star A211 meter for December 2019 and River Clyde samples. Samples for gravimetric TDS measurement were filtered through 1.2 μm glass fibre filter paper (Whatman, Maidstone, Kent) into pre-weighed porcelain evaporating dishes, which were then dried overnight at 180° C [101] before being cooled and weighed again. TDS concentration was calculated using the equation $((Final\_weight(g) - Initial\_weight(g)) * 1,000,000)/Sample\_volume(ml) = TDS(mg/l)$. Sample volumes for gravimetric TSS, TS, and TDS measurement (Table 5.3) were based on the sizes of the evaporating dishes and on the rate at which the filter paper became clogged. All gravimetric measurements were performed in duplicate.

Sample	**TSS Volume (ml)**	**TS Volume (ml)**	**TDS Volume (ml)**
May Input	80	10	21, 22
May Output A	160, 200	10	20
May Output C	160, 161	10	20
Aug Input	250	21	20
Aug Output A	200	20	20
Aug Output B	200, 203	20	20, 24
Aug Output C	200	20	20, 21
Aug Output D	200	20, 24	20
Dec Input	300	20, 21	-
Dec Output A	300, 400	20	-
Dec Output B	300	20	-
Dec Output C	300	20	-
Dec Output D	300	20, 22	-
River Clyde	300	23	-

Table 5.3: Sample volumes filtered for total suspended solids analysis. Where two values are listed, this indicates that different volumes were used for duplicates.

Samples for dissolved elements analysis and May/August 2019 total elements analysis were prepared as in Chapter 2. Details of the digestion mixtures and MARS 5 settings are shown in Tables 5.4 and 5.5. December 2019 and River Clyde total elements samples were digested in a MARS 6 microwave digestion system (CEM) using 4:1 HNO_3:HCl. Digestion mixtures and settings are shown in Tables 5.4 and 5.6. Elemental analysis using ICP-OES was performed as in Chapter 2.

	May19, Aug19	**Dec19, Clyde**
Sample	6 ml unfiltered water sample 3 ml HNO_3 1 ml HCl	45 ml unfiltered water sample 4 ml HNO_3 1 ml HCl
Blank	3 ml HNO_3 1 ml HCl	4 ml HNO_3 1 ml HCl

Table 5.4: Contents of sample and blank vessels used for acid digestion of samples destined for total elements analysis.

Step	**Pressure (psi)**	**Power (%)**	**Ramp (min)**	**Temperature (°C)**	**Hold (min)**
1	800	100	20	160	
2	800	100		160	20
3	800	100	20	180	
4	800	100		180	20

Table 5.5: Programme settings used for acid digestion in a CEM MARS 5 microwave.

Step	**Pressure (psi)**	**Power (W)**	**Ramp (min)**	**Temperature (°C)**	**Hold (min)**
1	800	400-1800	10	170	
2	800	400-1800		170	10

Table 5.6: Settings for acid digestion in CEM MARS 6 microwave, using the pre-installed US EPA 3015a programme.

Chemical oxygen demand (COD) was measured as in Chapter 2. Due to low COD values recorded in May, 0-150 mg/l test kits were used for both unfiltered and filtered samples for August 2019, December 2019, and River Clyde samples. Anions and ammonium were measured as in Chapter 2.

5.2.4 Bioavailability

Bioavailability was calculated using the M-BAT tool [102] as in Chapter 2.

The detection limits of 1.2, 1.0, 1.2, and 1.5 μg/l were used in place of the nickel concentrations for River Clyde, May, August, and December samples respectively in which the measured concentration was below the detection limit. The detection limit of 2 μg/l was used as the copper concentration for the Clyde.

As in Chapter 2, dissolved organic carbon (DOC) values were not determined for these samples, so the recommended value of 2 mg/l was used in the M-BAT calculator.

5.2.5 Microbial Community Analysis

Water samples were stored at 4° C prior to vacuum filtration through Nalgene Analytical Filter Test Funnels (0.2 μm, cellulose nitrate). The volume of water filtered was 100 ml for all samples with the exception of August Input (200 ml) and May Output A (53 ml). In May, a single replicate of each sample was filtered. For August, December, and River Clyde samples, three replicates of each sample were filtered. The funnels were stored at 4° C until DNA extraction.

DNA extraction, sequencing, and bioinformatics were performed as in Chapter 4. DNA concentrations are shown in Appendix Tables A.2, A.3, A.4, and A.5. The final reads per sample summary statistics for the 32 samples (60,515 ASVs) used in this study were [Minimum: 9,761; 1st Quantile: 20,384; Median: 76,752; Mean: 88,535; 3rd Quantile: 142,943; Maximum: 253,526].

5.3 Results & Discussion

5.3.1 Water Appearance

Figure 5.4 shows water samples taken from the rain gardens in May, August, and December 2019 and from the River Clyde in March 2021. In May 2019, all water samples were brown and turbid. The influent was darker than the effluent and appeared to contain more suspended solids. In August 2019 and December 2019, the water samples were significantly lighter in colour and less turbid than in May. The output samples were similar in colour or slightly darker than the input samples. This suggests that the rain gardens are successfully removing solids when concentrations in the influent are high but may be contributing some colouration as a result of the water filtering through the soil – visible when the influent is comparatively clean.

Water from the River Clyde sampled in March 2021 was slightly darker in colour than the August effluent, with similar levels of visible suspended solids.

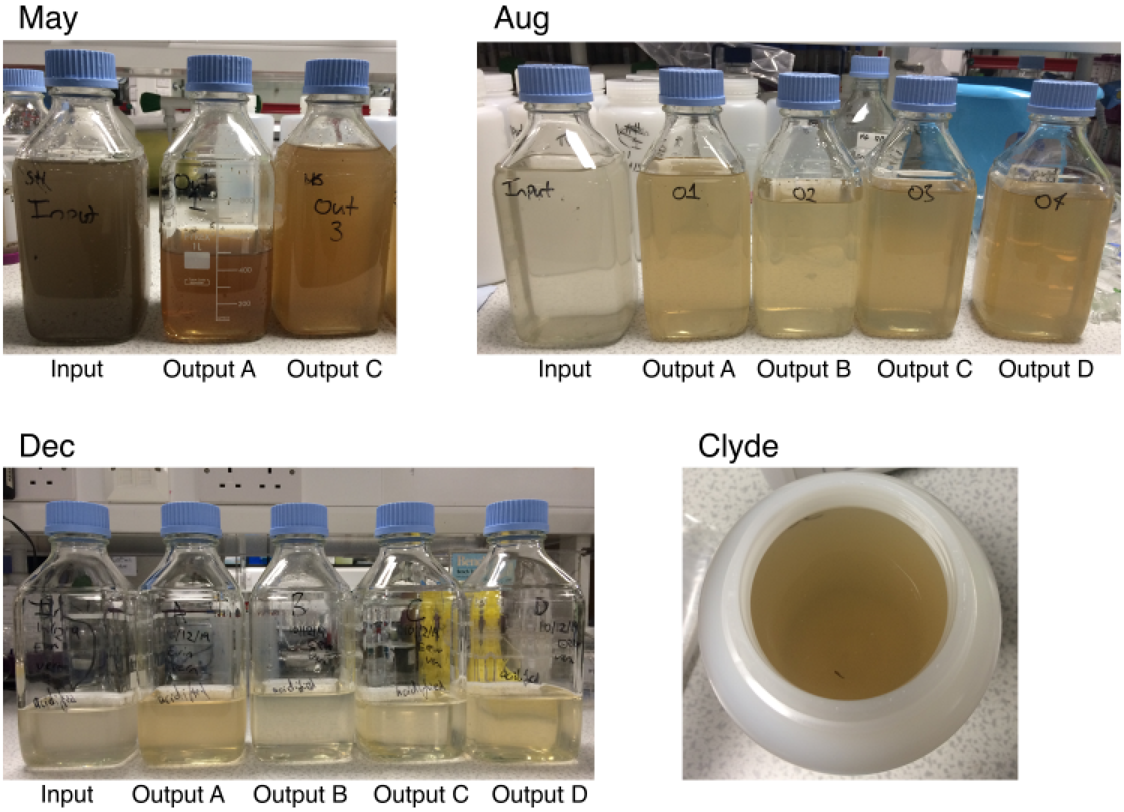

Figure 5.4: Water samples taken from the rain gardens in May, August, and December 2019, and from the River Clyde in March 2021.

5.3.2 Solids

The concentration of total suspended solids (TSS) was very low overall (Table 5.7). With the exception of the May input, all remaining samples had TSS concentrations of 25 mg/l or below.

During May, when the input TSS concentration was at its highest, the rain gardens were able to reduce suspended solids significantly - from the input TSS of 134 mg/l to output TSS of 2 mg/l and 16 mg/l (Outputs A and C respectively). In August, TSS concentrations were similar between the input and outputs. In December, input TSS was only 25 mg/l but was reduced to below detection limits in all outputs. This suggests that the rain gardens are capable of removing TSS even when the input is relatively dilute.

When compared to Glasgow stormwater data (discussed in Chapter 2) the TSS in all influent samples is significantly lower than that of Sauchiehall Street (2,827 mg/l) and Hope Street (1,015 mg/l). Both of these streets are to be redesigned as part of the Avenues programme, therefore it is important to ensure that any SUDS installed are also able to cope with high TSS concentrations. As noted with the May samples, the rain gardens were able to significantly reduce TSS when it was high, so it is expected that this would also be the case for any rain gardens installed on Hope Street or Sauchiehall Street. However, the extent of possible reduction should be investigated. TSS in the River Clyde was also significantly lower than in Sauchiehall Street, Hope Street, and the May 2019 rain garden input, and was of a similar concentration to the rain garden effluent. This is notable as it indicates that the installation of rain gardens across Glasgow could therefore minimise the impact of TSS pollution on the river ecosystem.

In addition to TSS, total dissolved solids (TDS) and total solids (TS) were also measured. TS (Table 5.8) and TDS (Table 5.9) were both highest in the December output samples. This is notably different from the TSS results in this month, which were very low, indicating that the dissolved fraction made up the vast majority of solids in these samples. Additionally, there was a significant increase in TDS between the input and output in December.

The high TDS in December is likely due to winter road salting. Chloride is not only a contributor to TDS itself, but high salt concentrations can cause the desorption of ions that were previously adsorbed to the soil grain surface [1], resulting in increased TDS in the effluent. Rain garden D was also subjected to additional salting by idverde, at a concentration of 10 g/m^2 every other working day between December 2019 and March 2020. This is the likely reason why output TDS was highest in Output D.

TDS also increased between the input and some outputs in May and August, when road salting was not taking place. This suggests that material in the rain gardens may be dissolving and contributing to TDS and this should be taken into account when choosing materials for rain garden substrate. Further testing could be carried out to determine the composition of the dissolved material.

Sample	Total Suspended Solids (mg/l)							
	May19	SD	Aug19	SD	Dec19	SD	Mar21	SD
Input	134	0.0097	8	0	25	0.01	-	-
Output A	2	0.0004	8	0.011	BDL	-	-	-
Output B	-	-	BDL	-	BDL	-	-	-
Output C	16	0.0004	10	0.007	BDL	-	-	-
Output D	-	-	12	0.004	BDL	-	-	-
River Clyde	-	-	-	-	-	-	14	0.005

Table 5.7: Total suspended solids measurements for each sample and standard deviations (SD) for duplicates. Concentrations below detection limits are labelled “BDL”.

Sample	Total Solids (mg/l)							
	May19	SD	Aug19	SD	Dec19	SD	Mar21	SD
Input	185	0.120	48	0	136	0.0091	-	-
Output A	330	0.28	25	0.035	437	0.0247	-	-
Output B	-	-	50	0.071	388	0.0318	-	-
Output C	75	0.021	BDL	-	457	0.0035	-	-
Output D	-	-	46	0.006	755	0.0064	-	-
River Clyde	-	-	-	-	-	-	98	0.0092

Table 5.8: Total solids measurements for each sample and standard deviations (SD) for duplicates. Concentrations below detection limits are labelled “BDL”.

Sample	Total Dissolved Solids (mg/l)							
	May19	SD	Aug19	SD	Dec19	SD	Mar21	SD
Input	79	0.016	BDL	-	108	-	-	-
Output A	300	0.014	50	0	442	-	-	-
Output B	-	-	92	0.012	500	-	-	-
Output C	BDL	-	BDL	-	396	-	-	-
Output D	-	-	BDL	-	640	-	-	-
River Clyde	-	-	-	-	-	-	98.09	-

Table 5.9: Total dissolved solids measurements for each sample and standard deviations (SD) for duplicates. Concentrations below detection limits are labelled “BDL”.

5.3.3 pH & Electrical Conductivity

Electrical conductivity (EC) varied significantly amongst the samples, ranging from 53 μS/cm in August’s Output C to 1301 μS/cm in December’s Output D (Table 5.10). EC generally stayed at similar levels or increased between the influent and effluent. Input EC was highest in December and increased significantly in all four outputs, likely due to the leaching of road salt and dissolved metals from the soil [63].

The pH of the samples remained broadly similar across both sample source and time, ranging from a minimum of 7.43 (Input, Aug19) to a maximum of 8.82 (Output A, May19) (Table 5.10). The input pH was highest in May and somewhat lower in August and December, suggesting that stormwater pH is being affected by factors such as traffic levels or rainfall. The pH of the stormwater increased after passing through the rain gardens, regardless of the soil mix or initial water pH. Soil samples were taken in November 2019 from leftover soil mix that had been stored in open bags next to the rain gardens. The pH values of soil mixes A, B, C and D were 8.25, 7.89, 8.14 and 7.98 respectively. The pH of a soil sample taken from rain garden B at the same time was 7.91, indicating that the pH of the soil had not been significantly impacted by planting or runoff in the approximately 6 months since rain garden installation. The observation that soils with higher pH cause a greater increase in pH in the effluent suggests that the pH of the chosen soil mix will directly impact the pH of any water released to the environment. A soil mix with a pH closer to neutral could therefore be preferable to reduce the chance of impacting the pH of local surface water. In this case, the pH of the River Clyde is 8.23, which is similar to that of the output from the rain gardens.

Sample	**EC (μS/cm)**				**pH**			
	May19	Aug19	Dec19	Mar21	May19	Aug19	Dec19	Mar21
Input	149.9	55.9	189.1	-	8.46	7.43	7.66	-
Output A	466	65.9	886.5	-	8.82	8.24	8.07	-
Output B	-	68.6	1011	-	-	7.82	7.77	-
Output C	139.3	53.0	796.5	-	8.47	8.07	8.11	-
Output D	-	84.1	1301	-	-	7.94	7.82	-
River Clyde	-	-	-	198.5	-	-	-	8.23

Table 5.10: Electrical conductivity (EC) and pH measurements for each sample.

5.3.4 Organics

Full chemical oxygen demand (COD) results are shown in Table 5.11 alongside the percentage change in COD after filtering. The highest COD was seen in the May input (134 mg/l), which was reduced to 87 mg/l and 49 mg/l in Outputs A and C respectively. COD in the May effluent was the same as or higher than that of the River Clyde, suggesting that when influent COD is high the rain gardens may not be able to reduce it to levels lower than in local surface waters. However, the rain gardens were still able to significantly reduce the input COD and therefore lower the impact of COD contamination. The rain garden effluent is many orders of magnitude lower in volume than the River Clyde and so will undergo significant dilution upon entering the river, further reducing any negative impact. COD measurements in August and December samples were much lower and quite consistent between the influent and effluent, ranging from 11 to 25 mg/l, suggesting that when input COD is low the rain gardens may not be able to reduce it beyond this minimum. The COD in Glasgow stormwater analysed previously (Chapter 2) ranged from 32 mg/l in Montague Street to over 1000 mg/l in Sauchiehall Street, so organic contamination in the rain garden influent was comparatively low. As with TSS, further testing should be carried out to determine the extent to which rain gardens could remove COD at the high concentrations seen in other areas of Glasgow.

Filtering of the water samples led to reductions in COD concentration of up to 97%, suggesting that much of the organic material in the samples was bound to suspended solids rather than dissolved. Output COD could therefore potentially be reduced further via the use of filters or geotextiles. Alternatively, reducing soil particle size would both increase COD capture by filtering and would also reduce soil permeability, therefore increasing the amount of time available for sedimentation of COD. However, care must be taken to avoid reducing infiltration to the point that overflow of the garden occurs.

Site		**COD (mg/l)**		**Percentage Difference**
		Unfiltered	Filtered (0.45 μm)	
Input	May19	133	4	97.0%
	Aug19	13	4	69.2%
	Dec19	22	-	-
Output A	May19	87	38	56.3%
	Aug19	14	13	7.1%
	Dec19	19	-	-
Output B	May19	-	-	-
	Aug19	14	14	0.0%
	Dec19	11	-	-
Output C	May19	49	6	87.8%
	Aug19	19	14	26.3%
	Dec19	14	-	-
Output D	May19	-	-	-
	Aug19	25	22	12.0%
	Dec19	14	-	-
River Clyde	Mar21	49	43	12.2%

Table 5.11: Chemical oxygen demand measurements for each of the samples, and the percentage difference resulting from filtering the samples through 0.45 μm filters.

5.3.5 Anions & Ammonium

The concentrations of ammonium and nitrite decreased or remained below detection limits between the input and outputs in most samples. In aerobic conditions, ammonium and nitrite may be converted to nitrates by nitrifying bacteria in the soil before these nitrates are taken up and used by plants [185] [63], and this may be the reason for the diminished ammonium and nitrite concentrations in the outputs. Nitrate concentrations increased in all outputs in May and in Outputs B and C in August, which could have been a result of nitrate generation from nitrite and ammonium. However, given that the concentration of phosphate also increased between the May influent and effluent while remaining low in other samples, nutrient loss from the rain garden soil soon after installation could also have contributed to the higher concentrations of nitrate in the May effluent.

Output water quality should be monitored closely after any future fertilisation of rain gardens, as the release of high concentrations of nutrients to the environment could cause eutrophication and algal blooms in receiving water bodies. Slowing the flow rate of water through the system by using different soil types or growing plants with deeper root structures (especially those that continue to grow year-round) are two possible methods of increasing nutrient removal in rain gardens [66].

Concentrations of fluoride, bromide, chloride, and sulphate increased or remained consistent between the input and all outputs in May, August and December. This suggests that the rain gardens are unable to remove these contaminants from the runoff and in addition could be leaching these substances from the soil.

Chloride concentrations were highest in December, particularly in Output D. As with TDS and EC, this is likely due to the impact of winter road salting as well as the additional salting of rain garden D. Chloride concentration, TDS and EC were lower in December's Outputs A and C than in Output B, suggesting that their finer soil mixes may be slightly better at adsorbing salt due to the higher surface area of the particles.

Output chloride concentrations in December exceeded SEPA's limit for freshwater (250 mg/l). Although in this case the River Clyde already contains some saltwater [115] and a significant dilution effect will be seen upon discharge, high concentrations of chloride being released to freshwater environments can increase metal mobilisation and harm local wildlife [25]. As many SUDS are not designed for salt removal, changes in road salting strategies such as reduced quantities or plant-based additives [186] may be required to minimise the impact of chloride on surface water. Chloride contamination is a particularly important consideration in infiltration-based SUDS, like many rain gardens, as this could result in contamination of groundwater [187].

Full results for anion and ammonium concentration measurement can be seen in Table 5.12.

		Ion Concentration (mg/l)					
		Input	Output A	Output B	Output C	Output D	River Clyde
F^-	May19	0.08	0.30	-	0.17	-	-
	Aug19	0.03	0.10	0.07	0.12	0.15	-
	Dec19	0.03	0.09	0.07	0.06	0.13	-
	Mar21	-	-	-	-	-	0.11
Cl^-	May19	6.54	23.94	-	8.00	-	-
	Aug19	0.52	1.06	1.48	1.13	1.10	-
	Dec19	49.79	335.42	411.91	294.81	533.56	-
	Mar21	-	-	-	-	-	23.75
NO_2^-	May19	0.12	0.05	-	0.02	-	-
	Aug19	0.011	0.001	BDL	0.004	0.017	-
	Dec19	BDL	BDL	BDL	BDL	BDL	-
	Mar21	-	-	-	-	-	0.061
Br^-	May19	0.08	0.30	-	0.17	-	-
	Aug19	BDL	0.043	0.077	0.063	0.106	-
	Dec19	0.026	0.379	0.353	BDL	0.539	-
	Mar21	-	-	-	-	-	0.032
NO_3^-	May19	0.27	3.66	-	1.01	-	-
	Aug19	0.13	BDL	0.29	0.15	BDL	-
	Dec19	BDL	BDL	BDL	BDL	BDL	-
	Mar21	-	-	-	-	-	3.18
PO_4^{3-}	May19	BDL	0.18	-	0.14	-	-
	Aug19	0.52	1.06	1.48	1.13	1.10	-
	Dec19	BDL	BDL	BDL	BDL	BDL	-
	Mar21	-	-	-	-	-	BDL
SO_4^{2-}	May19	1.68	15.57	-	2.43	-	-
	Aug19	0.32	1.28	1.31	0.88	2.95	-
	Dec19	5.42	7.31	8.74	6.35	10.33	-
	Mar21	-	-	-	-	-	11.33
NH_4^+	May19	-	-	-	-	-	-
	Aug19	0.06	BDL	BDL	BDL	BDL	-
	Dec19	0.022	0.017	0.015	0.029	0.0015	-
	Mar21	-	-	-	-	-	-

Table 5.12: Concentrations of anions in the samples as measured by ion chromatography, and ammonium concentration as measured using a KONE analyser. Blank values have been subtracted from measured values. Concentrations below detection limits are labelled “BDL”.

5.3.6 Elements

Tables 5.13, 5.14, and 5.15 show the dissolved concentrations of key potentially toxic elements (PTEs) that are commonly found in stormwater or are regulated in surface water, namely aluminium, arsenic, cadmium, chromium, copper, iron, lead, mercury, manganese, nickel, and zinc. The bioavailable concentrations of copper, manganese, nickel and zinc were determined for comparison with SEPA water quality standards, and the results are shown in Table 5.17. Full results of total and dissolved elements analysis and standard deviations are available in Appendix Tables A.11 - A.21.

In May, the dissolved concentrations of all PTEs decreased between the input and outputs or remained below detection limits, with the exception of arsenic (increased in both Outputs A and C) and manganese (increased in Output A). Similarly, in December, dissolved concentrations decreased or remained below detection limits for all outputs and all PTEs. Winter road salting can cause the desorption of PTEs from soil [1], resulting in the release of contaminated water to the environment. However, in this case it appears that the addition of salt has not caused significant desorption, as high concentrations of PTEs were not observed in the December effluent.

In contrast to May and December, dissolved concentrations of PTEs in August either increased or remained below detection limits in all outputs with the exception of mercury and zinc, which decreased in all outputs. Although August effluent concentrations were higher than those of the August influent, they remained lower than or comparable to the effluent concentrations seen in May and December. Differences in water treatment over the three months indicate that the rain gardens are effectively removing metals when input concentrations are higher (as in May and December) but are having less impact when concentrations are already low (as in August).

Concentrations of PTEs decreased the most in Output C in May and in Output B in August. In December, Outputs B, C, and D were similar in quality. As there is no clear pattern in PTE removal, it cannot be determined which, if any, of these soil particle size distributions is most suitable for treatment of PTE-contaminated stormwater.

The majority of PTE concentrations were higher in the rain garden effluent than in the River Clyde (Table 5.16 [Full total and dissolved results and standard deviations for the River Clyde are shown in Appendix Tables A.22 & A.23]). SEPA does not regulate SUDS output, so concentrations have instead been compared to SEPA's environmental quality standards (EQS) for surface freshwater [103], which are shown in Table 5.18.

According to these standards, concentrations of arsenic, manganese and zinc were acceptable in all outputs. Iron concentrations were acceptable in all outputs except May's Output A, and zinc concentrations were acceptable in December's Output D and all August outputs. Zinc concentrations exceeded the EQS in May and December's Outputs A, B, and C, cadmium and iron exceeded the limits in May's Output A, and copper concentrations exceeded the limits in all outputs. The detection limit of the measurement technique used was greater than the EQS for mercury and cadmium, so it cannot be determined whether these concentrations exceeded the EQS.

Although SEPA's EQS were exceeded in some cases, these standards apply to the surface water into which the rain gardens drain, rather than to the SUDS output itself. The effluent will be diluted significantly upon reaching the river. This fact, in combination with the facts that metal concentrations were often lowered by the rain gardens and many output concentrations did not exceed the EQS, suggests that the installation of SUDS like this is likely to reduce the negative impact of stormwater discharge on local surface water.

Element	**Concentration (mg/l)**		
	Input	Output A	Output C
Al	1.51	1.33	0.70
As	BDL	0.0061	0.0017
Cd	0.0031	0.0003	BDL
Cr	0.006	0.002	0.002
Cu	0.064	0.039	0.016
Fe	2.74	1.42	0.84
Hg	BDL	BDL	BDL
Mn	0.094	0.058	0.031
Ni	BDL	BDL	BDL
Pb	0.04	0.03	0.02
Zn	0.24	0.04	0.03

Table 5.13: Average dissolved concentrations of elements in the May 2019 samples. Samples were analysed in duplicate, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled "BDL".

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	0.202	0.447	0.329	0.494	0.519
As	BDL	0.0023	BDL	0.0052	0.0048
Cd	BDL	BDL	BDL	BDL	BDL
Cr	BDL	BDL	BDL	BDL	0.0009
Cu	0.0087	0.0091	0.0077	0.0096	0.0127
Fe	0.390	0.5942	0.4426	0.6497	0.7012
Hg	0.0002	BDL	BDL	BDL	BDL
Mn	0.011	0.014	0.0125	0.0191	0.0178
Ni	BDL	BDL	BDL	BDL	BDL
Pb	0.005	0.0076	0.0063	0.0081	0.0099
Zn	0.019	0.005	0.0023	0.0051	0.0088

Table 5.14: Average dissolved concentrations of elements in the August 2019 samples. Samples were analysed in duplicate, and blank values were subtracted from the measured values. Analysis of the input sample duplicate failed, so the input sample data displayed above is from a single sample rather than an average of a pair of duplicates. Concentrations below detection limits are labelled “BDL”.

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	1.16	0.56	0.19	0.21	0.19
As	BDL	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL	BDL
Cr	0.0059	0.0017	BDL	0.0019	BDL
Cu	0.026	0.016	0.008	0.006	0.005
Fe	2.530	0.688	0.259	0.251	0.244
Hg	BDL	BDL	BDL	BDL	BDL
Mn	0.048	0.011	0.005	0.004	0.005
Ni	0.0019	BDL	BDL	BDL	BDL
Pb	0.0134	0.0058	BDL	BDL	BDL
Zn	0.091	0.034	0.017	0.013	0.011

Table 5.15: Dissolved concentrations of elements in the December 2019 samples. Due to low sample volume, data displayed above is from single samples rather than pairs of duplicates. Blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”.

Element	**Concentration (mg/l)**
Al	0.182
As	0.0008
Cd	BDL
Cr	0.0024
Cu	BDL
Fe	0.3794
Hg	BDL
Mn	0.0245
Ni	BDL
Pb	0.0069
Zn	0.0224

Table 5.16: Average dissolved concentrations of elements in the March 2021 River Clyde samples. Samples were analysed in duplicate, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”.

May

Element	Bioavailable concentration (μg/l)		
	Input	Output A	Output C
Cu	17.99	13.2	4.5
Mn	93.7	57.9	31.3
Ni	1.0	1.0	1.0
Zn	188.22	27.11	26.1

August

Element	Bioavailable concentration (μg/l)				
	Input	Output A	Output B	Output C	Output D
Cu	1.05	2.01	1.11	1.74	2.04
Mn	11.30	14.00	9.01	19.10	16.43
Ni	0.64	1.11	0.85	0.99	0.96
Zn	18.8	4.21	1.89	4.15	6.79

December

Element	Bioavailable concentration (μg/l)				
	Input	Output A	Output B	Output C	Output D
Cu	3.37	2.99	1.13	1.15	0.73
Mn	25.08	11	3.27	4	3.62
Ni	1.16	1.41	1.09	1.36	1.13
Zn	85.45	25.24	12.49	10.23	8.15

Clyde

Element	Bioavailable concentration (μg/l)
Cu	0.46
Mn	24.5
Ni	1.2
Zn	16.09

Table 5.17: Bioavailable concentrations of dissolved elements as calculated using the M-BAT bioavailability tool. The detection limit was used in place of the measured nickel concentration when the measured value was below detection limts (1 μg/l for May samples, 1.2 μg/l for August and River Clyde samples, 1.5 μg/l for December samples).

Element	**EQS (μg/l)**
As	50 (total)
Cd	0.08 (dissolved)
Cu	1 (bioavailable, dissolved)
Fe	1,000 (dissolved)
Hg	0.07 (dissolved)
Mn	123 (bioavailable, dissolved)
Ni	4 (bioavailable, dissolved)
Zn	10.9 (bioavailable, dissolved)

Table 5.18: SEPA environmental quality standards (EQS) for discharge to surface waters [103].

5.3.7 Microbial Community Composition

Figure 5.5 shows relative abundances of the top 20 phyla and genera in each of the samples. There was a noticeable difference between the taxonomy of the influent and the effluent, indicating that microbial community composition was significantly altered by passing through the rain garden. However, effluent communities appear generally similar to one another, suggesting that the differences in soil particle size distribution between gardens are not having a notable impact on community composition in the output. The composition of all communities varied based on sampling date, which may be due to seasonal differences such as temperature and pollutant concentration (e.g. road salting in winter). Variation in effluent communities over time is also likely caused by the maturation of rain garden soil microbial communities as they become more established.

At phylum level, the influent was dominated by *Proteobacteria* at all sampling times. In May, *Patescibacteria* was more abundant than in August and December. Little is known about *Patescibacteria* ecology and behaviour [177] [173], but the phylum has been found in hydrocarbon-contaminated soils and petroleum-associated communities [168] [177]. The May influent had the highest COD of all the input samples, so the high abundance of *Patescibacteria* may be due to the availability of organic pollutants for degradation. In the May effluent (Outputs A & C), *Patescibacteria* was the most abundant phylum, but its abundance decreased in August and fell again in December – a similar trend to that seen in the influent.

The abundances of *Actinobacteriota*, *Acidobacteriota*, and *Bacteroidota* in the effluent increased consistently over the sampling period. *Acidobacteriota* can tolerate metals and hydrocarbons [86], so increasing concentrations of captured pollutants in the rain gardens may be leading to a greater abundance of *Acidobacteriota* in the soil and subsequently in the output of the gardens. Liguori et al. (2021) also identified *Actinobacteriota* and *Bacteroidota* as prevalent phyla in the influent and effluent of stormwater treatment devices [86].

At genus level, abundances of *Sphingomonas*, *Noviherbaspiriullum*, and *Methylobacterium-Methylorubrum* were greater in the influent than effluent though their relative abundances varied between the three sampling dates. *Sphingomonas* is associated with hydrocarbon degradation [168], *Noviherbaspirillum* has been found in oil-contaminated soils [188], and *Methylobacterium-Methylorubrum* may be involved in stress-resistance in extreme conditions [189]. The presence of these genera the influent is therefore likely associated with contamination in the urban stormwater.

In the August and December effluent, an uncultured member of the *Acidiferrobacteraceae* family was the most abundant genus. Some members of this family have been found to tolerate metals and oxidise iron and sulphur [190], so could be arising from the contaminated rain garden soil.

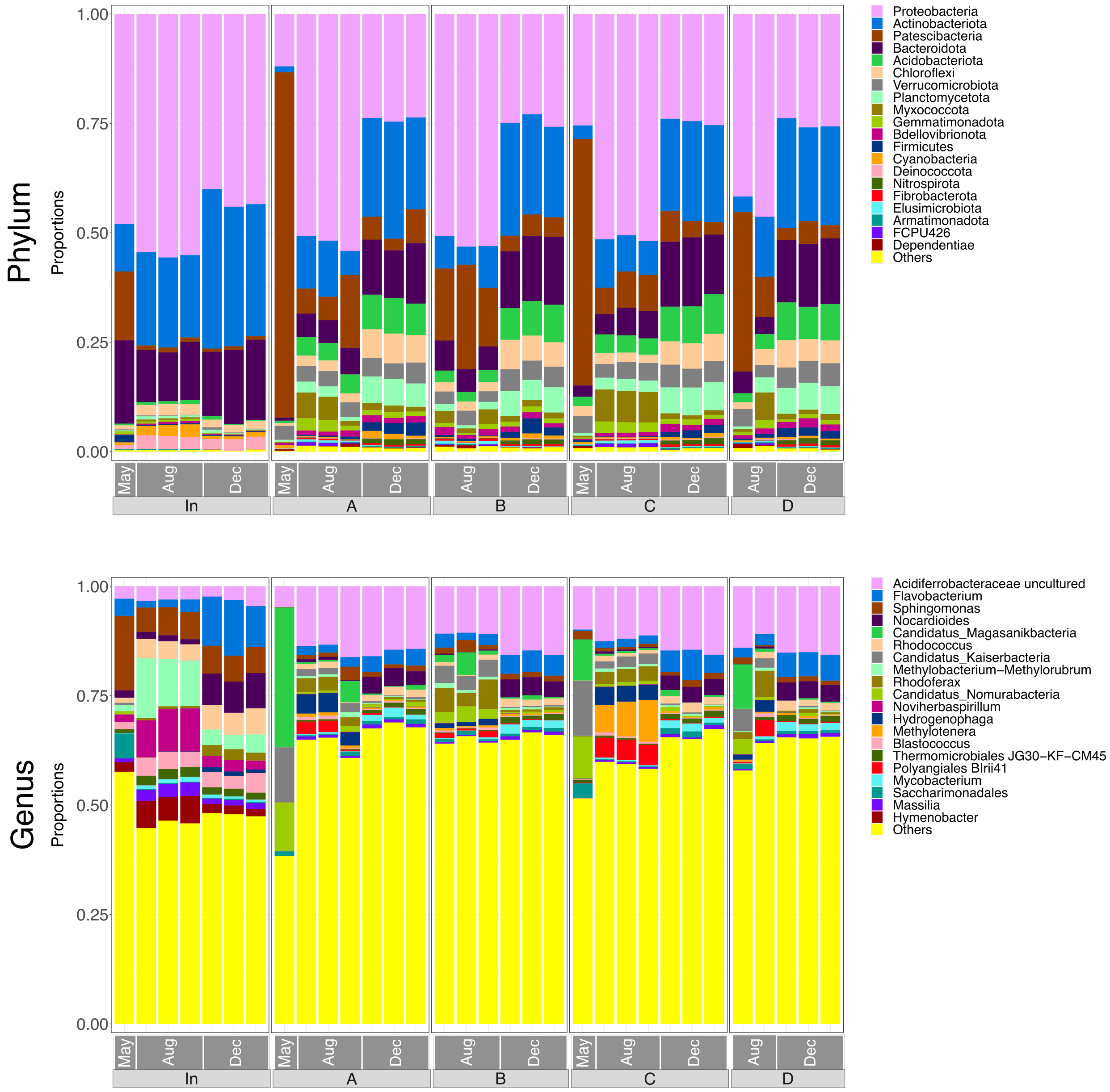

Figure 5.5: Bar plots representing the top 20 most abundant taxa at phylum and genus level. Taxa outside the top 20 are labelled "Others".

Appendix Figures A.3 - A.7 show the core microbiomes (defined as genera present in at least 95% of samples) of the input and the output from each rain garden. An uncultured *Acidiferrobacteraceae* genus was the most abundant member of the core microbiome in the output from each of the four rain gardens. This genus was not found in the core microbiome of the influent, suggesting it has arisen from the rain garden soil.

The most abundant core genera in the influent included *Sphingomonas*, *Rhodococcus*, *Flavobacterium*, and *Nocardioides*, all of which were also abundant in the effluent core microbiomes. These four genera are associated with hydrocarbon contamination and degradation [168] [175] so their presence is likely a result of stormwater pollution due to traffic.

5.3.8 Taxonomic & Functional Diversity

Taxonomic

No statistically significant difference in rarefied richness was found between any of the water samples (Figure 5.6). The input Shannon entropy was significantly lower than that of each of the four outputs, and Pielou's evenness and Simpson's index were also significantly lower in the input compared to Outputs B, C, and D. This suggests that although the overall number of species in the water remained consistent between the input and outputs, the community becomes more even after passing through the rain gardens – i.e. the species in the effluent are present in similar abundances rather than a few species dominating.

PCoA plots based on Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac showed output samples clustering by sampling date rather than by garden (Figure 5.7). This is significant as it illustrates that the differences in soil particle size distribution are having a smaller impact than changes occurring in the rain garden over time e.g. maturation of the microbial community in the rain garden or variation in input microbiology. Using all three distance measures, the input samples cluster separately from the outputs and also show some variation based on sampling date, indicating that there were seasonal changes in influent microbial communities that could impact the soil and effluent communities.

PERMANOVA identified date (May vs Aug vs Dec) followed by water source (In vs A vs B vs C vs D) as the factors having the greatest impact on beta diversity using Bray-Curtis and Weighted UniFrac, and vice versa for Unweighted UniFrac (Appendix Table A.26). This again illustrates that rain garden maturation and/or seasonal differences in input microbiology are having a significant impact on community composition in the effluent.

Functional

Functional richness was significantly lower in the influent than in the effluent (Figure 5.6). This suggests that organisms within the rain garden soil are capable of performing a wider range of functions than those in the original input stormwater, and that this is then impacting the output communities. No significant difference was found in Pielou's evenness between samples, but Shannon entropy and Simpson's index were significantly different between the input and Outputs B, C, and D, likely as a result of increased richness in the outputs.

PCoA using Hierarchical Meta-Storms to determine variation in functional diversity between samples yielded distinct clusters for the August and December output samples (Figure 5.7). May output samples neither clustered closely with one another nor with other outputs. Broadly speaking, the clustering appeared to become tighter over time, which suggests that the rain gardens became more functionally similar to each other over the sampling period. However, due to the low number of May samples, firm conclusions can't be drawn.

PERMANOVA identified sampling date as having the greatest impact on functional beta diversity (Appendix Table A.26). Other significant contributors included water source, COD, EC, nitrate, pH, phosphate, and sulphate. This could indicate that water chemistry is influencing the functions present in effluent communities. However, it may also be the case that the functional diversity of the water community (and likely also the soil community within the rain gardens) is influencing the water chemistry.

Disparity between functional and taxonomic beta diversity was also measured, giving an indication of community robustness (Figure 5.8). Overall, robustness was similar in each of the five samples. This indicates that the functions of each community are equally resilient to changes in taxonomy and therefore also equally resilient to changes in the environmental pressures that impact taxonomy.

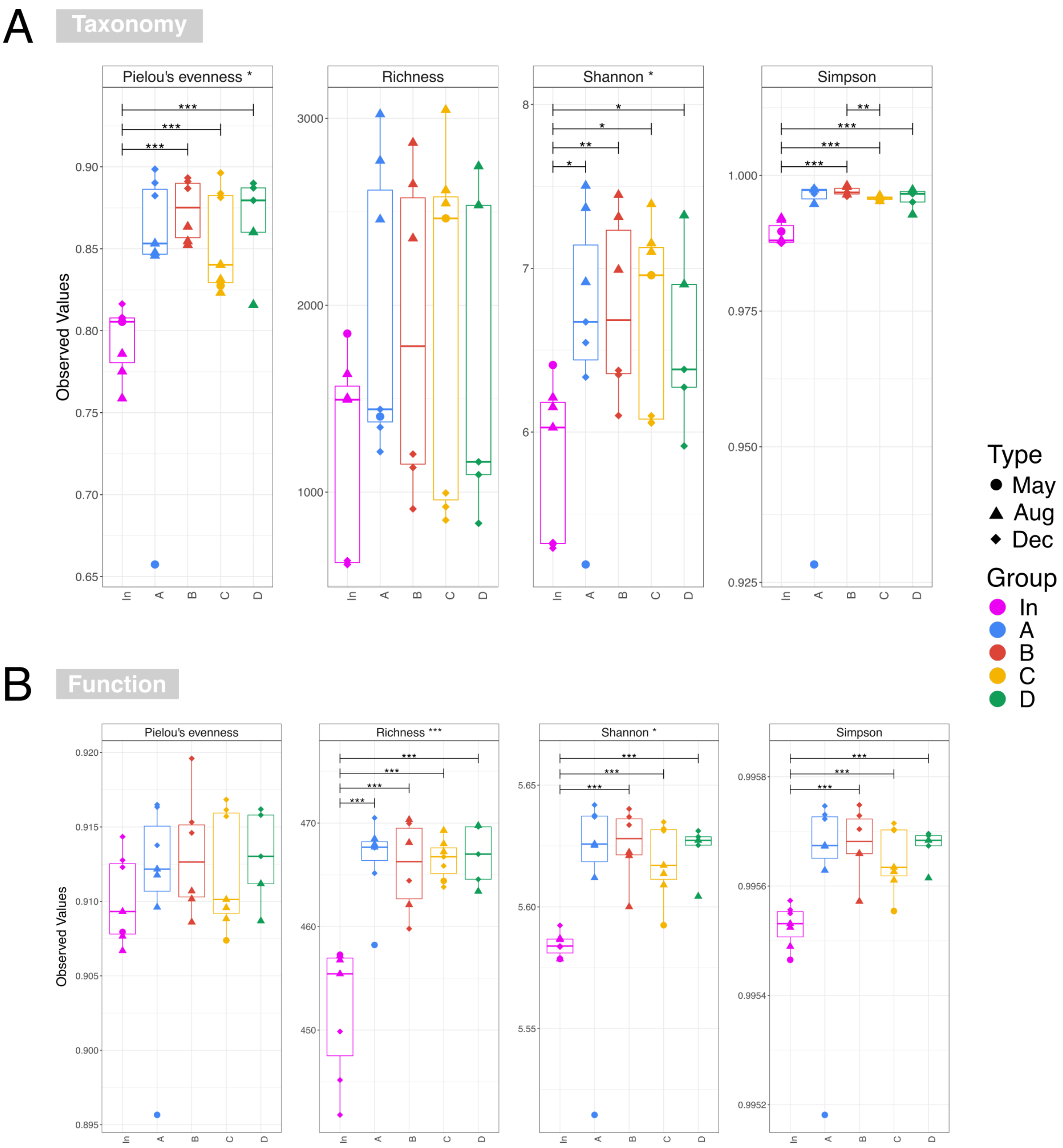

Figure 5.6: Alpha diversity estimates for (A) taxonomy and (B) function based on the PICRUSt2 prediction of the KEGG metagenome. Overall significance taking into account all five water sources is shown in the strip titles, and significant pairwise differences are represented by annotated lines connecting two categories.

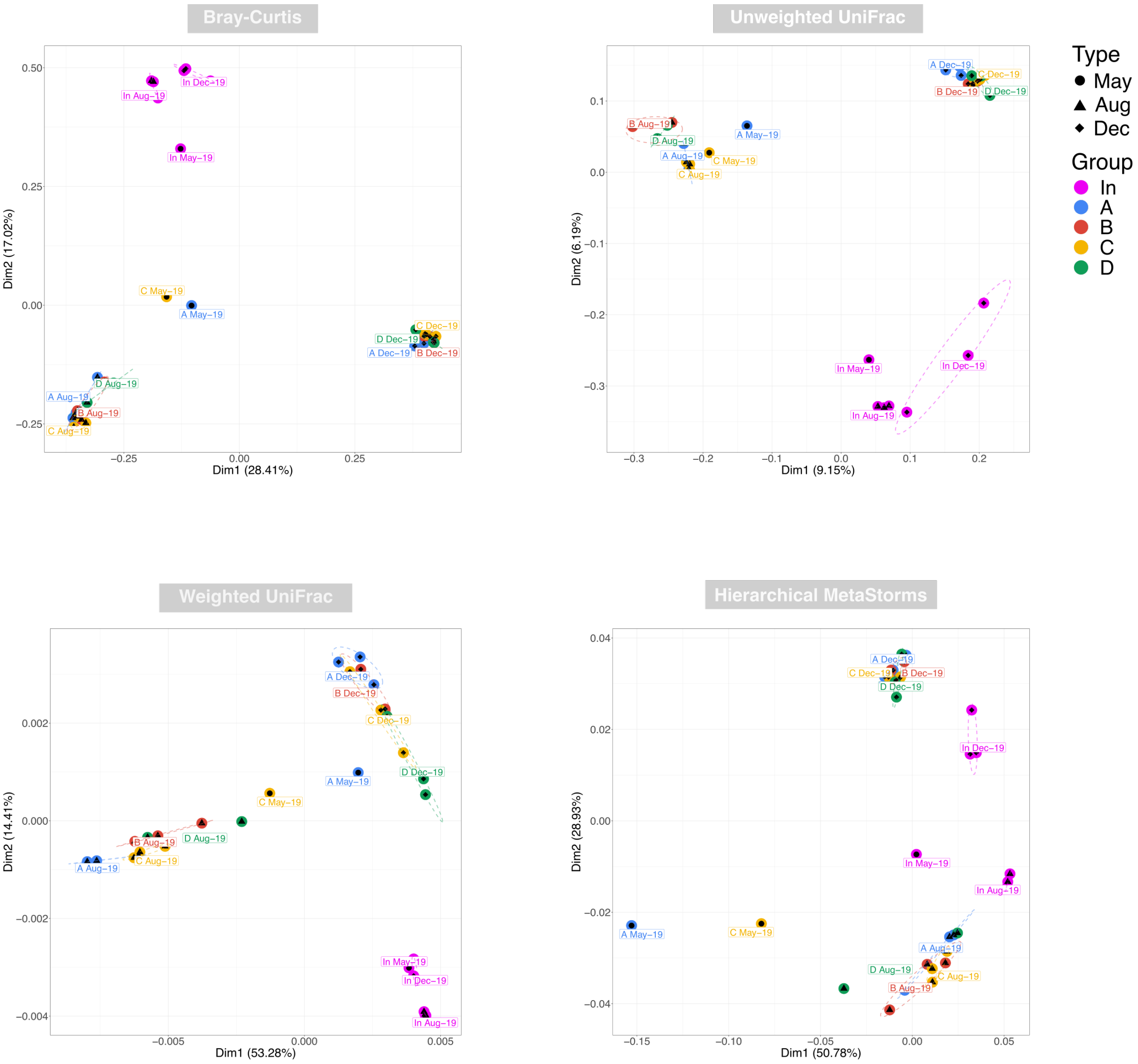

Figure 5.7: Principal co-ordinate analysis (PCoA) using Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac distances to represent taxonomic beta diversity and Hierarchical Meta-Storms to represent functional beta diversity. Ellipses for were drawn using 95% confidence intervals based on standard error of the ordination points for each category.

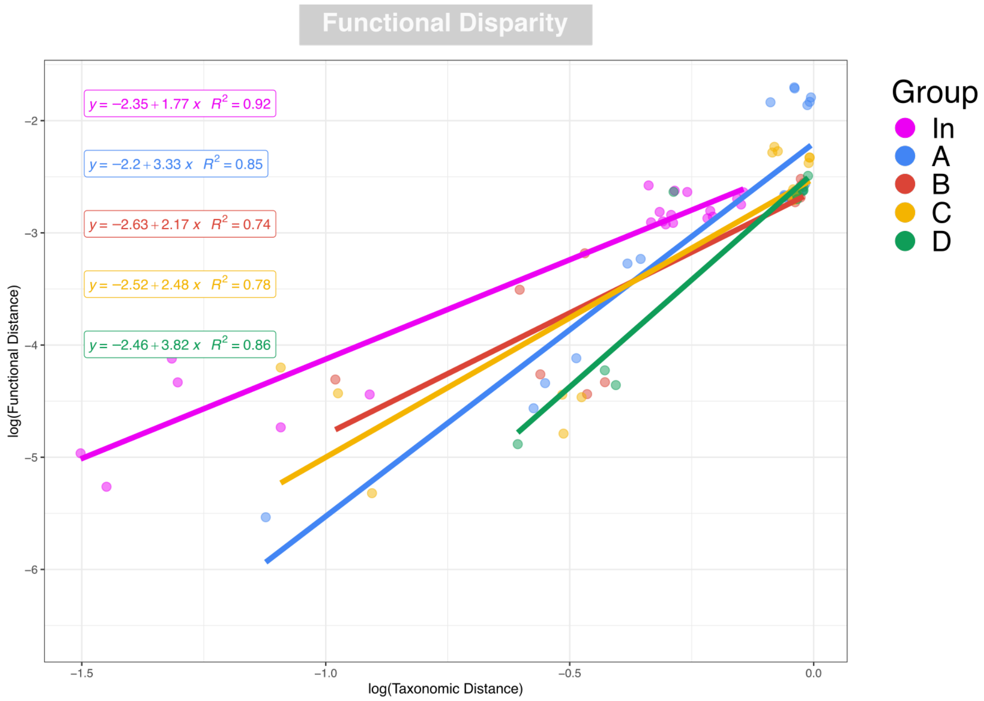

Figure 5.8: Functional disparity between taxonomic and functional distance is shown for each water source. Line gradient indicates the overall robustness of each community against perturbation.

In summary, the results of both taxonomic and diversity analysis indicate that effluent microbial communities are predominately impacted by sampling date. This could be due to maturation of the rain garden soil communities over time, but may also be a result of factors such as variation in input contamination or temperature/rainfall patterns. Effluent communities were distinct from those of the influent but not from other effluent communities from the same date, indicating that although passing through the rain garden significantly alters the water's microbial community, the four soil types are having a similar impact on community composition. Several of the prevalent members of the effluent communities can tolerate or degrade metals and hydrocarbons, suggesting that the soil community may have adapted to the contaminated conditions.

5.4 Conclusions

Each rain garden was able to effectively treat polluted stormwater, although some differences in removal were seen based on influent quality. In general, greater decreases in contaminant concentrations were seen when influent concentrations were high and less change was seen when influent concentrations were lower, suggesting that there may be minimum level to which rain gardens are able to reduce pollutants. Winter road salting appeared to increase TDS, EC, and chloride in the stormwater, but did not seem to lead to associated leaching of metals from the rain garden soil. Some variation in treatment effectiveness was seen between rain gardens, but no clear trend could be identified based on soil type. Future analysis should involve samples taken throughout each rainfall event and should make use of event mean concentrations to allow greater comparison between influent and effluent and between each of the four rain gardens.

Bacteria resistant to metals and capable of hydrocarbon degradation were found in both the influent and effluent, indicating that contamination in the stormwater and rain garden soil may influence community composition. Passing through the rain gardens increased the taxonomic evenness and overall diversity of the microbial community, as well as increasing the number of functions the community is capable of performing. Microbial community composition and function differed between the influent and effluent but showed little difference between output from different rain gardens. Therefore, soil particle size distribution may not be a significant contributor to community variation. Instead, the communities showed variation by date, suggesting that time of year, age of the rain garden (and associated community maturation), or varying pollutant levels may have more impact on effluent microbiology than soil type within the rain garden.

Variation in Contamination & Microbial Community Composition in Rain Garden Soil

Abstract

Rain gardens are sustainable urban drainage systems designed to reduce the volume of stormwater runoff while also providing in situ water treatment. The composition of the soil media used in rain gardens affects both their hydrology and their treatment capacity. Despite this, guidelines for soil composition vary worldwide and are often quite unspecific. In addition, although biodegradation is a key mechanism in rain garden water treatment, there has been little research into the impact of soil type on microbial community composition. This study analysed the chemistry and microbiology of soils from four rain gardens in Glasgow, Scotland, with the aim of determining how soil particle size distribution affects pollutant accumulation and microbial community structure. Chemical analyses included measurement of soil moisture content, potentially toxic elements, pH, and total petroleum hydrocarbons. Microbial analysis included alpha and beta diversity analysis of both taxonomy and function of the soil microbial communities. Clear differences between soils were found in both chemistry and microbiology, though further work is required to determine the precise reasons for these differences. Comparison to a control soil suggested that pollutants including

hydrocarbons and potentially toxic elements from stormwater influent were being successfully captured by the rain gardens. Comparison to soil guideline values shows that pollutant concentrations within the soils were overall low and not a cause for concern, although regular monitoring is advised to prevent unsafe build-up. Microbial analysis identified genera including *Flavobacterium*, which is known to degrade hydrocarbons and tolerate high concentrations of metals, both useful features for rain garden functioning. Soil pollutants and soil morphology were found to impact both taxonomic and functional diversity, which in turn could affect water treatment in the rain gardens.

6.1 Introduction

Rain gardens, also referred to as bioretention systems or bioretention cells, are a versatile form of sustainable urban drainage system (SUDS). They typically consist of a landscaped depression containing plants and soil media atop an aggregate drainage layer. Some rain gardens also contain underdrains for water transport (in cases where infiltration to the soil below is undesirable) or internal water storage to increase the removal of nitrogen [191].

Rain gardens are able to reduce peak flow rates and overall volumes of stormwater runoff while also improving water quality through adsorption, filtration, plant uptake, and biodegradation [71] [72] [191]. The extent to which they do this is affected by the composition and chemistry of the soil media within the rain garden. For example, using coarse material such as sand and gravel results in faster infiltration [40] [61]. Although this reduces the chance of the system overflowing during heavy rain, the high infiltration rate limits the time available for sequestration and degradation of pollutants. Conversely, fine media such as clay lower the infiltration rate, allowing more time for water treatment [182]. The greater surface area of clay and silt compared to coarser material also provides more opportunity for adsorption of metals and other contaminants [183]. Organic matter such as compost can increase water retention, metal adsorption, and biodegradation of organic contaminants [191]. The addition of amendments including biochar [191], coated sand [78], chitosan (sugar produced from shellfish exoskeletons) [79], and by-products of drinking water treatment [192] [193] can also enhance the removal of pollutants from stormwater.

As well as directly impacting pollutant removal through filtration and adsorption, soil composition and chemistry also affect the efficacy of water treatment by soil microbes. For example, biodegradation can be impacted by the availability of nutrients, soil pH, and the bioavailability of contaminants [194]. Hong et al. (2018) studied two rain gardens taking in either roof or car park runoff and found differences between rain garden microbial communities and those of nearby control soil, as well as differences between the rain garden communities. This included the presence of metal tolerant species in the rain garden that took in the more polluted car park runoff, indicating that soil chemistry and pollutant accumulation affect the resident microbial community [53]. Liguori et al. (2021) examined the microbial communities in two stormwater quality improvement devices (SQID), designed with different filter media but taking in the same stormwater. They found noticeable different communities in the two SQIDs, despite both systems receiving the same influent [86]. This suggests that filter media composition – or soil composition in the case of rain gardens – could have a significant impact on community composition and function.

Despite the known impact of soil media composition on hydrology and treatment capability, guidelines for rain garden design vary significantly across the world and can be vague – for example, by not defining the constituents of compost or topsoil [191] [39]. Local land use, surrounding soil types, and climate are also often not taken into account when choosing media [58]. An understanding of how the chosen soil media impacts pollutant removal is important from both an environmental standpoint (i.e. is the rain garden successfully treating stormwater prior to its release to local water bodies?) and a maintenance one (i.e. is the media likely to become saturated with contaminants and unable to provide further treatment?). Capture of pollutants in rain gardens generally occurs near the surface of the soil (specifically the top 10cm) [195] [66], so upper layers of soil may have to be removed if they become saturated [40]. However, processes such as biodegradation and phytoremediation can reduce concentrations of accumulated pollutants such as PTEs and hydrocarbons, meaning soil removal is required less frequently. Therefore, choosing appropriate soil media that is capable of both capturing pollutants and maximising the function of plants and the soil microbiome is key to designing effective rain gardens.

As part of the Glasgow Avenues programme (discussed previously in Chapter 5) [136], a pilot set of four rain gardens was installed in Washington Street, Glasgow, Scotland in early 2019. Each rain garden received the same influent and contained the same plant species but varied in soil particle size distribution. This study presents the results of chemical and microbial analysis of soil samples taken from the rain gardens in March 2021, with the aim of determining the impact of soil type on pollutant accumulation and microbial community composition.

6.2 Materials & Methods

6.2.1 Rain Garden Construction

Four rain gardens containing different soil mixes were constructed on Washington Street, Glasgow in early 2019 by Civic Engineers and idverde. Full details of the system are discussed in Chapter 5. Briefly, soil mixes A and C contained greater proportions of fines whereas mixes B and D were coarser (Figure 6.1, detailed table available in Chapter 5).

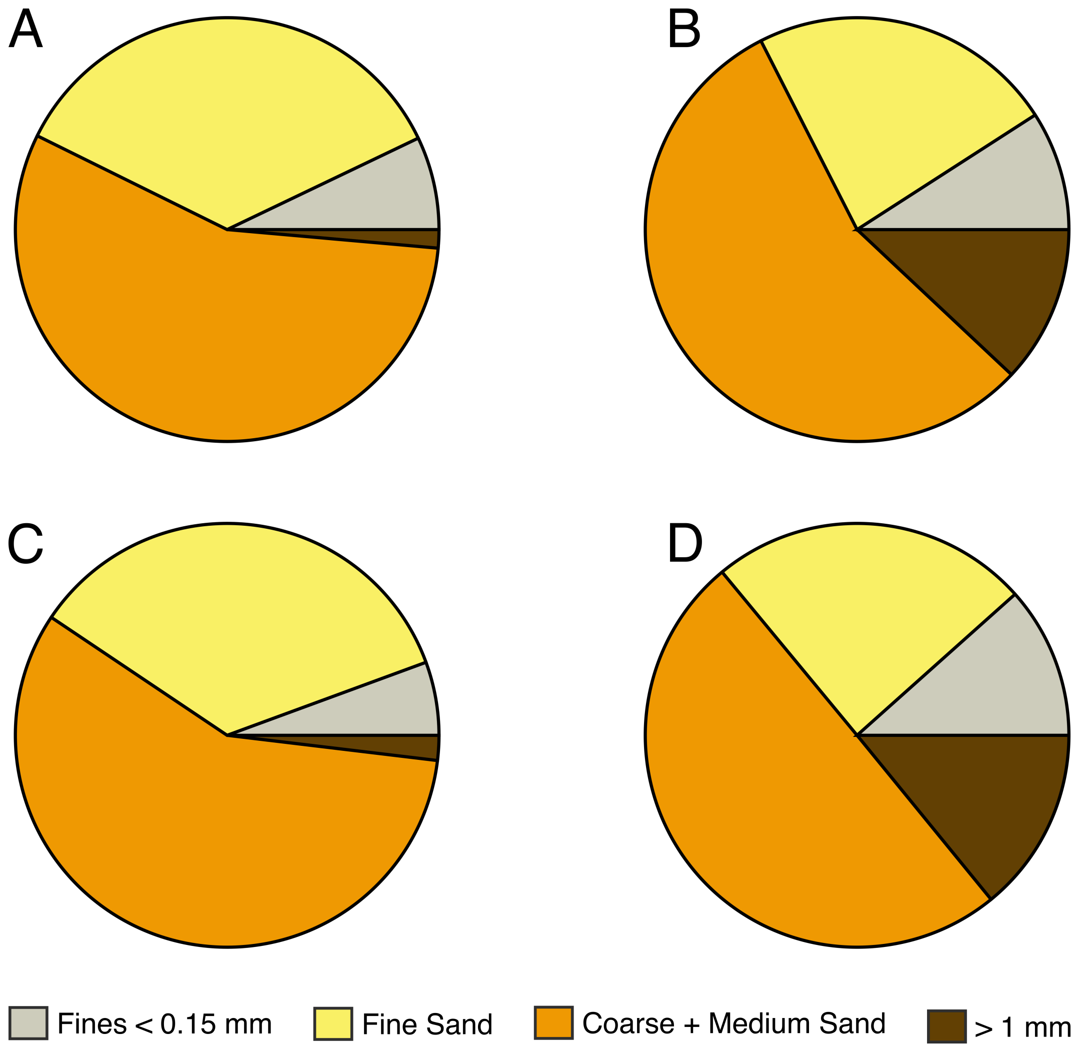

Figure 6.1: Visual summary of particle size distribution in each rain garden soil. Data provided by European Turfgrass Laboratories/idverde.

6.2.2 Sampling

Soil samples were collected from the Washington Street rain gardens on the 11th of March 2021, after approximately two years of rain garden operation. Samples were collected from the top 10 cm of each rain garden, at the output end of the bed (soils A, B, C, D). Soil was also collected from the top 10 cm of unused soil mix B, which had been stored in an open bag on Washington Street since rain garden installation (soil BB). Samples were stored at 4° C until analysis.

6.2.3 Chemical Analysis

Electrical conductivity (EC) and pH were measured at room temperature using a Mettler Toledo MPC277 pH/EC meter. Soil samples were air-dried and approximately 10 g of sieved (2 mm) soil was added to 20 ml of ultrapure (18.2 MΩ-cm) water. The soil and water were mixed intermittently for 30 minutes then allowed to settle for 1 hour before EC and pH measurement.

Elemental analysis was performed using inductively coupled plasma – optical emission spectroscopy (ICP-OES). Samples for total elements analysis were prepared by acid digestion of soil that had been oven-dried at 105° C overnight and sieved (2 mm). Acid digestion was carried out in a MARS 6 microwave digestion system (CEM), using the pre-installed programme US EPA 3051A. Samples were allowed to "pre-digest" with the acid mix in the microwave vessels for 1.5 hours before microwave digestion. Digested samples were made up to 50 ml in ultrapure water before a 10 ml subsample was filtered through a 0.45 μm syringe filter (mixed cellulose esters) for ICP-OES analysis. Digested samples were stored at 4° C. Details of the digestion mixtures, soil weights, and MARS 6 settings are shown in Tables 6.1, 6.2, and 6.3.

ICP-OES was carried out using iCAP 6000 Series ICP Emission Spectrometer (Thermo Scientific).

	Total elements
Sample	0.5 g soil + 9 ml HNO3 + 1 ml HCl
Blank	9 ml HNO3 + 1 ml HCl

Table 6.1: Contents of sample and blank vessels used for acid digestion.

Soil	**Weight (g)**	
A	0.4959	0.4906
B	0.4960	0.4948
C	0.5021	0.4954
D	0.4885	0.4952
BB	0.4895	0.5055

Table 6.2: Weights of sieved soil used for acid digestion.

Step	Pressure (psi)	Power (W)	Ramp (min)	Temperature (°C)	Hold (min)
1	800	900-1050	5.5	175	
2	800	900-1050		175	4.5

Table 6.3: Settings for acid digestion in CEM MARS 6 microwave, using the pre-installed US EPA 3015a programme.

To determine moisture content, soil samples in porcelain crucibles were weighed before and after heating in an oven overnight at 105° C. Moisture content (%) was calculated using the equation $((Final\_weight(g) - Initial\_weight(g))/Initial\_weight(g)) = Moisturecontent(\%.$

Soil samples for total petroleum hydrocarbon (TPH) analysis were dried overnight at 105° C, sieved (2 mm), and extracted into hexane:acetone using a MARS 6 microwave. Sample B was prepared in duplicate (B2). An additional sample from rain garden A was sieved and extracted without drying (referred to as “A_wet”). A sand control and a solvent-only blank were also prepared. Soil weights are shown in Table 6.4.

Sample	Weight (g)
A	9.802
B	9.9195
B2	9.9089
C	9.9518
D	9.7109
BB	9.9796
A_wet	9.8189
Sand	9.7254
Blank (solvent only)	-

Table 6.4: Weights of soil used for solvent extraction.

Prior to the addition of solvent, 20 μl of 1000 ppm tricosane was added to each soil sample to produce a 20 ppm tricosane concentration in the final 1 ml extract. 25 ml of 1:1 hexane:acetone was added to each sample. The MARS 6 programme US EPA 3546 115C (Table 6.5) was used for extraction. Extracted samples were made back up to 25 ml with hexane:acetone before being concentrated to 1 ml using a nitrogen blowdown evaporator. After concentration, 40 μl of 500 ppm chlorooctadecane was added to each sample to produce a final concentration of 20 ppm. Samples were further concentrated to the volumes shown in Table 6.6. Samples were stored at 4° C until analysis.

Step	Pressure (psi)	Power (W)	Ramp (min)	Temperature (°C)	Hold (min)
1	800	980-1060	15	115	
2	800	980-1060		115	15

Table 6.5: Settings used for solvent extraction in CEM MARS 6 microwave, using the pre-installed US EPA 3546 115C.

Sample	Final Volume (ml)
A	0.1
B	0.2
B2	0.2
C	0.5
D	0.5
BB	0.1
A_wet	0.5
Sand	0.1
Blank (solvent only)	0.1

Table 6.6: Extract volumes used for GC-FID analysis of TPH.

GC-FID was carried out using a Finnigan Focus GC (Thermo Scientific), using the settings shown in Table 6.7.

Inlet temperature (°C)	250
Detector temperature (°C)	340
Split flow (ml/min)	20
Split ratio	10
Carrier (ml/min)	2
Ramp rate (°C/min)	10
Ramp to temperature (°C)	300
Hold (min)	20

Table 6.7: GC-FID settings used for TPH analysis of hexane:acetone extracts.

6.2.4 Microbial Community Analysis

DNA extraction was performed using the QIAGEN DNeasy PowerSoil kit, according to the manufacturer's instructions. Soil weights are shown in Table 6.8. Soil was sieved (2 mm) prior to weighing and extraction. Five replicates were prepared from each sampling site. An extraction blank containing no soil was also prepared. DNA was eluted into 50 μl of elution buffer and stored at -80° C. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. DNA concentrations are available in Appendix Table A.6.

Soil	Mass (mg)				
A	243.1	240.3	273.8	272.3	271.7
B	239.9	249.3	244.0	239.4	264.4
C	253.8	254.8	258.7	269.3	264.9
D	280.2	251.3	259.4	260.4	271.0
BB	241.7	263.7	281.2	253.3	263.1

Table 6.8: Weights of sieved soil samples that were then extracted using a PowerSoil kit.

Sequencing and bioinformatics were carried out as in Chapter 4. Final summary statistics for the 25 samples (20,355 ASVs) used in this study were [Minimum: 14,109; 1st Quantile: 19,350; Median: 24,426; Mean: 23,730; 3rd Quantile: 26,626; Maximum: 35,133].

Statistical analyses were performed in R (version 4.1.2), as described in Chapter 4. All figures in this study were generated using R's ggplot2 package [158]. The following annotations are used to denote significance: '***' ($p \leq 0.001$), '**' ($p \leq 0.01$), '*' ($p \leq 0.05$), and '.' ($p \leq 0.1$).

6.3 Results & Discussion

6.3.1 pH & Electrical Conductivity

Soil pH was similar in the five samples, ranging from 6.93 in soil B to 7.44 in soil D (Table 6.9). Soil pH within the rain gardens impacts plant health and water treatment, with higher pH generally resulting in better adsorption of metals [196]. Woods-Ballard et al. (2015) suggest a pH range of 5.5 to 8.5 for rain garden soil [1] and the United States Environmental Protection Agency states that a soil pH of 6.0 to 7.5 is optimal for green infrastructure [197]. The pH values measured in the four Washington Street rain gardens were within these recommended ranges. Soil pH should be monitored regularly to ensure the continued health of plants and the soil microbiome, as well as to prevent leaching of captured metals if the soil pH falls.

Electrical conductivity (EC) was slightly lower in soil B than in the other four soils (Table 6.9). This may have been due to differing levels of salt washout between soils, or due to variations in salt accumulation throughout the soil, which could be detected by further sampling. Garden D had been subjected to additional salting during the winter of 2019-2020, at a concentration of 10 g/m^2 every other working day. Despite this, soil D's EC was only slightly higher than that of the unsalted rain gardens, indicating that the majority of the salt had either not been trapped by the soil D at all or had been washed out in the months since salt application. The EC of the Washington Street soils was lower than that seen in urban soils in Seville, Spain [198] and Chicago, USA [199], and similar to the lowest values seen in Maribor, Slovenia [200]. The low EC values observed in the rain gardens suggest that salt accumulation is not occurring to a significant extent. However, high levels of road salt in winter stormwater may result in leaching of metals and nitrate [201] or acute damage to plants even if the salt is not retained by the soil in the longer term. Further soil sampling over a greater length of time could indicate whether low EC is due to lack of salt capture or due to capture and subsequent leaching.

Sample	**EC (μS/cm)**	**pH**
A	40.73	7.14
B	22.34	6.93
C	42.95	7.34
D	49.31	7.44
BB	39.49	7.24

Table 6.9: Electrical conductivity (EC) and pH measurements for each sample.

6.3.2 Soil Moisture

Soil moisture was similar in all four rain garden samples and was slightly lower in soil BB (Table 6.10). This difference is likely due to the fact that the rain gardens receive additional water in the form of road runoff, whereas soil BB receives only rainwater. Some moisture is required for biodegradation of hydrocarbons but the presence of too much water can reduce oxygen availability and inhibit degradation [202] [66].

While some variation in moisture content over time is unavoidable due to the impact of rainfall and evaporation/evapotranspiration, biodegradation can be maximised by choosing soil media with a balance between water retention and infiltration, keeping moisture present as often as possible while avoiding constant ponding.

Sample	Moisture Content %)
A	12.3
B	11.6
B2	12.1
C	13.1
D	11.6
BB	9.6

Table 6.10: Moisture content of soil samples.

6.3.3 Elements

Table 6.11 shows the total concentrations of elements in the five soils. Concentrations of all measured elements were lowest in soil BB, with the exception of arsenic (lowest in soil B) and silicon (lowest in soil A). This indicates that the rain garden soil is trapping contaminants from the input stormwater, resulting in increased elemental concentrations. Potentially toxic elements (PTEs) commonly found in stormwater include aluminium, arsenic, cadmium, chromium, copper, iron, lead, mercury, manganese, nickel, and zinc. In the four rain gardens, concentrations of all PTEs except mercury were lowest in soil B. Mercury concentrations were below detection limits in soils C and D and highest in soil A. The concentration of arsenic was highest in soil D and concentrations of all other PTEs were highest in soil C.

Element	**Concentration (mg/kg)**				
	A	B	C	D	BB
Al	654	494	1295	1164	344
As	0.62	0.34	0.55	0.83	0.47
Ba	6.70	4.80	13.40	9.59	3.02
Ca	291	288	671	535	168
Cd	0.25	0.14	0.64	0.29	0.08
Co	0.62	0.44	1.21	0.98	0.24
Cr	2.46	1.89	4.24	3.75	1.48
Cu	15.4	11.6	19.2	17.0	7.0
Fe	1784	1361	3180	2694	1266
Hg	0.39	0.11	BDL	BDL	BDL
K	80.8	59.2	162.7	176.3	45.0
Li	0.88	0.63	1.85	1.89	0.47
Mg	343	230	797	532	100
Mn	32.3	17.1	47.3	45.9	11.9
Mo	0.36	0.19	0.34	0.28	0.14
Na	BDL	BDL	BDL	BDL	BDL
Ni	1.53	1.24	3.02	2.84	0.56
P	53.6	45.8	104.1	88.3	29.3
Pb	7.15	5.46	12.54	12.51	4.56
S	85.6	51.5	180.5	103.7	23.6
Sb	0.045	0.045	0.251	0.204	0.005
Se	BDL	BDL	BDL	BDL	BDL
Si	58.2	76.9	59.3	69.2	89.8
Sn	0.99	0.63	1.85	1.25	0.42
Sr	1.66	1.29	3.61	3.01	0.77
Ti	30.0	21.1	69.3	35.1	7.9
V	3.18	2.04	6.49	4.30	1.26
Zn	22.6	12.8	52.0	26.1	4.4

Table 6.11: Average total concentration of elements in the soil samples. Samples were analysed in duplicate and blank values were subtracted from the measured values. Concentrations below detection limits are labelled "BDL". Standard deviations are shown in Appendix Table A.24

In the UK, soil guideline values (SGVs) are used as an indicator of long-term risks to human health and can be used to identify sites where further analysis and risk assessment are necessary [203]. The SGVs for arsenic, cadmium, and selenium are shown in Table 6.12. Previous SGVs for mercury and nickel are also shown for comparison, although these values are no longer used for official measurements. SGVs are shown for both residential and commercial areas. Concentrations of these elements in all five soils were well below the SGVs, indicating that the rain garden soil is unlikely to pose a threat to human health and would not have to be treated as contaminated waste in the event of its removal.

Element	**SGV (residential, commercial)(mg/kg)**
As	32, 640
Cd	10, 230
Se	350, 13000
Hg*	1, 26
Ni*	130, 1800

Table 6.12: Soil guideline values (SGVs) for arsenic, cadmium, selenium, mercury, and nickel [203]. *Note that SGVs for mercury and nickel are not currently in use.

Table 6.13 shows PTE concentrations in urban soils in Glasgow and Scotland measured by the British Geological Survey (BGS) and the UK Soil and Herbage Survey (UKSHS) respectively [204]. Concentrations in the rain garden soils were generally either below or similar to the lower end of the ranges found by the BGS and UKSHS. This again indicates that the rain garden soil is unlikely to pose any additional threat to human health.

Element	**BGS range (Glasgow) (mg/kg))**	**UKSHS range (Scotland) (mg/kg)**
Arsenic	1 - 283	NA
Cadmium	0.25 – 16	0.11 – 0.62
Chromium	38 – 4286	17.8 – 60
Copper	14 – 3690	15.5 – 62.7
Lead	13 – 5001	39.8 – 290
Mercury	NA	0.07 – 0.78
Nickel	6 – 1038	9.9 – 51.3
Zinc	39 – 1781	51 - 212

Table 6.13: Concentrations of PTEs in urban soils in Glasgow and Scotland, measured by the British Geological Survey (BGS) and UK Soil and Herbage Survey (UKSHS). Data from Dobbie, 2011 [204]

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Analysis of PTE concentrations in rain garden soils has been carried out in South Korea by Hong et al. (2018) (As, Cd, Cr, Cu, Pb, Zn) and in Australia by Al Ameri et al. (2018) (Cd, Cu, Pb, Zn). Concentrations of all PTEs except arsenic were significantly lower in the South Korean rain gardens than in this study, although the systems in both cases were of a similar age (2-4 years) [53]. However, the South Korean rain gardens received their input from roof and car park runoff, which is likely to be less polluted than the road runoff received by the Washington Street rain gardens. The concentrations in this study were within the range seen in the Australian rain gardens, which were aged between 9 and 16 years old and covered a variety of land uses/urban densities [67]. The concentrations of PTEs in rain garden soils may therefore also be impacted by input concentrations rather than by age alone. This should be taken into account when designing maintenance regimes, to avoid either premature removal of unsaturated soils or delayed removal of saturated, toxic soils.

6.3.4 Organics

The concentration of total petroleum hydrocarbons (TPH) was lowest in soil BB at only 3 mg/kg, comparable to the 2 mg/kg in the sand control. Of the rain gardens, garden B contained the lowest concentration of TPH with an average of 27 mg/kg. The increase in TPH concentration between soil BB and soil B suggests that TPH contamination is mainly arising from the road runoff rather than through atmospheric deposition. The highest concentration of TPH was 132 mg/kg in soil C, approximately double the next highest concentration. This could indicate that soil C is best at trapping TPH or that less biodegradation is taking place in this soil. All TPH concentrations are shown in Table 6.14.

Le Fevre et al. (2012) analysed 71 rain garden soils and found that TPH concentrations were below detection limits or less than 3 μg/kg, significantly lower than the concentrations found in this study [205]. This difference could be due to variation in input TPH concentrations to the rain gardens or due to differing levels of biodegradation. Further analysis of Washington Street soil over a longer time period combined with microbiological analysis could indicate at what level biodegradation of organics is occurring. Analysis of urban soils in Glasgow [206] and London [207] yielded TPH concentrations ranging from 79 – 2505 mg/kg (median 272 mg/kg) and 72 – 4673 mg/kg (median 373 mg/kg) respectively. Of the TPH concentrations measured in the Washington Street rain gardens, only that of soil C fell within these ranges. The TPH concentrations in all four rain gardens were below the median concentrations found in Glasgow and London soils. This may be due to the relatively recent installation of the rain gardens compared to the age of urban soils or due to TPH biodegradation by plants and microbes in the rain garden. Further analysis of the rain gardens and nearby urban soils over time could be done to determine the reason for this difference in concentration.

Future analysis of organic contamination should also consider determining the concentration of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), both of which can cause significant damage to the environment as well as human health [206] [207].

Sample	TPH Concentration (mg/kg)
A	68
B	28
B2	26
C	132
D	42
BB	3
A_wet	59
Sand	2

Table 6.14: Concentrations of total petroleum hydrocarbons in each sample

6.3.5 Microbial Community Composition

Figure 6.2 shows relative abundances in each sample of the overall top 20 phyla and genera. At phylum level, community composition was broadly similar, with *Proteobacteria* and *Actinobacteriota* making up the greatest proportion of all five soils. *Bacteroidota* and the common soil phyla *Verrucomicrobiota* [162] & *Planctomycetota* [165] were slightly less abundant in soil BB, while *Patescibacteria* was more abundant. *Patescibacteria* has been found in soils contaminated with hydrocarbons [168] but, given the low TPH concentration in soil BB, it may be that other as yet undiscovered aspects of *Patescibacteria* behaviour are driving the phylum's increased abundance in this soil [177]. The proportion of *Firmicutes* was greater in soils D and BB, and *Cyanobacteria* was more abundant in soil B than the other four soils.

At genus level, *Saccharimonadales* was more abundant in soil BB than in the other soils and *Flavobacterium* was highest in soil C. *Flavobacterium* is tolerant of metals and hydrocarbons [193] [168], both of which were highest in concentration in soil C. An uncultured *Rhizobiales Incertae Sedis* genus was the most abundant in all five soils. This was also the most abundant genus in each soil's core microbiome (defined as genera present in at least 95% of samples, Appendix Figures A.8 - A.12). The family *Rhizobiales* contains common soil bacteria that are often associated with plant roots. *Rhizobiales* species play various roles in the soil including nitrogen fixation and hydrocarbon degradation [176] [177].

Other abundant members of the core microbiome were similar in each soil and included *Nocardioides*, *Flavobacterium*, and *Sphingomonas*, all of which are associated with hydrocarbon degradation [175] [168]. This suggests that hydrocarbon degradation may be carried out in each soil type, despite the wide range of TPH concentrations found (3 mg/kg - 132 mg/kg).

Cyanobacteriia Sep-B3 was the third most abundant core organism in soil B and did not appear in the core microbiome of any other soil. Of the top 10 most abundant core taxa, *Cyanobacteriia Sep-B3* is the only genus to be unique to a particular soil. *Cyanobacteria* produce oxygen via photosynthesis, which can allow aerobic degradation of contaminants including TPH, and some species are able to fix nitrogen, which degraders can use as a nutrient source [208]. It is unclear why *Cyanobacteriia Sep-B3* was identified only in soil B's core microbiome and why the abundance of the phylum *Cyanobacteria* was higher in this soil.

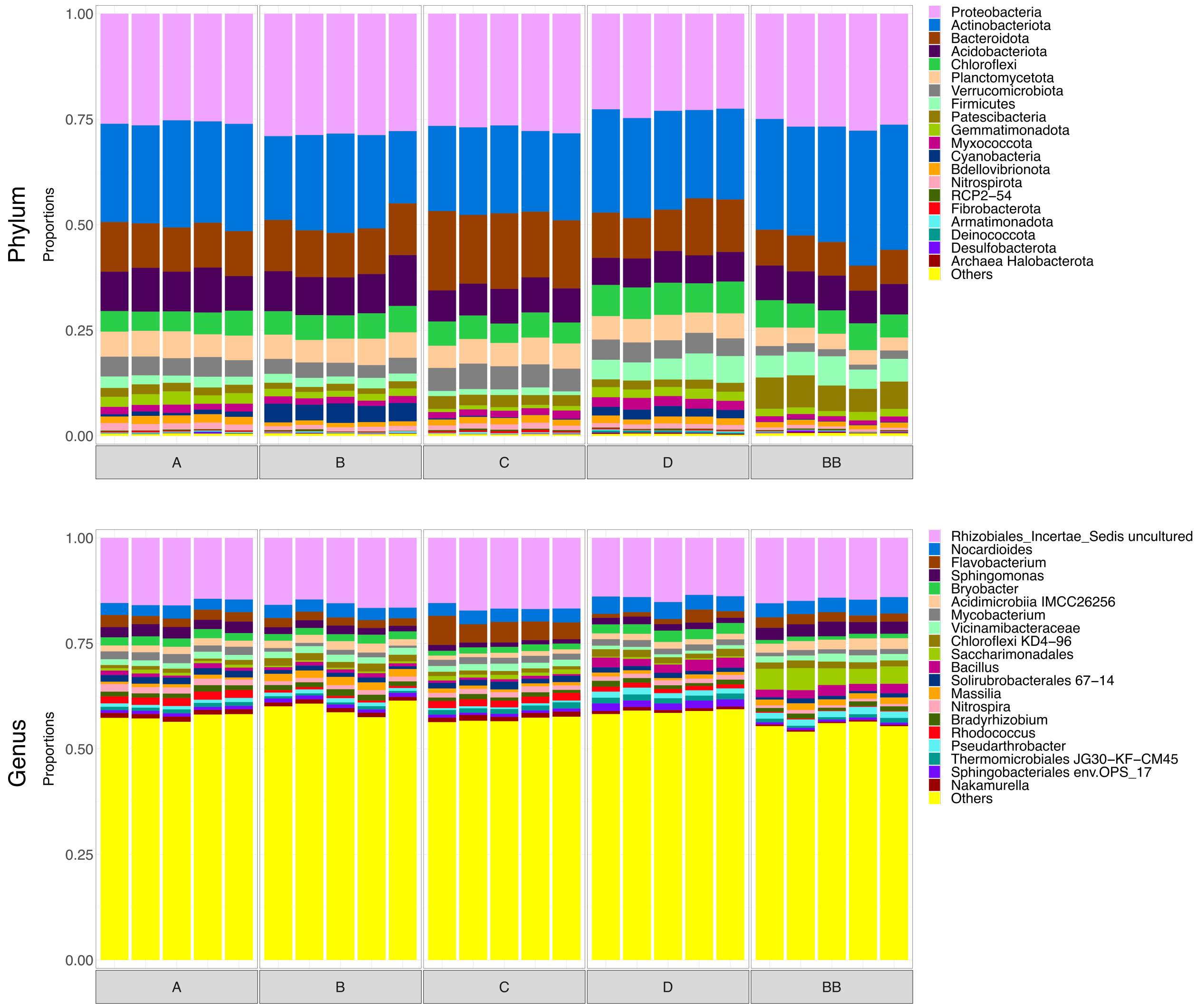

Figure 6.2: Bar plots representing the top 20 most abundant taxa at phylum and genus level. Taxa outside the top 20 are labelled “Others”.

6.3.6 Taxonomic & Functional Diversity

Taxonomic

No statistically significant difference was observed in rarefied richness or Shannon entropy, indicating that overall diversity and number of species was not highly impacted by soil particle size distribution (Figure 6.3). However, significant variation in Pielou’s evenness and Simpson’s index was seen between the five soil types. Pairwise differences in Pielou’s evenness were present between several soil types, with soils B and BB having the lowest evenness and C the highest. Simpson's index was also significantly higher in soil C than B and BB. This indicates that soil C's community is more balanced in terms of abundance whereas communities in soils B and BB are dominated by a small number of ASVs. The similarity in evenness and Simpson's index between soils B and BB suggests that the addition of plants and stormwater to the soil may not be having a significant impact on these aspects of diversity. This is important as the diversity of the initial microbial community in a soil could then play a key role in future diversity. More even and more diverse communities have been shown to be able to remove pollutants more quickly and effectively as well as potentially being more robust against variations in input water quality [84] [85], so choice of soil could significantly affect water treatment.

Principal co-ordinates analysis (PCoA) plots based on Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac distance measures showed distinct clustering of soil types (Figure 6.4). PERMANOVA (Appendix Table A.27) confirmed that soil source, soil chemistry, and soil morphology all made significant contributions to variation in beta diversity, with soil source (i.e. A vs B vs C vs D vs BB) having the greatest impact. The choice of soil mix used in rain gardens may therefore have to be considered more carefully in order to encourage the growth of a suitable microbial community.

Functional

Alpha diversity analysis of predicted function based on Kegg Orthologs (KOs) was also carried out (Figure 6.3). Significant variation in all four measures was observed between the five soil types. Soil C had the lowest Pielou's evenness, Shannon entropy, and Simpson index, while D had the highest. Richess was lowest in soil D and statistically similar between the four other soils. The regular application of salt directly to garden D during the winter of 2019-2020 may have limited the types of species able to tolerate the soil conditions, reducing the number of potential functions.

PCoA using Hierarchical Meta-Storms for functional beta diversity analysis yielded distinct clusters for soils B, C, and BB, while soils A and D showed functional overlap (Figure 6.4). As with taxonomic beta diversity, PERMANOVA identified soil source as having the greatest impact on functional beta diversity (Appendix Table A.27). Of the environmental covariates tested, only hydraulic conductivity had no significant impact on functional beta diversity. This suggests that the type of soil and its ability to capture pollutants have a significant impact on the functions that develop within that soil's microbial community. Further analysis of community function (e.g. quantitative polymerase chain reaction [qPCR]) for detection of specific degradation genes) could yield insights into key functions that differ or are shared between the soils, allowing more effective design of treatment systems.

Disparity between functional and taxonomic beta diversity was also measured, giving an indication of community robustness (Figure 6.5). R^2 values for disparity in soils A, D, and BB were deemed too low to determine robustness accurately. Soil B's steeper gradient indicates that its community is less robust than that of soil C – it takes less change in taxonomy to result in a change of function. This indicates that there is more overlap in function between species in soil C and this redundancy allows the community to retain functions even when some species may be lost e.g. due to changes in environmental conditions. Greater robustness is beneficial in SUDS communities as it maximises the chances of pollutant removal continuing even in the event of external pressures and stressors.

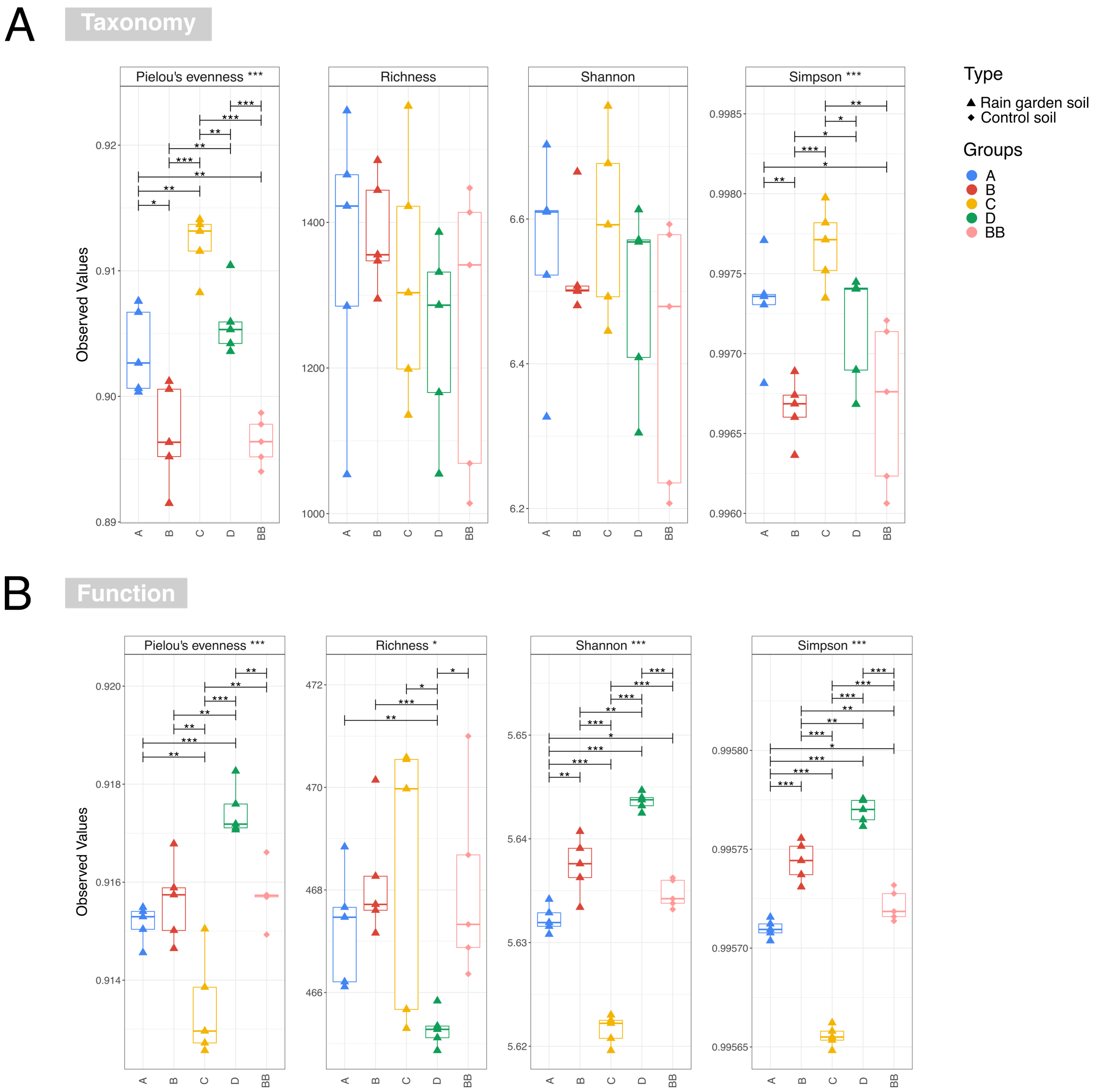

Figure 6.3: Alpha diversity estimates for (A) taxonomy and (B) function based on the PICRUSt2 prediction of the KEGG metagenome. Overall significance taking into account all five soil types is shown in the strip titles, and significant pairwise differences are represented by annotated lines connecting two categories.

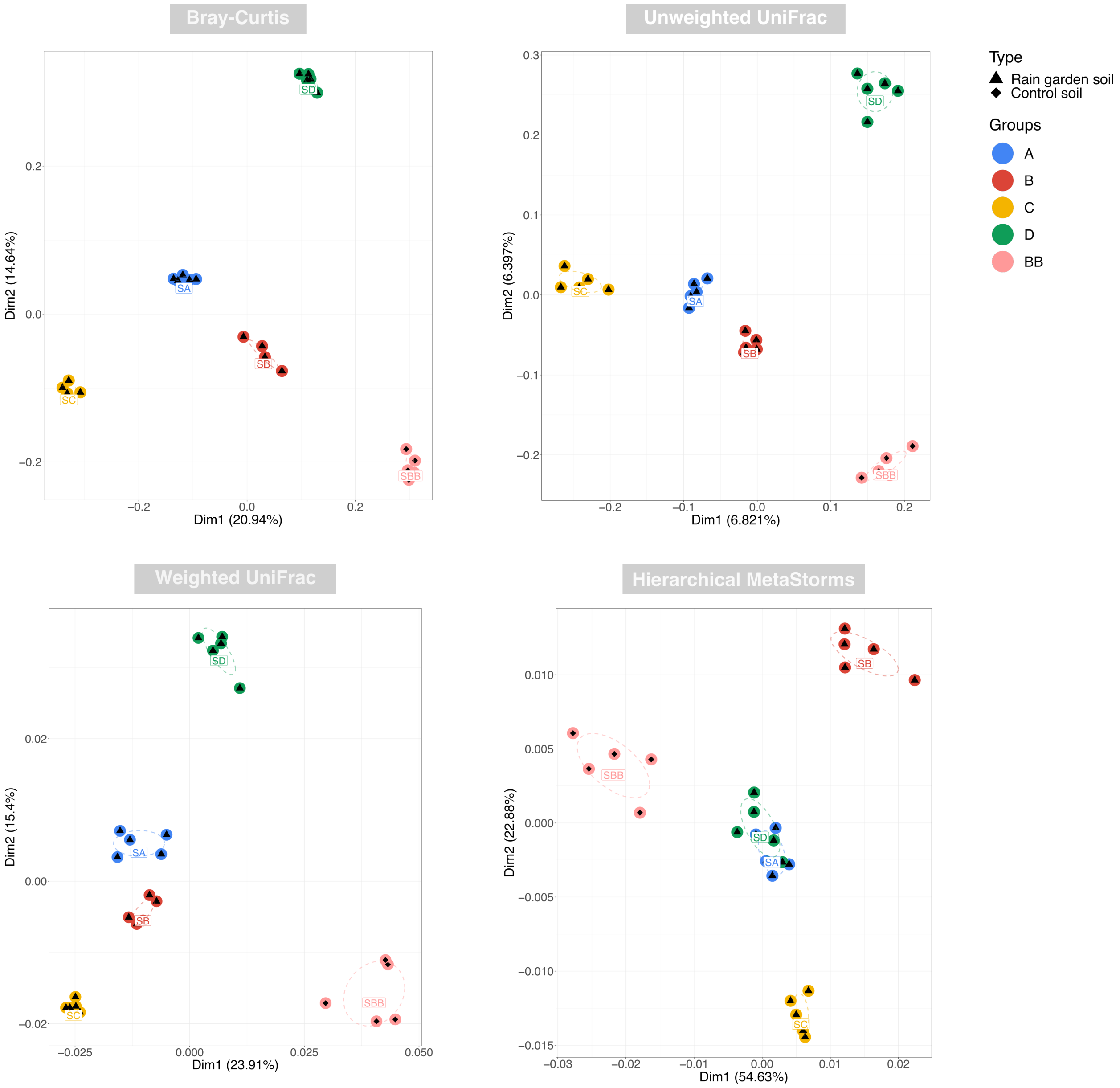

Figure 6.4: Principal co-ordinate analysis (PCoA) using Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac distances to represent taxonomic beta diversity and Hierarchical Meta-Storms to represent functional beta diversity. Ellipses for were drawn using 95% confidence intervals based on standard error of the ordination points for each category.

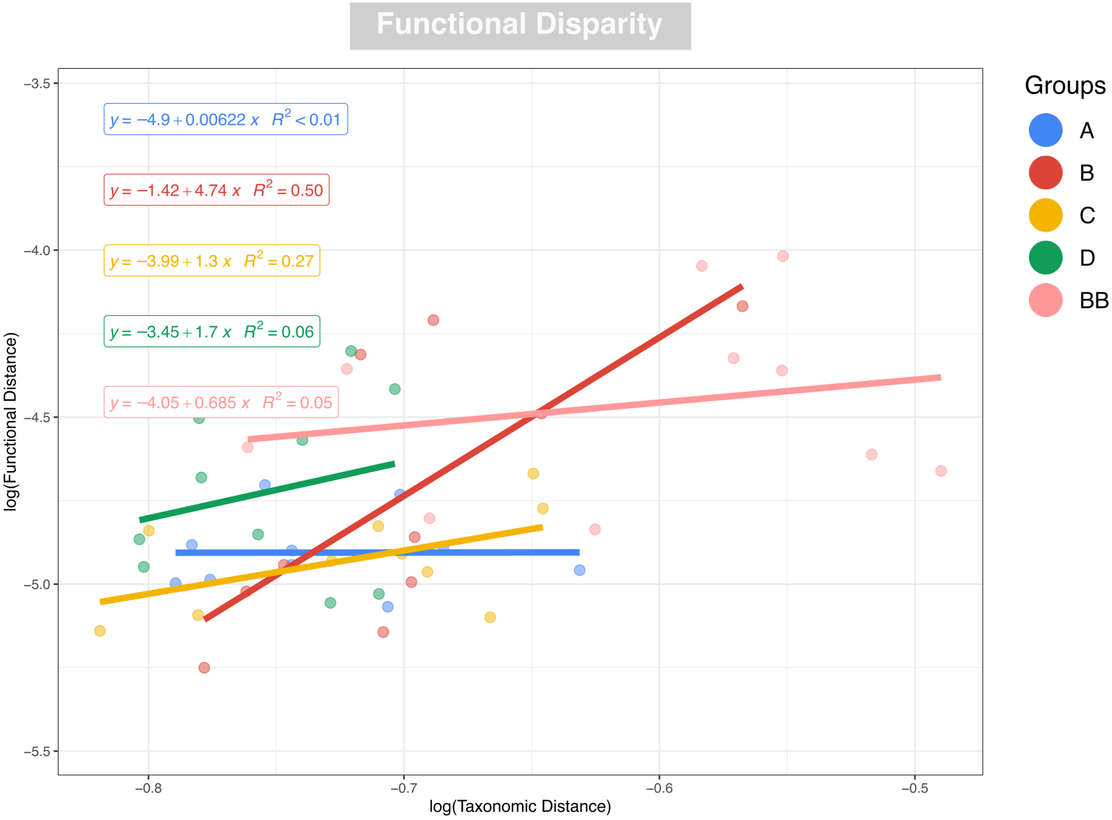

Figure 6.5: Functional disparity between taxonomic and functional distance is shown for each soil type. Line gradient indicates the overall robustness of each community against perturbation.

6.4 Conclusions

This study aimed to determine the impact of soil type on pollutant concentrations and microbial communities within rain gardens. Clear differences in both chemistry and microbiology were identified between the rain garden soils, although further work is required to determine the aspects of soil composition or other factors that are causing these differences. TPH capture appeared to be higher in the finer soils A and C, but PTE capture did not appear to be based on soil coarseness. A wider range of soil types as well as soil amendments should be considered in order to determine the soil characteristics that are having the greatest impact. Further analysis of both soil and influent/effluent is also required to identify optimal soil mixes for removal of specific pollutants.

Concentrations of PTEs and hydrocarbons were greater in the rain gardens than in the control soil, indicating that stormwater pollutants are being successfully captured during water treatment. Overall concentrations of these pollutants were low, so long-term risks to health or saturation of the rain gardens are unlikely at this time. However, regular monitoring of urban rain garden soils is advisable to identify and deal with pollutant accumulation before it becomes a significant issue.

A number of shared organisms including *Flavobacterium*, *Sphingomonas*, and *Rhizobiales* species were found during analysis of abundant and core genera. Many of the identified genera are known to be capable of hydrocarbon degradation, which is highly beneficial to rain garden functioning. Taxonomic and functional diversity were significantly impacted by soil chemistry and soil morphology, indicating that the choice of soil in rain gardens and other SUDS should be considered carefully in order to maximise the effectiveness and stability of the resident microbial community.

Future work should include monitoring of both soil and influent/effluent chemistry over a longer time period, to identify how pollutant concentrations in water and soil affect one another. Soil microbiology should also be analysed over a longer period, to discover how microbial communities in the rain gardens mature and adapt to changes in soil chemistry. Laboratory biodegradation studies or qPCR testing could be carried out to gain greater understanding of the functional potential of the soil microbial communities.

Conclusions

7.1 Overview

Sustainable urban drainage systems (SUDS) such as rain gardens offer a solution to the wide range of issues caused by climate change and urbanisation, such as flooding, environmental contamination, and water shortages. In order to maximise the potential of these systems for water treatment and re-use, they must be designed to encourage pollutant removal by physical, chemical, and biological means. Although it is generally accepted that different soil types are able to capture pollutants to varying extents, few studies have provided field-scale comparisons of SUDS soils. This research provided some preliminary insights into the impact of soil type on water treatment in rain gardens and allowed direct comparison of systems that differed only in soil particle size.

7.2 Limitations & Recommendations for Future Work

- In this work, all water samples were grab samples taken at single timepoints. Future work should make use of automated sampling (flow-weighed or time-weighted) to allow calculation of event mean concentrations, better comparison between events, and identification of variation in pollutant concentration across an event (e.g. the identification of first flush effects).
- Future work should build on insights established by this study by analysing a

wider range of urban sampling sites and rain garden soil types. Additional samples, larger sample volumes, and longer sampling periods will enable further replication, increased reliability, and in-depth statistical analysis.

- Standardisation of techniques across sampling sites and time periods would also improve reliability, and the addition of analyses such as dissolved organic carbon measurement or coliform detection would allow comparison with a wider range of water standards. The addition of further microbial analysis such as metagenomics or qPCR would improve understanding of the functional potential of stormwater and rain garden communities.
- Longer term analysis would allow the identification of seasonal or age-related factors in urban pollution, treatment, and microbial communities. Understanding of relationships between these would be improved by testing of stormwater, rain garden output, and rain garden soil over the same period rather than at different times.

7.3 Key Conclusions

7.3.1 Chapters 2, 3, and 4

- Traffic density, proximity to green space, local architecture composition, and climate factors including rainfall level and necessity of winter road treatments impacted stormwater pollutant concentrations in Glasgow and Florianópolis.
- Abundant organisms found in stormwater included hydrocarbon degraders (e.g. *Rhodococcus*, *Sphingomonas*), soil/plant-associated genera (e.g. *Planctomycetales*, *Luteolibacter*), and salt-tolerant genera (e.g. *Haliangium*, *Bradymonadales*).
- Country of origin was identified as a significant factor identifying microbial community diversity in Glasgow and Florianópolis.
- In order to provide optimal physical and biological water treatment, SUDS design should take into account local geography and climate. Ideally, stormwater should

be characterised prior to SUDS installation to ensure that the planned treatment level is adequate.

7.3.2 Chapters 5 and 6

- All four rain gardens were able to effectively treat stormwater when input pollutant concentrations were high, with more limited impact when input concentrations were low.
- No clear trend in treatment efficacy between soil types was identified based on effluent quality. However, soil analysis found differences in the concentration of contaminants including potentially toxic elements and total petroleum hydrocarbons.
- Bacterial genera capable of hydrocarbon degradation and metal resistance were identified in influent, effluent, and soil microbial communities.
- Effluent microbial community composition and diversity varied by sampling date moreso than soil type.
- Taxonomic and functional diversity of the soil microbial communities were significantly impacted by soil chemistry and morphology.
- Choice of soil should be carefully considered in order to maximise treatment potential, as soil types vary in their ability to capture or degrade pollutants. Seasonal variation and rain garden age should also be taken into account when planning long-term stormwater treatment.

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Appendix

DNA Concentrations

Chapter 4

Sample	**Concentration** ng/μl
MS	4.2
GS	12.6
SH	27.1
KG	3.7
HS	3.55
Br1	5.85
BR^2	4.65
Br3	23
Br4	6.6

Table A.1: Concentrations of DNA extracted from Glasgow and Florianópolis stormwater samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Chapter 5

Sample	**Concentration** ng/μl
In	0.5
A	2.8
C	0.7

Table A.2: Concentrations of DNA extracted from May 2019 samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Sample	**Concentration** ng/μl
In1	4
In2	4.9
In3	4.3
A1	3.15
A2	6.45
A3	3.85
B1	2.85
B2	5.55
B3	2.8
C1	5.95
C2	2.35
C3	8.5
D1	3.75
D2	3.9
D3	3.1

Table A.3: Concentrations of DNA extracted from August 2019 samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Sample	**Concentration** ng/μl
In1	3.275
In2	4.375
In3	5.225
A1	2.875
A2	2.525
A3	3.125
B1	1.225
B2	2.225
B3	2.125
C1	1.275
C2	1.625
C3	3.225
D1	1.875
D2	2.375
D3	0.925

Table A.4: Concentrations of DNA extracted from December 2019 samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Sample	**Concentration** ng/μl
Clyde1	57.125
Clyde2	66.125
Clyde3	67.475

Table A.5: Concentrations of DNA extracted from River Clyde samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Chapter 6

Sample	**Concentration** ng/μl
A1	23.175
A2	17.375
A3	21.375
A4	27.525
A5	20.975
B1	22.725
B2	22.125
B3	20.775
B4	19.175
B5	23.425
C1	67.375
C2	65.225
C3	63.275
C4	82.875
C5	61.875
D1	21.375
D2	24.125
D3	24.325
D4	22.575
D5	25.725
BB1	9.775
BB2	10.425
BB3	11.275
BB4	12.125
BB5	7.625

Table A.6: Concentrations of DNA extracted from rain garden soil samples. DNA concentrations were measured using a Biotek Epoch Microplate Spectrophotometer. Concentrations shown are the average of two readings of the same sample.

Dissolved & Total Elements - Full Results & Standard Deviations

A.0.1 Chapter 2

Element	**Standard Deviation**				
	MS	GS	SH	KG	HS
Al	0.0004	0.0045	0.0016	0.0240	0.0290
As	-	-	-	-	-
Ca	0.16	0.02	0.14	0.12	0.20
Cd	-	0	-	0.00007	0.00014
Co	-	-	-	0.00007	0.00007
Cr	-	-	-	0.00106	0.00007
Cu	0	0.00007	0.00035	0.00057	0.00057
Fe	0.0016	0.0011	0.0011	0.0144	0.0410
Hg	-	-	-	-	-
K	-	-	0.021	0.058	0.083
Li	0.00007	0	0	0	0
Mg	0.008	0.008	0.012	0.030	0.049
Mn	0	0.00057	0.00007	0	0.00127
Mo	-	-	-	-	-
Na	0.014	0.039	0.269	0.085	1.160
Ni	-	-	-	0.00049	0.00007
P	0.0004	0.0049	0.0037	0.0024	0.0153
Pb	0.00049	0.00071	0	0.0011	0.0059
S	0.009	0.011	0.020	0.010	0.086
Sb	-	-	-	-	-
Se	-	-	-	-	-
Si	0.006	0.009	0.005	0.012	0.029
Sn	-	-	-	-	-
Sr	0.0002	0.0003	0.0003	0.0002	0.0001
Ti	0	-	-	0.0001	0.0008
Zn	0.0010	0.0018	0.0007	0.0033	0.0089

Table A.7: Standard deviations of Glasgow stormwater dissolved elements measurements. '-' denotes samples where all measurements were below detection limits.

Element	**Standard Deviation**				
	MS	GS	SH	KG	HS
Al	0.21	0.18	0.07	0.57	0.18
As	-	-	-	-	-
Ca	0.36	0.66	9.22	0.11	2.35
Cd	-	0.0024	0.0024	0.0006	0.0024
Co	-	-	-	0.0018	0
Cr	-	-	0.0018	0.0012	0.0006
Cu	-	-	0.029	0.013	0.007
Fe	0.17	0.09	0.18	0.67	1.38
Hg	-	-	-	-	-
K	-	-	-	-	0.32
Li	0.00059	0.00059	0.00118	0	0.00118
Mg	0.09	0.04	1.14	0.11	1.22
Mn	0.004	0.001	0.005	0.001	0.022
Mo	-	-	-	-	-
Na	0.65	0.49	7.29	0.27	4.18
Ni	0.176	0.008	0.063	0.143	0.008
P	-	0.012	0.814	0.036	0.068
Pb	-	0.008	0.011	0.008	0.015
S	0.35	0.69	5.34	0.05	1.12
Sb	-	-	-	-	-
Se	-	-	-	-	-
Si	0.42	2.53	0.53	1.97	1.48
Sn	-	-	-	-	-
Sr	0.006	0.003	0.027	0.002	0.005
Ti	0.017	0.026	0	0.032	0.039
Zn	0.038	0.051	0.058	0.070	0.072

Table A.8: Standard deviations of Glasgow stormwater total elements measurements. ’-’ denotes samples where all measurements were below detection limits.

A.0.2 Chapter 3

Element	**Standard Deviation**			
	Br1	Br2	Br3	Br4
Al	0.011	0.030	0.012	0.007
As	-	-	-	-
Ca	0.14	0.19	0.09	0.10
Cd	0.00007	-	0	0
Co	-	-	-	-
Cr	-	-	0.00014	-
Cu	0.00028	0.00205	0.00085	0.00050
Fe	0.0012	0.01061	0.0069	0.0012
Hg	-	-	-	-
K	0.07	-	0.17	0.04
Li	0.00014	0.00014	0.00007	0.00007
Mg	0.006	0.017	0.021	0.021
Mn	0.0004	0.0007	0.0002	0.0001
Mo	-	-	-	-
Na	0.11	0.08	0.27	0.04
Ni	0.00014	0.00007	-	-
P	0.0004	0.0007	0.0033	0.0008
Pb	0.0001	0.0010	0.0002	0
S	0.02	0.04	0.08	0.03
Sb	-	-	-	-
Se	-	-	-	-
Si	0.027	0.125	0.007	0.011
Sn	-	-	-	-
Sr	0.00035	0.00092	0.00035	0.00021
Zn	0.00134	0.00240	0.00049	0.00057

Table A.9: Standard deviations of Florianópolis stormwater dissolved elements measurements. ’-’ denotes samples where all measurements were below detection limits.

Element	**Standard Deviation**			
	Br1	Br2	Br3	Br4
Al	0.082	0.062	0.077	0.056
As	-	-	-	-
Ca	0.47	0.02	0.38	0.15
Cd	0	-	-	-
Co	-	-	-	-
Cr	-	-	-	0.0082
Cu	0.00176	0.00295	0.00176	0.00059
Fe	0.014	0.007	0.032	0.053
Hg	-	-	-	-
K	0.62	-	-	-
Li	0	0	0	0
Mg	0.094	0.010	0.009	0.024
Mn	0.0029	0.0006	0.0006	0.0006
Mo	-	-	-	-
Na	0.071	0.293	0.065	1.028
Ni	0.011	0.021	-	0.007
P	0.025	-	0.006	0.047
Pb	-	-	-	-
S	-	4.93	0.24	0.53
Sb	-	-	-	-
Se	-	-	-	-
Si	0.11	0.02	0.14	0.03
Sn	-	-	-	-
Sr	0.0035	0	0.0018	0.0006
Zn	0.004	0.068	0.011	0.001

Table A.10: Standard deviations of Florianópolis stormwater total elements measurements. '-' denotes samples where all measurements were below detection limits.

A.0.3 Chapter 5

Element	**Concentration (mg/l)**		
	Input	Output A	Output C
Al	1.51	1.33	0.70
As	BDL	0.0061	0.0017
Ca	7.97	30.36	10.18
Cd	0.0031	0.0003	BDL
Co	0.0019	0.0013	BDL
Cr	0.006	0.002	0.002
Cu	0.064	0.039	0.016
Fe	2.74	1.42	0.84
Hg	BDL	BDL	BDL
K	0.82	4.20	1.53
Li	0.003	0.002	0.001
Mg	2.39	4.91	1.72
Mn	0.094	0.058	0.031
Mo	BDL	0.0065	BDL
Na	11.49	40.58	14.59
Ni	BDL	BDL	BDL
P	0.15	0.26	0.14
Pb	0.04	0.03	0.02
S	0.76	5.52	0.98
Sb	BDL	BDL	BDL
Se	BDL	BDL	BDL
Si	2.45	4.50	1.75
Sn	BDL	BDL	BDL
Sr	0.02	0.24	0.08
Zn	0.24	0.04	0.03

Table A.11: Average dissolved concentrations of elements in the May 2019 samples. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled "BDL".

Element	**Standard Deviation**		
	Input	Output A	Output C
Al	0.016	0.119	0.002
As	-	0.00035	0.00007
Ca	0.073	0.049	0.057
Cd	0	0	-
Co	0.00014	0.00021	-
Cr	0.00007	0.00007	0.00021
Cu	0.00049	0.00007	0.00021
Fe	0.0120	0.0820	0.0075
Hg	-	-	-
K	0.0122	0.0007	0.0368
Li	0	0.00014	0.00007
Mg	0.027	0.039	0
Mn	0.00049	0.00057	0.00007
Mo	-	0	-
Na	0.042	0.113	0.071
Ni	-	-	-
P	0.00184	0.00156	0.00056
Pb	0.00014	0.00049	0.00028
S	0.0011	0.0233	0.0035
Sb	-	-	-
Se	-	-	-
Si	0.021	0.242	0.027
Sn	-	-	-
Sr	0.00014	0.00162	0
Zn	0.0019	0.0020	0.0023

Table A.12: Standard deviations of May 2019 dissolved elements measurements. ’-’ denotes samples where all measurements were below detection limits.

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	0.202	0.447	0.329	0.494	0.519
As	BDL	0.0023	BDL	0.0052	0.0048
Ba	0.010	0.0191	0.0206	0.0198	0.0309
Ca	1.658	5.8895	7.3745	6.768	9.858
Cd	BDL	BDL	BDL	BDL	BDL
Co	BDL	BDL	BDL	BDL	BDL
Cr	BDL	BDL	BDL	BDL	0.0009
Cu	0.009	0.0091	0.0077	0.0096	0.0127
Fe	0.390	0.5942	0.4426	0.6497	0.7012
Hg	0.0002	BDL	BDL	BDL	BDL
K	BDL	0.5367	0.5149	0.4046	0.8235
Li	0.001	0.0006	0.0012	0.0007	0.0007
Mg	0.337	1.0734	1.2894	1.2119	1.8514
Mn	0.011	0.014	0.0125	0.0191	0.0178
Mo	BDL	BDL	BDL	BDL	BDL
Na	1.472	5.35	5.237	4.351	6.431
Ni	BDL	BDL	BDL	BDL	BDL
P	0.017	0.0833	0.0716	0.1016	0.0865
Pb	0.005	0.0076	0.0063	0.0081	0.0099
S	0.085	0.41	0.5202	0.3453	1.1785
Sb	BDL	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL	BDL
Si	0.380	1.3589	1.4964	1.5014	1.8919
Sn	BDL	BDL	BDL	BDL	BDL
Sr	0.006	0.0553	0.0654	0.064	0.0946
Zn	0.019	0.005	0.0023	0.0051	0.0088

Table A.13: Average dissolved concentrations of elements in the August 2019 samples. Duplicate samples from each sampling point were analysed, and blank values were subtracted from the measured values. Analysis of the input sample duplicate failed, so the input sample data displayed above is from a single sample rather than an average of a pair of duplicates. Concentrations below detection limits are labelled “BDL”.

Element	**Standard Deviation**				
	Input	Output A	Output B	Output C	Output D
Al	-	0.0092	0.0081	0.0227	0.0086
As	-	0.0032	-	0.0004	0.0001
Ba	-	0.0005	0.0006	0.0010	0.0005
Ca	-	0.10	0.16	0.23	0.10
Cd	-	-	-	-	-
Co	-	-	-	-	-
Cr	-	-	-	-	0.0013
Cu	-	0.0004	0.0002	0.0004	0.0004
Fe	-	0.016	0.013	0.027	0.006
Hg -	-	-	-	-	
K	-	0.045	0.179	0.037	0.034
Li	-	0.00007	0.00007	0	0.00007
Mg	-	0.019	0.036	0.041	0.025
Mn	-	0.0004	0.0005	0.0010	0.0001
Mo	-	-	-	-	-
Na	-	0.088	0.089	0.153	0.078
Ni	-	-	-	-	-
P	-	0.002	0.021	0.004	0.004
Pb	-	0.00007	0.00014	0.00007	0.00035
S	-	0.0052	0.0233	0.0097	0.0021
Sb	-	-	-	-	-
Se	-	-	-	-	-
Si	-	0.032	0.035	0.072	0.028
Sn	-	-	-	-	-
Sr	-	0.0010	0.0018	0.0030	0.0013
Zn	-	0.0023	0.0033	0.0015	0.0025

Table A.14: Standard deviations of August 2019 dissolved elements measurements. ’-’ denotes samples where all measurements were below detection limits.

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	1.16	0.56	0.19	0.21	0.19
As	BDL	BDL	BDL	BDL	BDL
B	0.008	0.006	0.004	0.003	0.002
Ba	0.022	0.029	0.029	0.016	0.023
Ca	3.87	12.87	14.58	8.83	13.71
Cd	BDL	BDL	BDL	BDL	BDL
Co	BDL	BDL	BDL	BDL	BDL
Cr	0.0059	0.0017	BDL	0.0019	BDL
Cu	0.026	0.016	0.008	0.006	0.005
Fe	2.530	0.688	0.259	0.251	0.244
Hg	BDL	BDL	BDL	BDL	BDL
K	0.88	1.97	1.94	1.16	1.01
Li	0.002	0.001	0.001	0.0002	0.0003
Mg	1.43	1.69	1.92	0.99	1.71
Mn	0.048	0.011	0.005	0.004	0.005
Mo	BDL	BDL	BDL	BDL	BDL
Na	35.5	86.6	95.6	62.2	57.3
Ni	0.0019	BDL	BDL	BDL	BDL
P	BDL	BDL	BDL	BDL	BDL
Pb	0.0134	0.0058	BDL	BDL	BDL
S	1.96	1.66	2.03	1.04	1.06
Sb	BDL	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL	BDL
Si	2.74	1.75	1.01	0.98	0.82
Sn	BDL	BDL	BDL	BDL	BDL
Sr	0.019	0.076	0.098	0.059	0.108
Ti	0.034	0.008	0.003	0.003	0.003
V	0.0083	BDL	BDL	BDL	BDL
Zn	0.091	0.034	0.017	0.013	0.011

Table A.15: Dissolved concentrations of elements in the December 2019 samples. Due to low sample volume, data displayed above is from single samples rather than pairs of duplicates. Blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”.

Element	**Concentration (mg/l)**		
	Input	Output A	Output C
Al	5.34	6.41	3.59
As	BDL	BDL	BDL
Ca	8.63	29.34	10.49
Cd	0.0042	BDL	BDL
Co	BDL	BDL	BDL
Cr	0.015	BDL	BDL
Cu	0.097	0.056	0.038
Fe	11.12	6.02	3.33
Hg	BDL	BDL	BDL
K	1.11	4.30	1.07
Li	0.0063	0.0092	0.0050
Mg	4.91	6.59	2.77
Mn	0.18	0.08	0.05
Mo	BDL	0.011	BDL
Na	12.03	39.89	14.60
Ni	BDL	BDL	BDL
P	0.31	0.41	0.24
Pb	0.053	0.043	0.044
S	1.43	5.20	0.90
Sb	BDL	BDL	BDL
Se	BDL	BDL	BDL
Si	11.30	12.85	6.68
Sn	BDL	BDL	BDL
Sr	0.03	0.24	0.08
Zn	0.44	0.23	0.10

Table A.16: Average total concentrations of elements in the May 2019 samples. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”.

Element	**Standard Deviation**		
	Input	Output A	Output C
Al	0.274	0.057	0.009
As	-	-	-
Ca	0.41	0.61	0.14
Cd	0	-	-
Co	-	-	-
Cr	0.0029	-	-
Cu	0.013	0.008	0.002
Fe	0.27	0.18	0.06
Hg	-	-	-
K	0.94	0.32	0.06
Li	0.0006	0	0
Mg	0.10	0.14	0.02
Mn	0.0041	0.0024	0.0006
Mo	-	0	-
Na	0.43	0.90	0.16
Ni	-	-	-
P	0.006	0.001	0.012
Pb	0.004	0.012	0.009
S	0.44	0.15	0.05
Sb	-	-	-
Se	-	-	-
Si	0.52	0.19	0.10
Sn	-	-	-
Sr	0.002	0.008	0.002
Zn	0.21	0.20	0.04

Table A.17: Standard deviations of May 2019 total elements measurements. '-' denotes samples where all measurements were below detection limits.

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	0.44	1.42	1.24	1.59	2.37
As	BDL	BDL	BDL	BDL	BDL
Ba	0.047	0.020	0.021	0.022	0.034
Ca	1.65	5.62	7.10	6.77	9.78
Cd	BDL	BDL	BDL	BDL	BDL
Co	BDL	BDL	BDL	BDL	BDL
Cr	BDL	BDL	BDL	BDL	BDL
Cu	BDL	BDL	0.007	BDL	0.019
Fe	0.92	1.51	1.32	1.69	2.22
Hg	BDL	BDL	BDL	BDL	BDL
K	BDL	BDL	BDL	BDL	BDL
Li	0.002	0.002	0.003	0.003	0.003
Mg	0.48	1.40	1.55	1.63	2.40
Mn	0.017	0.019	0.016	0.026	0.025
Mo	BDL	BDL	BDL	BDL	BDL
Na	1.56	4.82	4.73	3.93	5.90
Ni	BDL	BDL	BDL	BDL	BDL
P	0.04	0.12	0.08	0.13	0.15
Pb	BDL	BDL	BDL	BDL	BDL
S	BDL	0.24	0.36	0.17	0.97
Sb	BDL	BDL	BDL	BDL	BDL
Se	BDL	BDL	BDL	BDL	BDL
Si	0.77	2.63	2.48	3.84	4.58
Sn	BDL	BDL	BDL	BDL	BDL
Sr	0.007	0.053	0.063	0.065	0.094
Zn	0.050	0.008	0.011	0.036	0.052

Table A.18: Average total concentrations of elements in the August 2019 samples. Duplicate samples from each site were analysed, and blank values were subtracted from the measured values. Analysis of the Output C sample duplicate failed, so the Output C sample data displayed above is from a single sample rather than an average of a pair of duplicates. Concentrations below detection limits are labelled “BDL”.

Element	**Standard Deviation**				
	Input	Output A	Output B	Output C	Output D
Al	0.008	0.019	0.063	-	0.002
As	-	-	-	-	-
Ba	0.0530	0.0006	0	-	0.0012
Ca	0.11	0.11	0.14	-	0.05
Cd	-	-	-	-	-
Co	-	-	-	-	-
Cr	-	-	-	-	-
Cu	0	0	0.0094	-	0.0006
Fe	0.008	0.031	0.008	- 0.041	
Hg	-	-	-	-	-
K	-	-	-	-	-
Li	0	0	0	-	0
Mg	0.011	0.004	0.003	-	0.044
Mn	0	0.0006	0	-	0.0006
Mo	-	-	-	-	-
Na	0.431	0.032	0.061	-	0.080
Ni	-	-	-	-	-
P	0.057	0.004	0.011	-	0.003
Pb	-	-	-	-	-
S	-	0.029	0.015	-	0.020
Sb	-	-	-	-	-
Se	-	-	-	-	-
Si	0.29	0.02	0.07	-	0.02
Sn	-	-	-	-	-
Sr	0	0.0006	0.0006	-	0.0012
Zn	0.022	0.001	0.006	-	0.004

Table A.19: Standard deviations of August 2019 total elements measurements. '-' denotes samples where all measurements were below detection limits.

Element	**Concentration (mg/l)**				
	Input	Output A	Output B	Output C	Output D
Al	1.1074	0.9856	0.3263	0.4669	0.6342
As	0.0014	0.0047	0.0015	0.0031	0.0028
Ba	0.0167	0.0402	0.0404	0.0298	0.0673
Ca	3.2039	18.054	20.627	16.31	37.332
Cd	0.0007	BDL	BDL	BDL	BDL
Co	0.0012	BDL	BDL	BDL	BDL
Cr	0.0047	0.0031	0.0017	0.002	0.0023
Cu	0.0177	0.0162	0.0064	0.0048	0.0082
Fe	2.5776	1.3903	0.5108	0.6416	0.9044
Hg	0.0017	0.001	BDL	BDL	BDL
K	0.6322	2.7921	2.6121	2.091	2.7932
Li	0.0027	0.0026	0.0019	0.0016	0.002
Mg	1.1748	2.2426	2.5648	1.7009	4.342
Mn	0.0479	0.0195	0.0082	0.0092	0.0147
Mo	BDL	BDL	0.0006	BDL	BDL
Na	30.688	124.57	136.95	116.62	157.73
Ni	0.0029	0.0032	0.0011	BDL	0.0034
P	0.0874	0.1662	0.0633	0.0969	0.1095
Pb	0.0186	0.0151	0.0064	0.0081	0.01
S	2.016	2.5616	3.1727	2.3782	3.6088
Sb	0.0054	0.0056	0.0053	0.0052	0.0082
Se	BDL	BDL	0.0033	BDL	BDL
Si	2.3653	2.6987	1.4842	1.9076	2.4564
Sn	0.0035	BDL	BDL	BDL	BDL
Sr	0.0158	0.1021	0.1328	0.1046	0.2822
Ti	0.0393	0.0199	0.0066	0.0098	0.0127
V	0.0033	0.0046	BDL	0.0026	0.0042
Zn	0.0907	0.0373	0.0152	0.0124	0.0231

Table A.20: Average total concentration of elements in the December 2019 samples. Samples were analysed in duplicate, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled “BDL”.

Element	**Standard Deviation**				
	Input	Output A	Output B	Output C	Output D
Al	0.059	0.036	0.016	0.006	0.002
As	0.0009	0.0008	0.0007	0.0002	0.0009
Ba	0	0.0011	0.0013	0.0011	0.0016
Ca	0.02	0.57	0.67	0.47	0.66
Cd	0.00008	-	-	-	-
Co	0.00008	-	-	-	-
Cr	0.00008	0.00039	0.00031	0	0.00008
Cu	0.00039	0.00055	0.00063	0.00008	0.00008
Fe	0.163	0.069	0.029	0.002	0.035
Hg	0.0024	0.0014	-	-	-
K	0.0043	0.1485	0.0416	0.0024	0.0008
Li	-	0.00008	-	-	-
Mg	0.057	0.072	0.014	0.045	0.087
Mn	0.0064	0.0005	0.0002	0.0001	0.0002
Mo	-	-	0.0008	-	-
Na	0.38	4.09	1.34	0.86	0.08
Ni	0.0007	0.0015	0.0016	-	0.0016
P	0.0063	0.0033	0.0033	0.0001	0.0009
Pb	0.0020	0	0.0003	0.0008	0.0002
S	0.018	0.037	0.141	0.010	0.005
Sb	0.0002	0.0005	0.0001	0	0.0002
Se	-	-	0.0046	-	-
Si	0.142	0.087	0.040	0.024	0.031
Sn	0.0007	-	-	-	-
Sr	0.0002	0.0028	0.0050	0.0032	0.0064
Ti	0.0011	0.0014	0.00039	0.00008	0.00047
V	0.000235702	0.0005	-	0.0003	0.0001
Zn	0.0088	0.0093	0.0007	0.0015	0.0076

Table A.21: Standard deviations of December 2019 total elements measurements. ’-’ denotes samples where all measurements were below detection limits.

Element	**Concentration (mg/l)**	
	Dissolved	Total
Al	0.182	0.4765
As	0.0008	0.0022
Ba	0.0395	0.0392
Ca	16.062	15.032
Cd	BDL	BDL
Co	BDL	BDL
Cr	0.0024	0.0037
Cu	BDL	0.0031
Fe	0.3794	1.4287
Hg	BDL	BDL
K	2.2635	2.0488
Li	0.0019	0.0022
Mg	4.307	4.1631
Mn	0.0245	0.06
Mo	BDL	BDL
Na	16.1	13.899
Ni	BDL	0.0022
P	0.0987	0.1836
Pb	0.0069	0.0112
S	4.2825	3.9877
Sb	BDL	0.0052
Se	BDL	0.0032
Si	2.4865	2.4798
Sn	BDL	BDL
Sr	0.0811	0.0764
Ti	0.0078	0.0141
V	0.0011	BDL
Zn	0.0224	0.0146

Table A.22: Average dissolved and total concentrations of elements in the March 2021 River Clyde samples. Samples were analysed in duplicate, and blank values were subtracted from the measured values. Concentrations below detection limits are labelled "BDL".

Element	**Standard Deviation)**	
	Dissolved	Total
Al	0.0071	0.0285
As	0.0006	0.0016
Ba	0.0016	0.0004
Ca	0.62	0.27
Cd	-	-
Co	-	-
Cr	0	0.0003
Cu	-	0.0012
Fe	0.014	0.042
Hg	-	-
K	0.14	0.12
Li	0.00007	0
Mg	0.17	0.09
Mn	0.0009	0.0058
Mo	-	-
Na	0.66	0.38
Ni	-	0.0005
P	0.0004	0.0060
Pb	0.00007	0.00094
S	0.008	0.038
Sb	-	0
Se	-	0.005
Si	0.09	0.06
Sn	-	-
Sr	0.003	0.001
Ti	0.0003	0.0009
V	0.001	-
Zn	0.0002	0.0087

Table A.23: Standard deviations of March 2021 River Clyde dissolved and total elements measurements. '-' denotes samples where all measurements were below detection limits.

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Element	Standard Deviation				
	A	B	C	D	BB
Al	89	10	75	49	12
As	0.162	0.014	0.108	0.035	0.032
Ba	0.94	0.46	1.08	0.47	0.19
Ca	45	51	29	106	16
Cd	0.027	0.014	0.020	0.017	0.027
Co	0.060	0.008	0.040	0.038	0.012
Cr	0.11	0.12	0.38	0.30	0.07
Cu	2.1	0.9	1.2	3.5	1.9
Fe	174	99	248	147	422
Hg	0.23	0.15	-	-	-
K	17.8	2.4	15.9	10.6	2.3
Li	0.158	0.049	0.003	0.097	0.075
Mg	47.0	2.7	28.1	23.9	1.5
Mn	14.76	0.83	1.93	2.50	0.82
Mo	0.18	0.01	0.04	0.02	0.02
Na	-	-	-	-	-
Ni	0.37	0.49	0.30	0.58	0.02
P	4.09	0.88	2.34	1.66	0.52
Pb	0.44	0.32	0.38	0.51	0.29
S	22.7	3.2	1.3	15.1	0.8
Sb	0.064	0.064	0.017	0.059	0.007
Se	-	-	-	-	-
Si	3.4	1.7	4.5	2.5	1.7
Sn	0.064	0.022	0.018	0.048	0.052
Sr	0.15	0.09	0.43	0.37	0.02
Ti	1.01	0.28	3.13	4.36	0.67
V	0.069	0.004	0.203	0.379	0.071
Zn	1.78	0.56	0.15	0.42	0.60

Table A.24: Standard deviations of total elements measurements in the Washington Street rain garden soil samples.'-' denotes samples where all measurements were below detection limits.

Core Microbiomes

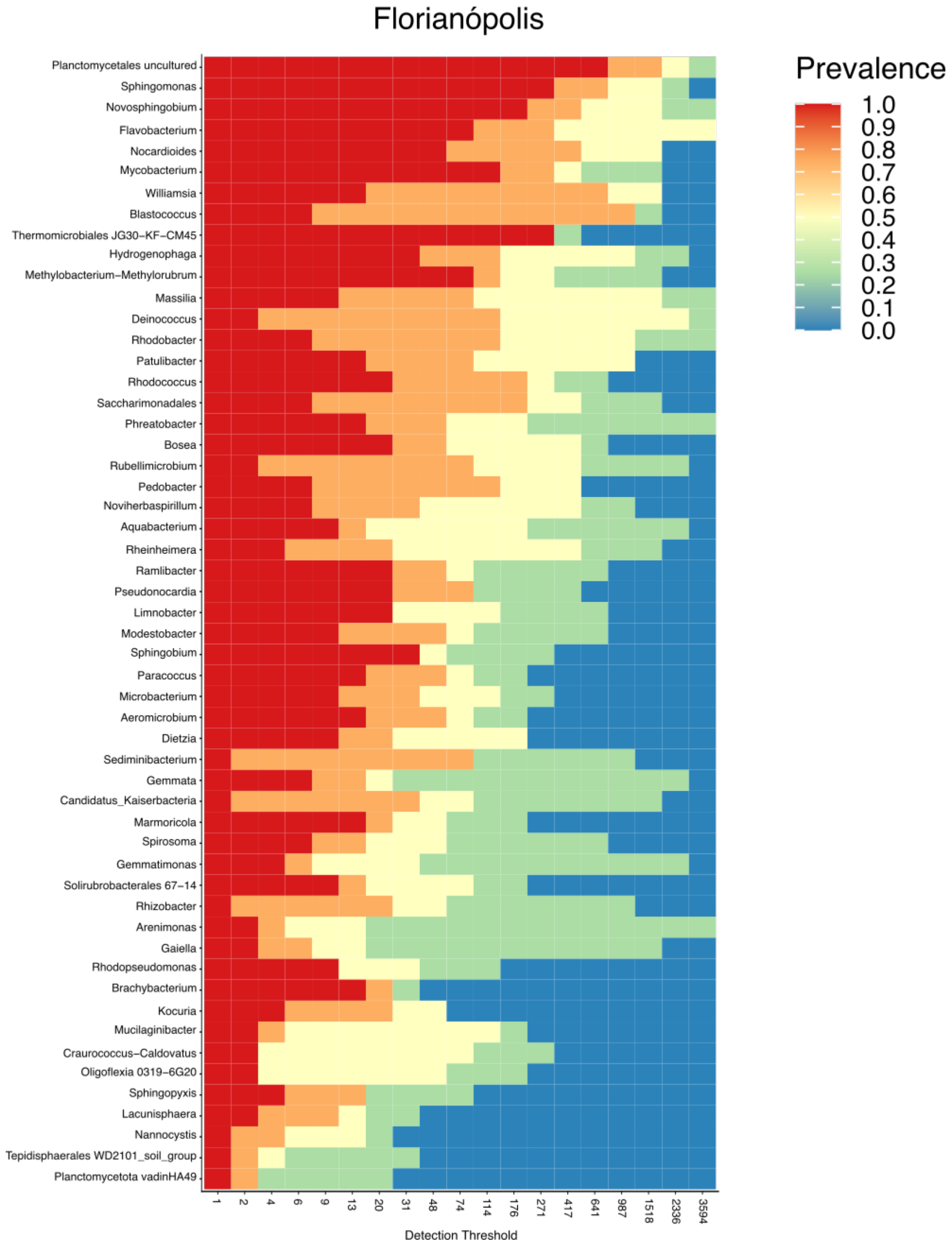

Figure A.1: Core microbiome of Florianópolis (defined as genera present in at least 95% of samples).

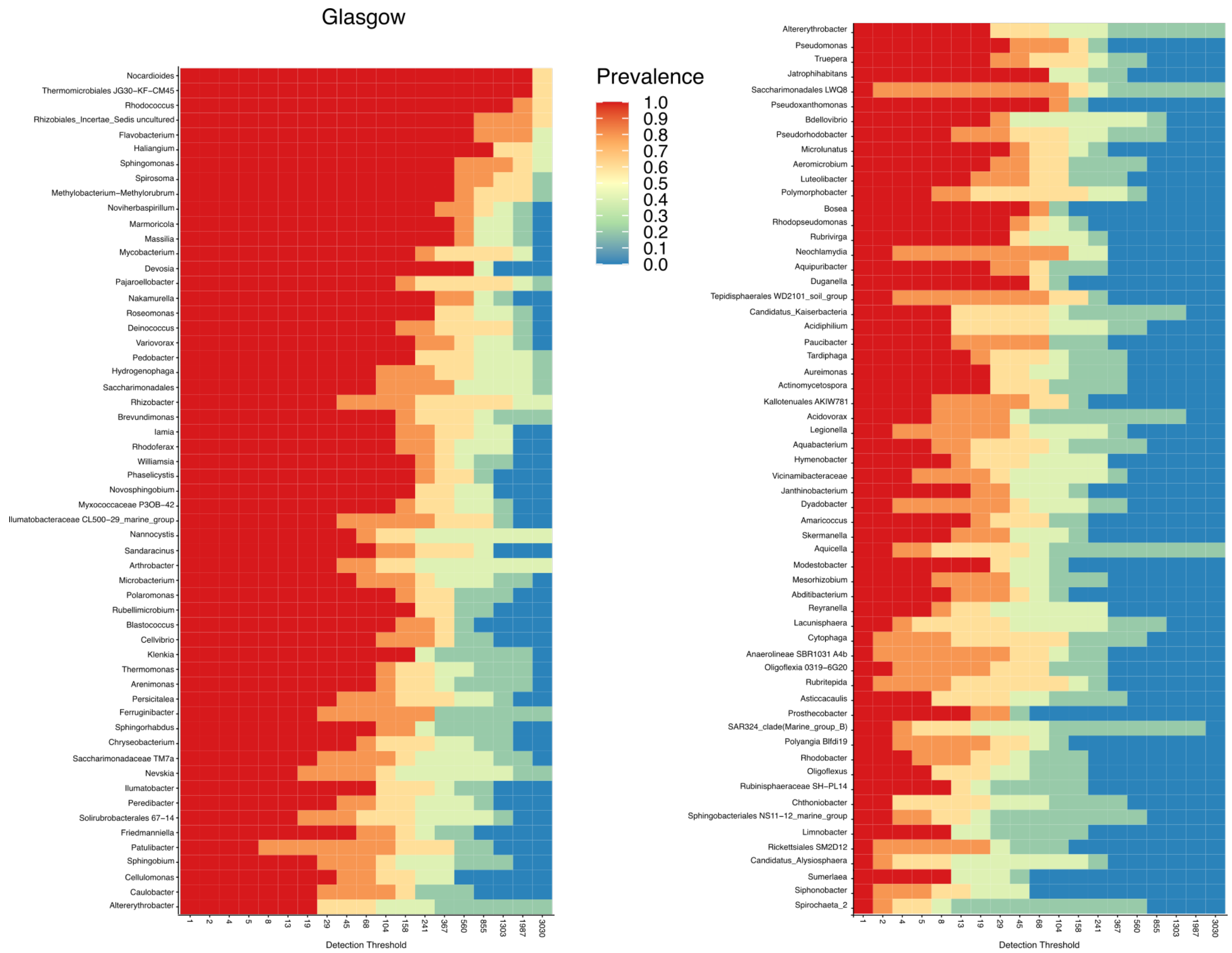

Figure A.2: Core microbiome of Glasgow (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

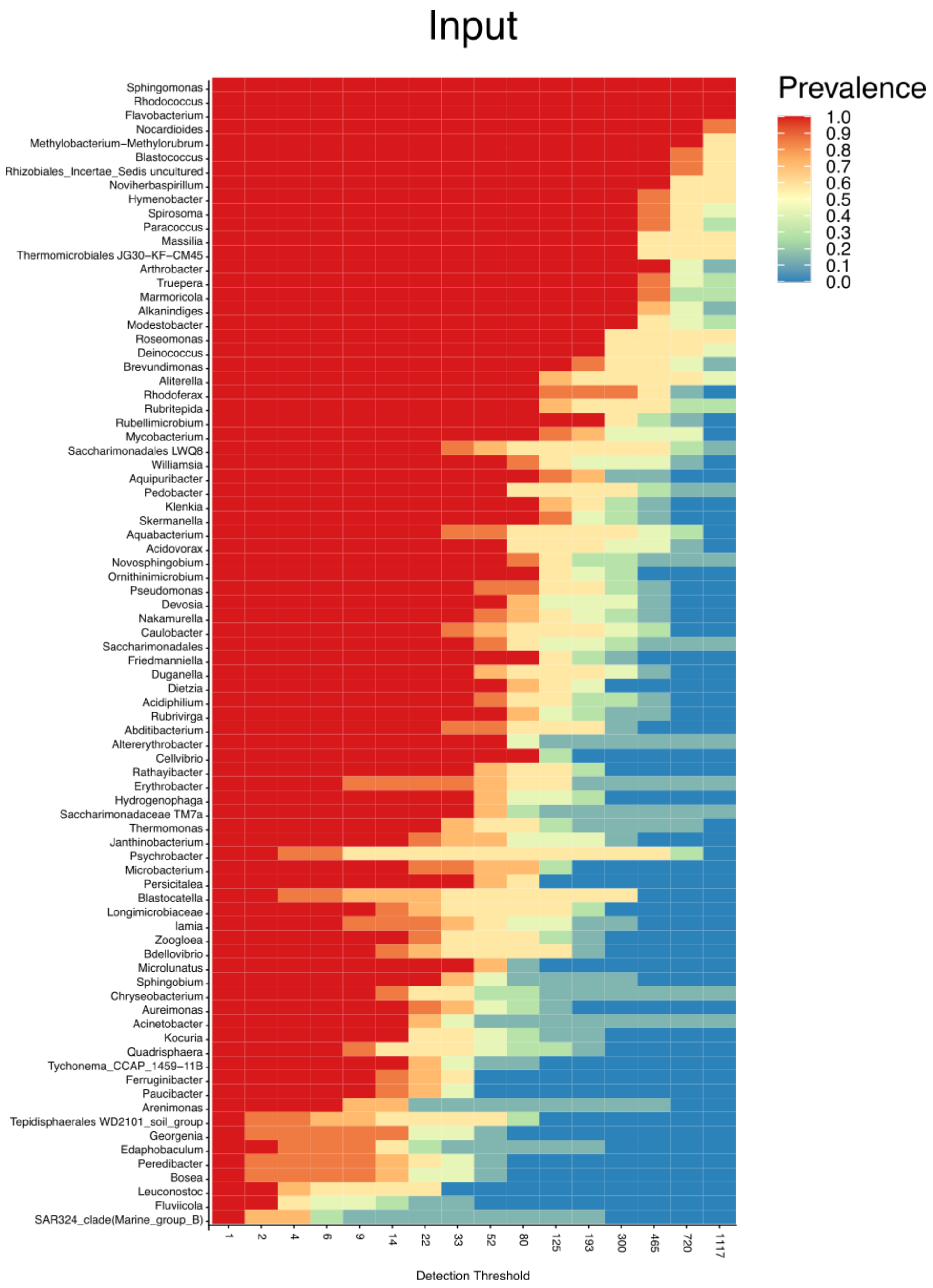

Figure A.3: Core microbiome of the rain garden Input (defined as genera present in at least 95% of samples).

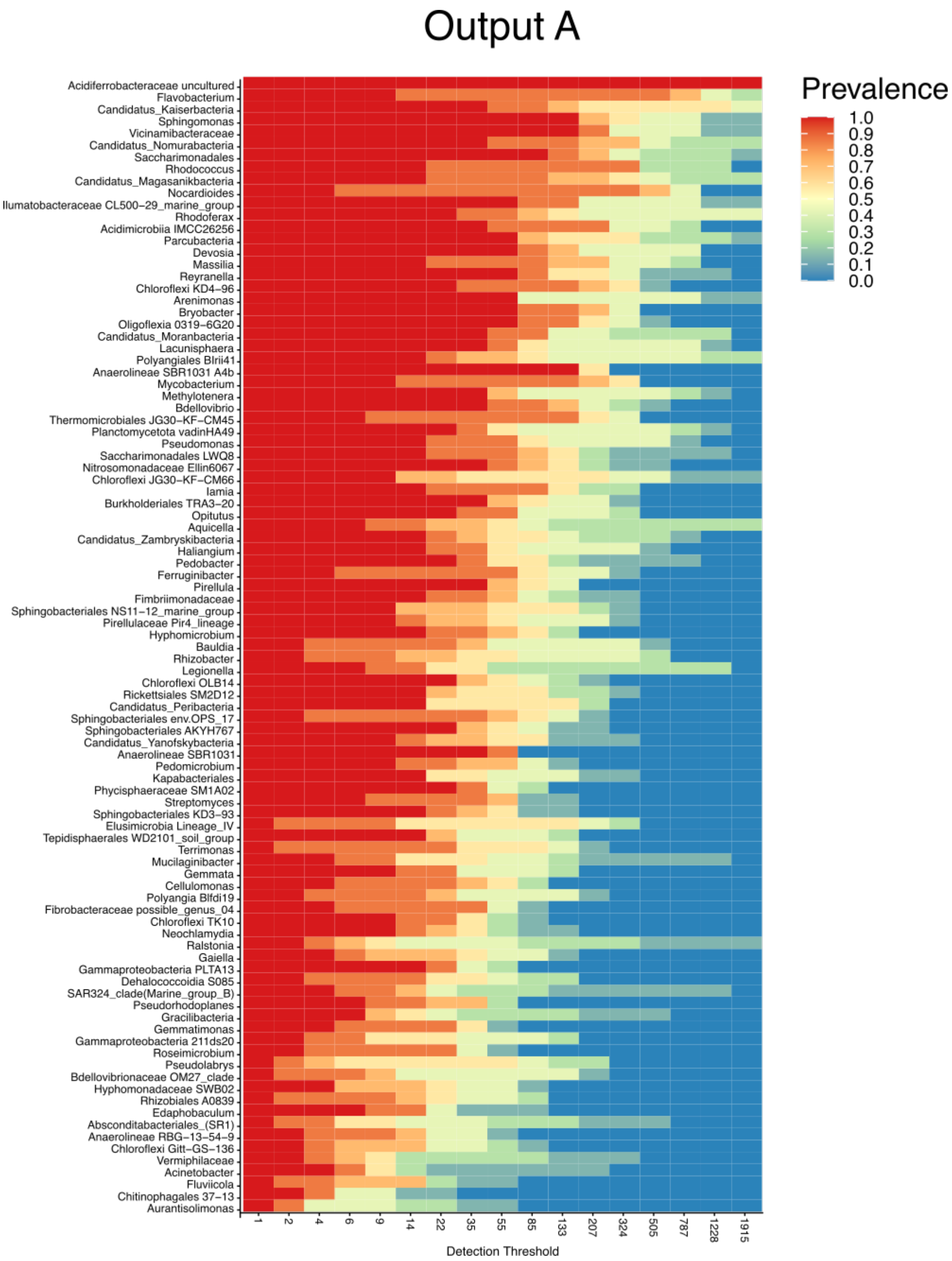

Figure A.4: Core microbiome of the rain garden Output A (defined as genera present in at least 95% of samples).

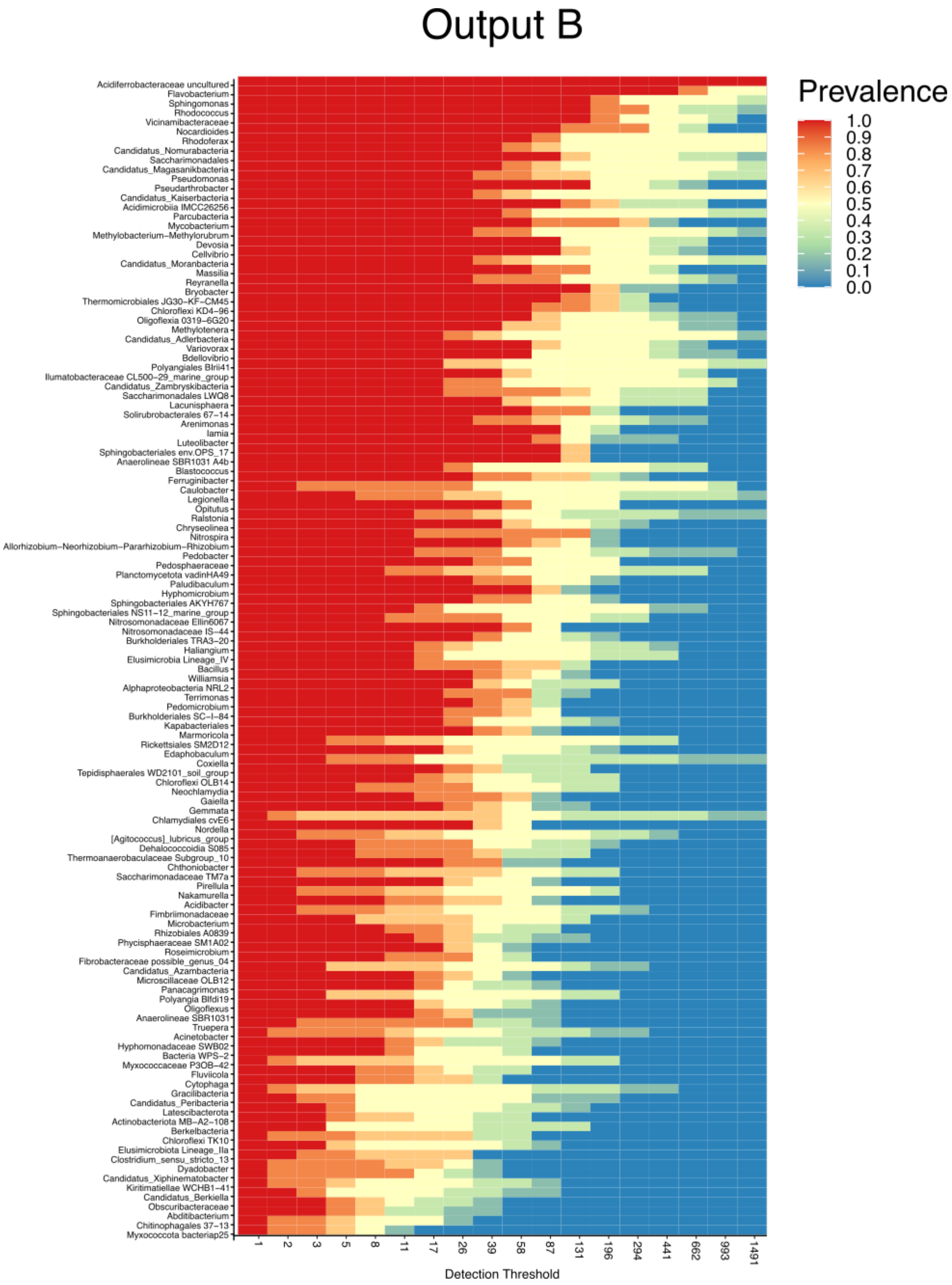

Figure A.5: Core microbiome of the rain garden Output B (defined as genera present in at least 95% of samples).

Figure A.6: Core microbiome of the rain garden Output C (defined as genera present in at least 95% of samples).

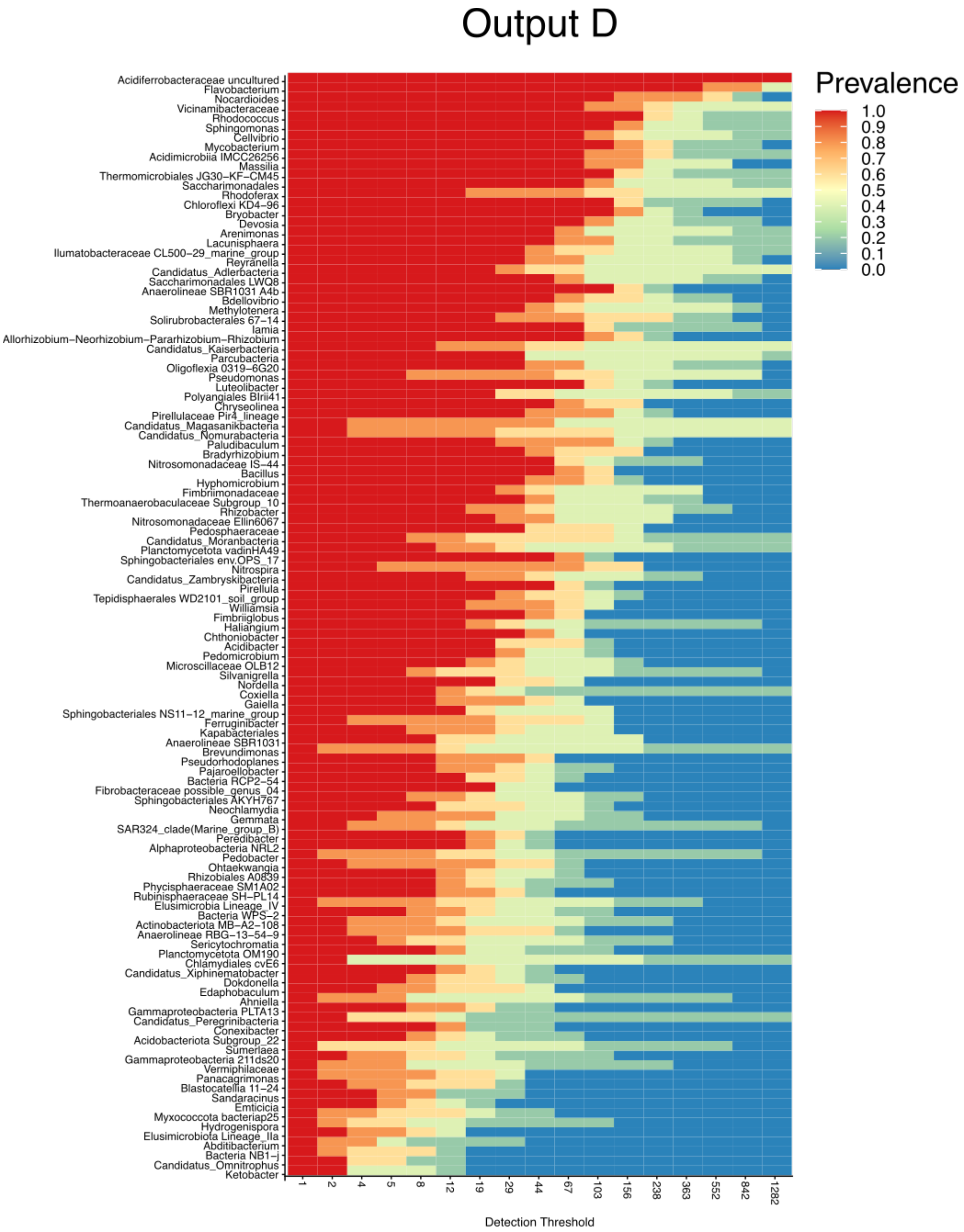

Figure A.7: Core microbiome of the rain garden Output D (defined as genera present in at least 95% of samples).

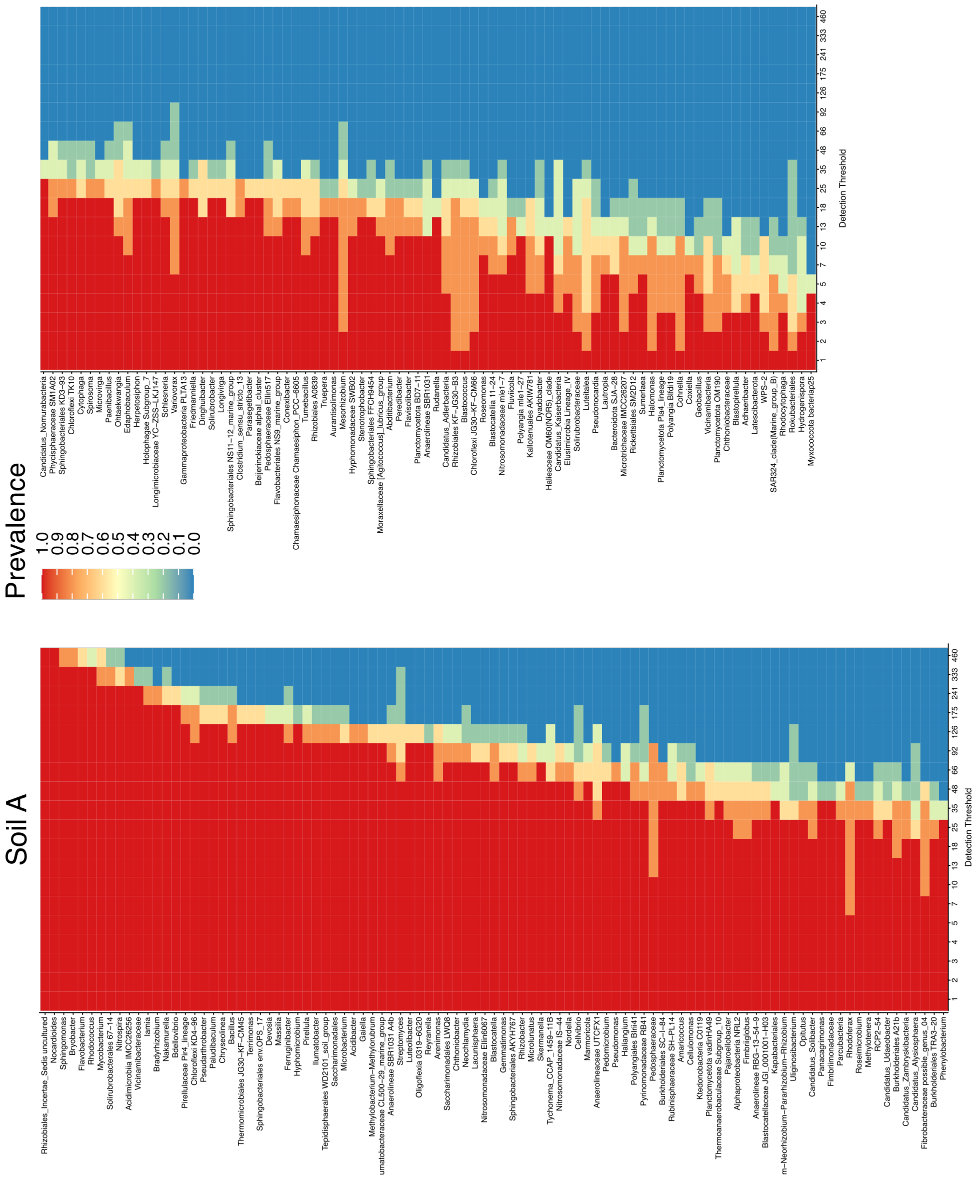

Figure A.8: Core microbiome of soil A (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

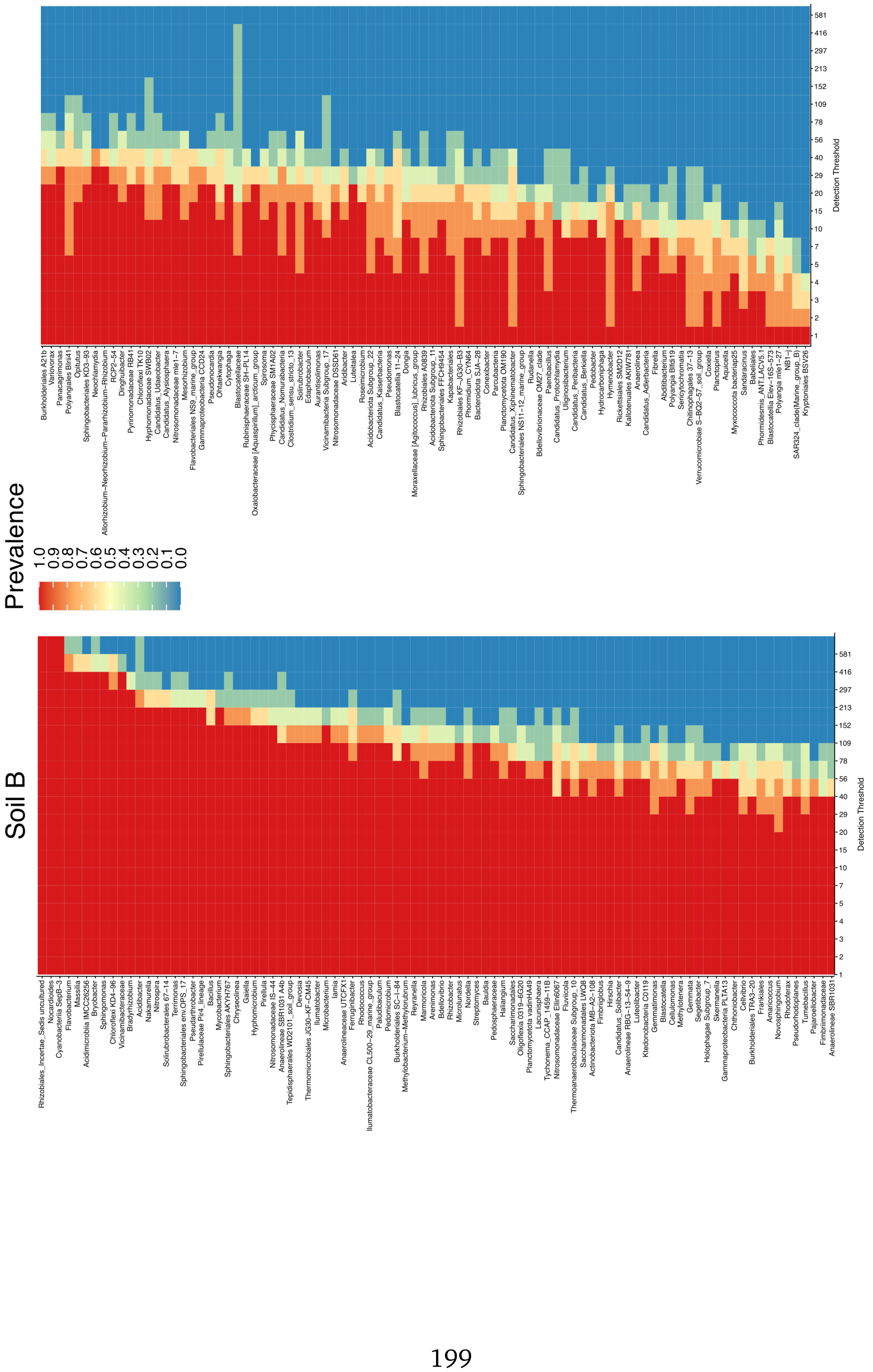

Figure A.9: Core microbiome of soil B (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

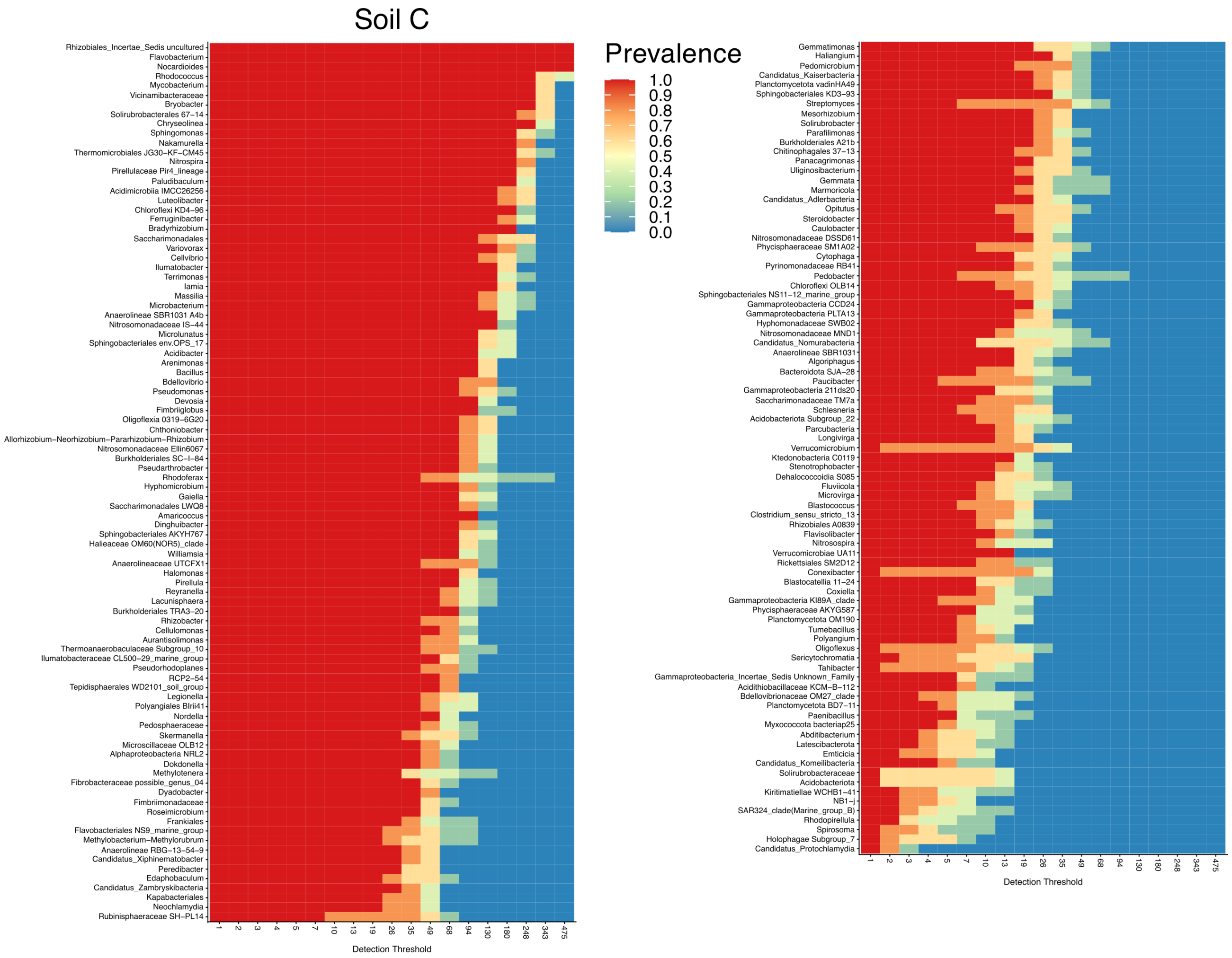

Figure A.10: Core microbiome of soil C (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

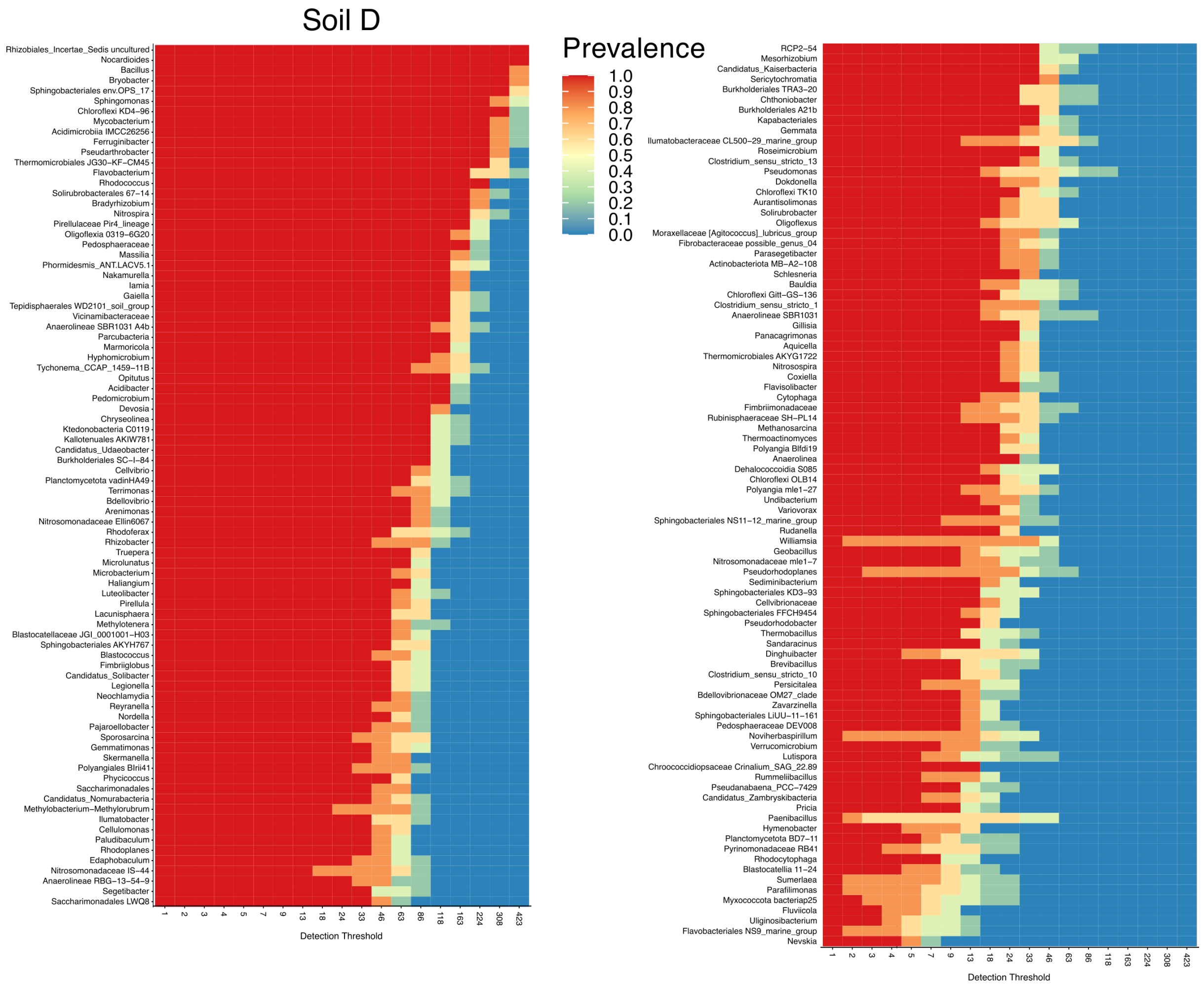

Figure A.11: Core microbiome of soil D (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

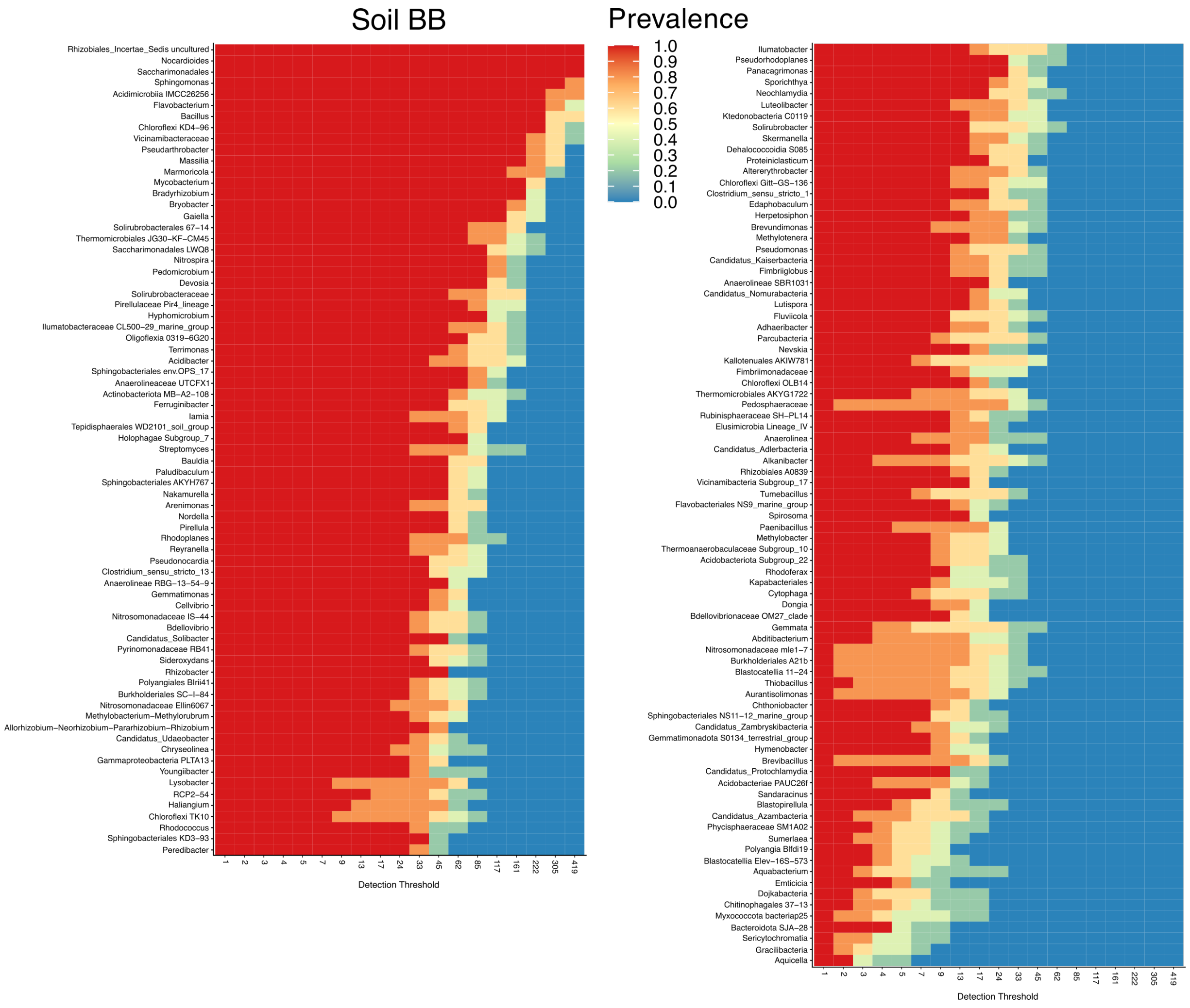

Figure A.12: Core microbiome of soil BB (defined as genera present in at least 95% of samples). Due to length, the image has been split and should be read left to right, top to bottom.

PERMANOVA Tables

Covariate	**Bray-Curtis**	**Unweighted UniFrac**
Country	R^2 = 0.20829 (p = 0.007 **)	R^2 = 0.14272 (p = 0.007 **)
Aluminium	N.S.	N.S.
COD	N.S.	N.S.
Copper	N.S.	N.S.
Iron	N.S.	N.S.
Nickel	N.S.	N.S.
Nitrite	N.S.	N.S.
Nitrate	N.S.	N.S.
Lead	N.S.	N.S.
Phosphate	N.S.	N.S.
Sulphate	N.S.	N.S.
TSS	N.S.	N.S.
Zinc	N.S.	N.S.

Covariate	**Weighted UniFrac**	**Hierarchical Meta-Storms**
Country	R^2 = 0.1913 (p = 0.012 *)	R^2 = 0.29176 (p = 0.016 *)
Aluminium	N.S.	N.S.
COD	N.S.	R^2 = 0.23646 (p = 0.02 *)
Copper	N.S.	N.S.
Iron	N.S.	N.S.
Nickel	N.S.	N.S.
Nitrite	N.S.	N.S.
Nitrate	N.S.	N.S.
Lead	N.S.	N.S.
Phosphate	R^2 = 0.16837 (p = 0.09 .)	N.S.
Sulphate	N.S.	N.S.
TSS	N.S.	N.S.
Zinc	N.S.	N.S.

Table A.25: Results of PERMANOVA testing on Glasgow & and Florianópolis samples using four beta diversity distance measures. The following annotations are used to denote significance: '***' ($p \leq 0.001$), '**' ($p \leq 0.01$), '*' ($p \leq 0.05$), '.' ($p \leq 0.1$), and 'N.S.' No significance. The R^2 value represents the proportion of variance explained by a given covariate.

Covariate	**Bray-Curtis**	**Unweighted UniFrac**
Water Source	R^2 = 0.24099 (p = 0.001 ***)	R^2 = 0.15411 (p = 0.002 **)
Date	R^2 = 0.31178 (p = 0.001 ***)	R^2 = 0.11545 (p = 0.001 ***)
Aluminium	R^2 = 0.08723 (p = 0.005 **)	R^2 = 0.04073 (p = 0.013 *)
COD	R^2 = 0.05779 (p = 0.022 *)	N.S.
Copper	R^2 = 0.06901 (p = 0.008 **)	R^2 = 0.03823 (p = 0.029 *)
EC	R^2 = 0.26124 (p = 0.001 ***)	R^2 = 0.06958 (p = 0.001 ***)
Iron	R^2 = 0.10682 (p = 0.001 ***)	R^2 = 0.04528 (p = 0.001 ***)
Nickel	R^2 = 0.10986 (p = 0.001 ***)	R^2 = 0.04827 (p = 0.001 ***)
Nitrite	R^2 = 0.05969 (p = 0.006 **)	N.S.
Nitrate	R^2 = 0.0637 (p = 0.001 ***)	R^2 = 0.03835 (p = 0.015 *)
Lead	R^2 = 0.10488 (p = 0.001 ***)	R^2 = 0.04347 (p = 0.008 **)
pH	R^2 = 0.07609 (p = 0.012 *)	R^2 = 0.04287 (p = 0.008 **)
Phosphate	R^2 = 0.07445 (p = 0.015 *)	R^2 = 0.04173 (p = 0.013 *)
Sulphate	R^2 = 0.18158 (p = 0.001 ***)	R^2 = 0.05713 (p = 0.001 ***)
TSS	R^2 = 0.0667 (p = 0.001 ***)	R^2 = 0.03668 (p = 0.054 .)
Zinc	R^2 = 0.07818 (0.002 **)	R^2 = 0.04337 (p = 0.001 ***)

Covariate	**Weighted UniFrac**	**Hierarchical Meta-Storms**
Water Source	R^2 = 0.26003 (p = 0.002 **)	R^2 = 0.23435 (p = 0.024 *)
Date	R^2 = 0.30116 (p = 0.001 ***)	R^2 = 0.53954 (p = 0.001 ***)
Aluminium	R^2 = 0.0849 (p = 0.017 *)	R^2 = 0.08973 (p = 0.035 *)
COD	R^2 = 0.07044 (p = 0.037 *)	R^2 = 0.14486 (p = 0.03)
Copper	R^2 = 0.08074 (p = 0.024 *)	R^2 = 0.08568 (p = 0.047 *)
EC	R^2 = 0.19428 (p = 0.001 ***)	R^2 = 0.26049 (p = 0.001 ***)
Iron	R^2 = 0.09647 (p = 0.009 **)	R^2 = 0.07095 (p = 0.085 .)
Nickel	R^2 = 0.08112 (p = 0.025 *)	R^2 = 0.10519 (p = 0.033 *)
Nitrite	R^2 = 0.05995 (p = 0.067 .)	R^2 = 0.08626 (p = 0.053 .)
Nitrate	R^2 = 0.09149 (p = 0.01 **)	R^2 = 0.33911 (p = 0.002 **)
Lead	R^2 = 0.09959 (p = 0.006 **)	R^2 = 0.1476 (p = 0.004 **)
pH	R^2 = 0.0591 (p = 0.085 .)	R^2 = 0.28473 (p = 0.001 ***)
Phosphate	R^2 = 0.07189 (p = 0.036 *)	R^2 = 0.20928 (p = 0.003 **)
Sulphate	R^2 = 0.16164 (p = 0.001 ***)	R^2 = 0.31475 (p = 0.001 ***)
TSS	N.S.	R^2 = 0.05451 (p = 0.076 .)
Zinc	R^2 = 0.08794 (p = 0.016 *)	R^2 = 0.06956 (p = 0.07 .)

Table A.26: Results of PERMANOVA testing on rain garden influent & effluent samples using four beta diversity distance measures. The following annotations are used to denote significance: '***' ($p \leq 0.001$), '**' ($p \leq 0.01$), '*' ($p \leq 0.05$), '.' ($p \leq 0.1$), and 'N.S.' No significance. The R^2 value represents the proportion of variance explained by a given covariate.

Covariate	**Bray-Curtis**	**Unweighted UniFrac**
Soil Source	R^2 = 0.54258 (p = 0.001 ***)	R^2 = 0.23536 (p = 0.001 ***)
Aluminium	R^2 = 0.15646 (p = 0.001 ***)	R^2 = 0.06201 (p = 0.001 ***)
Copper	R^2 = 0.18499 (p = 0.001 ***)	R^2 = 0.06426 (p = 0.001 ***)
EC	R^2 = 0.11573 (p = 0.001 ***)	R^2 = 0.05736 (p = 0.001 ***)
Iron	R^2 = 0.15689 (p = 0.001 ***)	R^2 = 0.06174 (p = 0.001 ***)
Moisture	R^2 = 0.20045 (p = 0.001 ***)	R^2 = 0.06485 (p = 0.001 ***)
Nickel	R^2 = 0.15892 (p = 0.001 ***)	R^2 = 0.06234 (p = 0.001 ***)
Lead	R^2 = 0.14988 (p = 0.001 ***)	R^2 = 0.06193 (p = 0.001 ***)
pH	R^2 = 0.11807 (p = 0.002 **)	R^2 = 0.05891 (p = 0.001 ***)
TPH	R^2 = 0.19996 (p = 0.002 **)	R^2 = 0.06464 (p = 0.001 ***)
Zinc	R^2 = 0.18638 (p = 0.002 **)	R^2 = 0.06349 (p = 0.001 ***)
Hydraulic Conductivity	R^2 = 0.12051 (p = 0.001 ***)	R^2 = 0.05659 (p = 0.001 ***)
% Coarse Gravel	R^2 = 0.14828 (p = 0.001 ***)	R^2 = 0.06207 (p = 0.001 ***)
% Coarse + Medium Sand	R^2 = 0.15877 (p = 0.001 ***)	R^2 = 0.06472 (p = 0.001 ***)
% Coarse Sand	R^2 = 0.17032 (p = 0.001 ***)	R^2 = 0.06079 (p = 0.001 ***)
% Fine Gravel	R^2 = 0.16636 (p = 0.001 ***	R^2 = 0.06147 (p = 0.001 ***)
% Fine Sand	R^2 = 0.17192 (p = 0.001 ***)	R^2 = 0.06108 (p = 0.001 ***)
% > 1 mm	R^2 = 0.1703 (p = 0.001 ***)	R^2 = 0.06161 (p = 0.001 ***)
% < 0.15 mm	R^2 = 0.17861 (p = 0.001 ***)	R^2 = 0.06452 (p = 0.001 ***)
% Medium Sand	R^2 = 0.17754 (p = 0.001 ***)	R^2 = 0.06337 (p = 0.001 ***)
% Silt + Clay	R^2 = 0.13932 (p = 0.001 ***)	R^2 = 0.06278 (p = 0.001 ***)
% Very Coarse Sand	R^2 = 0.17213 (p = 0.001 ***)	R^2 = 0.06164 (p = 0.001 ***)
% Very Fine Sand	R^2 = 0.19172 (p = 0.001 ***)	R^2 = 0.06443 (p = 0.001 ***)

Covariate	Weighted UniFrac	Hierarchical Meta-Storms
Soil Source	R^2 = 0.58127 (p = 0.001 ***)	R^2 = 0.87135 (p = 0.001 ***)
Aluminium	R^2 = 0.1146 (p = 0.013*)	R^2 = 0.21294 (p = 0.003 **)
Copper	R^2 = 0.14672 (p = 0.001 ***)	R^2 = 0.29532 (p = 0.001 ***)
EC	R^2 = 0.14983 (p = 0.002 **)	R^2 = 0.23689 (p = 0.001 ***)
Iron	R^2 = 0.11185 (p = 0.011 *)	R^2 = 0.20837 (p = 0.002 **)
Moisture	R^2 = 0.21506 (p = 0.001 ***)	R^2 = 0.40661 (p = 0.001 ***)
Nickel	R^2 = 0.11758 (p = 0.004 **)	R^2 = 0.23063 (p = 0.001 ***)
Lead	R^2 = 0.11729 (p = 0.006 **)	R^2 = 0.19116 (p = 0.003 **)
pH	R^2 = 0.16689 (p = 0.001 ***)	R^2 = 0.21584 (p = 0.002 **)
TPH	R^2 = 0.17337 (p = 0.001 ***)	R^2 = 0.27309 (p = 0.001 ***)
Zinc	R^2 = 0.13921 (p = 0.002 **)	0.26653 (p = 0.001 ***)
Hydraulic Conductivity	R^2 = 0.17859 (p = 0.001 ***)	N.S.
% Coarse Gravel R^2	= 0.2214 (p = 0.001 ***)	R^2 = 0.09665 (p = 0.061 .)
% Coarse + Medium Sand	R^2 = 0.2134 (p = 0.001 ***)	R^2 = 0.10515 (p = 0.05 *)
% Coarse Sand	R^2 = 0.1543 (p = 0.005 **)	R^2 = 0.19787 (p = 0.002 **)
% Fine Gravel	R^2 = 0.2084 (p = 0.001 ***)	R^2 = 0.14882 (p = 0.01 **)
% Fine Sand	R^2 = 0.18494 (p = 0.001 ***)	R^2 = 0.17964 (p = 0.004 **)
% > 1 mm	R^2 = 0.2039 (p = 0.001 ***)	R^2 = 0.16098 (p = 0.009 **)
% < 0.15 mm	R^2 = 0.21962 (p = 0.001 ***)	R^2 = 0.15345 (p = 0.01 **)
% Medium Sand	R^2 = 0.21646 (p = 0.001 ***)	R^2 = 0.16217 (p = 0.013 *)
% Silt + Clay	R^2 = 0.20834 (p = 0.001 ***)	R^2 = 0.08393 (p = 0.094 .)
% Very Coarse Sand	R^2 = 0.20037 (p = 0.001 ***)	R^2 = 0.16784 (0.013 *)
% Very Fine Sand	R^2 = 0.18786 (p = 0.001 ***)	R^2 = 0.20847 (p = 0.004 **)

Table A.27: Results of PERMANOVA testing on rain garden soil samples using four beta diversity distance measures. The following annotations are used to denote significance: '***' ($p \leq 0.001$), '**' ($p \leq 0.01$), '*' ($p \leq 0.05$), '.' ($p \leq 0.1$), and 'N.S.' No significance. The R^2 value represents the proportion of variance explained by a given covariate.