THE UNIVERSITY OF HULL

Investigating Sustainable Solutions for Roadside Gully Pot Management

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by

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Abstract

Roadside gully pots are an important component of urban drainage, with over 17 million examples in service throughout England and Wales. Their main purpose is to retain sediments from road runoff, leaves and organic litter in order to avoid blockage or hydraulic restriction of the drainage system. Gully pots require regular mechanical cleaning to prevent blockages; indeed, blocked gullies were partially blamed for exacerbating flooding in the city of Kingston upon Hull in 2007. The rate at which mechanical cleaning and emptying of individual gully pots is required depends in part on the decomposition rate of the waste contained within. However, the physical and chemical processes which dominate decomposition processes are poorly understood. Understanding these internal processes, and whether climate and catchment area have the potential to affect them, is an important factor in developing sustainable solutions for managing gully pots, thus reducing the likelihood of future blockages.

In order to establish a basic understanding of internal gully pot processes, waste was collected from a range of catchment areas and across different seasons. This allowed temporal and spatial variability to be assessed. Model gully pots were then set up under laboratory condition to monitor the effects of moisture and temperature in situ over a six month period. Additionally, the effect of substrate addition, including glucose, Tween 80 and itaconic acid, was assessed within these model gully pots. A composting trial was also executed under mesophilic and thermophilic conditions assessing the effect of a substrate addition (starch) on the waste. In order to assess the processes within the waste, the organic matter content, moisture content and pH were all measured throughout the study. The effects of these variables on the microbial community were assessed using Biolog EcoPlatesTM, along with the assessment of enzyme activity using a fluorogenic approach.

Seasonality has little influence upon the waste, whereas geographical location exhibits a stronger influence. This can be attributed to the variable levels of foliage in the areas. Under laboratory conditions the waste was significantly affected by temperature, showing greater degradation at higher temperatures. Varying moisture levels, however, had little to no effect. Furthermore, slight increases in degradation were observed upon the addition of a substrate to the waste; this increase varied not only with the choice of substrate, but was also temperature dependant. The starch addition to the compost trial confirmed the waste's ability to compost under both thermophilic and mesophilic conditions.

The results demonstrated the gully pot waste was able to decompose at a slow rate under replica field conditions. Using a substrate additive only increased this rate minimally, indicating that it would not be worthwhile for local authorities to use this as a substitute for, or in addition to, manual cleaning. However, the positive confirmation from the composting trial could be valuable when considering sustainable gully pot management in the future.

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Publications and Presentations

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Figures and Tables

1.0 Introduction

This thesis presents an assessment of the nature of decomposition processes within roadside gully pots in order to assess the efficacy of these decomposing environments and determine the potential for enhancing decomposition with a view to ensuring the efficient functioning of these components of urban drainage systems.

1.1 Background, rationale and justification

Roadside gully pots are an important component of urban drainage. Gully pots (also known as catch basins) are small sumps that are located in the roadside gutter which act as runoff inlet points to surface water sewers, combined sewers and drainage networks. Their main purpose is to retain sediments from road runoff, leaves and organic litter before entry into drains and sewers in order to avoid blockages or hydraulic restriction in the drainage system (Butler *et al*., 1995; Butler and Memon, 1999; Deletic *et al*., 2000; Memon and Butler, 2002a; 2002b; Osborne *et al*., 1998). They are extensively used in urban drainage networks, with over 17 million gully pots in service in England and Wales (Butler and Karunaratne, 1995; Memon and Butler, 2002b), with approximately 73,000 of these in Hull (*pers. comm.* Hagar, 2009). Gully pots collect significantly large amounts of material and therefore require regular mechanical cleaning (Karlsson and Viklander, 2008) to prevent them becoming blocked, or partially blocked. Blocked gullies may cause flooding (Osbourne *et al*., 1998) and blocked gullies were partially blamed for exacerbating the 2007 Hull floods (Coulthard *et al*., 2007).

The rate of decomposition of matter trapped within the gully pots may impact upon how often they are cleaned, yet we know little of the physical and chemical processes operating within the gully pots. Previous research on the gully pot has concentrated on pollution effluent (Fletcher and Pratt, 1981; Grottker, 1990), water runoff quality (Memon and Butler, 2002b), sediment supply (Deletic *et al*., 2000; Ellis and Harrop, 1984), solid trapping efficiency (Butler and Karunaratne, 1995), gully pot sediment aging (Clegg *et al*., 1993) etc. but not on the decomposition process occurring within the gully pot.

1.2 Aims and Objectives

The main aims of this research are:

- To investigate the decomposition processes that occurs within gully pots
- Identify whether seasonal and catchment area variations have any impact upon the processes
- Investigate methods to assist with speeding up decomposition within the gullies to prevent/remediate blockages.

Understanding the processes that occur within gully pots and determining if weather and catchment area affect these processes, is an important element in developing sustainable solutions for managing the pots. It is only with prior knowledge of the processes involved, and an understanding of what is actually collected in the gully pots, that methods can be developed to assist with the acceleration of the decomposition process. If the decomposition process inside the gully pot can be improved, the risk of

blockage could be reduced and future blockages could potentially be prevented, or at least avoided to some degree. This could also reduce the amount of time required in the emptying of the gullies thus saving local authorities time and money.

1.3 Outline of the thesis

This thesis consists of eight chapters, with this introduction forming the first. Chapter 2 reviews the relevant literature within the scope of this study. This chapter focuses on the gully pot system and current management of the waste. It then progresses onto looking into *in situ* and *ex situ* decomposition processes within wastes, composts and soils, and the variables which can control it and the means of increasing it. Chapter 3 outlines the main methodologies used throughout the study on various components of the data collection plan. This includes sample collection, and physical and microbial analysis; providing justification throughout. Chapters 4, 5, 6, and 7 elaborate on method adaptations, presents results and discusses these results in relation to the original research questions. Chapter 4 presents the results from *in situ* analysis in the field, assessing the effects of geographical location and seasons on the physical processes and extracellular enzyme activity. Chapter 5 presents the results from *in situ* analysis of model gully pots within the laboratory environment assessing the effects of time, temperature and moisture on the physical processes and microbial community. Chapter 6 presents the results from *in situ* analysis of gully pot waste amended with additives within the laboratory environment assessing the effects of time, temperature and moisture on the physical processes and microbial community. Chapter 7 presents the results from *ex situ* analysis of gully pot waste within the laboratory environment assessing the effects of time, temperature and moisture on the physical processes and extracellular enzyme activity. Chapter 8 contains the main conclusions of the research,

providing suggestions for further work and for alternative gully pot waste management strategies.

2.0 Literature Review

2.1 Roadside Gully Pot

2.1.1 Definition and function

A roadside gully pot, also known as a catch basin in North America and Canada (Osborne *et al*., 1998), is a small settling chamber or sump provided along the kerb of the road (Memon and Butler, 2002a; 200b). Historically, the purpose of a gully pot was to prevent sewers clogging by trapping coarse debris and to prevent odour emanations from the sewer by providing a water seal (Lager *et al*., 1977). The prevention of sewer clogging was especially important prior to the existence of good quality street pavements (Lager *et al*., 1977). For storm and combined sewer systems the roadside gully pot forms an important and integral part of the surface water collection infrastructure (Butler *et al*., 1995). They are extensively used in urban drainage networks, primarily to prevent surface runoff from carrying solids and sediment into drains and sewers, and so causing blockage or hydraulic restriction in the drainage system (Butler *et al*., 1995; Butler and Memon, 1999; Deletic *et al*., 2000; Memon and Butler, 2002a; 2002b; Osborne *et al*., 1998). Gully pots also have important secondary functions; providing a water seal to prevent odours from combined sewers escaping to the atmosphere (as mentioned above) (Butler and Davies 2004; Butler *et al*., 1995; Osborne *et al*., 1998); retaining some fine sediment and so reducing the polluting load (Begum *et al*., 2008; Ellis and Harrop, 1984; Osborne *et al*., 1998); retaining oil and other floating pollutants (Osborne *et al*., 1998) and reducing the risk of accidents caused by the build up of water on the roads and in the gutters (Begum *et al*., 2008).

Being a ubiquitous component of many drainage systems, there are more than 17 million gully pots in service in England and Wales (Butler and Karunaratne, 1995; Memon and Butler, 2002b), with approximately 73,000 in Hull (*pers. comm.* Hagar, 2009).

2.1.2 Design

Gully pots are available in a range of diameters and depths and made from a variety of materials (Grottker, 1990; Osborne *et al*., 1998). In older, urban areas it is common to find square or rectangular brick-built gully pots, however most new pots are constructed from plastic, ordinary or sulphate-resistant concrete, or clay (Osborne *et al*., 1998). The most common pots installed in the UK are circular with a diameter of 450mm and a capacity of 90 litres (Osborne *et al*., 1998). A typical gully pot consists of a sump which acts as a settling basin to trap sediment, with an outlet pipe to the sewer, which is generally below the water level (Lager *et al*., 1977; Osborne *et al*., 1998) (see Figure 2.1).

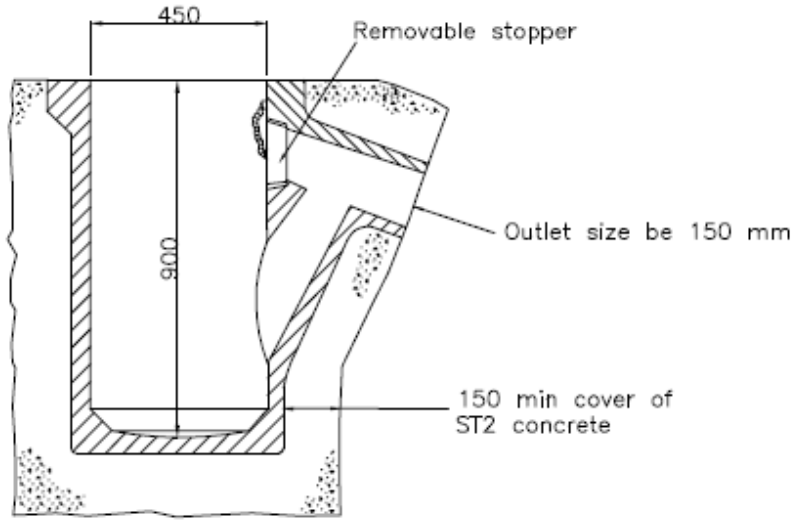

Figure 2.1 Diagram of a large square gully pot used by Hull City Council

These sumps are normally built under the inlet grating, or alternatively the openings are either under the gutter or just at the back of the curb (Davis *et al*., 1996; Lager *et al*., 1977). Inlet gratings vary in design, with the older gully pots having diamond gratings and the newer ones having gratings with slats. Due to the inefficient design of the older gully pot gratings, these are slowly being replaced in the city of Hull, as the holes in the gratings are relatively small (see Figure 2.2), therefore restricting the amount of leaf litter and detritus entering the gully pot, and causing the litter to block the entry to the pot (*pers. comm.* Hagar 2009).

Figure 2.2 Detritus and leaf litter collecting on the diamond gully pot lid.

Occasionally, one gully pot will serve two or more standard inlets (Lager *et al*., 1977). If the gully pot is connected to a foul- or combined-sewer, a water seal is incorporated to act as an odour trap (Butler and Davies, 2004; Davis *et al*., 1996; Lager *et al*., 1977). During rainfall, surface water is directed into the pot via a grating or inlet and typically

falls vertically to impact the surface of the pot reservoir below (Butler *et al*., 1995). The solids trapping efficiency is high for particles in excess of 300μm, but poor for smaller particles which carry proportionately more of the pollutant load (Butler *et al*., 1995).

The position and number of gullies installed in a highway will depend on the rainfall regime, the area being drained, the gradient of the carriageway, and the type of surface (Osborne *et al*., 1998). Gullies are provided to cope with the maximum discharge the channel is capable of delivering without over topping (Davis *et al*., 1996). In current practice their spacing is dictated primarily by the hydraulic requirements of the inlet grating in draining the road surface effectively (Butler and Karunaratne, 1995) rather than that of the gully pot or the pipes in the drainage system, as these are usually designed to cope with more water than the grating can deliver. Another factor controlling gully spacing is the amount of water flowing along the road edge, the width of flow tolerated depending on the nuisance which it would cause (Davis *et al*., 1996). In the UK it is conventional to use a catchment area per gully of 200m^2 (Butler and Karunaratne, 1995; Osborne *et al*., 1998).

2.1.3 Operation in dry weather and wet weather

The gully pot operates under two distinct regimes, dry weather and wet weather. Biochemical processes dominate when the gully pot is operating under dry weather conditions, and physical processes dominate during wet weather (Butler *et al*., 1995). During dry weather biochemical reactions dominate, with rapid drops in dissolved oxygen (DO) concentrations, particularly in the summer, resulting in the establishment of anoxic conditions and anaerobic degradation (Butler *et al*., 1995; Memon and Butler 2002a). The depressed DO levels result in the build up of anoxic conditions and

anaerobic degradation of the bottom sediments (Butler *et al*., 1995). Re-aeration will occur only when sufficient rainfall passes through the pot (Butler *et al*., 1995). After a storm event inflow into the gully pot ceases and conditions become rapidly quiescent (Butler *et al*., 1995).

In wet weather, physical processes predominate as incoming runoff rapidly displaces the standing liquor, including its dissolved and suspended pollutant load (Butler *et al*., 1995). Typical wet weather processes include sedimentation, sediment bed build up, erosion of the top sediment layer, dilution and washout of the dissolved and suspended pollutants in the top liquor, and re-aeration of gully liquor (Butler and Memon, 1999). During storm events high runoff rates produce marked decreases in pH levels from the initial gully pot liquor values of pH 6.0-7.1 to values approaching typical rainfall levels (average rainfall pH=4.1) (Morrison *et al*., 1995).

2.1.4 Solid supply

Solids from above-ground surfaces gain access to the sewerage system during storm events mainly by suspension in runoff (Butler and Karunaratne, 1995). Other possible mechanisms include the action of wind and vehicle-generated turbulence, vibration, street sweeping and deliberate dumping of material (Butler *et al*., 1995; Butler and Karunaratne, 1995). The rate of the material supply into the highway gullies is highly variable, both spatially and temporally, within the catchment area (Pratt *et al*. 1987). Pratt *et al*. (1987) found evidence that variability exists both between measurements of the contents of the gullies in the same 14 day period and between the results of one gully obtained in different periods of the year. The spatial variability may be associated with land use and human activity (Pratt *et al*. 1987).

Seasonal variation has been observed in a catchment in North London where a peak in material supply was found to occur in June, when soil surfaces dried and water- or wind-mobilised material were readily available because of gardening and other human and animal activities (Pratt *et al*. 1987). In the months from June to the following February, solid material supply to the gully generally decreased as outdoor activities decreased; soils became wetter, binding particles to surfaces, and the available plant matter was limited (Pratt *et al*. 1987). The leaf fall in November to December may be significant in some catchments and can represent another clear peak in material supply (Pratt *et al*. 1987). Around February to March snow and freezing conditions resulted in an increase in supply, either as a result of road gritting or during the thaw when frost-loosened material was transported to gullies (Pratt *et al*. 1987).

Different locations may lead to different timings for peak material inputs depending on the local climate and environment. For example, leaf fall in November to December may be significant in some catchments and represent another clear peak in supply (Pratt *et al*. 1987). This seasonal variation was supported by Grottker (1990), who found that the loss on ignition analysis, which measures organic matter, from samples of dry gully pots were 6% – 10% greater in autumn than spring. Furthermore, Ellis and Harrop (1984) found that the rate of sediment removed showed a strong seasonal variation which was clearly influenced by both rainfall and surface flow characteristics. Total sediment weight removed from the road surface during the spring period was only 18-20% of the summer loadings, which showed a close correspondence to both seasonal rainfall and flow characteristics (Ellis and Harrop, 1984).

Gully pots are also prone to gross pollutants, contaminants, soil and grease which often form a layer of scum on the top of the liquid within the pot, as well as the accumulation of larger particles and rubbish such as paper, confectionary wrappers, tins, sticks and cigarette-stubs (Begum *et al*., 2008; Grottker, 1990; Hepp, 1995) as seen in Figure 2.3.

Figure 2.3 Large particle and rubbish littered within a gully pot.

Depending on the interval between storms, this liquid within the gully pot may also be highly turbid or less so if the sediment contained in the liquid has had a chance to settle (Hepp, 1995). Morrison *et al*., (1995) found that solids washed into the gully pots contained organic fractions of up to 40%, and that these possessed a size composition in which 75% of particles were less than 250μm in diameter. These results were consistent with those reported by Ashley and Crabtree (1992). Several studies (for example Ellis and Harrop, 1984; Pratt and Adams, 1984; Sartor *et al*., 1974) have attempted to intercept the solids prior to the point of entry in order to determine size distribution. The

smaller particles are more important in terms of water quality control as they contain the majority of pollutants of concern (Butler and Davies, 2004). Although one role of gully pots is to remove solids from surface runoff, there will be a transfer of these solids into the sewerage system during high flow events (Clegg *et al*., 1993). The sediments which accumulate in the gully pots must, therefore, be thought of as precursors to sewer sediments (Clegg *et al*., 1993).

When road runoff conveying sediment enters a gully pot, the sediment mixes with the gully liquor and the following can occur: (a) deposition of some of the input sediment and overall build-up of gully sediment deposits, or (b) erosion of part of the existing sediment deposits, with a reduction in the depth of deposits (Deletic *et al*., 2000). During low flow rates deposition tends to occur, whilst erosion will occur only during the most intense storm events (Deletic *et al*., 2000). Roadside gully pots have been identified as potential sources, and can make significant contributions to stormwater pollutant loadings (Fletcher and Pratt, 1981; Morrison *et al*., 1988). Between storm events the gully pot sediments and liquor undergo changes to composition as a result of biochemical reactions (Morrison *et al*., 1995), as described in Section 2.3.1 (below).

2.1.5 Maintenance and cleaning

As the primary purpose of a gully pot is to trap solids that would otherwise enter the sewer, the material that has been trapped must be removed if the gully pot is to perform in its designed manner (Lager *et al*., 1977; Karlsson and Viklander, 2008). This is typically carried out periodically once or twice a year depending on the road use (Osborne *et al*., 1998). Failure to clean gully pots can lead them to becoming blocked, or partially blocked, resulting in surface flooding (Osborne *et al*., 1998). As when they

become clogged, storm water backs up and spreads over the pavement and adjacent areas (e.g. Figure 2.4), and serious property damage can occur (Lager *et al*., 1977).

Figure 2.4 Blocked gully showing water backing up.

The most commonly used method to clean gully pots is to use an eductor truck (see Figure 2.5), which uses hydrodynamic pressure and a vacuum to loosen and remove solids and the standing liquids from the gully pot (Karlsson and Viklander, 2008; Lager *et al*., 1977). The eductor will usually not pass large debris so larger items require manually removing with long gully grabs prior to cleaning (Lager *et al*., 1977). Manual cleaning is also used when the eductor truck is unable to reach the gully pots. Gratings, openings, traps, and outlets must also be kept free so that they will not interfere with, or prevent, the flow of storm water (Lager *et al*., 1977). The expenses and hazards involved in cleaning clogged gully pots during storm conditions make a regular cleaning program an attractive alternative (Lager *et al*., 1977).

Figure 2.5 A gully pot being cleaned by an operative using the eductor truck.

When the gully pot is cleaned the solids and standing liquid are vacuumed into the eductor truck, and this mixture becomes a slurry that has to be disposed of (Karlsson and Viklander, 2008). The waste was previously classed as a 'Controlled Waste' as defined in Section 75(4) of The Environmental Protection Act 1990 (Osborne *et al*., 1998), in practice, this means that the residue must be disposed of at a licensed disposal site (Osborne *et al*., 1998). However, the waste is now classed as a waste code '20 03 03: street cleaning residue' which is a code for non-hazardous waste, although it may have a hazardous nature (Environment Agency, 2012). This means that the waste no longer has to be sent to landfill and can be disposed of in a more sustainable manner, such as composting or dividing and reusing the waste (as described in Section 2.1.5.2).

2.1.5.1 Gully pot cleaning frequency

Gully pot cleaning is regarded as a cyclic activity based on emptying at a predetermined number of times per year. Due to this the content of the gully pot is extracted once or twice a year and transported for disposal (Butler *et al*., 1995; Memon and Butler, 2002b; Nanbakhsh *et al*., 2007; Osborne *et al*., 1998). After the severe flooding event that impacted upon Hull in 2007, the level of recommended gully cleaning (being 12 months or every six months for gullies on major routes) was outlined by the Independent Review Report (Coulthard *et al.*, 2007).

2.1.5.2 Operation and maintenance costs

The operation costs include gully cleaning and debris removal, maintenance cost of sump and trap, and operation and maintenance cost of gully pot cleaning equipment etc. The cleaning costs involved with gully pot cleaning can vary depending on the methods used by the local authority, the frequency in which they are required to be cleaned, the amount of debris removed and the cost to dispose of the debris (Lager *et al.*, 1977). During 2010 it cost Hull City Council, the local authority, approximately £94 per tonne just to send the waste to the landfill, a figure which increases on a yearly basis as the landfill tax increases (*pers. comm.* Thomas, 2011).

Due the high costs incurred though the use of the landfill, it is in the best interest of the local authority to look for cheaper, more sustainable methods for managing gully pot waste. Hull City Council have recently conducted a one-year trial where the gully waste, mixed with street sweepings, was sent to an external company where the waste

was divided and the organic material was sent off to external composters. In doing so, the council are saving approximately £56 per tonne for waste processing, a figure which would be the premium incurred for sending this material to landfill (*pers. comm.* Thomas, 2011); as well as enabling the waste to be managed in a more sustainable manner.

2.2 Decomposition

Decomposition is defined as the process of separation of materials into their constituent parts, and it represents the biodegradation of organic materials (Paul and Clark, 1996). The study of decomposition is not a unified scientific discipline – it draws upon the subject matter of ecology, soil science, agriculture, forestry, microbiology, physiology, biochemistry and zoology (Swift *et al*., 1979). Decomposition has in the past been most closely tied to ecosystems processes and soil fertility (Paul and Clark, 1996).

2.2.1 Soil decomposition processes

Decomposition is an enzyme mediated biological process carried out by bacteria and fungi (Palmer and Troeh, 1995). When organic material in the soil decomposes, carbon dioxide and nutrients are released due to the mineralization of carbon (Palmer and Troeh, 1995). The way which organic inputs in soil are decomposed depends primarily on their quality, which is dependent on the type of compounds that are present within them (Bardgett, 2005).

Obtaining a stable product from biological oxidative transformation effectively mirrors the degradation processes which occur naturally in the soil (de Bertoldi *et al*., 1983).

While soils are usually characterised by a steady-state situation, composting substrate is a fast changing system (Mondini *et al*., 2004). Composting and soil processes share a variety of simultaneously assimilated substrates, and organisms (Kaiser, 1996). However, they are characterised by different properties (Mondini *et al*., 2004) and the method of matter transfer in composting is to achieve fast substrate degradation in an aerobic environment, and this characterises the process as a solid-state fermentation (Kaiser, 1996).

Composting is largely an aerobic process, but anaerobic microenvironments may develop (Tuomela *et al*., 2000). As the quickest way to produce high quality compost, aerobic composting is a widely accepted way of stabilizing organic wastes and converting them to useable, and value added compost product (Liang *et al*., 2003). Aerobic composting is the process where decomposition takes place in the presence of oxygen (Liang *et al*., 2003; Kulcu and Yaldiz, 2004) and the principle aeration methods providing O_2 during composting are: physical turning of the mass, natural convection, and forced aeration (Kulcu and Yaldiz, 2004).

Temperature, moisture content, C/N ratio (nutrient balance), pH and available nutrients have been shown to have significant impact on composting performance (Liang *et al*., 2003). Microbial activities measured in biosolids blended at controlled temperatures and moisture settings show that moisture content has a greater influence on activity than temperature (Liang *et al*., 2003; Margesin *et al*., 2006). However, moisture and oxygen levels often have an inverse relationship, as when soil moisture is high, deficiency of oxygen may restrict decomposition, whereas when the soil is dry, moisture but not oxygen will be the limiting factor (White, 1997). Composting under low temperature

conditions is thought to not be practicable because of low microbial degradation levels, however, successful composting of municipal sludge (Smith, 1984) or animal manure (Lynch and Cherry, 1996; McCartney and Eftoda, 2005) has been observed at ambient temperatures of 15 to 28°C (Margesin *et al*., 2006).

Composting passes through several stages, each of which is characterised by the activity of different microbial groups (de Bertoldi *et al*., 1983; Fogarty and Tuovinen, 1991; Tuomela, 2000). Under optimal conditions the phases, through which composting proceeds are the mesophilic phase leading to the thermophilic phase, which can last from a few days to several months, and the cooling and maturation phase, which lasts for several months (Fogarty and Tuovinen, 1991; Tuomela *et al*., 2000). The length of the composting phases depends on the nature of the organic matter being composed and the efficiency of the process, which is in turn determined by the degree of aeration and agitation (Tuomela *et al*., 2000).

Organic materials are varied in composition. For example, the litter of deciduous trees and animal faeces are rapidly decomposing materials that generally contain high amounts of labile substances, such as amino acids and sugars, and low concentrations of recalcitrant compounds such as lignin (Bardgett, 2005). In contrast, the litter of coniferous trees decomposes slowly, being rich in large, complex structural compounds such as lignin and defence compounds such as polyphenols; this material is also unpalatable to soil fauna, further slowing down its decomposition (Bardgett, 2005). Wood is degraded by bacteria under certain extreme environmental conditions, e.g. wood saturated with water, almost anaerobic conditions or wood with a high extractive content; however, the rate of degradation is very slow (Tuomela *et al*., 2000). The

degradation of lignin, an enzymatic aerobic transformation is restricted to a limited microbial group of higher fungi: basidiomycetes (de Badiane *et al*., 2001). Basidiomycetes degrade lignin slowly and do not reach their highest degree of activity until about one month after the starting of the compost process (de Badiane *et al*., 2001). Benner and Hodson (1985) have reported that an elevated temperature of 55°C enhances the anaerobic degradation of lignin.

2.2.1.1 Temperature

In temperate climates temperature is one of the most important variables controlling the rate of microbial degradation of organic matter to methane, carbon dioxide, and water in anaerobic environments, such as sediments and waterlogged soils (Bardgett, 2005; Westermann, 1996). The effects of temperature on soil biological activity are well known; it is generally accepted that there is an approximate doubling of microbial activity and enzyme-catalysed reaction rates in soil for each 10°C rise in temperature, up to around 30-35°C (Bardgett, 2005). Above this temperature, however, most enzyme-catalysed reactions decline markedly, as proteins and membranes become denatured (Bardgett, 2005). Low temperature limits the decomposition of organic-material accumulation in soil (Douterelo *et al*., 2009).

When composting, it is widely accepted that temperature is an important environmental variable (Liang *et al*., 2003). Composting under low temperature conditions is thought not to be practical because of low microbial degradation activities (Margesin *et al*., 2006). Microbial metabolism is highly temperature dependant, and the population dynamics (e.g. composition and density) of microbes are dramatically influenced by temperature (Liang *et al*., 2003). The achievement of maximum temperature levels is

essential to an effective composting process and contributes substantially to the high rates of decomposition achieved during processing (Liang *et al*., 2003; Miller, 1992). The temperature of composting material below 20°C has been demonstrated to significantly slow or even stop the composting process (Liang *et al*., 2003). However, temperature in excess of 60°C have also been shown to reduce the activity of the microbial community, and above this temperature, microbial activity declines as the thermophilic optimum of microorganisms is surpassed (Liang *et al*., 2003; Miller, 1992).

2.2.2.2 Moisture

Moisture content is one of the most commonly used analysis for soil studies (Topp, 1993), and is important as it provides a medium for the transport of the dissolved nutrients required for the metabolic and physiological activities of microorganisms (Liang *et al*., 2003). Variation in the levels of saturation in the soil represent a physiological stress and can reduce soil microbial diversity, favouring those species that are best adapted to deal with the given stress (Douterelo *et al*., 2009). Very low moisture content values would cause early dehydration during composting, which will arrest the biological process, therefore giving physically stable but biologically unstable composts (de Bertoldi *et al*., 1983; Liang *et al*., 2003). Alternatively, high moisture may produce anaerobic conditions from waterlogging, which will prevent and halt the ongoing composting activities (Liang *et al*., 2003; Schulze, 1962; Tiquia *et al*., 1996). Waterlogging in soil instantly sets in motion a series of chemical and microbiological processes that affect nutrient cycling and accumulation of toxins (Pulford and Tabatabai, 1988). In addition to the retardation of gaseous exchange between soils and air, waterlogging results in changes to microbial populations, a decrease in soil redox

potential (Eh), as well as electrochemical and chemical changes (Pulford and Tabatabai, 1988). Many investigators have conducted experiments and identified that 50-60% moisture content is suitable for efficient composting, for example Tiquia *et al*., (1998); Suler and Finstein, (1977). The continuous decrease in moisture content during composting is an indication of organic matter decomposition (Kulcu and Yaldiz, 2004). Furthermore the measurement of moisture content is a crucial initial requirement for the calculation of organic matter and other physical parameters (Hesse, 1971).

2.2.2.3 pH

Soil pH affects the availability of nutrients and as a result influences the composition and diversity of the microbial community; decomposition is slower in acid soils than in neutral soils due to reduced microbial activity (Douterelo *et al*., 2009). Matter with a high range pH (from 3 to 11) can be composted, however, optimum values are between 5.5 and 8 (de Badiane *et al*., 2001; Shi *et al.*, 2006). Whilst bacteria prefer a nearly neutral pH, fungi develop better in a fairly acidic environment (de Badiane *et al*., 2001). Generally the pH begins to drop at the initiation of the composting process (de Badiane *et al*., 2001).

2.3 Enzymes

Life is composed of a series of enzymatic reactions that are responsible for most of the reactions in nutrient cycling (Paul and Clark, 1996). Enzymes are biological catalysts; they increase the rate of chemical reactions taking place within living cells without themselves suffering any overall change (Palmer, 2001).

2.3.1 Enzymes in soil

Enzyme activity profiles are an essential part of the functional diversity in soils, which are driven by the genetic diversity of soil micro-organisms, plants and soil animals as well as environmental effects and ecological interactions (Stemmer, 2004). Enzymes are found in plant seeds, fungal spores, bacterial endospores, protozoan cysts and plant roots (de Badiane *et al*., 2001; Paul and Clark, 1996). Various pools of enzyme activities (intracellular, free extracellular, clay – and humus – absorbed enzymes etc.) contribute to the overall enzyme activity measured (Stemmer, 2004). The biochemical versatility of the soil bacterial population provides soil with the capability to degrade all natural compounds and most of the synthetic compounds which enter either deliberately, for example as pesticides to crops, or accidently, for example industrial pollution of land (Wood, 1995). Many of the enzymes involved in these reactions are located within the organisms (endocellular enzymes), however, soils also possess enzyme activity which persists after the microbial population has been inhibited or killed, termed extracellular or abiotic enzymes (Wood, 1995). One of the most important soil properties is pH which affects the activity of enzymes due to the pH sensitivity of the amino acid functional group that alter conformational and chemical changes of the amino acids essential for binding and catalysis (Dick *et al*., 2000).

Soil enzyme activates are attractive as indicators for monitoring various impacts (such as pollution) on soil because of their central role in the soil environment (Margesin, 2005), serving several important functions (de Badiane *et al*., 2001; Darrah and Harris, 1986; Dick *et al*., 2000; Marx *et al*., 2001). Enzymes are the main mediators of soil biological processes, such as organic matter degradation, and mineralisation (Marx *et al*., 2001). They are intimately involved in the cycling of nutrients, reflect the

microbiological activity in soil and act as indicators of soil change (de Badiane *et al*., 2001; Caldwell, 2005; Dick *et al*., 2000). Soil enzyme activities have been used as biological indicators of environmental pollution, as an index for microbial activity, an index of soil fertility (Darrah and Harris, 1986), pollution with heavy metals, pesticides, and hydrocarbons (Margesin, 2005). Enzyme activity should indicate the ability of compost to degrade a wide range of organic substances (Mondini *et al*., 2004). In addition, on the basis of the well established relationship between enzyme activity and quality of organic matter, enzymes could give information on compost stability, which is defined as the degree of decomposition of the readily biodegradable organic matter (Mondini *et al*., 2004). Hydrolytic enzymes are believed to control the rate at which substances are degraded and become available for microbial or plant uptake; as such they could be used as functional indicators (Marx *et al*., 2001).

As the above discussion indicates, the presence of enzymes can be used as an indicator of degradation potential and functionality. For example, the potential of litter and wood decomposition is reflected by xylanase activity, since this enzyme is one of the most important in primary litter degradation (Margesin *et al*., 2006). The degradation of lignin, an enzymatic aerobic transformation, is restricted to a limited microbial group, namely the higher fungi, basidiomycetes (de Badiane *et al*., 2001). Basidiomycetes degrade lignin slowly and do not reach their highest degree of activity until about one month after the commencement of the composting process (de Badiane *et al*., 2001).

2.4 Similar environments

Organic wastes resulting from solid wastes have an important role in environmental pollution (Kulcu and Yaldiz, 2004). Besides landfill, incineration and pyrolysis, biogas processes and composting are used to dispose of organic wastes (Kulcu and Yaldiz, 2004). Undesirable compounds are present in urban wastes, and some of these can be eliminated, or at least reduced by composting (García *et al*., 1995). Low energy consumption is a characteristic of the composting process and this permits the disposal of the organic fraction of the solid urban waste and sludge, which together represent quantitatively the greatest portion of refuse (de Badiane *et al*., 2001). It minimises the environmental damage and provides economically valuable products from the wastes (Kulcu and Yaldiz, 2004).

Transformation into compost of the biodegradable organic fraction of solid urban waste is one of the most validated methods of recycling (de Badiane *et al*., 2001). The high organic matter content of sewage sludge, a product of waste water treatment, means that the sludge is frequently employed for agricultural purposes as fertiliser and soil conditioner amendments (García *et al*., 1995; Margesin *et al*., 2006; Ming *et al*,. 2008). Modern wastewater treatment plants use a combination of biological, physical and chemical processes to treat the water, a by-product of this treatment is biosolids, which are simply dewatered sludge (a by product of the sewer treatment process) generated during the treatment of municipal wastewater (Liang *et al*., 2003).

Due to changes in legislation Yorkshire Water are using an alternative composting technique called sludge phyto-conditioning. This is a low technology process that involves growing grass on sewage sludge/green waste to produce a compost-like material, and reducing bacterial indicators to below detectable levels, while retaining the beneficial energy recovery aspects of anaerobic digestion (Thompson, 2007). In sludge phyto-conditioning soil moisture content, aeration and the availability of substrates and nutrients all influence the size and function of the microbial community, and the interaction of these factors creates an environment that is ideal for specific micro-organisms that are capable of degradation (Taylor, 2004). It was found that with limited management the results from the growth of rye grass over one growing season reduced levels of *Escherichia coli* below the limits of detection. In addition, the volume of sludge was reduced by 50% and the final product was a peat or compost like substance with a low/pleasant odour, which was populated with earthworms and other soil fauna (Taylor, 2004).

2.5 Additives

The popularity of composting has lead to a high market demand for composters of various scales, and compost related material such as bulking materials, and also compost accelerators that are intended to improve the process and the quality of the compost (Himanen and Hänninen, 2009). There are a wide range of additives/accelerators available on the market; for example, Global-life Bio-stimulant and advetec Bio-tech, both of which are advertised to be used for municipal and industrial waste degradation. Generally, compost additives are a mixture of different amounts of various microorganisms, mineral nutrients or readily available forms of carbon, enzymes, and pH balancing compounds that are meant to enhance microbial

activity when the additive is in contact with the waste material (Himanen and Hänninen, 2009). Dozens of patents can be found claiming the positive impacts of different mixtures on the composting process (Himanen and Hänninen, 2009), however, there is little evidence on the effectiveness of compost additives in scientific literature.

A number of studies have shown that certain strains of bacteria have been used as successful stimulants. Nakasaki *et al.* (1994) demonstrated that a thermophilic bacterium, *Bacillus licheniformis*, could effectively decompose protein, prevent the decreasing of initial pH values during composting, and significantly increase the rate of decomposition. It was also found that *Bacillus sp*., when used as a biological treatment of sewage treatment plant sludge, had higher degradation rates when compared to treatment with existing mixed microbes in a stirred tank bioreactor. Bacteria have also been shown to stimulate the rapid break down of fatty substances from restaurant districts (Shon *et al*., 2002). These authors found that in industrial food-processing and food restaurant areas, the degradation of fats, oils and grease by *Pseudomonas sp*. strain was 41% higher than that of the naturally occurring bacteria (Shon *et al*., 2002).

2.6 Research questions

Despite a significant amount of literature on gully pots, relating to pollution effluent (Fletcher and Pratt, 1981; Grottker, 1990), water runoff quality (Memon and Butler, 2002b), sediment supply (Ellis and Harrop, 1984; Deletic *et al*., 2000), solid trapping efficiency (Butler and Karunaratne, 1995) and gully pot sediment aging (Clegg *et al*., 1993), there is none concerning the decomposition processes within the pots themselves. Understanding how these processes are operating within gully pots can be an important tool in blockage prevention as it can help with the maintenance/cleaning

regime, but also with assessing how the introduction of additives such as stimulants can help to break down the gully pot waste.

As gully pot contents have received little attention, internal processes may be better characterised by relating them to environments such as soils and composts. Using soil processes as a starting point, it will not only be possible to look at the *in situ* and *ex situ* management techniques that are similar to composting, but also the possibility of introducing additives and measuring the decomposition rates and microbial activity of the gully pot waste.

Significant gaps in the research undertaken to date have allowed space for this study, thereby enabling the following research questions to be asked;

- What *in situ* decomposition and enzyme processes are occurring within the gully pot?
- Do seasonal factors and variations in geographical location have an impact upon these processes?
- Can methods be developed to assist with the speeding up of the decomposition of the gully waste *in situ* to assist in the remediation of blockages?
- Is management of the waste *ex situ* viable if *in situ* management is not possible?

This thesis aims to answer these questions through the means of field sampling of gully pots throughout the city of Hull, the laboratory analysis of waste collected, and through laboratory simulation of the gully pot. These various strands of investigation will be addressed in the subsequent chapters.

3.0 Methods

This chapter presents and assesses the field and laboratory techniques used throughout each study. Five phases of waste sampling and testing were carried out and the general procedure for each phase is described below. However, during certain phases of the study the methods used have been modified; this modification in approach will be explained in more detail in the relevant chapter/section. For consistency the term waste is used throughout the study to refer to the contents within the gully pot.

In order to estimate the gully pot waste processes, whether this was *in situ* or *ex situ,* or to determine the degradation process, waste samples were collected in collaboration with Hull City Council Street Scene Services Department. The sampling was performed for five phases of the project. The first phase (Chapter 4) was to monitor the processes occurring in the gully pots and evaluate if area and season affected them. The second phase (Chapter 5) was a pilot study to assess the *in situ* degradation rate of the waste through controlled methods. The third phase (Chapter 5) used model gully pots prepared under field conditions in a laboratory environment to monitor the effects of time, temperature and moisture on the physical processes and microbial community of the waste *in situ.* The fourth phase (Chapter 6) re-examined the degradation rate and processes of the waste inoculated with selective additives in a model gully pot environment. The fifth and final phase (Chapter 7) examined the degradation processes under *ex situ* conditions, assessing the effect of mesophilic and thermophilic conditions, using starch as a positive control.

3.1 Sample Collection

The gully pots were sampled as part of Hull City Council's maintenance regime, and were selected in collaboration with the Council Street Scene Services Department. To ascertain the variety of contents that may be found throughout the city during all phases, gully pots were selected from four different area types, as defined with the Street Scene Services Department, with these comprising:

- Areas with high foliage, where there was a substantially higher amount of vegetation, such as trees and hedges etc, as opposed to other areas in the city.
- Industrial areas, being in, or surrounded by, the industrial estates of Hull, where there is very little vegetation.
- Residential areas, being areas of residential housing estates with no busy roads but which may contain foliage due to vegetation.
- Areas with busy roads, where samples were taken from gully pots off roads that were known for having a high amount of traffic using them on a daily basis. Similarly to residential areas, these may also have areas of vegetation.

Prior to any samples being removed, pictures of the gully pot with the lid open were taken for photographic reference. The area type and location of the gully pots (street name and house number or lamp post number) were recorded, in order to map their location, as can be seen in Chapter 4. Every sample taken from the gully pot and its location were given unique identification numbers for use during laboratory assays and analysis. Samples were removed manually in accordance with the method applied by Hull City Council when emptying gully pots that could not be reached by the eductor

truck. The waste within the gully pot was stirred with the long gully grabs prior to sampling to homogenise the contents. The waste was then grab sampled using long gully grabs, with samples taken from the bottom of the pot and from near the top of the water level. This was performed to ensure a range of solids were collected in case layering within the gully pot had occurred, as this has been observed in previous studies (Memon and Butler, 2002a). Figure 3.1 shows a sample being removed from a gully pot using grab sampling method with the long gully grabs.

Figure 3.1 Sample from the gully pot being removed using the long gully grabs.

Once the samples were removed they were placed in individual polythene bags, which were then loosely tied to avoid spillage, and labelled with the gully location number. The samples were transported back to the laboratory where they were stored at 5°C until they could be analysed. Prior to the start of any analysis all inorganic litter, for example plastics, card, clothing and cigarette stubs, were removed from the samples.

3.2 Physical analysis

The Section outlines the physical analysis of the gully pot waste which can provide a crude indication of the quality of the gully pot contents and the likelihood of biological decomposition. During this study analysis occurred in the field and laboratory measuring temperature, pH, moisture content, dry matter and organic matter content. These physical analyses were carried out for all samples and phases.

3.2.1 Field analysis

3.2.1.1 Field pH and temperature

For all phases, before any sampling took place, gully pot pH and temperature was measured using a Hanna HI 8424 pH/mV/Temperature meter in accordance with the manufacturers' instructions. Both probes were rinsed with distilled water before use. They were then placed inside the gully pot, ensuring the pH electrode and temperature probe were fully immersed in the gully pot liquor. The liquor was briefly agitated until the reading stabilised, which was then recorded alongside the gully pot details.

The meter was calibrated using pH 4.0 and 10.0 buffering solutions on a weekly basis, while the temperature ranges were factory calibrated. The meter's accuracy was reported to be ± 0.01 pH and 0.4°C.

3.2.1.2 Gully waste accumulation

For the monthly monitoring exercise of phase one, the amount of waste collected within the gully was measured prior to the sample being taken. This was achieved using a device that measures the depth of the gully pot, then the depth of the content (see Figure 3.2).

Figure 3.2 Content depth measurer, showing the thin plate which lies on top of the waste, the tip of the retractable inner rod and the red arrow indicating the depth.

The thin retractable inner rod is placed in the centre of the gully pot while the flat horizontal plate lies on top of the contents within it. The inner rod is pushed through the contents to the base of the pot using the red arrow on the side of the outer shaft. The red arrow also lines up with markings on the outer shaft (see Figure 3.3) that indicated the depth between the top of the gully pot waste and bottom of the pot (in cm). This method was repeated two further times, towards the front and the back of the pot as some gullies are dipped in the centre, or may have had a false bottom where bricks or other large objects have sunk to the bottom.

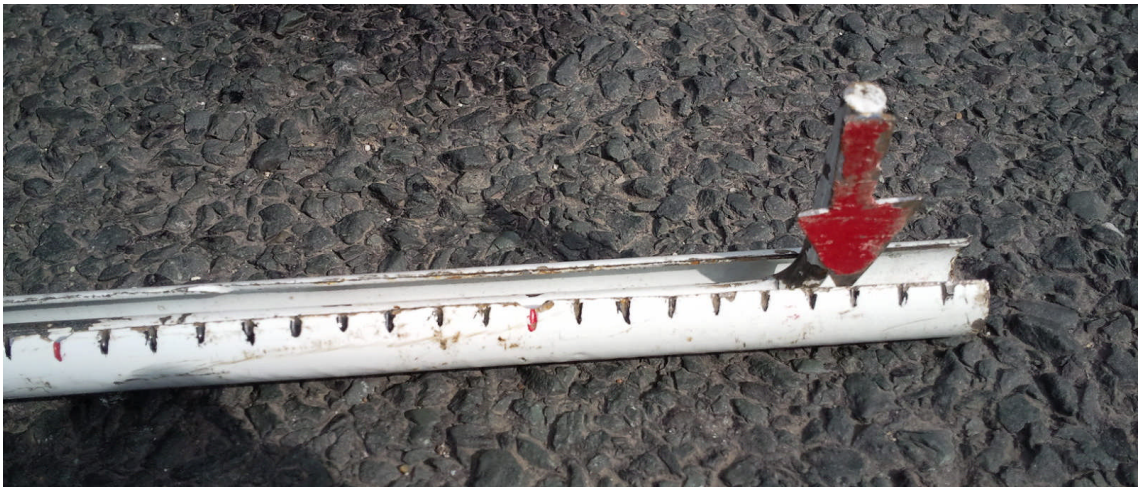

Figure 3.3 Contents depth measurer, displaying the red arrow which indicates the depth of the waste within the gully pot in cm.

3.2.2 Laboratory analysis

3.2.2.1 pH

Laboratory pH was measured using the same apparatus as described in Section 3.2.1.1 in accordance with the manufacturers' instructions. The pH for each sample was measured in a 10% (w/v) slurry sample as per Adams and Frostick (2008), which was created by adding 1g of the gully pot waste to 9ml of sterile distilled water. Both the temperature and pH probes were immersed into the slurry and were then agitated until the reading stabilised.

3.2.2.2 Moisture content analysis

Moisture content is one of the most commonly used analysis for soil studies (Topp, 1993) and is a crucial initial requirement for calculations of other physical parameters, such as loss on ignition (Hesse, 1971). There are numerous indirect and direct methods

in which moisture content can be measured, within the literature (e.g. Baize, 1993; Gardner, 1965; Topp, 1993), which all involve separating and measuring the amount of water removed. Gravimetric oven drying is the most frequent method used due to its simplicity (Gardner, 1965; Reynolds, 1970; Zazueta and Xin, 1994). However, there have been limitations observed within this method, e.g. soil samples not being dried to a constant weight (Topps, 1993).

During this study the moisture content was measured gravimetrically by weighing an empty, clean, ovenproof container to two decimal places prior to sample addition. Samples were then removed from their location (dependant on the phase) and stirred, ensuring there was an even homogenous mix. 100g of the waste was removed from the container (the storage containers depended on project phase) using plastic tongs and placed into the pre-weighed container. The container was then re-weighed; the new weight was recorded, and then placed into a pre-heated oven at 105°C overnight for approximately 16 hours. Once removed from the oven the container was allowed to cool in a dessicator, to prevent re-absorption, before re-weighing. The percentage of moisture within the sample was then calculated by dividing the amount of weight lost by the sum of the original wet sample minus the container. This procedure was repeated for every sample collected.

Using the moisture percentage calculated it was then possible to calculate the total dry matter of the sample. This was determined by multiplying the percentage of dry waste (percentage remaining after the percentage of moisture had been calculated) by the weight of the sample before drying.

3.2.2.3 Organic matter content

Gravimetric measurements to estimate the amounts of organic matter within samples have been widely used (as mentioned below). There are three main methods for measuring organic matter content through gravimetric calculations; ignition at a temperature of 550°C, ignition at a higher temperature of 850°C (Stantisteban *et al.*, 2004) and hydrogen peroxide treatment (Hesse, 1971). The lower temperature loss on ignition (LOI) method was selected for use in this study as it allows for the organic matter to be completely depleted, which occurs at around 550°C (Stantisteban *et al.*, 2004), and it is widely used due to its simplicity (e.g. Adams and Frostick, 2009; Adams and Umapathy, 2011; Grey *et al.*, 2012; Wang and Yi, 2011).

LOI, which here is used to determine organic matter content, was measured gravimetrically. The previously oven dried samples (see Section 3.2.2.2) were then mixed, once cooled, to create a homogenised sample. 5g of the mixture was placed in a previously weighed, clean and dry porcelain crucible using a spatula. The crucible was weighed again and placed in a furnace at 550°C for four hours. After ignition, the crucible was removed and transferred to a heat resistant plate using long handled tongs, allowed to air cool and then placed in a dessicator to allow it to cool fully before it was re-weighed. The percentage of weight lost on ignition was then calculated by dividing the weight lost after ignition with the dry sample weight (before ignition).

From the percentage LOI, and the total dry matter calculation (Section 3.2.2.2) it was possible to determine the total organic matter and total ash content of the samples, which are important when analysing the relative contents of the samples. The total

organic matter was calculated by multiplying the dry sample weight (before ignition) with the percentage LOI. The total ash content was calculated by removing the dry sample weight (before ignition) from the percentage LOI

3.2.2.4 Slurry preparation for replicate laboratory gully pots

Slurries were prepared for experimental phases 2 to 5 in Chapter 5, 6 and 7 where mesocosms were created to replicate gully pots under laboratory conditions. Following the results of phase one (Chapter 4) the waste was treated as a composite sample, as opposed to creating different mesocosms for different geographical area types. Additionally, to ensure a variety of contents were collected, the gully pot waste was sub-sampled equally from the four different areas, as previously described in Section 3.1. The slurries within the mesocosms were used to determine:

1. The likelihood of biological decomposition through physical parameters such as pH, moisture content and organic matter content.
2. Extracellular enzyme activity
3. Microbial community function using Biolog EcoPlates^{TM}.

Waste was removed from the selected gully pots as described in Section 3.1 and taken back to the laboratory. The samples were removed from the plastic sample bags and divided into three buckets. Whilst doing this, the waste was scanned for any inorganic material and this was removed. Items removed from the waste prior to analysis included cigarette butts, confectionary wrappers, money, keys, clothing, needles, bricks and spoons. The waste in each bucket was stirred to create a homogenous mix then removed using plastic tongs. The waste was placed on large ovenproof trays, creating a layer that

was approximately 2cm thick, and then placed into a pre-heated oven at 105°C overnight for 16 hours. The waste was then removed, fully cooled and then crushed using a pestle and mortar. A well mixed volume of dried, crushed waste was poured through a British Standard sieve with a mesh size of 2mm and vigorously shaken for 5 minutes. The sieved waste was collected in a clean container. Waste that was too large to sieve was then repeatedly re-crushed and sieved again, or discarded. The 2mm sieved waste was weighed to the desired weight depending on the phase (which will be described in detail in later chapters) and then added to 250ml conical flasks. A desired amount of sterile distilled water (which will also be described in detail in later chapters) was then added to each flask, this slurry was then mixed thoroughly in preparation for the desired assay.

3.3 Microbial methods

The Section outlines the two microbial approaches used during the current study. The extracellular enzyme analysis was used during the assessment of decompositional activity in the field (Chapter 4) and in the *ex situ* study (Chapter 7), whereas the Biolog EcoPlates™ were used to assess the microbial community under *in situ* modelled conditions (Chapter 5) and the substrate addition study (Chapter 6). Biolog EcoPlates™ was favoured instead of the extracellular enzyme analysis for chapters 5 and 6 to gain a more in depth idea of the microbial community changes within varied controlled environments.

3.3.1 Extracellular enzyme activity

As previously mentioned (Section 2.3.1) enzymes are the main mediators of soil biological processes, such as organic matter degradation and mineralisation (Marx *et al.,* 2001). Therefore, enzymes can be used as functional indicators when assessing the viability of organic substrate degradation (Mondini *et al.,* 2004) and are commonly used to measure potential activity of microbial communities in soils (Badiane *et al.*, 2001).

Extracellular enzymes are mainly derived from soil microorganisms; particularly those enzymes involved in the degradation of insoluble substrates such as proteins and carbohydrates, which are too large to enter the cell and must therefore be partially broken down outside the cell (Wood, 1995). Extracellular enzyme activity has been widely researched in a differing of environments, such as; the effects of seasonal changes on soils (Baldrian *et al.,* 2012; Bell *et al.,* 2010; German *et al.,* 2012; Wallenstein *et al.*, 2009), and monitoring organic matter stability and maturity in aerobic organic waste processing (e.g. Cayuela *et al.,* 2008; Komilis *et al.,* 2011; Mondini *et al.,* 2004).

Molecular-based methods provide valuable information about the microbial community, as opposed to only culture-based techniques (Kirk *et al.,* 2004). The importance of using fluorogenic substrates as an alternative, especially in environments which may contain low levels of extracellular activity, has been previously demonstrated using fluorogenic methylumbelliferyl substrates (MUF). There are many advantages in using the fluorogenic substrate techniques, as when compared to chromogenic substrates, the reaction products can be measured at very low concentrations making the assay suitable for use with very low substrate concentrations and for very short incubation times

(Darrah and Harris, 1986; Freeman *et al.,* 1995 and Niemi and Vepsäläinen, 2005). Furthermore, as similar methods can be used for each enzyme, the assay can be readily automated for routine analyses, and in addition, there are wide ranges of fluorogenic substrates available (Darrah and Harris, 1986) such as the ones used in this study.

Criteria for choosing enzyme assays were based on their importance in nutrient cycling and organic matter decomposition, with sulphatase and phosphatase taken as indicators of sulphur and phosphorus cycling respectively (Shackle *et al.,* 2000). The galactosidase and glucosidase were chosen for their critical role in releasing low molecular weight sugars that are important as energy sources for microorganisms (Bandick and Dick, 1999). β-glucosidase, α-glucosidase and β-xylosidase enzymes are also involved in soil organic matter degradation (German *et al.,* 2012). β-glucosidase, an enzyme involved in the final stage of cellulose degradation, plays an important role in the soil organic carbon cycle (Piotrowska and Koper, 2010), whereas α-glucosidase is one of the enzymes involved in starch degradation (Sun and Henson, 1992). β-galactosidase enzymes catalyze the hydrolysis of lactose, but are not present in soils in significant levels (Dodor and Tabatabai, 2005). β-glucuronidase catalyzes the breakdown of complex carbohydrates. Butyrate esterase is a hydrolytic enzyme involved in biochemical cycling of nutrients (Wittmann *et al*., 2004) whereas β-xylosidase is involved with Hemicelluloses degradation.

3.3.1.1 Reagent preparation

The enzyme activities were assayed using the model fluorogenic substrate with methylumbelliferone (MUF) as the fluorescing agent. The eight selected enzymes were obtained from the Sigma-Aldrich chemical company, and were; sulphatase potassium,

phosphatase, β -D-glucosidase (glucopyranoside), α-D- glucosidase (glucopyranoside), β-D- galactosidase, β-D-glucuronidase hydrate, butyrate and β-D-xylosidase. These were measured using the fluorogenic respective substrates; 4-Methylumbelliferyl sulphate potassium salt, 4-Methylumbelliferyl phosphate, 4-Methylumbelliferyl β -D-glucoside (glucopyranoside), 4-Methylumbelliferyl α-D- glucoside (glucopyranoside), 4-Methylumbelliferyl β-D- galactoside, 4-Methylumbelliferyl β-D-glucuronide hydrate, 4-Methylumbelliferyl butyrate and 4-Methylumbelliferyl β-D-xyloside.

Stock solutions of the eight substrates were prepared prior to the assay at a concentration of 5mmol l^{-1} in sterile, distilled water, and stored at -4°C until the day of the assay.

3.3.1.2 Fluorogenic enzyme assay

The enzyme activity assays were carried out on four consecutive days after sampling using a method based on the principles of Hoppe (1993); as described in Adams *et al*. (2008) and Adams and Umapathy (2011).

A slurry sample was prepared from each waste sample by adding 1g (wet weight) of sample to 99ml of sterile distilled water. From this 10ml of the slurry was added to 90ml of water to create a slurry with a final concentration of 1g l^{-1} wet weight. For each of the enzyme assays a measurement of 240µl of the slurry (1g l^{-1}) was pipetted into 1.5ml eppendorf microcentrifuge tubes, which were labelled with the sample identification number and which enzyme was to be tested. Additional samples were prepared for use as a control for each enzyme assay by pipetting 240µl of compost slurry (1g l^{-1}) into a 1.5ml eppendorf and boiling for ten minutes. This was undertaken

in order to correct for background fluorescence and destroy any enzyme activity. Once the controls were cooled, 10µl of the fluorogenic enzyme substrate (5mmol l^{-1}) was added to their respective microcentrifuge tubes (including blank controls) to give a final concentration 250µmol l^{-1}. All of the samples were then incubated at 30°C for two hours.

After incubation the samples were centrifuged for two minutes at 10,000 rpm. Using a micropipette, 200µl of the supernatant was removed from each sample and added to a 96 well microtiter plate. In addition to the sample, 16 µl of pH 9.5 borate buffer solution (Sigma Aldrich) was added to each well. The intensity of the fluorescence was quantified using a fluorometer (FLUOstar OPTIMA, BMG LABTECH Ltd., UK) fitted with an excitation filter at 350 nm and emission filter at 460 nm. The control fluorescence intensity reading was subtracted from the sample reading so any background information, such as florescent impurities, were not counted.

A straight line calibration curve was plotted using control concentrations of MUF 4-methylumbelliferone (Sigma Aldrich) at 0, 1, 2, 4, 6 and 8µmol 1^{-1} with sterile, distilled water. From this, the concentration of the enzyme substrate was determined. The quenching effect was also tested for each sample by running the same straight line calibration curve with standard solutions of 4-methylumbellifone (Sigma Aldrich) with the slurry sample, as opposed to sterile, distilled water.

3.3.2 Biolog EcoPlates™

The microbial community activity within the samples was explored using Biolog EcoPlates™. The Biolog system assesses the physiological profile of the microbial

community within a sample and characterises them using a pattern of substrates utilisation in a 96 well MicroPlate (Garland and Mills, 1991; di Giovanni *et al*., 1999). The EcoPlates™ contains 31 of the most useful carbon sources for soil community analysis and one control well, which were all replicated three times (Biolog, 2000; Kirk *et al*., 2004). The growth of aerobic, heterotrophic microorganisms in the wells is indicated by the oxidation of the substrate with the concomitant reduction of the tetrazolium dye. This reaction provides colour development which can be measured colourmetrically (Smalla *et al*., 1998). Studies demonstrating the utility of the Biolog EcoPlates™ in detecting population change have been carried out in soil, water, wastewater, activated sludge, compost and industrial waste (Biolog, 2000). The Biolog technique has been widely used in diverse studies of soil microbial communities, including plant cover (Grayston *et al*., 2001; Ritz *et al*., 2004; Singh *et al*., 2011), herbicide treatment (el Fantroussi *et al*., 1999), pollution (Avidano *et al*., 2005; Knight *et al*., 1997), composting treatment (Laine and Jørgensen, 1997), and aquifers (Röling *et al*., 2000).

3.3.2.1 Biolog EcoPlate™ assay

The 96 well Biolog EcoPlate™ contained 31 different carbon substrates and a control well with no added carbon substrate, in triplicate. Table 3.1 (reproduced from Biggs *et al*., 2011 and Jena *et al*., 2006) contains details of each well and the carbon source it relates to. The plate was divided into three sections and labelled with a unique number that related to the waste sample that was to be inoculated into each section.

A slurry sample was prepared from each waste sample by adding 1 g (wet weight) of sample to 9ml of sterile distilled water. From this 1ml of the slurry was added to 9ml of

sterile distilled water, this solution was then mixed and 1ml was removed and added to a separate beaker with 9ml of sterile distilled water. A slurry with a final concentration of 1g wet weight l^{-1} was created using this dilution series. For each waste sample 100µl of the slurry (1g l^{-1}) was pipetted into all 32 wells of its allocated section of the plate. The lid of the plate was then replaced and placed in the incubator for the desired amount of time, as required by the project phase. After incubation, the absorbance of each well was read on a MicroPlate reader (FLUOstar OPTIMA, BMG LABTECH Ltd., UK) which was set at a wavelength of 590nm.

The data from each microplate Section was assessed in three stages. Firstly the colour development in the control well (A1 water) was subtracted from the readings in the remaining 31 wells to account for utilisation of background dissolved organic carbon (Balser and Wixon, 2009). Substrate wells that had a negative value after the subtraction of the control, indicating they have no colour development, were set to 0. The substrates were then analysed based on the average well colour development, as described in Garland (1996) to normalise and indicate relative utilisation between samples. This was achieved by totalling the optical density of the wells for that section of the plate then dividing it by 31.

Table 3.1 Group arrangements of different carbon substrates found within the Biolog EcoPlates™ platform.

Classification of different carbon sources	**Carbon source**	**Key**
Reference well	Water	A1
Carbohydrates	βMethly-D-Glucoside	A2
Carbohydrates	D-Xylose	B2
Carbohydrates	i-Erythritol	C2
Carbohydrates	D-Mannitol	D2
Carbohydrates	N-Acetyl-D-Glucosamine	E2
Carbohydrates	D-cellobiose	G1
Carbohydrates	α-D-Lactose	H1
Polymers	Tween 40	C1
Polymers	Tween 80	D1
Polymers	Cyclodextrin	E1
Polymers	Glycogen	F1
Carboxylic acids	D-Galactonic Acid γ-Lactone	A3
Carboxylic acids	D-Galacturonic Acid	B3
Carboxylic acids	2-Hydroxy Benzoic Acid	C3
Carboxylic acids	4-Hydroxy Benzoic Acid	D3
Carboxylic acids	γ-Hydroxbutyric Acid	E3
Carboxylic acids	D-Glucosaminic Acid	F2
Carboxylic acids	Itaconic Acid	F3
Carboxylic acids	α-Ketobutyric Acid	G3
Carboxylic acids	D-Malic Acid	H3
Phosphorylated chemicals	Glucose-1-Phosphate	G2
Phosphorylated chemicals	D,L-α-Glycerol Phosphate	H2
Amino acids	L-Arginine	A4
Amino acids	L-Asparagine	B4
Amino acids	L-Phenylaianine	C4
Amino acids	L-Serine	D4
Amino acids	L-Threonine	E4
Amino acids	Glycyl-L-Glutamic Acid	F4

Amines	Phenylethyl-amine	G4
Animes	Putrescine	H4
Esters	Pyruvic Acid Methyl Ester	B1

3.4 Statistical analysis

All statistical analysis tests were performed using PASW Statistics 18, Release Version 18.0.0. A variety of statistical methods were used to measure the data collected throughout the study where confidence levels of 95% or 99.9% were tested and are described below.

Prior to the main statistical analysis the normality of the data was assessed using a Kolmogorov-Smirnov test for goodness of fit to assist in determining the correct test to use. If the data was assumed to be normally distributed, and several means were to be evaluated, a one-way analysis of variance (ANOVA) was performed. These were followed with a least significant difference (LSD) *post-hoc* test to determine where the differences were. If the data was assumed to not be normally distributed a Kruskal-Wallis tests was performed. These were further analysed with Mann-Whitney-U *post hoc* tests to determine where the differences were.

Multivariate analysis was only performed on the repeated monitoring exercise in Chapter 4, as each individual gully pot was repeatedly sampled. The data was tested for normality as previously described in this section. If the data was normally distributed a repeated-measure ANOVA was performed followed, with a least significant difference (LSD) *post-hoc* test. If the data was not normally distributed a Friedman test was

performed for the repeated exercise, and this was followed with a Mann-Whitney-U *post hoc* tests.

Hierarchical cluster analysis was used during the assessment of the microbial community under *in situ* modelled conditions (Chapter 5) and in the substrate addition study (Chapter 6) using Biolog EcoPlates^{TM}. This was used to initially identify grouping amongst the carbon substrate utilisation profile assessing for differences between the variables. Hierarchical cluster analysis was used over principle component analysis as visual interpretation was less complicated, and is widely used as an alternative to PCA (e.g. Biggs *et al.*, 2011; Kadali *et al.*, 2012; Tang *et al.*, 2010; Xue *et al.*, 2008).

3.5 Addition analysis

This Section describes further analysis which was trialled during the study, but which was not however used, for reasons discussed below.

3.5.1 Real time monitoring through the use of a datalogger

A datalogger was deployed in the field to measure the conductivity, pH, dissolved oxygen and temperature level in gully pots located on the University of Hull campus. The aim of this exercise was to gain an idea of the real time activity *in situ* over a year.

Designated gully pots were chosen depending upon their location as it was essential that the datalogger itself was hidden from public view to reduce the risk of vandalism. The first pot chosen was located due to the ability to bury the data logger next to the gully pot and had a capacity of 45l. The probes were inserted into the pot at each corner, in

order for them not to come into contact with each other. The wire was taped down to reduce the risk of movement within the pot.

The logger was first deployed on the 17th December 2009 and left to run for one month measuring the parameters every 30 minutes. However, due to the snow and low temperatures during this period the battery life was reduced. A back up battery was subsequently connected, however, this also failed, resulting in the data being corrupt and as such un-readable. The datalogger was taken back to the lab for calibration and reassessment and then deployed on the 3rd of February 2010 and again on the 17th February. Due to further unknown problems with the datalogger no results were able to be retrieved. Tests were carried out in the laboratory, measuring the exact parameters in model conditions using a container the same size of the original gully pot. Due to the results obtained from the laboratory model it was decided a larger gully pot (90l), with easier access to monitor for problems, would be better suited for the analysis as it appeared the probes were interfering with each other in such a confined space. The datalogger was then re-deployed in the new location with a 90l gully pot on the 28th April 2010, where only temperature and pH was measured. This was due to the results obtained during the laboratory trial, where it was decided to remove the dissolved oxygen and conductivity probes. The data logger was redeployed on the 12th May, and again on the 27th May, however, due to vandalism no results were able to be obtained on both counts.

Measuring the activity of gully waste in the field is difficult due to the open locations of the gully pots which are prone to vandalism (e.g. Pratt and Adams, 1984) and the inability to control the external environment. Due to this there has been very little

research surrounding the real time monitoring of gully pots in the field, with the majority of approaches being conducted within the laboratory e.g. Butler and Karuranatne (1995); Lager *et al.* (1977). However, a recent method has been piloted to measure the water level within the gully pot *in situ* through the use of wireless sensors (See *et al.,* 2012). This method is still in the prototype phase though could be an optimal way of measuring the *in situ* activity of the gully pot for future research, whilst avoiding the datalogger and wire being tampering with, or vandalised. From the lack of results obtained over a five month period, and the persistent vandalism, it was deemed impractical to collect real time data using this method, and it was decided that the gully pots were not a suitable environments to monitor these parameters.

3.5.2 CO_2 analysis to measure mass loss of organic content

Similar to mass loss, carbon dioxide (CO_2) release is regarded as a common indicator used to measure decomposition rates of litter (Song *et al*., 2010). Measurements of CO_2 efflux has not only been used to measure the rate of organic matter decomposition in leaf litter (e.g. Briones and Ineson,1996; Chemidlin Prévost-Bouré *et al.*,2010; Salamanaca *et al*., 1998; Zeng *et al*., 2010), but also in other environments, for example soils (e.g.Ngao *et al.,* 2012; von Lützow and Kögel-Knabner, 2009; and Zhu and Cheng, 2011), compost (Nakasaki *et al.*, 2005), and bio-waste compost (Eklind *et al*., 2007) . Although there is a large amount of literature using CO_2 to assess decomposition it was not the chosen method in the current study.

An attempt was made prior to this composting trial to measure the CO_2/respiration of the waste. However, the CO_2 release was relatively small, which could be a reflection of

the environment the waste has been subjected to. When the background CO_2 was measured for use as a control, it was found to be similar to that of the waste recorded, and as such it was deemed un-suitable for this type of waste measurement.

3.6 Method limitations and sampling bias

3.6.1 In the field

During the collection of samples from the field certain limitations were encountered which could have lead to biasing at a later stage. All of the samples were chosen and collected with the assistance of the Street Scene Services Department at Hull City Council. Although the sampling would not have been possible without their assistance it did lead to some restrictions. This was especially noted during the winter months when the sampling was not possible as the team was needed for alternative tasks, such as clearing the snow and gritting.

During the repeated sampling exercise in Chapter 4, there were only three gully pots repeatedly sampled in the industrial area compared to the other three geographical areas, which could have produced a biasing towards the industrial area. This was an unavoidable issue due to the lack of gully pots that had been emptied within the same fortnight as the other chosen ones. In order to maintain consistency a fourth gully was not selected for sampling after the 12 month exercise had started. The collection of more samples would have created a more representative study, and supported the findings, but unfortunately it was not possible. Due to the amount of time the sample collection, preparation and subsequent assessment took it was not possible to collect more samples than planned on a fortnightly basis. Tongs were used to grab sample the

waste as it enabled a wide variety to be collected, which as the project required, and has previously been used when sampling from gully pots (e.g. Clegg *et al.*, 1993). There was an initial thought that it may bias the waste away from the fine samples, however, the results of the ash experiments show that fine samples had been collected. These fine samples were also visible when homogenising the samples prior to analysis. An alternative method to this would have been to collect a core sample, which also could have tested for layering. This, however, was not the chosen method as grab sampling was a quicker method, which was needed due to the high amount of samples collected and the time restrictions of the project. Furthermore the initial stage was to only identify the contents, and not the layering ability.

3.6.2 In the laboratory

The laboratory experiments had unavoidable drawbacks in simulating the degradation and activity of the field conditions. For instance, the results from measuring the microbial community, referring to decomposition, in the laboratory experiments might not be equal to that observed in the field (He *et al.*, 2010). This could cause bias in the results, however, controlling the temperature and moisture in the field would be very difficult. Furthermore, some of the procedures used during the experiments, such as drying and crushing the waste, could also cause a bias in the results as it may reduce the amount of microorganisms within the samples. Measuring the microbial community in the field prior to laboratory procedure and then measuring after the samples had been set up in the laboratory could have identified if there were any differences between the two. Due to time and cost restrictions it was not possible to extend the experiments in this way, however, this was not deemed an issue as the methods used have been previously cited (e.g. Adams *et al.*, 2008). Furthermore, the results received from the laboratory

experiments (Chapter 5, 6 and 7) were similar to those observed in Chapter 4, from the field, and the results received were also comparable to those observed in other studies (e.g. Biggs *et al.*, 2011; Adams *et al.*, 2008).

As observed during the study there were fluctuations in ash content which indicates the inorganic material remaining in the waste and is assumed that it would remain constant. These spurious ash contents could be due to part of the preparation method. As there is no trend in the losses it is assumed that it is not a biological factor but potentially a result of the sampling procedure, perhaps where fine heavy particles (e.g. fine grit, small stones or sediment) passed through the sieve during screening. The sample was mixed thoroughly before preparing each mesocosm, some of which may have received more of the sand and small stones, as opposed to the others. This could have resulted in the higher inorganic matter in some of the mesocosms causing the fluctuations. As all of the samples were screened with a 2mm sieve to ensure any stones or grit were removed, similar methodological setups (e.g. Adams *et al.*, 2008) used a similar screening sieve size. However, particles <2mm have previously been observed in gully samples (e.g. Ellis and Harrop, 1984; Morrison *et al*., 1995; Pratt and Adams, 1984; Sartor *et al*., 1974) and could have passed through the sieve. Subsequent work shown that the analysis of total carbon by loss of ignition could have identified the amount of silt and clay within the sample, however time restrictions meant it was not possible to re-analyse for this. Furthermore reducing the sieving size would have also been too time consuming and may have not enabled the collection of enough gully waste to fulfil the needs of the trial. Therefore in order to assist in the reduction of biasing during the sample preparation the bucket of dried sieved waste was homogenised before each

sample was weighed out, thereby attempting to ensure a consistent organic: inorganic ratio throughout the study.

During this study the Biolog EcoPlates™ were only incubated for 24 hours, which was mainly due to high demands for the equipment. Previous research has observed this method being employed but with longer incubation times e.g. Biggs *et al.* (2011); Douterelo *et al.* (2010). Preliminary assays were initially trialled to examine the effects of incubation time on the average well development. Little differences were observed between the 24 hour study and the 48 hour study, justifying the project allowing only 24 hours incubation. This however, does not mean that it is the optimal incubation time for this type of waste. Therefore, in order to gain the most from this exercise, it would probably be best to measure repeatedly over a week-long period, and examine the results to define this. Though as previously mentioned, there was high demand for this MicroPlate reader and this option was not available. The method employed has displayed results which complement previous research, particularly in the temperature variations, which can also be used as a justification for the method employed under the circumstances.

3.7 Conclusions

Various features and objectives of the data collection programme implemented in this study have been discussed in this chapter. An overview of the methods adopted for sample collection and laboratory analysis have also been described. These techniques measured pH, temperature, waste accumulated, moisture content, organic matter content, extracellular enzyme activity and microbial community. The methods were validated for their use through a consideration of previous studies from better

characterised environments such as soils and composting, as previously mentioned. The methods will be further refined, if needed, and the results of the analysis of the decompositional activity in the field, the *in situ* modelled conditions, the effects of substrate addition and the *ex situ* activity will be discussed in the next four chapters.

4.0 Influences on decompositional activity in roadside gully pot

4.1 Introduction

The rate at which organic matter within gully pots decomposes can impact upon how often they need to be cleaned. However, we know very little about the physical and biochemical processes operating within the gully pots. Previous research on gully pots has concentrated on pollution effluent (Fletcher and Pratt, 1981; Grottker, 1990), water runoff quality (Memon and Butler, 2002b), sediment supply (Ellis and Harrop, 1984; Deletic *et al.,* 2000), solid trapping efficiency (Butler and Karunaratne, 1995), and gully pot sediment aging (Clegg *et al.*, 1993) etc., but not on the decomposition processes occurring within the gully pot; especially the enzyme activity which can be used to measure organic matter dynamics (Marx *et al*., 2001; Stemmer, 2004). As a consequence, the development of an understanding of the general character of these processes was determined to be a fundamental initial stage in the current research project.

As the previous discussion has indicated, it is apparent that fluctuating temperatures and alternating wet/dry spells have the potential to directly affect the conditions within gully pots. The dominant effect of climate and substrate quality on litter decomposition has been well documented (e.g. Aerts, 1997; Coûteaux *et al.*, 1995; Heim and Frey, 2004; Trofymow *et al.*, 2002), however, this area of study has not previously been targeted specifically at developing our understanding of gully pot environments. Fundamentally, understanding how the waste collected inside gully pots can be affected, and in turn influenced by seasonal change is an important aspect in understanding the decomposition processes occurring within gully pots; as can an understanding of the

potential effects of geographical location. For instance, more organic matter may be found in gully pots in areas with high foliage, compared to those in industrial areas with no trees.

The type of location can significantly influence the quality of the substrate and waste entering the gully pot, therefore potentially affecting the decompositional processes. Understanding how each location and season affects the processes within the pots can assist with gully pot management, as this can indicate optimum cleaning times for the gullies in various areas. Better characterised environments, in terms of microbial activity and decompositional processes, such as soils and composting, were used to generate background information on the potential processes occurring within the gully pots, as a baseline dataset was unavailable for use in the current study. Understanding these processes is an important element in developing sustainable solutions for managing gully pots more efficiently and potentially reducing the likelihood of drainage system blockages.

4.2Aim

The main aim of this chapter is to survey the status of gully pot waste and investigate the geographical and seasonal controls on decompositional processes that occur throughout the city of Kingston upon Hull (Hull), U.K. Assessing the waste over a set time period and from different areas allowed the waste to be analysed to identify whether seasonal (air temperature and runoff temperature, pH, biological activity) and geographical variations (e.g. contributions from surrounding foliage in-wash, detritus , rubbish from urban areas) had any impact upon such *in situ* degradation processes occurring.

4.3 Method

The monitoring phase comprised of two elements. The first of these was a random sampling exercise, which took place over two years, and which examined gully pots throughout the city, in an unsystematic manner. This was to facilitate a greater understanding of the potential range of activity and assess any variability that was in evidence (see Section 4.3.1.1). The second element was a repeated monitoring exercise which took place over one year, examining the same 15 gully pots during each assessment in order to monitor how waste accumulates over a yearly cycle, and how this may affect processes occurring *in situ* (see Section 4.3.1.2). In order to develop a more nuanced understanding of the variety of waste which can be found in the gully pots throughout the city, the pots were selected from four different types of locations; i.e., areas with high foliage, industrial areas, residential locations, and locations with busy roads, as described in Section 3.1. The datasets were compared to determine if any patterns were present between gully pots that were evaluated during the 12 month monitoring assessment, starting directly after cleaning until their next scheduled clean, and those assessed during the random monitoring assessment which was conducted over a two year period.

4.3.1 Sample selection and collection

All samples were collected systematically to ensure consistency and replicability, as previously described (Section 3.1). The gullies were sampled as an integral part of the Hull City Council maintenance regime, and were collected on a bi-monthly basis (randomly sampled at the beginning and repeatedly sampled at the end of the month) to comply with the operatives' work load. Sample collection commenced in October

2009, and entailed working with a team of Street Scene Services operatives from Hull City Council for the duration of the monitoring exercise.

4.3.1.1 Random sampling

Starting in October 2009 samples were collected at the beginning of the month (n=180 gullies sampled out of approximately 70,000), ensuring the same amount of gullies were sampled in each season of the year. In order to obtain a wide spectrum of samples for this exercise, 15 different gullies per month were sampled by Hull City Council Street Scene Services operatives from the four survey areas (see 3.1). The address of each gully pot sampled and the characterisation of the location (as previously described in 3.1) was recorded and plotted on an ordinance survey map (see Figure 4.1).

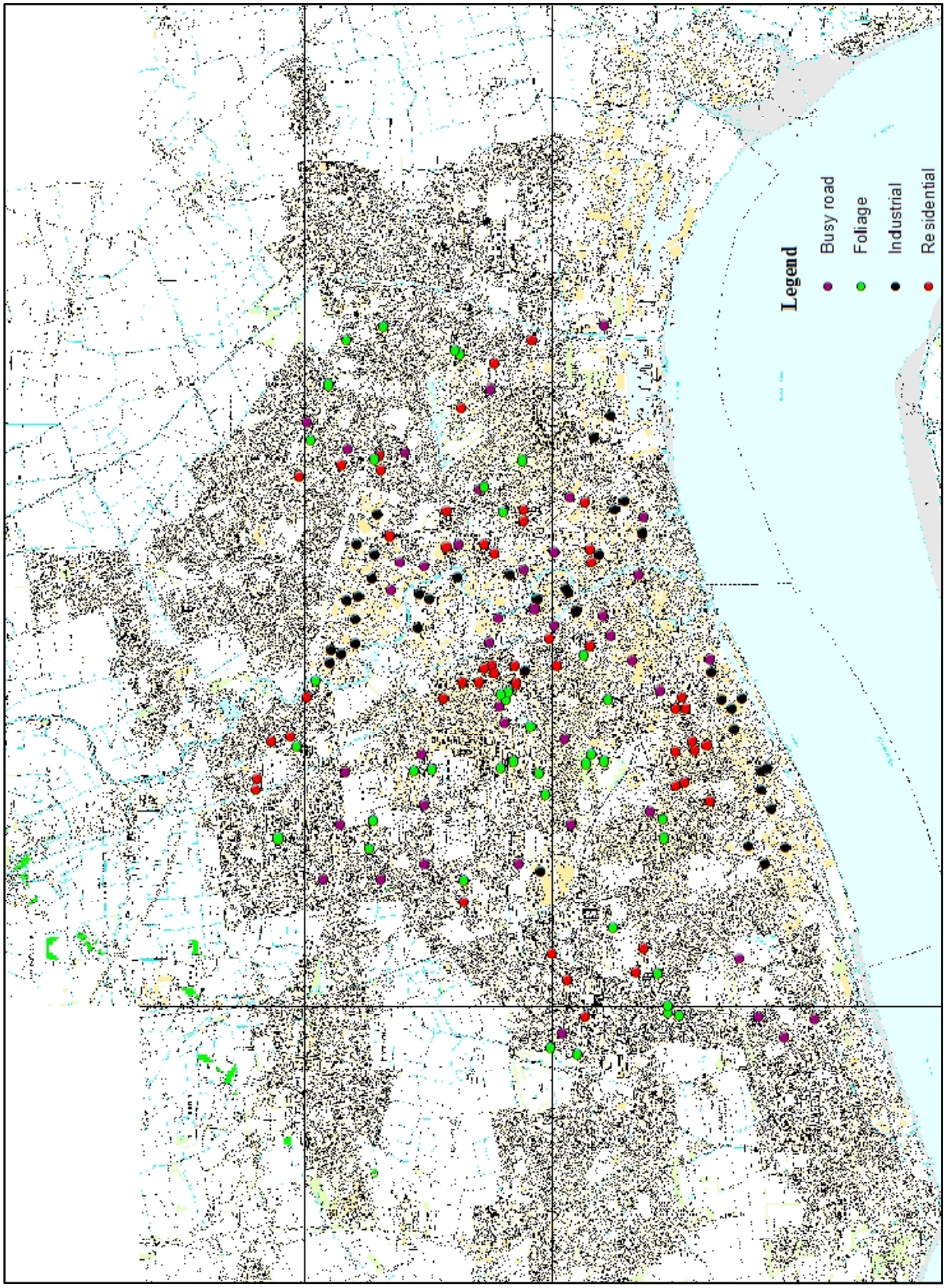

Figure 4.1 The locations of the gully pot sampled in the city of Hull during the random monitoring exercise, the colouration indicated the area type as illustrated in the legend.

4.3.1.2 Repeated sampling

For the repeated monitoring exercise, 15 gullies that had been cleaned using an eductor truck in September 2009 were selected for monitoring by Street Scene Services operatives. As previously described (see Section 3.1) the gullies were chosen from the four different characterised areas, sampling four gullies from locations with high foliage, busy roads and residential location, and three being sampled from the industrial locations. Sampling started in the third week of October 2009, and took place on the third week of each subsequent month for a year after this date. The only exception was January 2010, when there was very heavy snow, which restricted access to the desired gullies, and the team which assisted with the sampling were unavailable. All of the gully pots were grab sampled, as previously described (see Section 3.1). As with the random sampling strategy, the location characterisation and address of each gully pot sampled and the location was recorded and plotted on an ordinance survey map (see Figure 4.2).

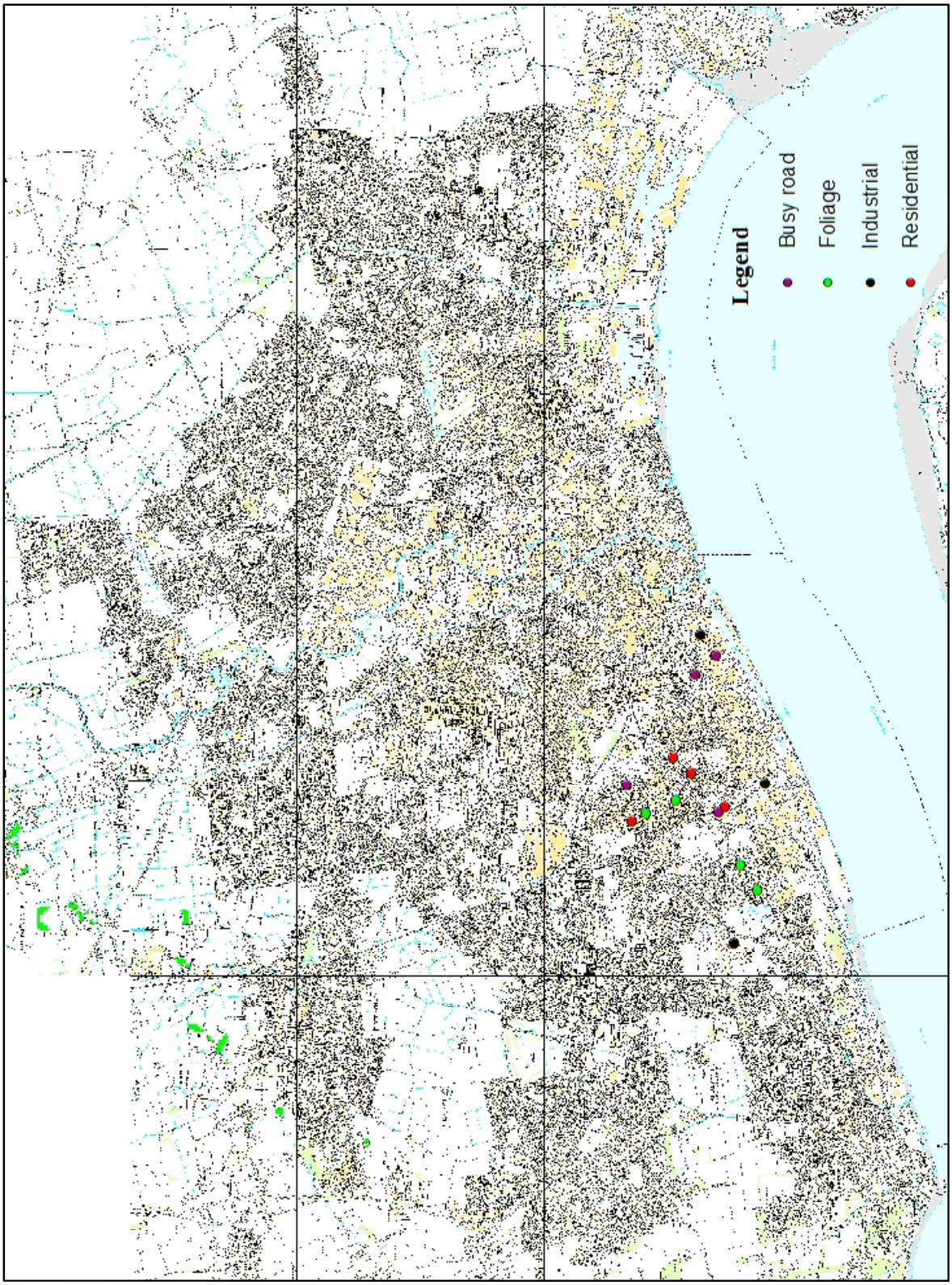

Figure 4.2 The location of each gully pot sampled in the city of Hull during the repeated monitoring exercise, the colouration indicated the area type as illustrated in the legend.

4.3.2 Overall gully pot waste degradation activity

For the repeated and random monitoring exercises the same suite of analyses was performed to assess the degradation, aside from depth accumulation which was only measured on the repeated monitoring exercise. All of the laboratory analyses were carried out on four consecutive days following sampling.

4.3.2.1 Physical parameter analysis

Prior to the removal of the sample from the gully pots, during both exercises, the pH and temperature of each gully pot was measured (as described in Section 3.2.1.1). For the repeated monitoring exercise, the depth of the waste that had accumulated in the pots was also measured for each gully pot (see Section 3.2.1.2) to give an insight into the accumulation of waste within the pots over a year (Figure 4.3 shows the depth of the waste being measured). This was important when looking at seasonal variability as it identified seasonal differences in accumulation rates and facilitated an indication of degradation activity over a year.

The pH, moisture content and organic matter content were measured gravimetrically (as described in Section 3.2.2.1, 3 2.2.2 and 3.2.2.3 respectively). These parameters were measured for every sample as they provide a crude characterisation of the waste from the gully pots, and can indicate its ability to decompose.

Figure 4.3 A Street Scene Services operative using the content depth measurer

4.3.2.2 Extracellular Enzyme Activity

For both monitoring exercises, the activity of all eight selected enzymes (sulphatase, phosphatase, β-glucosidase, α-glucosidase, β-galactosidase, β-glucuronidase, butyrate esterase and β-xylosidase) were analysed as described in Section 3.3.1 The enzyme activities were assessed as they can indicate the ability of a microbial population to degrade a wide range of organic substrates (Mondini *et al.*, 2004), and this can be used as an index for microbial activity (Darrah and Harris, 1986).

4.3.4 Statistical Analysis

An initial assessment of the normality of the data was undertaken using a Kolmogorov-Smirnov test for goodness of fit. Statistical tests were used to determine if there were significant differences between the gullies sampled from different locations, and between the seasons in which they were sampled. If the data was normally distributed a one-way analysis of variance (ANOVA) was performed for the random sampling data, whilst for the repeated exercise data a repeated-measure ANOVA was performed. This was followed by a post-hoc test of least significant difference (LSD) to determine where the differences were occurring. If the data was not normally distributed a Kruskal-Wallis test was performed for the random sampling data and a Friedman test was performed for the repeated exercise. These were further analysed with a post hoc Mann-Whitney-U tests. All tests were performed using PASW statistics, version 18.

4.4 Results

To establish if seasonal change and geographical location characteristics had an effect on the internal processes of gully pots, each dataset, repeated (n = 150) and random (n = 180), was investigated individually. These results were then compared between the datasets to determine if any patterns were present between gully pots that were evaluated during the 12 month monitoring assessment, starting directly after cleaning until their next scheduled clean, and those evaluated during the random monitoring assessment over a two year period.

For both monitoring assessments the temperature of the sample was recorded within the gully pot and compared to the external air temperature, to allow for assessment of the

thermal range. The results of the sample temperature assessment demonstrated that the gully pots showed little difference between location and season, when compared to the trend of the external air temperature. The mean gully pot temperature was 14.9°C, whereas the mean external air temperature was 16.7°C, with the maximum *in situ* gully temperature being 21°C and the minimum being 0°C. This 2°C difference in temperature between external and *in situ* environments was observed throughout both exercises, the exception being when the external air temperature dropped to below 0°C, in this situation the gully pot temperature did not mirror this trend, and remained at 0°C.

4.4.1 Physical parameters

Prior to the results being analysed for seasonal and geographical effects, the waste from the pots were initially examined to give an overview of the full range of parameters being assessed. The aim behind this approach was to allow for an evaluation of the potential range of physical and enzyme activity occurring in the waste. When looking at the physical processes of all the samples there appeared to be a relatively wide range of variability in evidence throughout the monitoring exercise for all of the waste examined during the random experiment, especially in relation to moisture and organic matter content.

4.4.1.1 Organic matter content

Of particular interest were the results for the organic matter content, which showed significant seasonal variation in the random dataset. The random exercise indicated a very large range of 2.17% - 96.54% organic matter content, with a mean of 45.79% for all locations studied. The repeated monitoring exercise displayed a smaller range of

22.53% - 72.43% for the organic matter content, with a mean of 44.51%. There was a significant difference observed between summer and the other three seasons in the random exercise ($p<0.05$), with the summer months having a higher organic matter percentage than the remainder of the seasons. This result was surprising as it was assumed that autumn would have the higher organic matter values, being the optimum time for organic supply. This distinct summer peaking is visible in Figure 4.4, and is seen to be following on from a drop in spring, and a subsequent drop in the autumn. The organic matter content results for the repeated dataset did not show any significant differences between the four seasons; however, as seen in Figure 4.5, a slight peaking in the summer months was observed, which is broadly similar to the random exercise.

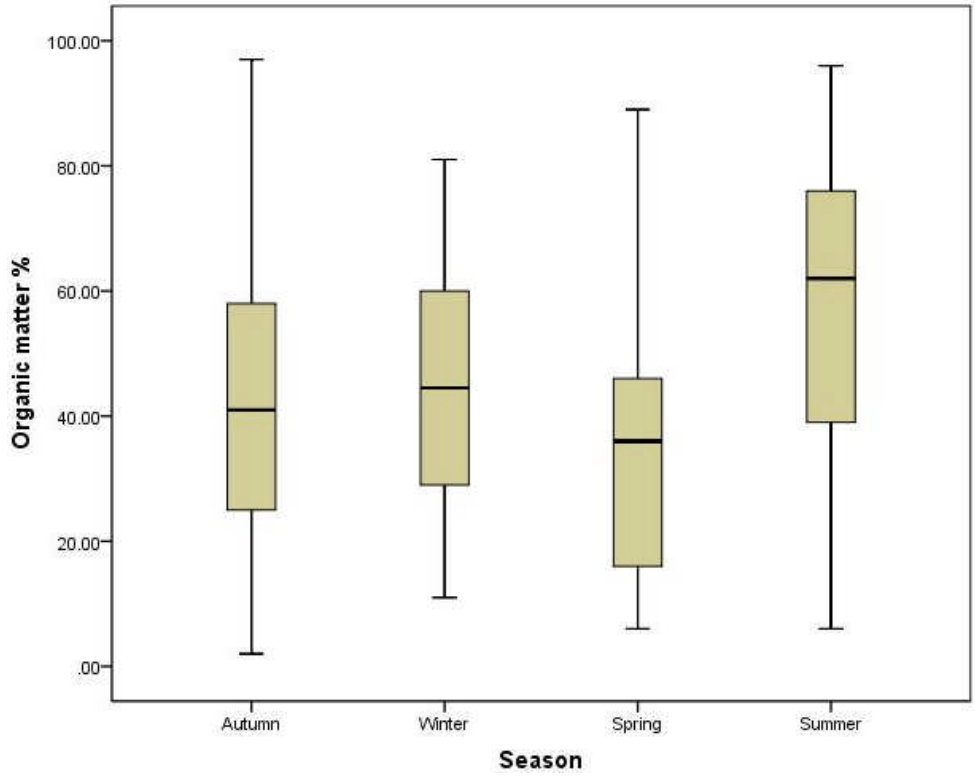

Figure 4.4 Box plot for the percentage organic matter content between seasons for the random sampling exercise (showing the range, interquartile range, and the mean (n=180).

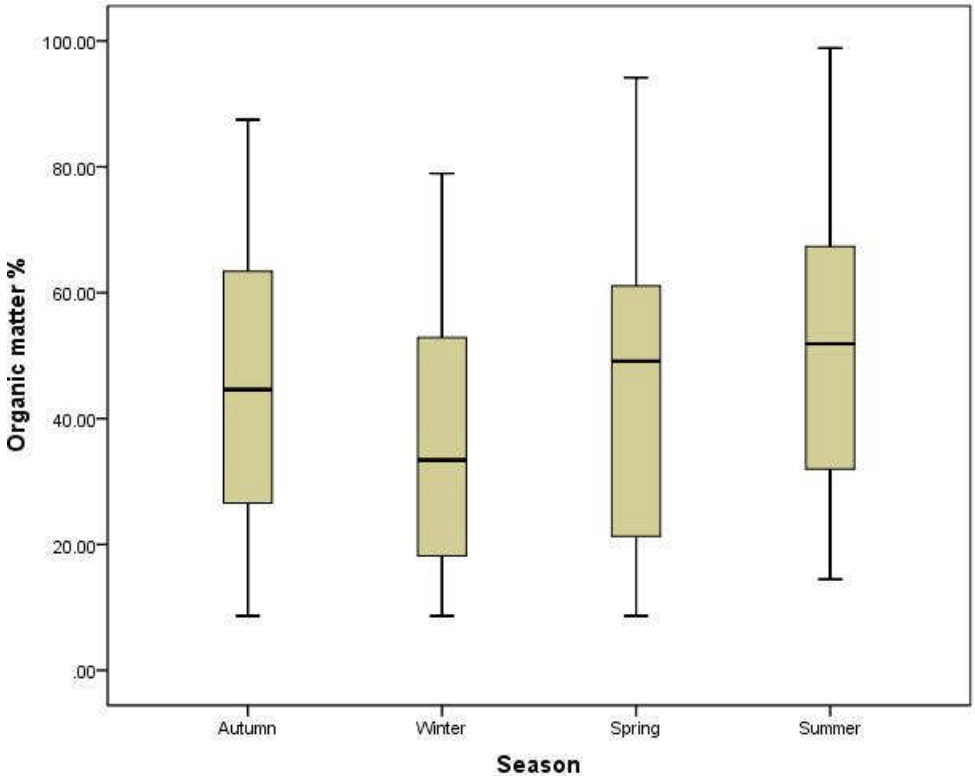

Figure 4.5 Box plot for percentage organic matter content between seasons for the repeated sampling exercise (showing the range, interquartile range, and the mean) (n=150).

Geographical locations had a visible an effect on organic content in the random dataset (see Figure 4.6). The percentage organic content for busy roads, residential and high foliage areas (which had the highest organic matter content), showed no significant differences. However, samples taken from industrial areas were statistically different ($p<0.05$) to the other three locations, displaying lower organic matter content. This was also visible in the repeated exercise, where industrial areas had lower organic matter content when compared to the higher organic matter values observed in areas with busy roads, residential areas, and high foliage (see Figure 4.7). This, unlike the random exercise result was not statistically significant ($p>0.05$).

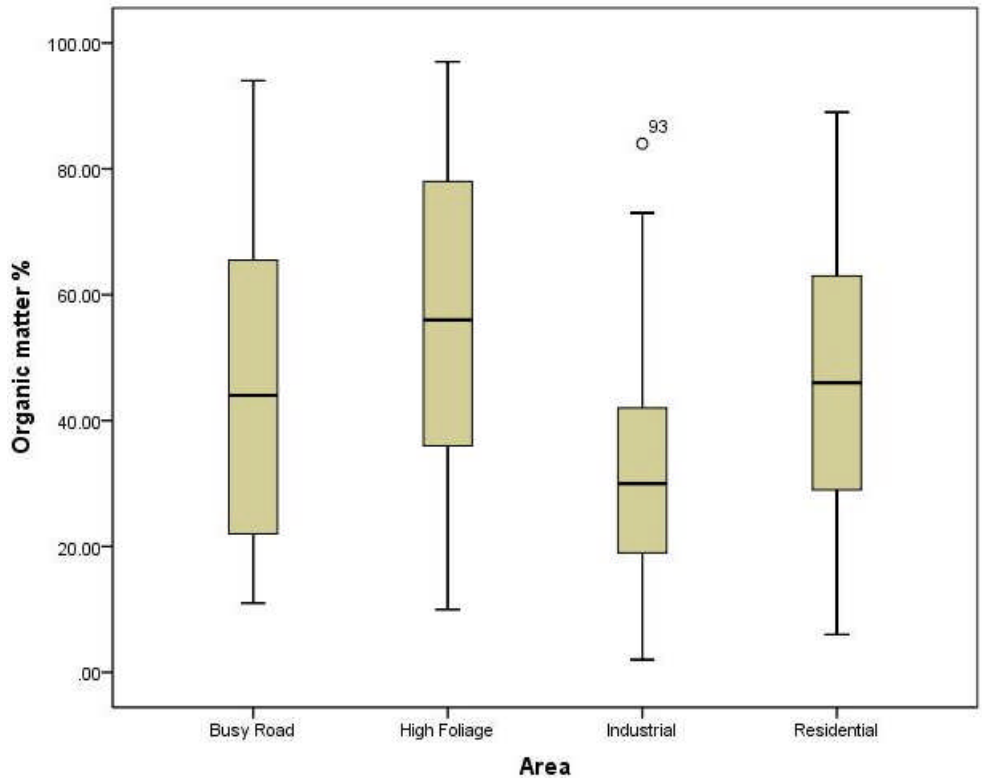

Figure 4.6 Box plot for percentage organic matter content between areas for the random sampling exercise (showing the range, interquartile range, and the mean)) anomalies indicated as 93 in the graph (n=180).

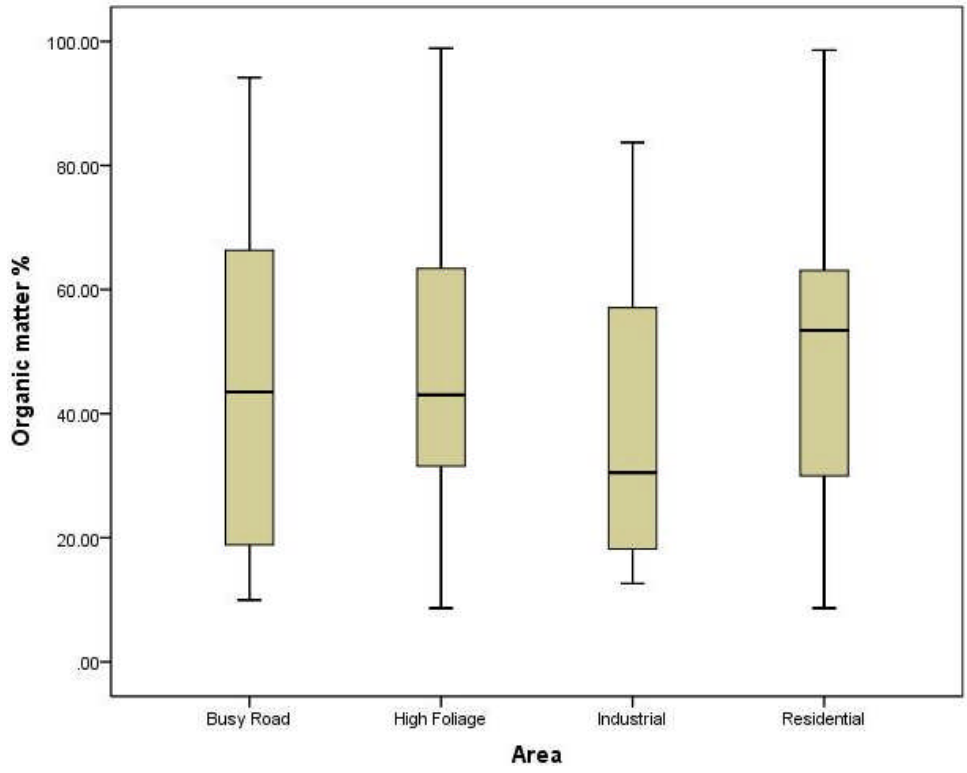

Figure 4.7 Box plot for percentage organic matter content between areas for the repeated sampling exercise (showing the range, interquartile range, and the mean) (n=180).

4.4.1.2 pH

The random sampling exercise produced pH values ranging from 7.32 to 10.73, with one very high alkaline reading of 13.22. This anomalous reading came from a gully pot that had been contaminated with concrete and cement from a house renovation. The mean pH of all the gully pots in the random sampling exercise was 8.80. During the repeated exercise the pH ranged from 8.02 – 10.08, the mean being 9.09, which was slightly higher than in the random sampling exercise.

Significant seasonal differences were also observed in pH ($p<0.05$), where the autumn and winter sampling displayed lower pH levels when compared to spring and summer during the random exercise. This was similar to the results observed in the repeated exercise where significant seasonal differences ($p<0.05$) were observed in pH levels. In this exercise however, only the autumn data displayed significantly lower levels when compared to spring and summer.

Significant differences ($p<0.001$) were observed in the random monitoring exercise when comparing the pH levels of the four areas. It was noted that both busy roads and areas with high foliage had lower pH values when compared to industrial and residential areas. During the repeated monitoring exercise the pH of gullies sampled in residential areas was significantly higher ($p<0.001$) than those of the other three areas.

4.4.1.3 Moisture content

The moisture levels recorded during the random exercise ranged from 28.24% - 97.38%, with a mean of 65.70%. During the repeated monitoring exercise the moisture levels ranged from 51.43% - 75.98%, which was a much smaller range than the random dataset, but the moisture content had a very similar mean value of 64.10%.

No statistical differences were observed when assessing the moisture levels over the four seasons for both data sets. This was also the case when assessing the effect of geographical location on the moisture levels, as no significant differences were observed during both exercises.

4.4.4.4 Depth accumulation

When analysing the depth accumulated over the year during the repeated exercise statistically significant differences were observed between the seasons ($p<0.05$). Autumn was shown to have a larger accumulation compared to winter and summer. This result was initially expected due to high leaf fall during autumn; however, the organic matter content does not reflect this result, having a lower value in this season. This was not the case with spring as the mean measurement for areas with high foliage spiked to 0.21m (as can be seen in Figure 4.8).

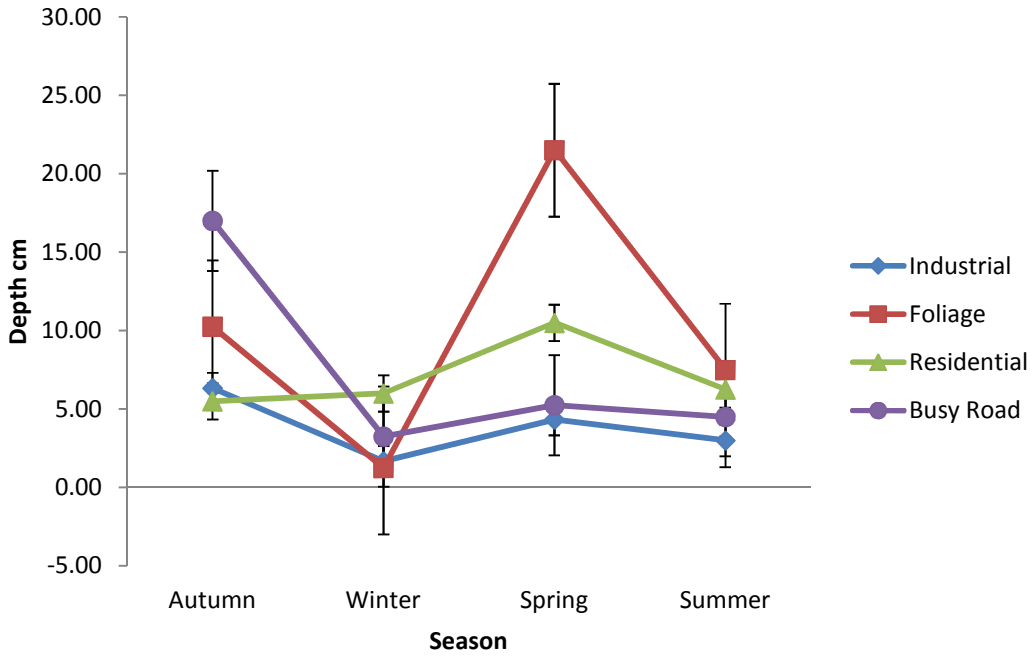

Figure 4.8 Line graph showing the mean waste accumulated each season in the four areas over the year period with error bars (n=150).

When assessing the depth of waste accumulated over the year for the repeated sampling exercise, there were no significant differences observed between the locations analysed ($p>0.05$). Despite this, there were obvious visible differences. For instance, the maximum increase in waste depth in one month for a busy road location was 0.31m compared to 0.28m for high foliage areas and 0.21m for residential areas, but only 0.08m for industrial locations. These latter areas also had the smallest mean input throughout the monitoring period accumulating only 0.15m of waste, as opposed to the high foliage areas that exhibited a mean total input of 0.40m.

4.4.2 Extracellular Enzyme activity

As can be seen in Table 4.1, seasonal change seemed to have had little effect on enzyme activity over the whole period for both of the datasets. However, both datasets appeared

to be influenced more by geographical location than seasonal change. The enzyme activity, as with the physical factors, showed a similar trend when examining the effects of area types. During both exercises the locations with high foliage had larger influence on the enzyme activity when compared to the other three location types. For the random dataset six out of the eight enzymes examined displayed significant differences between areas (see Table 4.1). By contrast, only four of the eight enzymes were significantly affected by location type during the repeated monitoring exercise.

Table 4.1 Results of ANOVA and Kruskal-Wallis tests for gully pot waste activity from the random and the repeated monitoring exercise.

	Repeated dataset (*n*=150)		Random dataset (*n*=180)	
	Season	Area	Season	Area
pH	0.007*	0.000*	0.003*	0.000*
Moisture %	0.575	0.912	0.932	0.300
Organic matter content %	0.101	0.325	0.003*	0.001*
Sulphatase	0.012*	0.123	0.876	0.403
Phosphatase	0.094	0.557	0.501	0.288
β-glucosidase	0.219	0.008*	0.100	0.000*
α-glucosidase	0.560	0.000*	0.046*	0.000*
β-galactosidase	0.273	0.543	0.744	0.068
β-glucuronidase	0.269	0.283	0.310	0.003*
Butyrate esterase	0.310	0.002*	0.058	0.000*
β-xylosidase	0.246	0.002*	0.774	0.000*

* Indicates a significant level of $p<0.05$

For both the repeated and the random exercises β-glucosidase displayed significantly higher activity in areas with high foliage compared to the other three locations ($p<0.05$). Season did not appear to significantly affect the activity of β-glucosidase in either dataset ($p>0.05$).

Significantly higher activity of α-glucosidase ($p<0.001$) was observed in areas with high foliage in both datasets when compared to the other three location types. Furthermore, during the random sampling exercise, α-glucosidase showed a significant difference

($p<0.05$) between the seasons. By contrast, the winter data showed significantly less activity when compared to the summer and autumn monitoring ($p<0.05$). This was the only enzyme that was observed to be affected by seasonal influences, and this situation was not observed in the repeated dataset.

Butyrate displayed significantly higher activity ($p<0.05$) in the locations with high foliage during both the random and the repeated exercises. However, seasonal influences did not appear to affect the activity in either dataset for this particular enzyme.

Areas with high foliage clearly influenced the activity of xylosidase, which was seen to exhibit significantly higher levels of activity ($p<0.001$) when compared to its activity in the other three areas monitored during the random sampling exercise. This, however, was not observed in the repeated exercise where β-xyloside displayed significantly lower levels of activity ($p<0.05$) in areas with high foliage when compared to the other three locations studied.

During the random sampling exercise, β-glucuronidase displayed significantly lower activity ($p<0.05$) in areas with busy roads when compared to the other areas. This was not observed in the repeated monitoring exercise where no significant differences between area types occurred.

Area type did not seem to affect the activity of sulphatase, which shows no significant differences in either the repeated or the random sampling exercises. During the repeated monitoring exercise only one enzyme appeared to be affected by seasonal change.

Sulphatase showed significantly different activity ($p<0.05$) between the seasons where spring and summer had higher levels of activity when compared to autumn and winter. This seasonal variation was not observed in the samples from the random monitoring exercise.

In the current study only phosphates and β-galactosidase show no significant differences between the geographical location types ($p>0.05$) or seasons ($p>0.05$) for both datasets.

4.5 Discussion

The physical factors (organic matter content, pH and moisture content) showed similar trends throughout both datasets showing significant differences between geographical areas, but less statistically significant differences between seasons. However, when examining the ranges observed during the physical analyses the random sampling exercise displayed a much larger range of pH, organic matter content, and moisture content than the repeated exercise. This could be a consequence of the sampling size as for the random exercise a total of 180 gullies were analysed once, while the repeated exercise entailed repeatedly sampling the same 15 pots over the period of a year. Although the ranges of pH, organic matter content, and moisture content differed between monitoring exercises, the means for all three were similar between both exercises (8.80-9.09, 44.51-45.79% and 64-65.70% means respectively).

4.5.1 Seasonal effects

Given previous observations it was anticipated that there would be large differences observed in selected parameters across the seasons, for example reductions of enzyme

activity due to low temperatures in winter (Fekete *et al.,* 2007), and alterations in organic matter input due to leaf fall in autumn (Pratt and Adams, 1984). However, the effect of seasonality seemed to have very little impact upon the processes monitored in both studies; as will be discussed in further detail below.

No statistical differences between the seasons were observed when monitoring the moisture levels during both studies. A range of values were recorded, but as the pots were constantly full of water, unless there had been a crack in the gully pot allowing water to drain, the moisture level was not expected to vary. The observed variability in the ranges between the two exercises could be due to the type of waste collected within the pots, and its ability to retain water. For example, organic matter in soils enhances water holding capacity, therefore the higher the organic matter content the more water that can be retained (Dubbin, 2000). As the random exercise sampled more pots, there was a greater probability of recovering a wider variety of contents.

Autumn displayed statistically lower pH values than spring and summer for both sampling exercises, alongside winter having lower pH levels in the repeated monitoring exercise. Generally the pH begins to drop at the initiation of the degradation process (de Badiane *et al*., 2001), the drop in pH could be a result of the microbial activity as decomposition occurs. However, this is a weak argument as the low pH levels were not observed in the spring or summer months, which had similarly high organic matter levels. The near neutral pH could be due to the addition of salt and grit to the roads during the late autumn and winter months. The sodium chloride in the salt has a neutral pH value, which when the snow and ice melts will naturally drain into the gully pots where it would mix with the gully liquor. Previous studies on gully pot pH by Morrison

et al. (1995) noticed increased gully liquor pH during dry weather, and these authors associated this with the alkalinity caused by the release of calcium ions from the cement-concrete structure of the gully pot. This could provide an explanation for the elevated pH values observed in the spring and summer months when contrasted to the autumn and winter periods. To gain a better idea of how the pH and other parameters such as temperature, conductivity and dissolved oxygen were affected by seasons, a real time monitoring trial was implemented. However, the few results obtained during the trial suggested that the gully pots did not have ideal environmental conditions to test for this seasonal variation, and as the equipment was subjected to persistent vandalism it was decided terminate this area of the study and to concentrate instead on the routine single field measurements and laboratory tests (see Section 3.4.1).

Statistically significant differences were not observed in organic matter contents during the repeated exercise; however, they were in evidence during the random monitoring exercise where the spring season generally exhibited higher organic matter contents when compared to the other three seasons. This was a surprising result, as autumn was anticipated to have the higher organic matter content due to the heavy leaf fall, as observed by Grottker (1990). However, a peak in material input may not always indicate a higher organic matter content, and if it did, different locations may have different timings for peak material inputs, according to local climate and environment (Pratt *et al.*, 1987). For instance Ellis and Harrop (1984) observed larger sediment supply in summer when compared to spring, whereas Pratt *et al.* (1987) observed a peak in material supply in June, which they associated with gardening and other human activity allowing wind or water-mobilised material to be readily available. If higher input is related to higher organic matter then these results could be due to early leaf fall

or human activity, which would be in general agreement with the observed patterns on Clifton Grove, London, and as such elevated organic matter, might be anticipated (Pratt *et al.,* 1987)

During the repeated monitoring exercise the autumn, spring and summer samples all had relatively similar organic matter levels, with an insignificant drop observed in the winter months (see Figure 4.5). When this was compared to the mean waste accumulated from the repeated exercise, it was clear that the autumn data had the higher intake. The exception to this general trend was the occurrence of elevated levels in the high foliage areas in spring. This spring result could be due to anomalies caused by heavy littering occurring on two occasions; one pot in April had a 0.28m accumulation increase due to sand being deposited, and a different gully pot in May had an accumulation increase of 0.23m due to concrete being deposited. This resulted in both gullies being emptied before their due date. The long term effect of littering within the gully pots, apart from the two examples previously mentioned, were not measured and this could have had a larger effect on the accumulation monitored over the seasons. The accumulation was only compared to the organic matter values however, the peak in the organic matter differed to the peak in litter accumulation. Larger litter accumulation within gully pots due to deposition of other waste material was evident throughout both monitoring stages, and has been widely recorded within the gully pots over the years during their mechanical cleaning (*pers. comm.* Hagar, 2009). Litter observed within the gully pot during this study has ranged from small items such as confectionary wrappers and socks, to large items such as car and bike parts. Without emptying the gully pots each time they were sampled it was impossible to omit the litter, therefore this data was gathered and included in the accumulation data. However, this task was only performed

to measure the rate in which the gullies filled up. Though littering from the public can clearly affect the performance of the gully pot by causing them to fill up more rapidly or become obstructed. The littering observed during the sampling occurred throughout the gully pots studied, although the older diamond lids restricted the amount of litter allowed into the pots when compared to the slatted lids (see Section 2.1.2) due to the reduced size of the diamond shaped holes. The shortcoming of the smaller holes in the diamond lids is that they block externally more readily affecting the drainage of the roads. This in turn can lead to surface water flooding. As noted above, it is due to this factor that the diamond lids are gradually being replaced with slatted lids across the city of Hull (*pers. comm.* Hagar, 2009), partially due to the need to let litter in, thereby reducing surface flooding and encouraging biodegradation processes within the gully pot waste.

It is, however, important to consider the sampling times when comparing these two exercises. Re-sampling of the same gully pots for a year could be an important factor when understanding the seasonal results during the repeated exercise. As the repeated monitoring study ran from October 2009 to September 2010, samples collected during the autumn in 2009 would have remained in the pot until it was emptied the following year, which could produce a bias in the data when assessing organic matter and waste accumulation. This would be especially noticeable when looking at the data across a seasonal timescale. By contrast, during the random monitoring study, seasonal data was collected as an amalgamation over two years, with different gully pots being grab sampled at random intervals during the annual cleaning regimes. As such, it is uncertain as to how long the waste had been accumulating within the gully pot, a factor which could affect the assessment of the contents.

As previously mentioned, similar trends were observed for the physical parameters, such as pH and organic matter, between the repeated and the random monitoring exercises, however, this was not the case for enzyme activity. During the repeated exercise, sulphatase was the only enzyme that displayed a difference in activity between the seasons, being higher in spring and summer. This however, was not the case for the random exercise, where α-glucosidase had lower activity during the winter months when compared to summer and autumn.

Previous research has shown that sulphatase activity can remain relatively stable throughout the year e.g. in planted and arctic tundra soils (Spier and Ross, 1978). This observation is supported by the findings for sulphatase activity in the current study from the random dataset, but these results are not replicated in the repeated data. At present the reason for this variability is unclear but it was observed that the pH levels were higher during the spring and summer months, although this cannot perhaps be attributed to the higher activity, as the optimal pH for sulphatase in soil is 6.2 (Speir and Ross, 1978; Whittmann *et al*., 2004). However, just because a pH level of 6.2 is optimal, this does not mean that it is not an influencing factor. The higher organic matter content observed during these months may have impacted upon the observed enzyme activity. The high sulphatase activity could be an indication of sulphate reduction which can occur during anaerobic organic matter degradation (Hastings and Emmerson, 1988). Although autumn had similarly high organic matter levels, the waste would not have remained stagnant for over half a year, which could explain the lower sulphatase activity. The accumulation of waste that occurred over two seasons could, therefore, be the reason why it was not statistically observed in the random exercise, as the waste

accumulation date was unknown. Whilst these observations offer possible insights into the reasons for the variability in sulphatase activity, in order to verify these observations it is clear that further analysis, such as the monitoring of redox potential and a more targeted assessment of the inherent variables, would need to be undertaken. It is also important to consider the possibility that the high sulphatase activity could be due to disturbances within the gully pot, as similar issues have been observed in soils, where wet disturbed soils displayed consistently less activity when compared to the activity recorded in undisturbed soils (Neal and Herbein, 1983). Heavy rain or snow fall, or increases in the waste accumulating in the gully pots during the autumn and winter months could cause disturbances, thereby resulting in lower activity.

Low α-glucosidase activity was observed during the winter season in the random dataset when it was at its lowest temperature of 0°C, although the organic matter was not at its lowest level. Low pH has been previously observed to cause higher α-glucosidase activity, as well as higher β-glucosidase and β-xylosidase activity, in soils (Niemi and Vepsäläinen, 2005). However, this was observed at a pH level of 5.5, which was much lower than the levels observed during this experiment. Furthermore, the pH had a lowering effect, on enzyme activity, though as we were not assessing soil, a direct relationship could not be demonstrated. As the organic matter levels were similar to that of autumn, and they were not observed in the repeated dataset, it is assumed that the pH had not affected the enzyme activity. By contrast it is possible that temperature may have influenced enzyme activity as low temperature is known to affect the activity of enzymes within these conditions. However, it is unclear if this is a defining factor, as no other enzymes were affected during this season. This low α-glucosidase activity was not observed during the repeated exercise or in previous studies (e.g. Whittmann *et al*.,

2004) and so this could be due to sampling technique or anomalies within the dataset. Temperature was measured as this can influence rates of enzyme activity. It is of fundamental importance to generate baseline data for future monitoring so that any changes, such as those that are climatically induced, can be contrasted against the data generated in the current study.

The remaining six enzymes did not seem to be affected by seasonal change. Previous research has shown that these enzymes in soils are relatively stable across the seasons e.g. β-glucosidase (Bandick and Dick, 1999; Whittmann *et al*., 2004), phosphatase (Kang *et al.*, 2009), α-glucosidase, β-xylosidase, and butyrate-esterase (Whittmann *et al*., 2004). The low seasonal effects observed in the current study were initially considered anomalous due to the fact that temperatures increase during summer, decreases during winter and leaf litter supply would be increased during autumn were expected to affect the physical environment and enzyme activity. The current study has shown that this was not case in respect to the gully pots being investigated.

As the approaches adopted in the current study have not previously been utilised in the study of gully pot environments, investigating these seasonal and geographical variables over the one and two year time scales adopted, does not rule out the possibility that the results that are generated simply reflect the normal conditions within gully pots. It is likely that the *in situ* environment may remain semi-constant throughout the year due to moisture levels and other parameters remaining largely unchanged. Overall the general physical factors (i.e. moisture and organic matter content) that have indicated differences between the seasons have all displayed similar trends. It is generally unclear as to what the precise biological significance of these parameters is, and what effect

they may have on enzyme activity during the season. The differences observed during the seasons, for organic matter and pH, did not seem to relate to the known activity of the enzymes that have been shown to respond to seasonal influences. Therefore, it is important to understand that although differences were observed due to seasonal changes, these did not impact on the gully pot systems overall. This implies that they are in a general state of equilibrium, regardless of climate conditions. Importantly this suggests that the gully pots may create their own microenvironment *in situ.* Microenvironments behaving in this self regulating way can be found in both wetland and paddy soils, which are either sites of oxidation reactions or sites of reduction reactions mediated by a host of soil microorganisms (e.g. Douterelo *et al.*, 2009; Dommergues, 1978). Therefore, due to the prevailing *in situ* conditions within the gully pot, the microenvironments may self regulate to provide very similar, and possibly efficient, environmental conditions within which they can operate.

4.5.2 Geographical effects

Geographical location, and setting, appeared to have a more significant influence on the activity of the waste found in the gully pots. This result was not entirely unexpected as previous studies have shown differences in certain parameters, such as organic and sediment content, when measured between different catchment locations e.g. Grottker (1990); Pratt *et al.* (1987). In general lower activity was found to occur in waste from gullies in industrial areas as opposed to the other three areas due to the lack of organic input. As such, trends in the data generated between both monitoring exercises were observed, and these differences were often shown to mirror the findings from previous studies e.g. increases in sediment/waste supply to gully pot depending on area type (Pratt *et al.* 1987).

As with the seasonal data, no differences in moisture levels were observed between geographical areas for both the repeated and the random monitoring exercises. This can largely be attributed to the pots being constantly full of water (Section 4.5.1). For the random samplings, the pH was observed to be higher in areas with high foliage, and at the busy road locations, but not for the repeated exercise where pH was observed to be higher in the residential areas. The high pH levels could be due to the type and amount of waste accumulating within the pots. However, this finding is not definite as during the random sampling exercise the residential area had pots with similar organic matter contents to those in the busy road areas, yet the pH was not affected. It is possible the pH increase in the repeated sampling exercise could be due to the littering that was occurring in the residential areas. As previously described, there were two instances where gully pots were littered with sand, cement and concrete, all contaminants that could have affected the overall pH of the pot. Furthermore, as the gullies were manually emptied, once they had been discovered to be littered the liquor inside them was not removed, so the high pH (potentially from the inputs of cement and sand) could have been residual. Furthermore not all of the debris would have been removed, thereby increasing the alkalinity, as previously described.

No statistically significant differences were observed between the four areas for organic matter content in the repeated exercise. However, it can be seen in Figure 4.6, where the waste from industrial areas exhibits lower organic matter content when contrasted with the other areas, an observation that is similar to the random sampling exercise. It is reasonable to conclude that the low organic matter contents identified in the current study could be attributed to the limited vegetation surrounding the industrial area. As a

result, waste containing organic matter was clearly not being transported to the gully pot on a regular basis. In the random sampling exercise higher percentages of organic matter was observed in the pots that were surrounded by high foliage, as might be anticipated. This observation is supported by the work of Grottker (1990), who has previously reported higher organic matter contents when trees or bushes were located near the sampled gully pot.

Although the discrete study areas were distinguished by their surroundings, on occasion overlapping of environmental parameters occurred, and this may well have affected the area results, especially in relation to organic matter content. A clear example of this can be found in the busy road areas, where the busy roads were occasionally tree lined or had grassy verges. The same issue could apply for residential areas, which would help explain the results for the organic matter content and the depth of organic matter accumulation recorded. It was speculated, at the start of the study, that this overlap of areas could potentially cause anomalous results, however, this was not considered to be a major issue when developing the method as the results generated should provide insights into any variation that occurred as a result of areal overlaps. Furthermore, to isolate the areas with small overlaps would have been difficult, as there are a large number of green areas in the city, especially around the large residential areas. Had we decided to omit areas of overlap this would have reduced the amount of samples we could obtain for the study. Therefore, wherever possible, samples were taken from areas that had the least amount of overlap. We should also note that the observed overlaps could also help explain the variance in the rate of accumulation identified in the repeated monitoring exercise, where residential areas and busy roads sometimes had higher accumulation rates than the areas with high foliage (see Figure 4.7). Industrial

areas generally had the lowest mean waste accumulation levels, which is probably due to the lack of vegetation, and people, around those areas.

The data indicated that enzyme activity is influenced by geographical location more than season factors. Through the repeated exercise four out of the eight enzymes analysed (α-glucosidase, β-glucosidase, butyrate and β-xylosidase) showed statistically different levels of activity between geographical areas. These four enzymes also displayed statistical differences in activity between areas for the repeated exercise, as well as β-glucuronidase. All of the differences observed between geographical areas followed similar patterns, with areas of high foliage having an elevating effect upon the levels of enzyme activity. For example, in both datasets, increased activity of butyrate and α-glucosidase was observed in samples taken from areas with high foliage. In addition, from the random sampling dataset, β –glucuronidase, β-glucosidase and β-xylosidase all showed higher levels of activity in this area. This elevated enzyme activity might be linked to the large amount of organic content in the samples as well as to substrate availability, with most of the organic substrate entering the gully pot as leaf litter. This has been observed in soil decomposition studies of forest floors, where β -xylosidase, α -glucosidase, and butyrate-esterase were higher on the top of the soil floor, where the leaf litter would fall (Whittmann *et al.*, 2004). This elevated activity could be due to the initial stages of decomposition, which has been observed in leaf litter decomposition (Šnajdr *et al.,* 2010), where enzyme activity increases with organic source, regardless of the nature of that source (Bandick and Dick, 1999). There were, however, two enzymes that did not follow this general trend. In the high foliage areas, β-xylosidase had significantly lower levels of activity when compared to the other areas in the repeated exercise. This differed from the random sampling exercise and could be

attributed to the accumulation of waste over time in, what can be a largely stagnant environment, a context which may have caused the enzyme activity to differ from the 'norm'. In the random dataset, the observed levels of activity of β-glucosidase were significantly lower in areas with busy roads. This area has a lower organic matter content, clearly a factor that could lead to lower rates of activity. However, this correlation is unclear as the organic matter content of the pots was also lower in the industrial areas, but the enzyme activity for β-glucosidase was higher, suggesting a more complex interplay of variables.

Sulphatase, phosphatase and β-galactosidase displayed similar levels of activity throughout the experiment. These enzymes showed no significant variance between geographical locations, suggesting that they may be more adaptable to the changing environments of the gully pots. Of these three enzymes only sulphatase exhibited seasonal differences, and this variability was assumed to be attributable to the higher rates of litter accumulation within the gully pot. If this observation is upheld it could be assumed that the high foliage areas would create differences in the observed levels of enzyme activity. However, this was not in fact the case, suggesting that the result for the sulphatase activity is weakly correlated to the observed variables and that this enzyme is more adaptable to the environment than originally perceived.

Although there were more observed differences in geographical location when compared to seasonal influences, the differences did not appear to have a major effect on the levels of physical variables and enzyme activity recorded. The industrial areas did have lower values in most of the measured parameters when compared to the other locations, however, the waste still displayed some form of activity. Due to the low

organic matter values observed in the waste, the enzyme assays are more reliable in determining how active the waste is in a degradation sense. Therefore, monitoring extracellular enzymes may be more useful than organic matter weight loss as an approach to estimate microbial activity (Tank *et al.*, 1998). Whilst enzyme activity has been extensively researched using a variety of assays e.g. colormetric assays (Verchot and Borelli, 2005; Whalen and Warman 1996), and spectrophotometrically assays (Kähkönen *et al.*, 2008; Negoita *et al.*, 2002), fluorogenic substrates have been used in abundance e.g. Adams and Umpathy, (2011), Hoppe (1993), and are shown to provide greater sensitivity, when compared to other assays (Freeman *et al.*, 1995; Niemi and Vepsäläinen, 2005), which was the main reason in choosing this method for the current study.

4.6 Conclusion

This study has shown that the contents of the gully pot can vary considerably, displaying wide ranges of organic matter, moisture levels and extracellular enzyme activity. The effect of seasonality appears to have very little impact upon the processes monitored in both studies. However, geographical location and setting appeared to have a more significant influence on the waste found in the gully pots. In general lower physical and microbial activity processes were found to occur in waste from gullies in industrial areas as opposed to other areas due to the lack of organic inputs however, the waste still displayed some form of activity, but not as high as the other three locations. It is important to understand that although differences were observed due to seasonal or geographical changes, these did not impact on the gully pot systems overall, suggesting that they are in a general state of equilibrium, regardless of external conditions. Examining these seasonal and geographical variables has enhanced our understanding

of the processes occurring *in situ*, and has also provided insights into variability in response to variations in environmental factors, particularly geographical location.

Whilst significant differences in the parameters monitored between gullies were recorded, it has proven difficult to determine any distinct causes for the observed variability in the nature of the gully pot waste reactions from different locations. It is conceivable that the *in situ* environment may remain semi-constant throughout the year, due to moisture levels and other parameters remaining relatively unchanged. As a consequence it may be possible to treat gully waste in the next phases of the current project in a homogenous manner for each area of study, rather than individually, especially when considering the data in a seasonal context.

These results can greatly assist future research aimed at investigating the physical and microbial processes in the waste *in situ.* This could be undertaken via replica systems in a laboratory, where the environment can be controlled to assess external variables, and the data generated can be used as a baseline when examining sustainable solutions for gully pot waste management.

5.0 Assessing the effects of moisture and temperature on the degradation processes and microbial community under *in situ* modelled conditions

5.1 Introduction

Previous research has demonstrated that the transformation of the biodegradable organic fraction of solid urban waste into compost is one of the most validated methods of recycling. It is a process with low energy consumption that permits the disposal of the organic fraction of the solid urban waste and sludge, which together represent quantitatively the greatest portion of refuse (de Bertoldi *et al.*, 1983). Given this, it is apparent that using composting environments as analogies for developing an understanding of the processes occurring within gully pots has the potential to assist the current study in determining the decay rates that may be occurring in the gully pot waste environment.

In the previous chapter, the seasonal influences on biological activity within gully pots were investigated over a yearly cycle suggesting that seasonality had little influence on the rates of decay observed. However, in order to understand the effects of various environmental parameters on *in situ* degradation, a tighter controlled experiment was required (e.g. Smith, 2005) and for this purpose a series of laboratory based mesocosms were created as a way to control the environmental parameters of interest to the current study and stimulate gully pot processes.

Given the inherent gaps in our understanding of *in situ* processes in relation to degradation within gully pots it is apparent that developing an understanding of the

effects of temperature and moisture over a long term study has the potential to further enhance the current study through the generation of data that is of direct relevance to these *in situ* processes.

5.2 Aim

This chapter aims investigate the potential range of physical and microbial activity within the gully pots from the perspective of controlled *in situ* environments, using model gully pots constructed in the laboratory. These mesocosms were subjected to differing temperature and moisture regimes in an attempt to assess the potential range of influences on decomposition rates over a 20 week period. A range of methods are employed in order to measure decomposition rates; through physical parameters such as dry matter and organic matter decay, and also through an assessment of the effects of temperature and moisture on the microbial community. This latter element will be assessed through the use of Biolog EcoPlates™.

5.3 Method

5.3.1 Sample collection

Samples of typical gully materials were collected and stored as described in Section 3.1. These samples were then prepared and mesocosms were created (see Section 3.2.2.4) for use in the analysis which started the day after collection. Due to the results of phase one (Chapter 4.0), which indicated the pots from different areas behaved in general equilibrium, the waste was treated as a composite sample as opposed to creating different mesocosms for different geographical areas. Therefore to ensure that a variety of contents were collected the gully pots were sampled equally, and 10 samples of the

same size were collected from the four different geographical areas, as previously described in Section 3.1.

5.3.2 Pilot i*n situ* degradation trial

5.3.2.1 Experimental setup

The experiment consisted of 35 individual, meso-scale model gully pots, which were set up in the laboratory (as described in Section 3.2.2.4). These mesocosms were prepared by adding 40g of dried sieved gully waste and 60ml of distilled sterile water, into a 250ml conical flask of known weight. The mesocosms were created with 40g dry waste and 60ml water to account for the 60% average moisture level observed within the gully pots at the time of preparation. Tin foil was placed over the top of the conical flask, tightening it around the neck, before incubation to prevent excessive moisture escaping. The final weights of the mesocosms were recorded before incubation to ensure that an assessment of weight loss during incubation could be determined. All of the mesocosms were incubated at 30°C +/- 1°C for five weeks to replicate the mesophilic temperatures over a short time period (as observed in Adams and Umapathy, 2011). Analysis took place three times a week, where one mesocosm per sampling point was selected at random and sampled to destruction.

5.3.2.2 Physical parameter analysis

Throughout the experiment at each sampling point, moisture content (see Section 3.2.2.2), dry matter and organic matter content (see Section 3.2.2.3) were measured, and used to determine the rate of degradation.

5.3.3 Modelled *in situ* monitoring

5.3.3.1 Experimental setup

In total, 168 meso-scale model gully pots were set up in the laboratory (as described in Section 3.2.2.4) to measure the effects of two moisture levels; 60% and 80%, which were assessed in four different temperature environments; 5°C, 16°C, 24°C and 30°C (+/- 1°C) over a 20 week period. The range of temperatures and moisture levels observed in the field (Chapter 4) directed the choice of the moisture and temperature categories to be simulated within this part of the study. This method has been employed in previous studies investigating the seasonal effects of municipal solid waste (e.g. Cecchi *et al.*, 1992). A wide range of external field temperatures were considered, ranging from 5°C to 30°C covering a range of expected temperatures including those that may be expected under a warming climate, which was also included to model the impacts of potential future temperature increases (i.e. climate induced). A wide range of moisture levels were recorded in the field, with these averaging at 65%. The lower moisture level was determined to be 60%, with an upper level of 80% moisture to represent the higher moister levels observed from the fieldwork element of the project. To maintain the same volume across all of the mesocosms two different dry waste weights were used to produce the 60 and 80% moisture levels, this approach also served to reduce biasing and ensure the tests were equitable.

The mesocosms were prepared in two batches on the same day; 84 of these were monitored with 60% moisture levels by adding 40g of dried sieved gully waste and 60ml of distilled sterile water into a 250ml conical flask of known weight. The following 84 were monitored with 80% moisture levels by adding 20g of dried sieved

gully waste and 80ml of distilled sterile water into a 250ml conical flask of known weight. Tin foil was placed on the top of the conical flask and the final weights of the mesocosms were recorded before incubation. From each batch 21 mesocosms were incubated at 5°C, 16°C and 24°C, and the remaining 21 from each batch were incubated at 30°C. Over a 20 week period, three mesocosms from each temperature regime and moisture level were sampled to destruction for further analysis.

To maintain the effects of moisture, the total fresh weight of the remaining mesocosms were maintained by adding sterile distilled water (Adams and Umapathy, 2011). It is assumed that gully pots behave mainly in an anaerobic manner (Butler *et al., 1995)* but to avoid total anaerobic environments in the laboratory study, the moisture levels were only topped up with sterile distilled water if they fell below 80% of their original moisture content. The first sampling period occurred after 7 days of incubation, and sampling continued on a weekly basis for a further 3 weeks, sampling subsequently occurred in the 9th and 13th weeks of the experiment, with the final sampling date occurring after 21 weeks of incubation.

5.3.3.2 Physical parameter analysis

During sampling, the total weight (including flask) of the mesocosms was recorded to determine any loss which may have occurred during incubation; subsequently, one gram of waste material was removed for slurry preparation for use in the Biolog assay. The pH (see Section 3.2.2.1), moisture content (see Section 3.2.2.2), dry matter and subsequently organic matter content (see Section 3.2.2.3) were measured using the remaining material from the mesocosms.

5.3.3.3 Biolog analysis

Sampling for the Biolog EcoPlate™ occurred on the same day as the general analysis, and these were analysed as described in Section 3.3.2.1. Three samples from each temperature regime and moisture level were inoculated into one Biolog EcoPlate™, for example 30°C/60% moisture in triplicate. This was repeated for each temperature and moisture regime. The plates were then incubated for 24 hours at the same temperature that the initial sample was incubated at. The absorbance of each well was read on a MicroPlate reader (FLUOstar OPTIMA, BMG LABTECH Ltd., UK) at 590nm. The relative utilisation of each well was calculated as mentioned in Section 3.3.2.1, removing the control well (A1) value from all wells.

5.3.4 Statistical analysis

The effect of moisture and incubation setting were tested separately by analysis of variance (ANOVA) of each mesocosm and the sampling time, followed by a least significant difference (LSD) test if the data was normally distributed. For non-parametric data Kruskal-Wallis tests were performed followed by Mann-Whitney-U tests. All tests were performed using PASW statistics, version 18.

5.4 Results

5.4.1 *In situ* degradation trial

The results from the five week laboratory trial indicate that the contents of the gully pots are in fact able to be degraded *in situ*. Total organic content decreased significantly

over time ($p<0.001$), decreasing at an approximate rate of 0.008g of organic matter/per day (see Figure 5.1).

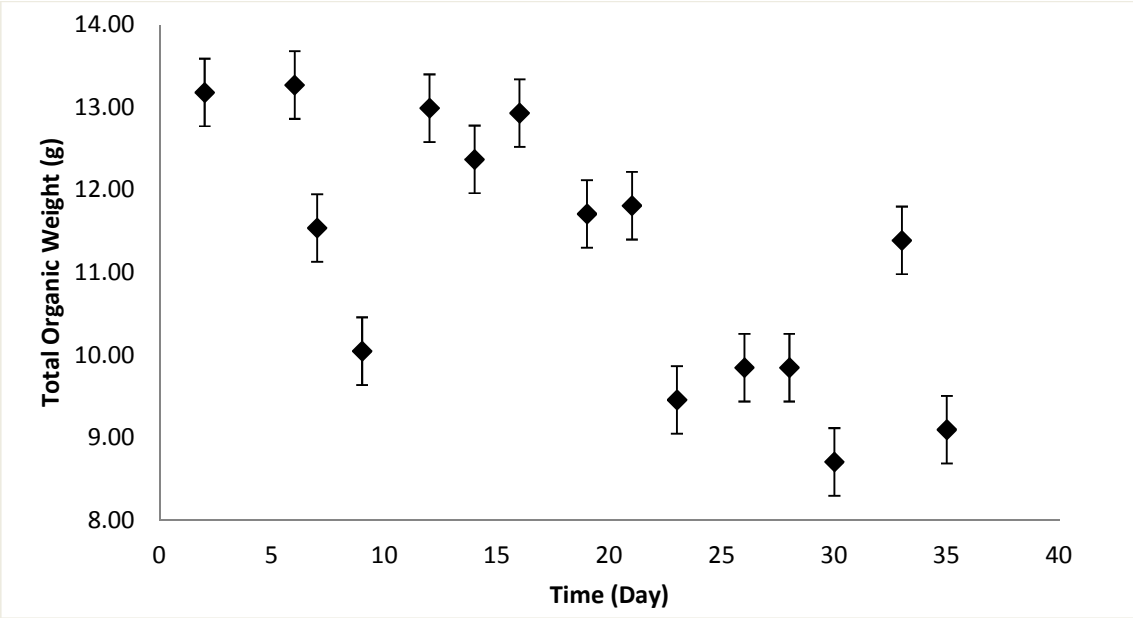

Figure 5.1 Mean total organic content reductions over a five week gully waste in situ trial with error bars fitted (30°C temperature and 60% moisture) (n=35).

The mean amount of weight lost after incubation increased over the five week trial. A small proportion of this loss was due to evaporation, however, throughout the entire trial moisture levels remained relatively constant (see Figure 5.2).

The total ash content was not a constant weight throughout this pilot study (see Figure 5.2), fluctuating between 25.45g and 29.58g. This result was unexpected as the ash content should remain constant and could therefore be due to limitations in the methodology. However, these fluctuations in ash were shown not to be significantly relevant ($p>0.05$).

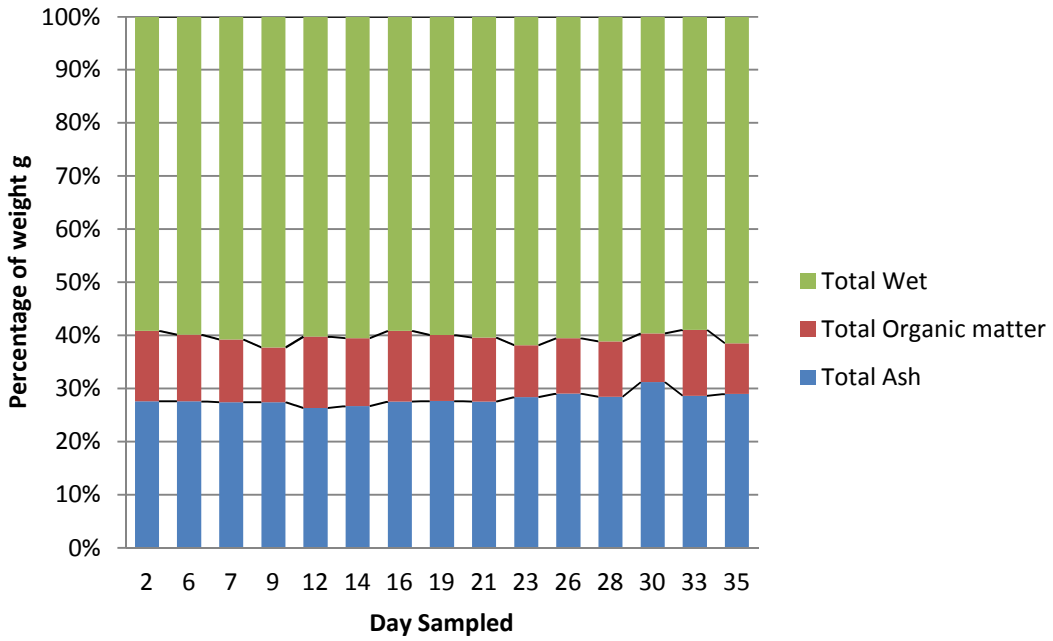

Figure 5.2 Percentage of the overall total wet, total organic matter and total ash weights over the five week trial (30°C temperature and 60% moisture) (n=35).

5.4.2 Modelled long term *in situ* decomposition monitoring

5.4.2.1 Physical parameter analysis

5.4.2.1.1 pH

The pH ranged from being very weakly acidic (pH 6.33) to low alkaline (pH 8.65), however, the mean pH observed throughout the whole study was a neutral pH 7.55. Temperature appeared to have no significant effect on the pH of the samples throughout the study ($p>0.05$) however, time ($p<0.05$) and moisture percentage ($p<0.05$) did.

5.4.2.1.2 Total dry matter decay

Dry matter content appeared to be significantly affected over time ($p<0.05$), displaying a lower mean value at the end of the 20 weeks when compared to the beginning of the

experiment. This time element was also observed when the different moisture levels, 60% and 80%, were assessed separately ($p<0.05$).

Due to the two different sample sizes that were added to the mesocosms to assess the possible influence of percentage moisture level, the dry matter was assessed separately for the effects of time and temperature at 60% and 80% (i.e. for each mesocosm moisture level that was employed).

There appeared to be a significant effect from temperature on the dry matter content at 60% moisture, where significant differences were observed between all four temperatures used in the experiment ($p<0.05$). This statistically significant difference ($p<0.05$) was also observed in the mesocosms with 80% moisture. Overall, a greater mean loss of dry matter was observed in the two higher temperatures (i.e. 25°C and 30°C see Figures 5.5 and 5.6) when compared to the lower temperatures (i.e. 5°C and 16°C see Figures 5.3 and 5.4). Statistical differences were observed over time in the mesocosms incubated at 25°C and 30°C with 60% moisture levels ($p<0.05$), and also with those incubated at 25°C with 80% moisture.

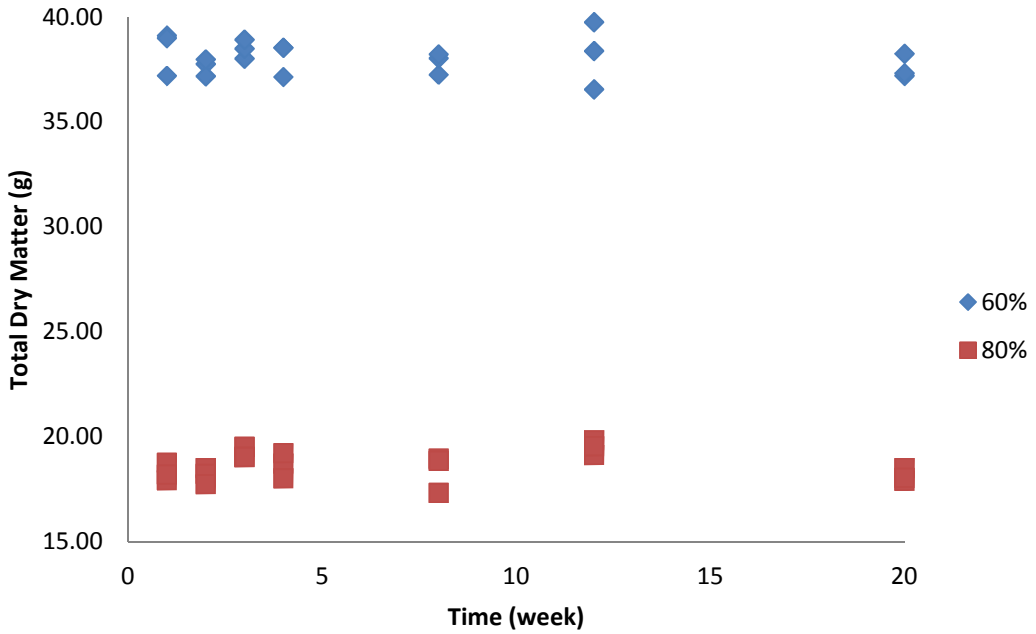

Figure 5.3 Total dry matter content over the 20 week trial for the mesocosms incubated at 5℃ with 60% and 80% moisture levels (n=42).

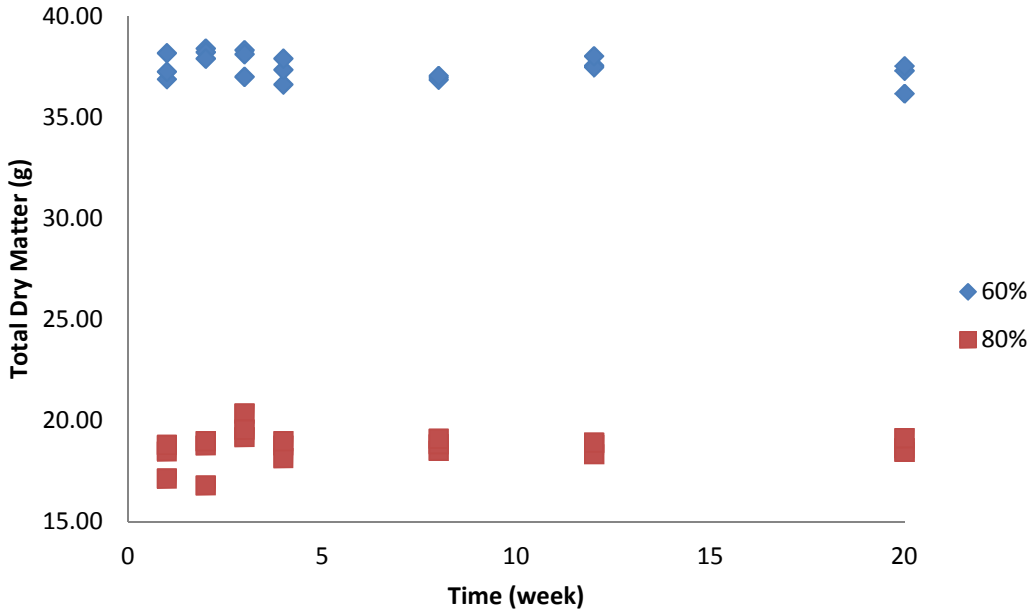

Figure 5.4 Total dry matter content over the 20 week trial for the mesocosms incubated at 16℃ with 60% and 80% moisture levels (n=42).

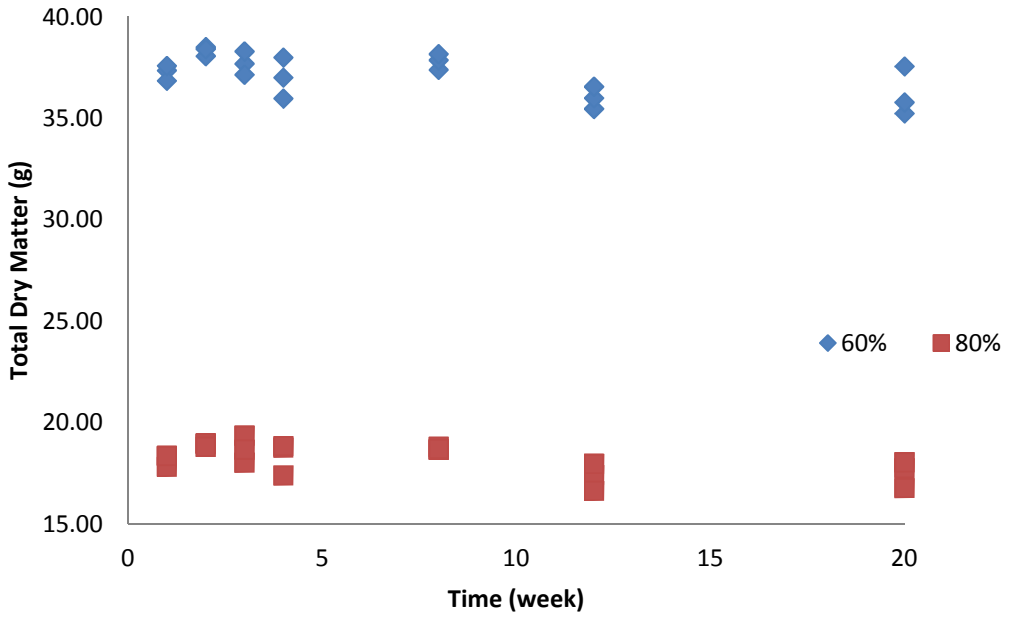

Figure 5.5 Total dry matter content over the 20 week trial for the mesocosms incubated at 25℃ with 60% and 80% moisture levels (n=42).

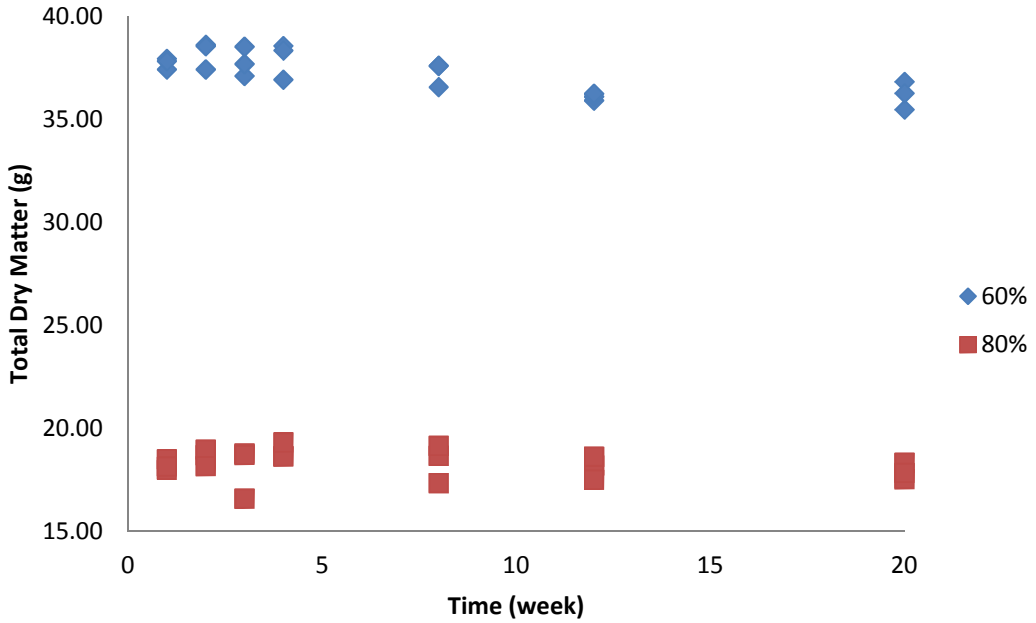

Figure 5.6 Total dry matter content over the 20 week trial for the mesocosms incubated at 30℃ with 60% and 80% moisture levels (n=42).

5.4.2.1.3 Total organic matter decay

Time was shown to have a significant effect on the mean organic matter content of the experiments ($p<0.05$). However, this was not observed when the mesocosms were assessed at each moisture level. An assessment of both moisture levels (60% and 80%) has indicated that no significant effect in relation to time is occurring ($p>0.05$). This is thought to be due to fluctuations in organic matter content throughout the 20 weeks of the experiment (see Figures 5.7 to 5.10).

It might be anticipated that the organic matter would follow a similar pattern as the dry matter, in terms of decay rates, though this has been shown to not be the case in this experiment. In contrast to the dry matter results, the above discussion has shown that the differing temperature regimes did not appear to effect organic matter content ($p>0.05$). These results suggest that moisture levels have no influence on organic matter decay, due to the similarities in evidence between the observations at 60% and 80% moisture contents. In addition, time did not appear to have any statistical effect upon the organic matter results when assessed against the different temperature levels ($p>0.05$).

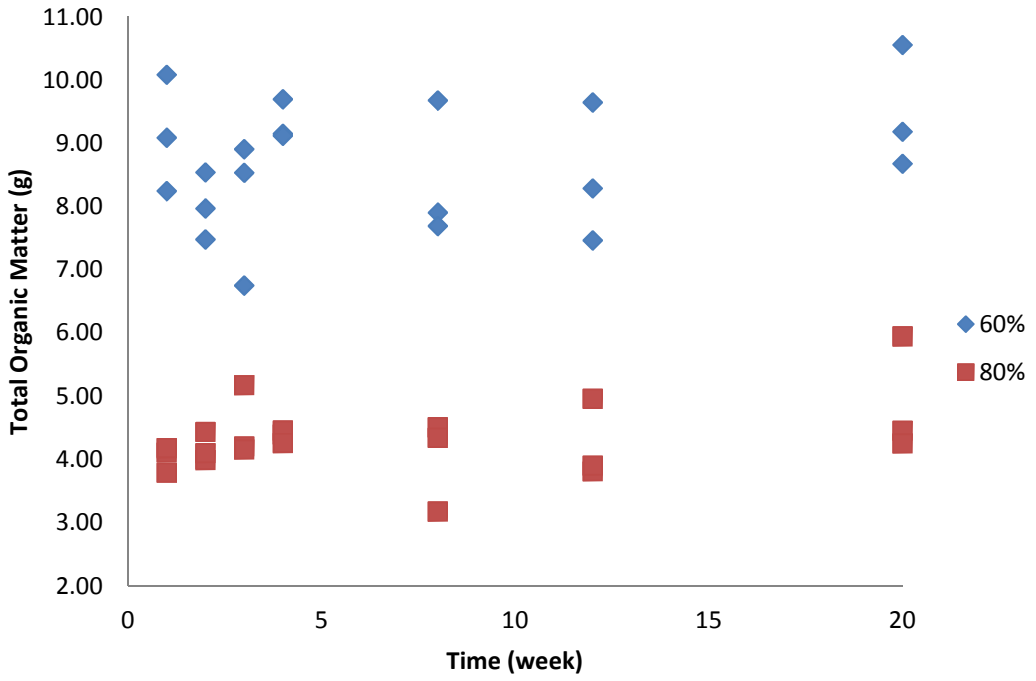

Figure 5.7 Total organic matter content over the 20 week trial for the mesocosms incubated at 5°C with 60% and 80% moisture levels (n=42).

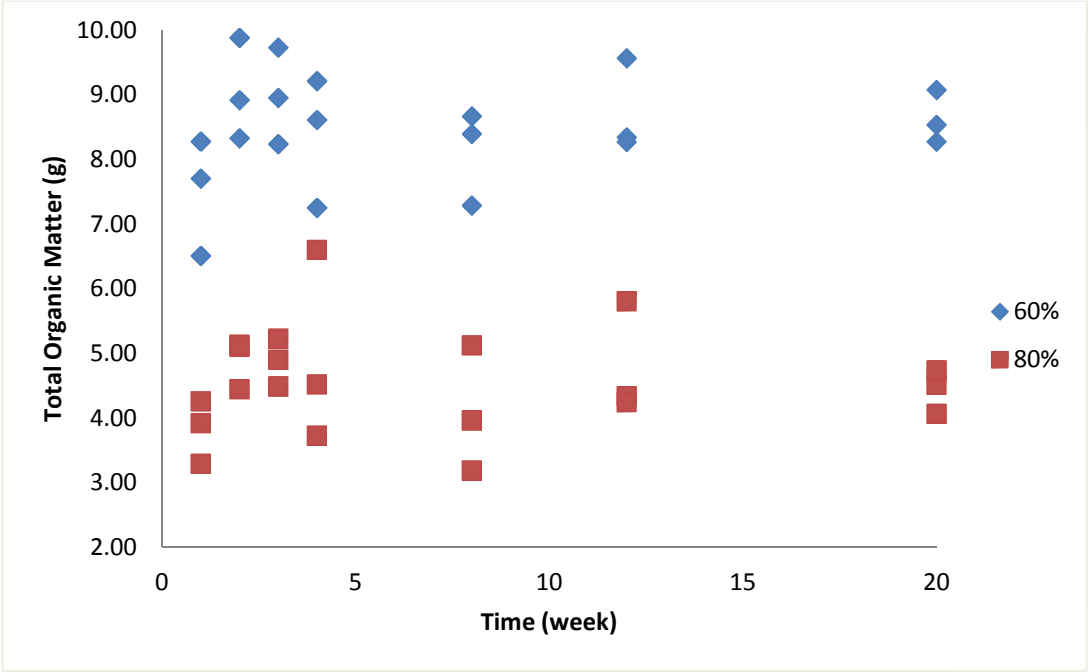

Figure 5.8 Total organic matter content over the 20 week trial for the mesocosms incubated at 16°C with 60% and 80% moisture levels (n=42).

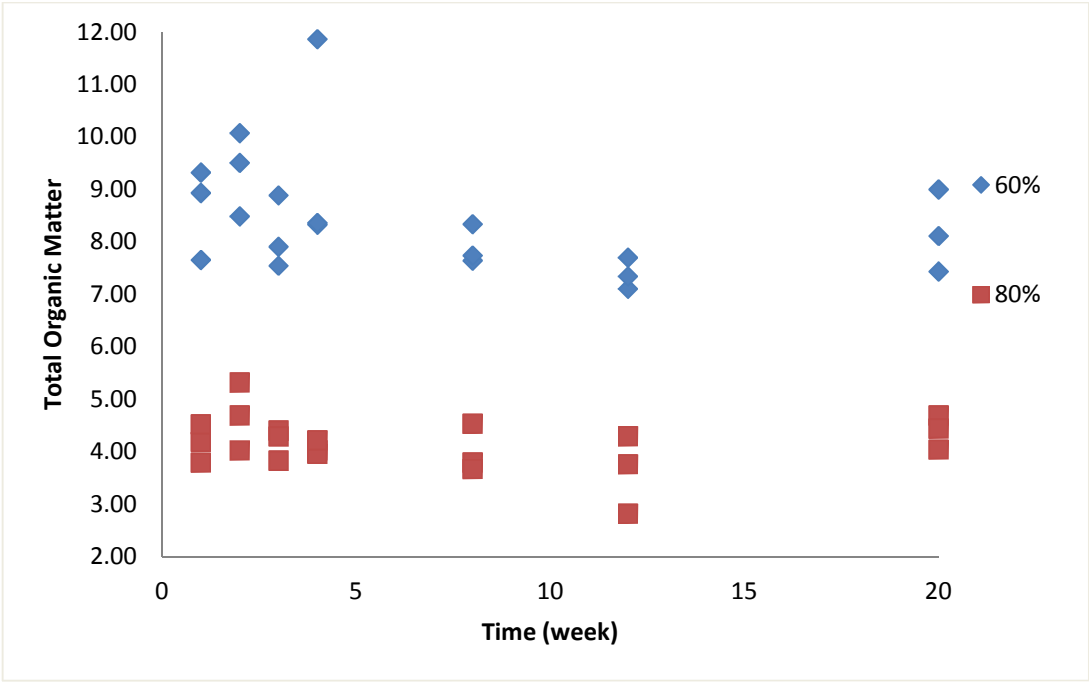

Figure 5.9 Total organic matter content over the 20 week trial for the mesocosms incubated at 25°C with 60% and 80% moisture levels (n=42).

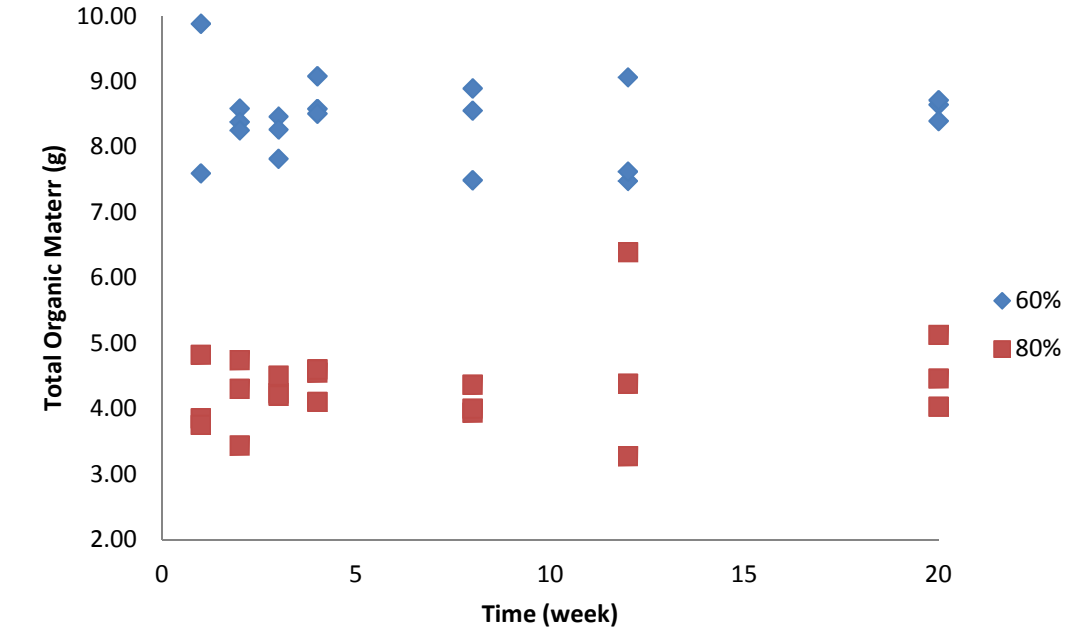

Figure 5.10 Total organic matter content over the 20 week trial for the mesocosms incubated at 30°C with 60% and 80% moisture levels (n=42).

5.4.2.1.5 Total ash content

When analysing the total ash content variability between samples was observed. However, when analysed, these variations were not statistically significant, showing no effect in relation to time ($p>0.05$), temperature ($p>0.05$) or moisture level ($p>0.05$) variability within the experiments.

5.4.2.1.5 Mould growth

Mould was initially observed growing at week two for both sets of moisture levels when incubated at 16°C, 25°C and 30°C. By contrast, mould growth was initially observed for both moisture levels at 5°C at week three. The growth remained very low at 5°C throughout the duration of the experiment when contrasted with those incubated at 30°C (where much heavier growth rates were observed), although this rate was not quantified (see Figure 5.11). The level of moisture did not appear to affect the rate of mould growth.

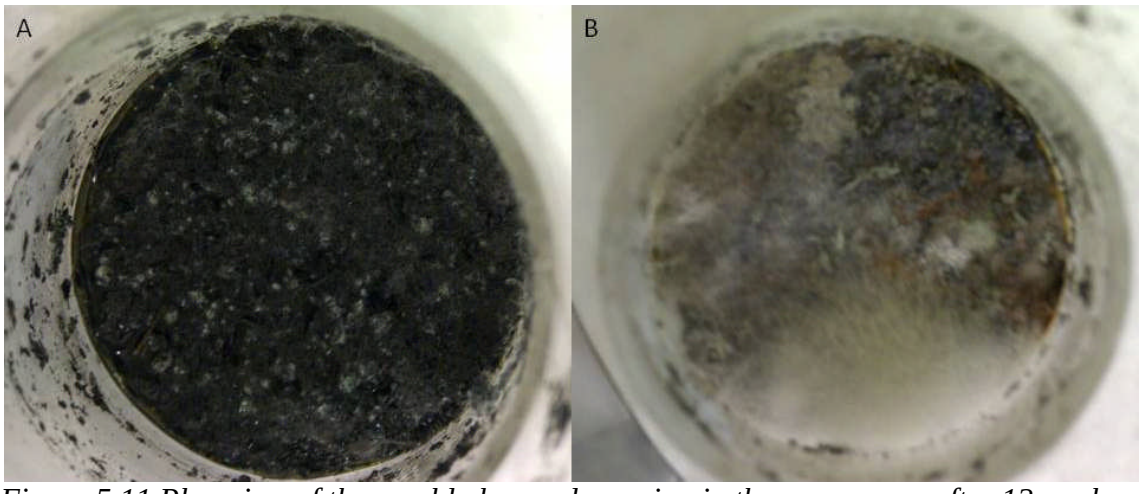

Figure 5.11 Plan view of the mould observed growing in the mesocosms after 12 weeks of incubation at (a) 5°C and (b) 30°C.

5.4.3. Biolog analysis

When the relative utilisation of the individual carbon substrates were analysed separately, it was apparent that time did not affect all of the substrates equally ($p>0.05$) as only 13 substrates, C1,H1, A2, D2, F2, G2, H2, A3, E3, G3, H3, A4 and B4, showed significant differences ($p<0.05$). As visible from Table 5.1 the substrates that did exhibit differences show very little trend in utilisation over time: C1, A2 and D2 decreased over time whilst the remaining substrates fluctuated, both increasing and decreasing. The colouration of each well on the Biolog EcoPlate^{TM} plate can also be used as an indicator to show how well the carbon substrates had been utilised. Visual assessment of the Biolog plate after incubation indicated that colouration decreased over time, which is indicative of the carbon substrates being better utilised in the earlier stages of the assay.

Furthermore, Table 5.1 indicates the carbon substrates that were able to be better utilised throughout the study. High utilisation, whether throughout or gradually, was observed in substrates C1, D1, G1, D2, E2, and F3.

Table 5.1 The mean relative utilisation of each carbon substrate over time (20 weeks) indicating those in bold that showed significant differences.

Carbon substrate ID	Time (weeks)						
	1	**2**	**3**	**4**	**8**	**12**	**20**
B1	0.89%	0.64%	0.87%	0.46%	0.35%	0.46%	0.60%
C1	**3.01%**	**2.70%**	**2.74%**	**2.17%**	**1.30%**	**1.24%**	**1.14%**
D1	2.49%	2.90%	3.41%	1.77%	1.44%	1.46%	1.59%
E1	0.95%	1.45%	1.21%	1.15%	1.64%	1.34%	1.20%
F1	1.50%	1.39%	1.36%	1.56%	1.77%	1.29%	1.03%
G1	3.23%	2.99%	2.48%	2.09%	2.71%	2.32%	2.30%
H1	**0.65%**	**0.99%**	**1.27%**	**1.01%**	**1.03%**	**0.49%**	**0.73%**
A2	**2.31%**	**2.19%**	**1.62%**	**0.74%**	**0.29%**	**0.12%**	**0.29%**
B2	1.43%	1.41%	1.80%	1.26%	1.72%	2.08%	2.00%
C2	0.56%	0.90%	1.18%	1.03%	1.43%	1.03%	0.53%
D2	**3.16%**	**3.39%**	**1.55%**	**1.78%**	**1.43%**	**1.30%**	**1.11%**
E2	4.33%	3.85%	3.37%	3.16%	2.81%	3.04%	2.82%
F2	**0.79%**	**1.29%**	**1.92%**	**1.82%**	**1.37%**	**1.47%**	**0.99%**
G2	**1.21%**	**1.84%**	**1.40%**	**1.43%**	**1.89%**	**1.31%**	**0.86%**
H2	**1.02%**	**1.71%**	**1.68%**	**1.59%**	**0.56%**	**0.44%**	**1.13%**
A3	**1.46%**	**1.25%**	**0.58%**	**0.36%**	**0.34%**	**0.27%**	**0.95%**
B3	1.93%	1.40%	1.37%	1.51%	1.41%	1.05%	1.32%
C3	0.27%	0.09%	0.45%	0.26%	0.23%	0.39%	0.24%
D3	1.69%	1.56%	1.76%	1.58%	1.33%	1.01%	1.06%
E3	**0.36%**	**1.17%**	**1.78%**	**1.10%**	**0.91%**	**1.17%**	**0.64%**
F3	2.05%	2.50%	2.30%	1.60%	2.97%	1.82%	2.17%
G3	**1.30%**	**1.91%**	**2.16%**	**1.47%**	**1.29%**	**1.23%**	**1.12%**
H3	**1.31%**	**1.92%**	**1.94%**	**1.43%**	**0.87%**	**0.41%**	**1.14%**
A4	**0.13%**	**0.20%**	**0.58%**	**0.48%**	**0.50%**	**0.25%**	**0.09%**
B4	**1.72%**	**1.57%**	**1.70%**	**0.73%**	**0.74%**	**0.45%**	**0.58%**
C4	0.41%	0.50%	0.70%	0.46%	0.38%	0.35%	0.16%
D4	1.14%	1.18%	1.32%	1.51%	1.73%	1.03%	0.91%
E4	0.67%	1.01%	1.49%	1.29%	1.54%	0.81%	0.98%
F4	1.15%	1.44%	2.44%	1.72%	1.76%	1.37%	1.13%
G4	0.66%	0.85%	1.30%	1.08%	1.42%	0.67%	0.87%
H4	1.03%	1.43%	1.42%	0.92%	1.41%	1.23%	1.17%

Obvious differences in microbial community functioning were observed between temperatures, where raised temperatures showed an increase in the relative utilisation observed in the carbon utilisation profiles. Statistical differences ($p<0.05$) were observed in the carbon substrate utilisation profiles of the microbial communities

between the four different temperatures for the majority of substrates when looking at the substrates individually. Temperature did not appear to affect the carbon substrate utilisation of F1, C2, A3, B3, C3, D3, F3, D4, F4 AND H4 ($p>0.05$). Increased temperatures (24°C or 30°C) are associated with an increase in the number of different carbon substrates that were able to be utilised. Better growth, indicated through higher relative utilisation, was also observed at these higher temperatures when compared to the lower temperatures, with 5°C showing lower relative utilisation. These differences were also observed in the control water well (A1) which indicated very low microbial utilisation at times, although as this is the control it is assumed there would be no utilisation. Figure 5.12 highlights the colour formation observed on a plate which had been incubated at 30°C, indicating the very pale colouration in cells A1, in contrast to the dark purple colour of the other cells.

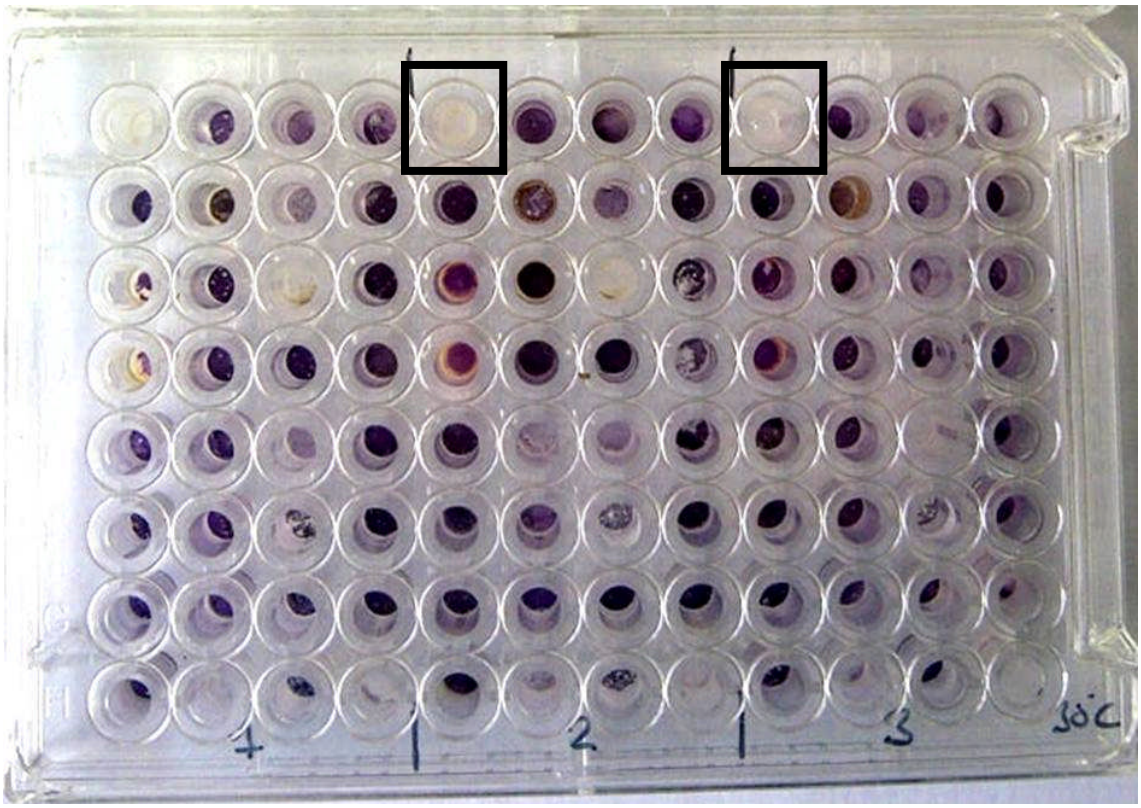

Figure 5.12 Biolog EcoPlate™ incubated at 30ºC with a 60% moisture level showing the formation of a purple colour which occurs when the microbes utilise the carbon source and begin to respire, the black squares highlight the colouration formation occurring in wells A1.

When hierarchical cluster analysis is undertaken on the samples analysed (Figure 5.13) it is immediately apparent that reduced branches of clustering occur as a result of the identification of larger groups containing similar carbon substrate utilisation patterns. These larger groups were observed to form as the temperature increased to 24°C and 30°C during incubation. There are obvious differences between the relative substrate utilisation levels when assessed over the four temperatures, which is due to a shift in the microbial community. The grouping observed in the higher temperatures indicates that more carbon substrates are able to be utilised as the microorganisms are work in a similar manner, regardless of the substrate.

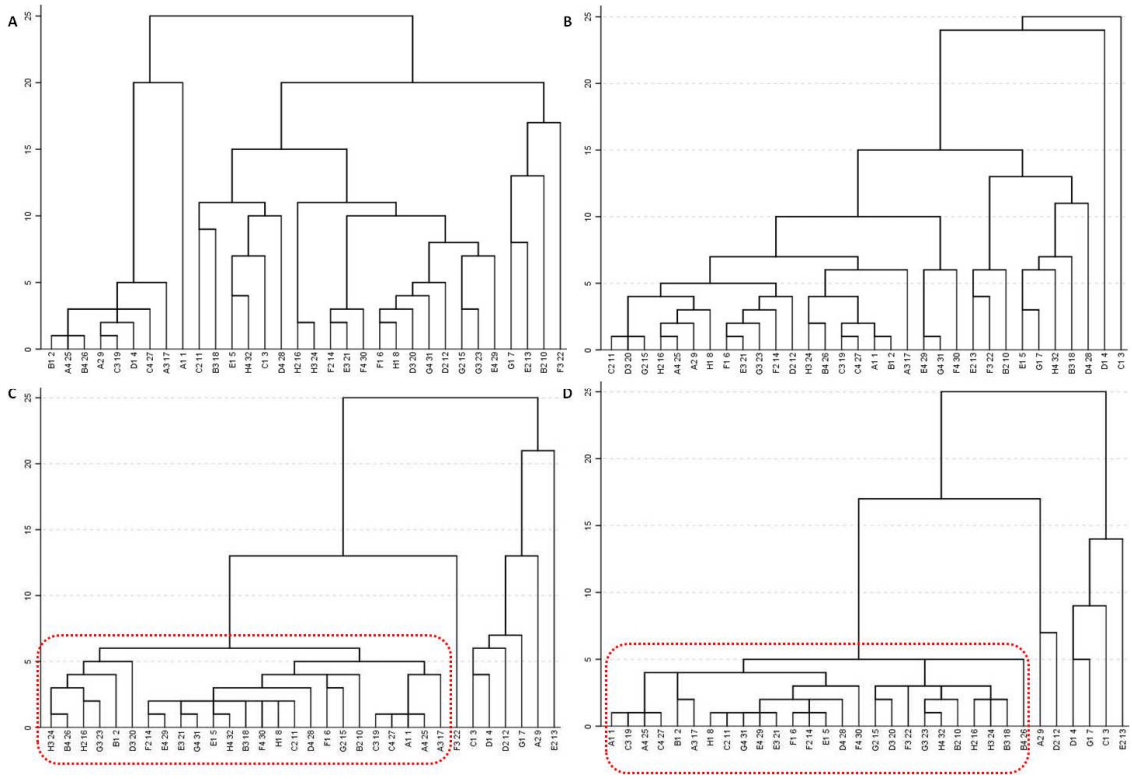

Figure 5.13 Hierarchical cluster analysis of the different carbon substrates for the in situ gully pot contents at (a) 5°C (b) 16°C (c) 24°C and (d) 30°C.

When observing the total relative utilisation of the plate, time appeared to have little effect on carbon utilisation when incubated at the four different temperatures ($p>0.05$). In addition, there were no differences observed when analysing the effect of moisture on the mesocosms incubated at the four different temperatures ($p>0.05$).

The different moisture levels had little to no effect on the microbial communities within the roadside gully pot waste. In terms of the relative utilisation of the individual carbon substrates analysed, the majority showed no significant differences ($p>0.05$) between slurries made up of 60% and 80% moisture levels. Only five of the 31 substrates, C1, G1, G2, C3 AND E4, show a significant difference ($p<0.05$) between the two moisture levels. Hierarchical cluster analysis reinforces the observed lack of difference between

the two moisture levels. In general, small group clustering was observed throughout both moisture regimes (see Figure 5.14), although the samples at 80% moisture appeared to have larger clusters toward the lower levels of the clusters highlighted by this analysis.

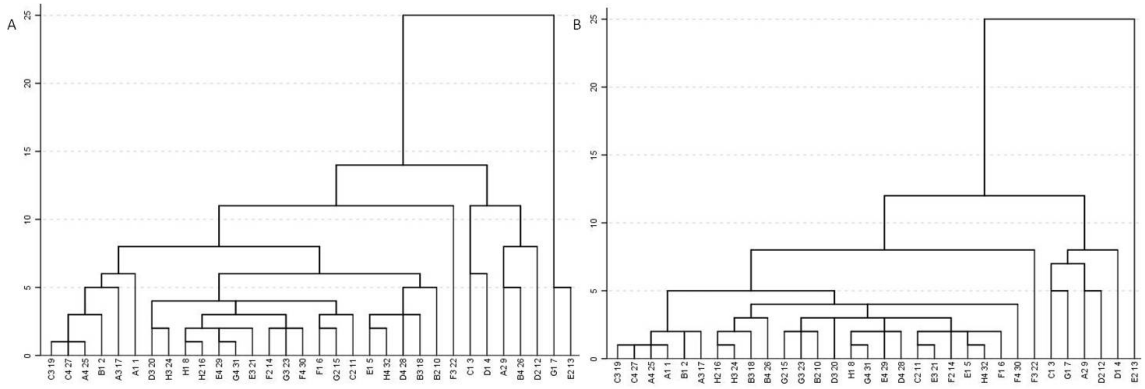

Figure 5.14 Hierarchical cluster analysis of the different carbon substrates for the in situ gully pot contents at (a) 60% moisture and (b) 80% moisture.

Time appeared to have little effect on the carbon utilisation potential of the microbial communities in the mesocosms prepared with 60% and 80% moisture ($p>0.05$). Furthermore, no differences were observed when analysing the effect of temperature on the mesocosms with 60% and 80% moisture levels ($p>0.05$).

5.5 Discussion

This chapter has assessed the potential of the gully pot waste to degrade under modelled conditions. Although no apparent impact from seasonal variations were observed in the study of gully pot environments at roadside locations in Hull (Chapter 4), the effect of moisture and temperature on degradation potential has been assessed as part of the laboratory experiment undertaken above.

5.5.1 Pilot *in situ* degradation trial

The preliminary five week *in situ* degradation experiment discussed above has indicated that the contents of the gully pots were able to decompose under a modelled laboratory environment, thereby providing a quantification of a previously unknown degradation potential and the processes involved in this. To date, *in situ* composting of gully pot waste has not been documented. As such, it was considered to be an important element of the current study to determine whether or not the waste was able to decompose in this manner.

Removal to landfill and incineration have been the most widely used means of solid waste disposal throughout the world, but in the 1980s interest in disposal methods which took recycling into consideration developed (de Bertoldi *et al.,*1983). Composting is a way of obtaining a stable product from biological oxidative transformation, similar to that which naturally occurs in the soil (de Bertoldi *et al.,* 1983). The use of urban wastes as organic amendments not only improves soil fertility and crop yield but also provides a useful way to dispose of wastes such as sewage sludge (Garcia *et al.,* 1995).

The recycling of gully waste mixed with street sweepings in an aerated environment has become a common disposal method, with local councils sending their waste off to external contractors; for example Hull city council are currently running a trial with Transwaste, whilst Tayside contractors working alongside Living Waters are assisting Dundee city council and surrounding areas. With the ever increasing landfill tax, this not only assists with cost saving but it also reduces damage to the environment (Duncan, 2003). The waste goes through a treatment process where the solid fraction is

separated and dried. The waste is then mixed with green waste to form compost; this part of the process is either performed on site, or the waste is transported to a different contractor. Composting has also been used as a successful strategy for the sustainable recycling of organic wastes and composting of similar municipal wastes such as sewage sludge; the processes of which have been widely documented e.g. Fermor, (1993); Ingelmo *et al.* (1998); Margesin *et al.* (2006); Tuomela *et al.* (2000).

This approach to waste management is also being investigated in developing countries (Taiwo, 2011). The high organic matter content of municipal solid waste and sewage sludge means that these elements are able to be employed for agricultural purposes as organic amendments (Cooperband, 2002; Garcia *et al.*, 1995; Singh *et al.*, 2011). However, the low organic matter content observed in the current study, and previous research stating that gully pot sediments are known to be deficient in organics (Clegg, *et al*., 1993), could be amongst the reasons why the waste is not employed in this manner. Nevertheless, the current pilot experiment has shown that the low organic waste component can be composted under conditions similar to those in which the waste would be subjected to in the field.

Although the gully pot waste was shown to decompose in the laboratory experiment, the actual rates of decomposition were low and sporadic in nature. Bardgett (2005) has stated that the rate at which soils decompose depends primarily on organic input quality, which is in turn dependent on the type of compounds that are present within them. The organic matter content of the gully waste was relatively low, being at least half that of the remaining inorganic material, which could be a factor to consider when evaluating the low decomposition rates observed.

By measuring total organic matter and dry weights, it was possible to monitor decay over time. Similar to mass loss, carbon dioxide (CO_2) release is regarded as a common indicator used to measure decomposition rates of litter (Song *et al*., 2010). Although there is a large amount of literature using CO_2 to assess decomposition (e.g. Chemidlin Prévost-Bouré et *al.*, 2010; Zeng *et al*., 2010; Zhu and Cheng, 2011) it was not the chosen method in the current study (see Section 3.5).

To attempt to understand organic content and its ability to degrade, it is important to understand the main input of the gully waste itself. The ease with which compounds degrade is determined by the complexity of the carbon compounds in the waste and generally follows the order: carbohydrates > hemicellulose > cellulose = chitin > lignin (Cooperband, 2002). From visual observations during preparation for the mesocosm experiment a relatively high amount of tree foliage appeared to be contained within the waste samples. This litter could have influenced the rate of decomposition in the experiment, depending on whether the litter was from deciduous or coniferous trees. The litter of deciduous trees are rapid decomposers as they contain high amounts of liable substances, such as amino acids and sugars, however, those of coniferous trees are slower decomposers as they are rich in large, complex structural compounds such as lignin (Bardgett, 2005). Salinas *et al.* (2010) also observed that leaf litter from certain species which are more resistant to decay had higher sensitivity to temperature.

As the mesocosms were replicated from observed gully pot environments (see Chapter 4) they were prepared with a 60% moisture level. The existing literature reports that a moisture content of 50 - 60% is suitable for efficient composting (Schulze, 1962; Tiquia

et al., 1998; Suler and Finstein, 1977). However, the slightly higher moisture level used in this study could have retarded the decomposition process. It has been shown that overall organic matter decomposition rates are slower in submerged soils (Sahrawat, 2003) as anaerobic conditions may be produced which will prevent and halt the ongoing composting activities (Liang *et al*., 2003; Schulze, 1962; Tiquia *et al*., 1996). Regardless of this observation, decomposition was observed in the current laboratory experiment, indicating that this type of waste is, in fact, able to decompose under these high moisture conditions.

The duration of the experiment was relatively short as it was designed to act as a pilot study aimed at indicating whether *in situ* decomposition was possible, prior to the long term study. Composting studies under similar conditions often run for longer periods of time, for example 122 days (Zhu and Cheng, 2011) and 9 months (Couto *et al*., 2010). However, five week composting studies using different wastes are not uncommon, and have a proven value (e.g. Adams and Umapathy, 2011), therefore enabling sufficient confidence in the viability of this assessment period in relation to the current study, and also supporting its efficacy as a preliminary trial.

The controlling variables that have generally been used in composting facilities, during the composting processes, include aeration, moistening and turning (Körner *et al*., 2003). However, as this was a preliminary short trial, it was deemed unnecessary to manipulate the variables at this stage. Furthermore, the trial samples were measured in mesocosms that were replicates of field conditions, and were therefore not aerated, as this was not recognised as an influencing parameter in the field. This is due to the

largely anaerobic environment found in a gully pot, apart from occasions when the contents may be aerated due to sufficient rainfall (Butler *et al.,* 1995).

The total ash content was not constant throughout the study (Figure 5.2), although at present the precise reasons for these fluctuations in the ash component of the waste remain to be established. This ash content represents a proportion of the inorganic material remaining in the waste, and as such it is assumed that this would remain constant throughout the study. As there is no trend in the losses of the inorganic fraction of the waste it is assumed that it is not a biological factor, but potentially a result of the sampling procedure, as described in Section 3.5.

Ultimately, the trial has demonstrated that the waste is able to degrade under *in situ* conditions similar to those that the waste would be subjected to in the field, thereby indicating the possibly of an *in situ* remediation potential for gully pots.

5.5.2 Modelled *in situ* decomposition monitoring

This experiment has allowed for an evaluation of both dry matter and organic matter decay over time, thereby investigating the effects of temperature and moisture on decay processes. In addition, the effect that these variables have had on microbial community functioning was analysed. The importance of these variables has been outlined previously e.g. He *et al.* (2010)

5.5.2.1 The effects of time

The monitoring of decay over time under laboratory conditions has been widely applied e.g. Adams *et al.* (2008); Guo and Sims (2001); Salamanca *et al.* (1998). However, in general, studies are usually undertaken in the field as replication of the numerous influences in a 'real world' scenario is difficult to achieve in the laboratory e.g. Ngao *et al.* (2012). In recent years external contractors have been able to produce compost from treated gully waste after approximately 20 weeks under aerobic conditions (*pers. comm*. Duncan, 2010). Examining untreated waste for this time period under *in situ* conditions has produced results which were previously unknown, such as if, and precisely how the waste is decomposed over this timescale. Such studies could assist with the comparison of the effects of *in situ* vs. *ex situ* influences on waste decomposition processes. Furthermore, the length of the current study was restricted to a 20 week period due to the gully pot cleaning regime stipulated by Hull City Council. Gully pots are generally cleaned out on an annual basis; however, those located on busy roads, or in the town centre, are cleaned out on a bi-yearly basis. As such, running the trial for 20 weeks was deemed sufficient to assess the *in situ* decomposition in the field.

5.5.3 The effects of temperature

5.5.3.1 Physical parameter analysis

The influence of temperature on mesocosms was monitored to investigate its effect on the physical parameters of the waste, and on microbial community functioning. Temperature appeared to have a more significant effect on the parameters studied than moisture. The range of temperatures observed in the field (see Chapter 4) directed the choice of temperature regimes to be monitored within this part of the study, thereby

allowing the complete range of external temperatures to be considered. This method has been employed in previous studies investigating the seasonal effects of municipal solid waste (e.g.Cecchi *et al*., 1992). A high temperature of 30°C was also included to model the potential impacts of future temperature increases on decomposition processes. Increases in temperature due to global warming (though not up to 30°C), have been hypothesised as having the potential to lead to increased litter decomposition rates if there is sufficient soil moisture (Aerts, 2006); therefore both of these parameters were studied.

pH remained unaffected by temperature throughout the study, having a mean circumneutral pH for the duration of the study. Similar conditions have previously been observed during the decomposition of sewage sludge, where pH maintained equilibrium at pH 7, independent of variations in the pH of the ingoing material (Schulze, 1962). However, this was during thermophilic conditions, which have much higher temperature conditions than were assessed during the current study.

Temperature had a positive effect on overall rates of dry matter decay, displaying significant losses through incubation in the higher temperature ranges used in the current study, especially when compared to the lower temperature ranges. This temperature effect did not alter when analysing the mesocosms incubated at the two different moisture levels used in the current study. The temperature influences identified during the current study reflect those identified in previous studies, as it is generally reported that temperature is an important driver in decomposition processes (e.g. Bardgett, 2005; Liang *et al*., 2003; Salinas *et al*., 2010; Ibrahim *et al*., 2010). However, variations on this general theme exist as He *et al.* (2010) observed that

temperature increase does not lead to a significant difference in mass loss of leaf litter. Prior to this study Adams *et al*. (2008) indicated that temperature was not critical in determining the rate of composting in green waste. The results of the current study agree with these earlier studies in that an increase in mass loss over time was apparent even in the lowest temperature range of 5°C. Whilst the losses at 5°C were not significant, they were observed. Lower temperatures have been observed to limit the decomposition of organic-material accumulation in soil (Douterelo *et al*., 2009), but as the current study emphasises, along with the work of Taylor and Jones (1990) decomposition still occurs at low temperatures. We have no data on lignin content, which has shown to be a good predictor of some leaf litter decomposition rates (Salinas *et al.*, 2010; Wieder *et al*., 2009), and this is unfortunate as this could be used to strengthen the argument that decomposition has occurred.

These effect of temperature observed in the dry matter results were, however, not apparent in the organic matter decay, where temperature had no overall effect on the organic matter content. All of the temperature regimes displayed fluctuating values in organic matter content, which varied somewhat throughout the experiment. These temperature results were unexpected, especially when assessing the ash contents, which also fluctuated over time in some of the samples. The fluctuating organic matter and ash content, corresponds with the loss of dry matter, however, it is unclear to why the fluctuating levels occurred. This may be due to some form of unknown elemental transformation or loss which is driving these unexpected results, a sampling bias due to the fine silt and sieving technique (as mentioned in Section 3.5). The levels of ash loss, however, are not great in terms of mass weight and these differences are not significant (ANOVA analysis). Future long term exploration will be needed to assess this factor.

Fungus growth was observed initially at week two for the mesocosms incubated at 16°C, 25°C and 30°C. It took a further week to observe fungus growth in the mesocosms incubated at 5°C, starting growth at week three. Growth in the higher temperature ranges appeared to be heavier and thicker than in those incubated at 5°C (as can be seen in Figure 5.11). This visible fungus growth has not previously been reported in gully pots, nor was it observed in the monitoring exercise (Chapter 4), which could be due to the conditions of the exercise. However, previous studies had identified a thin layer of 'scum' developing on the surface of the liquor of gully pots kept at 20°C (Memon and Butler 2002a). Memon and Butler (2002a) speculated that the scum development may have been attributed to the excessive growth of certain filamentous micro-organisms of the genus *Nocardia*, which entrain gas bubbles and become buoyed to the top liquor surface. This film was not observed in the current experiment. It is speculated that this is a mould belonging to the fungus family, however, without assessing the spores it would be erroneous to elaborate on this further here.

5.5.3.2 Biolog analysis

Besides climate, the microbial community itself is also one of the main factors controlling litter decomposition (He *et al*., 2010). The Biolog EcoPlate™ works on the principle that carbon is a key nutritional requirement for microbial growth and function, and that growth of micro-organisms related to specific organic carbon substrates can be used to assess the metabolic capabilities of micro-organisms (Biggs *et al.*, 2011). By understanding the ecological controls of microbial community composition, and the role of the microbial groups in the decomposition of different organic compounds it may be

possible to gain a more mechanistic view of the decomposition process (Brant *et al.*, 2006).

Individual assessment of the carbon substrates indicated that higher temperatures exerted a more significant influence on growth. The higher temperature inoculations (at 24°C or 30°C) highlighted an increase in the number of different carbon substrates that were able to be utilised. Response to temperature is dependent on the communities' habitual temperature regimes, including the magnitude of temperature changes (Balser and Wixon, 2009). This study agrees with previous work where patterns of carbon utilisation changed with temperature (e.g. Biggs *et al.*, 2011; Lipson, 2007). The lower temperatures, especially 5°C, displayed lower relative utilisation when compared to the higher values, suggesting that the microbial community is unable to utilise the substrate efficiently in low temperature conditions (Douterelo *et al.* 2009). This reduction in, or absence of utilisation has also been observed in a significant amount of carbon substrates in sewer sediments when incubated at 4°C (Biggs *et al.*, 2011). Furthermore the low microbial degradation activities due to low temperatures can result in composting being impractical (Margesin *et al*., 2006).

Time appeared to affect the microbial community as visible differences were observed in the colouration of the EcoPlate^{TM} over time. A darker purple colour was observed at the beginning of the 20 week period, which indicated utilisation, and this was seen to reduce over time. These differences were also observed in the water well (A1) which occasionally indicated very low utilisation. As this is the control it is assumed there would be no utilisation and could just be caused by background noise.

Reduced branches of clustering, visible in Figure 5.10, can be seen to be causing larger groups containing the same carbon substrates, as the temperature increased to 24°C and 30°C. This clustering indicates that the substrates are able to be utilised in a homogenous manner, showing that due to the increase in temperature the microbial community are able to utilise more carbon sources more effectively.

Substrates C1, D1, G1, E2 and F3 exhibit mean high utilisation when incubated at all temperatures, and over time they either had a progressive rise or they were constantly higher than the others. These consisted of carbohydrates, polymers and carboxylic acid substrates. These substrates may be easily utilised by adaptable microbes, allowing the substrate to be used over all temperatures. When soils are maintained at a constant temperature (e.g. in lab incubation studies), genetically better adapted soil microbes may progressively outcompete other less well-adapted microbes at each temperature level (Zhu and Cheng, 2011). Over time, the constant temperature regime may selectively favour microbes already genetically better adapted to the temperature level (Zhu and Cheng, 2011). Whilst the carbon sources in these plates may not represent the dominant carbon sources found *in situ*, oxidisation of these substrates may serve as a proxy for understanding utilisation patterns under various environmental conditions (Biggs *et al.*, 2011) found in gully pot waste.

The Biolog EcoPlate^{TM} is a robust and easy way to provide a fingerprint-type insight into microbial activity (Biggs *et al.*, 2011). The plates have been compared to other methods, such as phospholipid fatty acid analysis, for monitoring community and ecological changes and have been found to be more sensitive to changes in the environment and major determinants such as temperature and water (Biolog, 2000).

5.5.4 The effects of moisture

5.5.4.1 Physical parameter analysis

The moisture effects on mesocosms were monitored to investigate its effect on the physical parameters and on the microbial community. Moisture did not appear to have a major effect on the process occurring within the gully pot waste. As moisture levels observed in the field (Section 4.0) indicated a wide range of moisture levels in the waste, averaging at 65%, with the lower end of the moisture level range determined to be 60%, which has been widely cited to be a suitable level for efficient composting (Schulze, 1962; Tiquia *et al.*, 1998; Suler and Finstein, 1977). In the field the gully pots are constantly filled with water so an upper level of 80% moisture was decided on this premise.

Moisture levels appeared to have no effect on the on the overall dry matter loss and did not impact upon the process when evaluating the effect on different temperatures and over time. In addition the organic matter was not affected by the different moisture levels, and it was apparent over the course of the study that organic matter content varied with time and decay rate. The total organic matter levels were lower in the 80% moisture mesocosm as opposed to those with 60% moisture. This was to be expected though, as the overall waste going into the mesocosm was lower, therefore this is not a result from a biological aspect, purely a sampling one.

These results agree with previous research, which has suggested that moisture content is not as important as temperature in driving decomposition processes (e.g. Jurgensen *et al.*, 2006; Van Cleve and Sprague, 1971). By contrast however, it has been suggested

that moisture has a more important impact on compost activity than previously thought (Liang *et al.*, 2003). Low moisture content has been shown to be a more limiting factor for composting than low temperature for sewage sludge as it is an important parameter influencing biological activity and biochemical rates (Margesin *et al*., 2006). Decomposition is slow in saturated soils because anaerobic conditions develop. Due to this it was anticipated that the gully waste would have a quicker decomposition rate when incubated at 60% when compared to 80% moisture contents. However, this was not the case as moisture played no effect on the rate of decomposition. A significant difference may have been observed if the lower moisture levels had been examined, but this lower rate was deemed unnecessary due to the mean moisture levels observed in the monitoring exercise (Chapter 4) and the pots being constantly full of water.

The different moisture levels had no effect on the growth of mould over the 20 week incubation period. As mentioned above, when assessing the effects of temperature the mould increased over time, however, there was no difference observed between the two moisture levels.

5.5.4.2 Biolog analysis

Different moisture levels did not affect the microbial communities of roadside gully pot waste as only five substrates showed significant differences between the two moisture levels. Moisture had no effect on the utilisation of the substrates over time either. Contradictory to these findings, previous research has shown soil moisture to be a major control on microbial community structure in a variety of environments e.g. forest floor (Wagener and Schimel, 1998) and waterlogged soil (Douterelo *et al.,* 2010). The

reasons for this not occurring within the gully pot waste could be due to the levels of moisture used in the current study, as previously explained.

Soil pH affects the availability of nutrients, and therefore influences the composition and diversity of the microbial community (Douterelo *et al.,* 2010). The pH remained around neutral, with the highest reading being a low alkaline pH, so it is understood it would not impede the microbial community. Had the pH been acidic (Corfield, 1996) or extremely alkaline the decomposition would have been slower as the microbial activity is reduced.

5.6 Conclusion

This study demonstrates that temperature variations have a significant effect on the carbon utilisation profile of microbial communities within gully pot waste *in situ*. However, when examining moisture levels this was clearly not the case, as no differences were observed throughout the trial. Examining these variables has provided a better understanding of the processes occurring *in situ* and provided insights into the variability in response to fluctuations in environmental factors, indicating that temperature affected the overall activity of the waste. Furthermore the trial has shown that the waste is able to decompose, to a degree, under conditions similar to those that it would be subjected to in the field, therefore indicating the possibly of an *in situ* remediation for gully pots. Understanding the effect of temperature and moisture on the microbial community of gully pot waste is important when considering future research investigating more sustainable methods for managing urban drainage. The next stage of this analysis will further investigate the effects of specific substrates on these patterns of decomposition.

6.0 Assessing the effects of substrate additives on *in situ* degradation processes and microbial community function

6.1 Introduction

Enhancing degradation is becoming an increasingly popular method for managing waste matter, as longer term sustainable approaches are sought. This, and the popularity of composting, has lead to a high market demand for composters and compost related material; such as bulking materials and compost accelerators. These are intended to enhance microbial activity, improve the composting process and also the quality of the compost (Himanen and Hänninen, 2009). Compost additives are typically composed of a mixture of differing amounts of various microorganisms, mineral nutrients or readily available forms of carbon, enzymes, and pH balancing compounds (Himanen and Hänninen 2009).

Soil microbial activity is highly influenced by the carbon substrates that are present in the soil (Jonasson *et al.,* 1996). As such, the results obtained from the laboratory experiment undertaken in Chapter 5 can be used to provide insights into the potential range of carbon substrate additives that may be required in order to enhance *in situ* degradation processes. The Biolog EcoPlates^{TM} used in the previous study (Chapter 5) indicated that there were three carbon substrates (glucose, itaconic acid and Tween 80) that promoted elevated utilisation and growth in the gully pot waste under *in situ* laboratory conditions. Glucose is a simple carbohydrate with neutral chemical groups, and is a compound commonly found in soils (Rukshana *et al.,* 2010). This particular carbon substrate has been widely used in the priming of soils in order to enhance degradation (e.g. Shen and Bartha, 1996). Itaconic acid is excreted by fungi and used

exclusively in non-food applications. Its primary application is in the polymer industry where it is employed as a co-monomer at a level of 1-5% for certain products (Magnuson and Laure, 2004). Tween 80 (Polysorbate 80) is a soft non-ionic surfactant and emulsifier derived from sorbitol, which is obtained from various types of fruits, and it is widely used as an additive in the production of enzymes (Shi *et al.*, 2006). There is no literature on the use of itaconic acid as a soils amendment or as an accelerator; however, the high utilisation observed in the *in situ* experiment (Chapter 5) was of some interest, suggesting that if added to waste this substrate may also have the potential to enhance biodegradation processes.

6.2 Aims

The aim of the current chapter is to investigate the effects of carbon substrate additives on roadside gully waste using model gully pots constructed in the laboratory. A series of mesocosms were inoculated separately with one of the three substrate additives outlined above (i.e. glucose, itaconic acid or Tween 80), at three different temperatures to assess whether these substrates influence microbial community functioning and as a consequence enhance gully pot waste decomposition rates.

6.3 Method

6.3.1 Sample collection

The samples were collected and stored as outlined previously (Section 3.1), and prepared for the trial which started the day after collection. As previously described (Section 5.3.1) the waste was treated as a composite sample, and as such a variety of

contents were collected from gully pots from the four different urban areas that were used in the current study.

6.3.2 Experimental setup

In total, 144 small scale model gully pots (mesocosms) were set up in the laboratory (as described in Section 3.2.2.4), to measure the effects of the three different substrate additions (glucose, itaconic acid and Tween 80; all of which were obtained from the Sigma-Aldrich chemical company) on the degradation rate in different temperature environments (5°C, 16°C, and 25°C) over an 8 week period. Each mesocosm was measured, as described below, in triplicate for accuracy, and all temperatures were maintained to +/- 1°C during the incubation period. The mesocosms were prepared in four batches of 48, with the first batch being prepared without an additive; thereby acting as a control. All of the mesocosms in the second batch contained 5g of α-D-glucose, which was added to a 250ml conical flask of known weight with 70ml of distilled sterile water. The solution was mixed thoroughly before adding 30g of dried sieved gully waste then re-mixed until a homogenous slurry had been created. The third batch contained 5g of itaconic acid, and the fourth contained Tween 80 which was prepared in the same way. The concentration of the surfactant Tween 80 was above its critical micelle concentration (CMC) which was calculated to water at 20-25ºC (Hait and Moulik, 2001). An increased CMC was measured to allow for any fluctuation caused by temperature decrease or issues caused by the addition of waste to the water.

Tin foil was placed on the top of all of the conical flasks and the final weights of the mesocosms were recorded before incubation. From each batch, 12 mesocosms were incubated at 5°C, 16°C and 25°C. Over an 8 week period, three mesocosms from each

additive batch (including the control) and temperature regime were randomly selected and sampled to destruction. The first sampling period occurred after 7 days, and sampling was undertaken at 14 days, then in the fourth week on day 28, with the final sampling date occurring at week 8.

6.3.3 Physical parameter analysis

During sampling, the total weight (including flask) of the mesocosms was recorded to determine any loss which may have occurred during incubation. Subsequently, 1g of waste material was removed for slurry preparation for use in the microbial community assay. The pH (as described in Section 3.2.2.1), moisture content (Section 3.2.2.2), and subsequently total dry and organic matter content (Section 3.2.2.3) were measured using the remaining material from the mesocosms.

6.3.4 Microbial community analysis

Sampling for the microbial community analysis occurred on the same day as the general analysis outlined above, and followed the procedure outlined in Section 3.3.2.1 using Biolog EcoPlates^{TM}. Three samples from one batch and temperature regime were inoculated on one Biolog EcoPlate^{TM} (e.g. Tween 80 incubated at 5°C). This was repeated for the remaining three batches under each of the three different temperature regimes. The plates were then incubated for 24 hours at the same temperature as the incubation of the initial sample. The absorbance of each well was read on a MicroPlate reader (FLUOstar OPTIMA, BMG LABTECH Ltd., UK) at 590nm.

6.3.5 Statistical analysis

The data was tested for normality using a Kolmogorov-Smirnov test. If the data was normally distributed, an ANOVA model was used to test the effect of each batch and incubation setting for each mesocosm; this was followed by a LSD test. For non-parametric data Kruskal-Wallis tests were performed followed by Mann-Whitney-U tests. All tests were performed using PASW statistics, version 18. Hierarchical cluster analysis was used to look for similarities and differences in substrate utilisation profiles across the different batches and temperatures.

6.4 Results

6.4.1 Physical parameter analysis

6.4.1.1 pH

A highly statistical difference was observed ($p<0.001$) when analysing the overall pH between the four batches (control with no substrate addition, glucose addition, itaconic acid addition and Tween 80 addition). The mean pH for the control and Tween 80 batches remained near neutral throughout, being 7.41 and 7.25 respectively. By contrast, both the glucose and itaconic acid batches had slightly acidic mean pH's of 6.40 and 6.12 respectively (see Figure 6.1) with both having lower starting pH values. Temperature did not have any significant effect upon the pH of each batch examined ($p>0.05$), and neither did time ($p>0.05$).

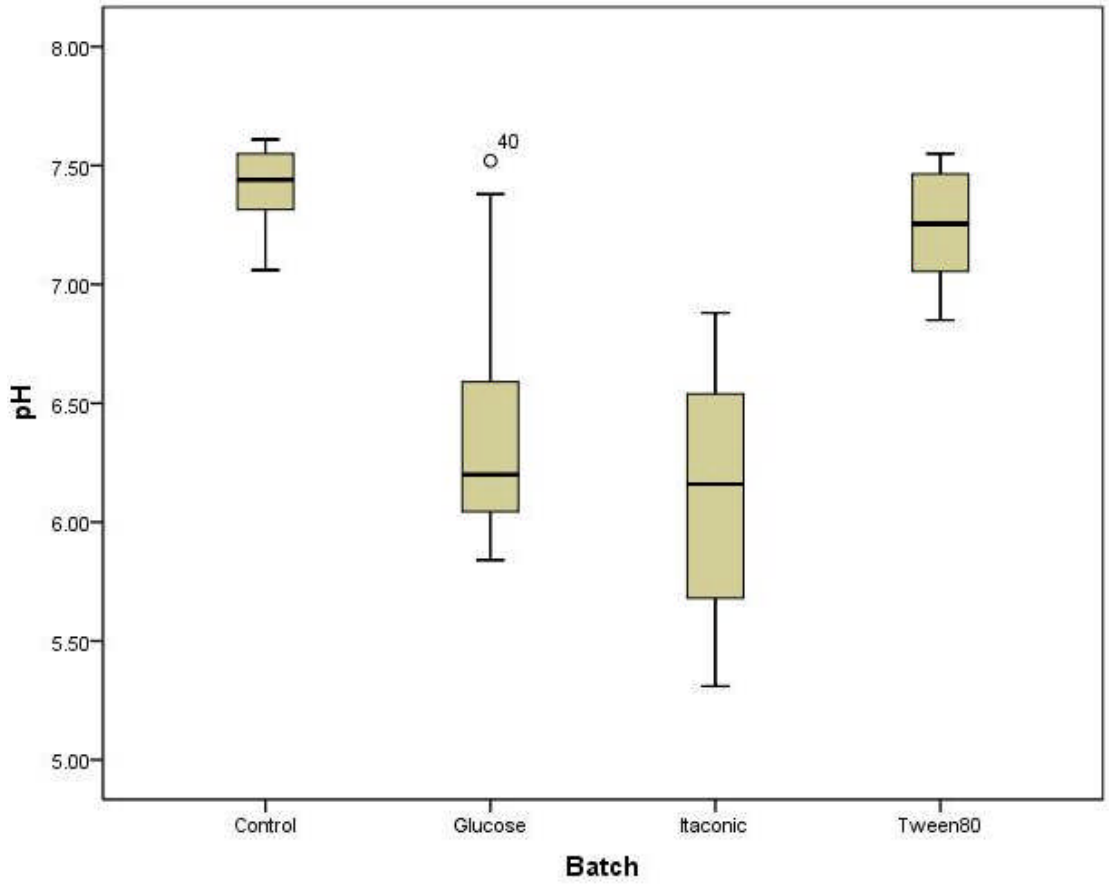

Figure 6.1 Box plot for the overall pH values for the four mesocosms batches (showing the range, interquartile range, and the mean), anomaly in the data indicated as 40 within the graph (n=144).

6.4.1.2 Moisture content

When assessing the effect of temperature ($p>0.05$) or time ($p>0.05$), no significant statistical difference to the moisture levels of all of the batches analysed were observed. Since the moisture levels did not drop excessively throughout the experiment, there was no need for these to be topped up over the duration of the study.

6.4.1.3 Total dry matter

Temperature, time and batch type appeared to have highly statistically significant effect on the decay of dry matter ($p<0.001$). In particular temperature, which significantly affected the overall decay of dry matter ($p<0.001$) with higher losses over time occurring at the higher temperature of 25°C. The mesocosms incubated at 5ºC had a mean loss of 2.01g over the eight weeks, which was similar to that of those incubated at 16ºC where a mean loss of 1.96g was observed. The mesocosms that were incubated at 25ºC had a mean loss of 2.70g. Time had a highly significant effect on the overall dry matter content ($p<0.001$), and decreases were observed throughout the eight weeks of the experiment. Furthermore, these significant effects were observed between batch ($p<0.05$) over time, with the final sampling point having a lower total dry matter value when compared to the first sampling values.

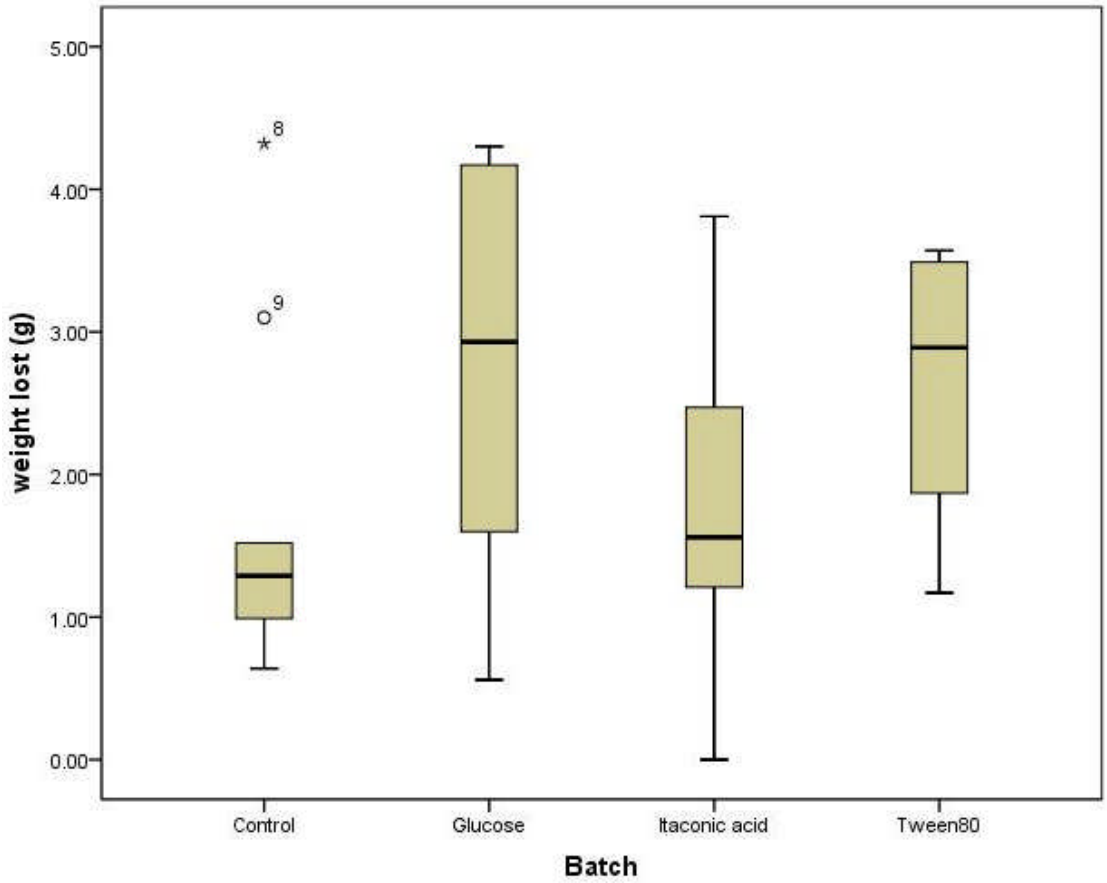

Figure 6.2 Box plot for the amount of total dry matter lost between the first and last sampling point for the four batches (showing the range, interquartile range, and the mean) anomalies indicated as 8 and 9 within the graph (n=144).

The batch type significantly affected the overall dry matter decay ($p<0.001$), where the control showed highly significant differences to the glucose batch ($p<0.001$), and also to the Tween 80 batch ($p<0.001$). Statistical differences were also observed between the glucose and Tween 80 batches ($p<0.001$), and between the itaconic acid and Tween 80 batches ($p<0.001$), with the glucose batches having lower overall levels of dry matter. The itaconic batch had similar overall dry matter losses to the control batch, both of which had lower levels of loss when compared to the other two batches (see Figure 6.2). However, this difference in loss was not statistically significant ($p>0.05$). Furthermore highly significant effects were observed between batch type and temperature ($p<0.05$). The control indicated greater losses occurring at the highest temperature monitored; as

did the itaconic batches. The glucose batches displayed greater losses at 16ºC, whereas Tween 80 had the greatest loss at the lowest temperature of 5ºC. The glucose batches that were incubated at 16ºC exhibited the greatest dry matter losses throughout the experiment.

6.4.1.4 Total organic content

When analysing the overall organic matter content highly significant statistical differences were observed between temperature, time and batches ($p<0.001$).

Temperature significantly affected the overall decay of organic matter in the samples ($p<0.001$). Greater losses over time were observed at the higher temperature of 25°C when compared to the lower temperatures (i.e. 5°C and 16°C). Further analysis indicated that there were also highly significant differences between the samples incubated at 5°C and 25°C ($p<0.001$) as well as between those at 16°C and 25°C ($p<0.001$), but there were no significant differences between 5°C and 16°C ($p>0.05$).

Time had a highly significant effect on the overall total organic content of the samples ($p<0.001$). Decreases were observed throughout the eight week study, with statistically significant differences occurring between each week of the experiment ($p<0.05$). These highly statistically significant effects were also observed between batch and time ($p<0.001$), where the final sampling point had a lower total organic matter value when compared to the initial sampling point.

The batch type significantly affected the overall organic matter value ($p<0.001$), indicating that the control was significantly different to the glucose batch ($p<0.001$),

itaconic acid batch ($p<0.001$) and also the Tween 80 batch ($p<0.001$). Statistical differences were also observed between the glucose and Tween 80 batches ($p<0.001$) and the itaconic acid and Tween 80 batches ($p<0.001$), with the glucose batches having lower overall levels of total organic matter content. The itaconic acid batch had the lowest level of loss throughout the experiment, at 0.87g per 105g, with the control losing 1.71g per 100g. The glucose batch had the highest level of loss at 3.03g per 105g.

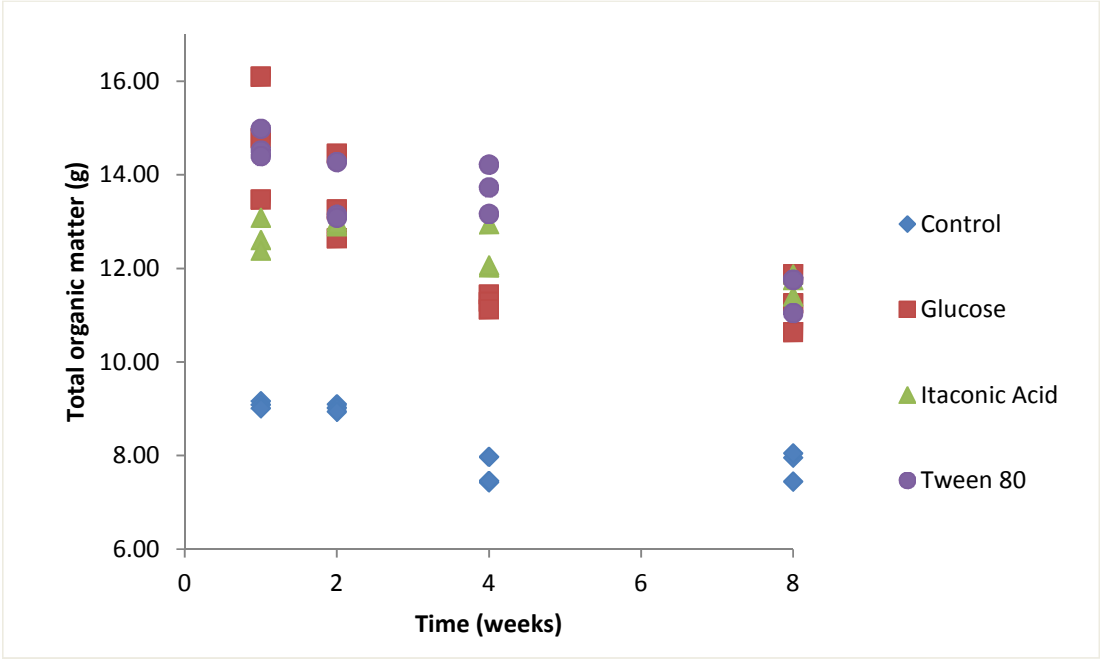

Figure 6.3 Change in total organic weight for the control, glucose, itaconic acid and Tween 80 batches incubated at 5ºC (n=48).

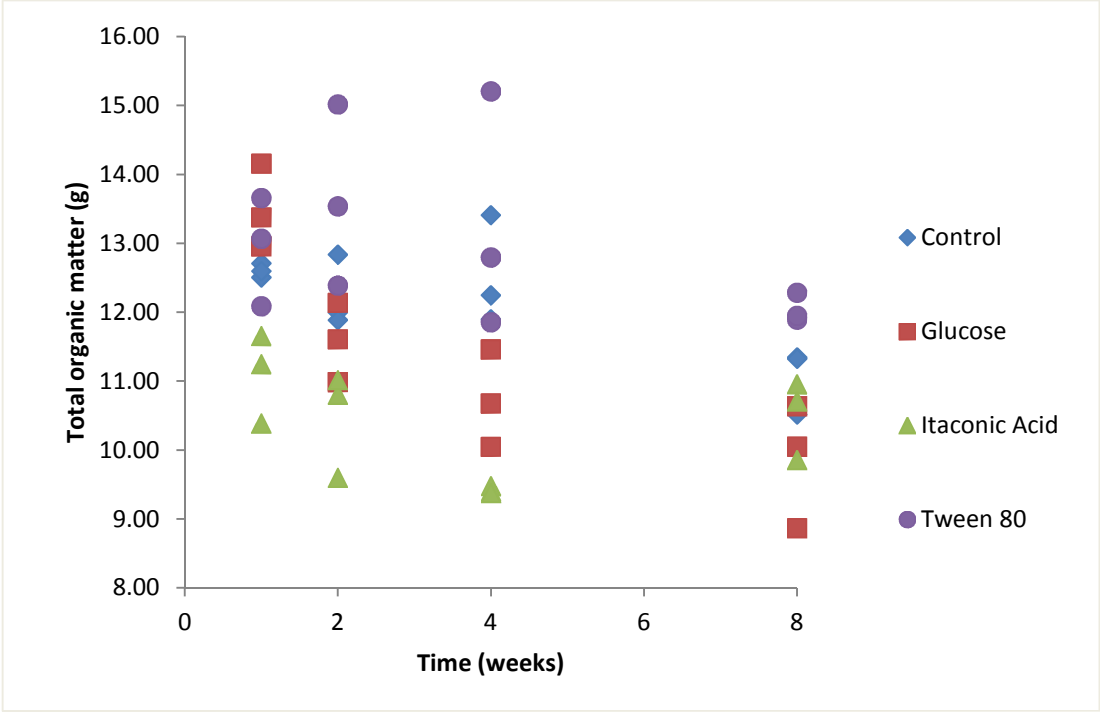

Figure 6.4 Change in total organic weight for the control, glucose, itaconic acid and Tween 80 batches incubated at 16ºC (n=48).

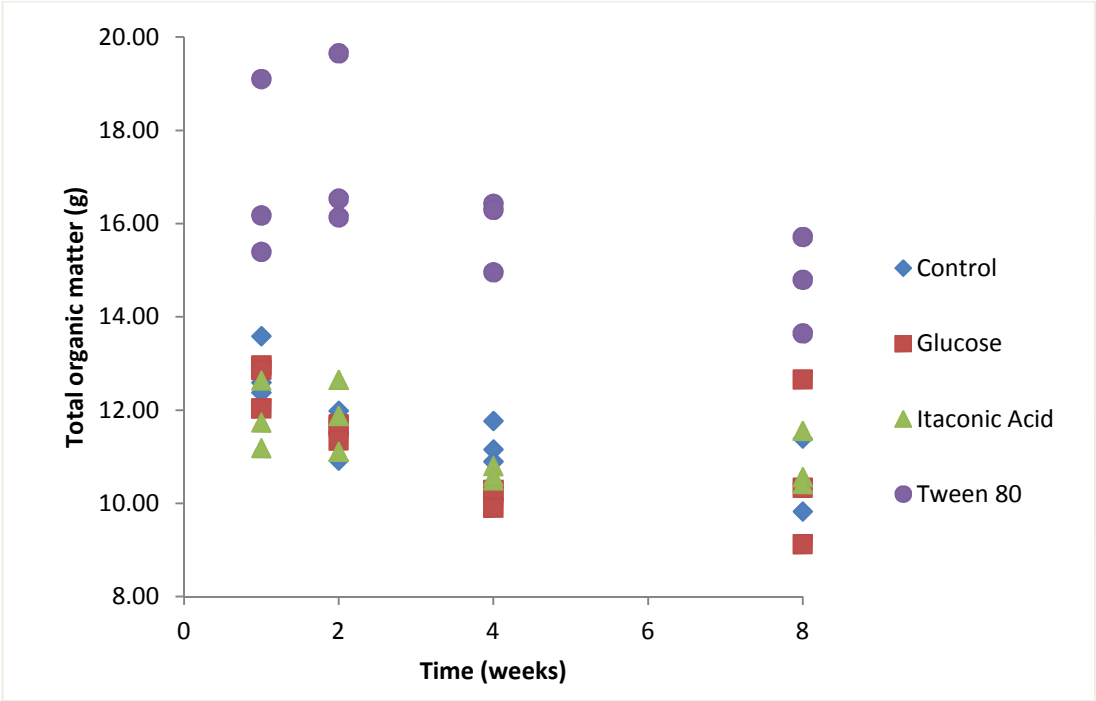

Figure 6.5 Change in total organic weight for the control, glucose, itaconic acid and Tween 80 batches incubated at 25ºC (n=48).

The rate at which each batch decomposed was highly statistically significant when correlated with temperature ($p<0.001$). The control exhibited greater losses at the highest temperature; however, the remaining three batches did not mirror this pattern (see Figure 6.5). The glucose batches displayed greater losses at 16ºC (see Figure 6.4); whilst the itaconic acid and Tween 80 batches both exhibited the mean greatest loss at the lowest temperature of 5ºC (see Figure 6.3). The glucose batches at 16ºC exhibited the greatest total organic content losses throughout the experiment.

6.4.1.5 Total ash content

When analysing the overall ash content, highly statistically significant differences were observed between temperature ($p<0.001$), and each of the batch types analysed ($p<0.001$). However, this was not observed over time ($p>0.05$). There was no significant pattern of loss over time for temperature ($p>0.05$) and the batches ($p>0.05$) respectively, indicating that the losses were not systematic in nature.

6.4.2 Microbial community analysis

An initial observation on how each factor effects substrate utilisation highlighted significant differences between the weeks ($p<0.05$) and temperatures ($p<0.05$), but not between the batches themselves ($p>0.05$). However, when the carbon substrates were analysed individually the batch type had more of an influence on substrate utilisation than time, whilst temperature had the largest overall influence.

When analysing the effect of time on the relative utilisation of the individual substrates, 17 out of the 31 were shown to have statistical differences ($p<0.05$). Of the substrates showing these differences, two were identified from the carbohydrate group (A2 and

B2), two from the polymers group (D1 and F1), seven from the carboxylic acids group (A3, B3, F2, F3, G3, C3 and D3), one from the Phosphorylated chemicals group (G2), four from the amino acid group (C4, E4, F4 and A4) and one from the amines group (G4). As previously noted (Chapter 5), there is no apparent consistency in the observed changes in substrate utilisation over time; for example when looking at the differences between the initial sampling point and the final sampling point there was an observed increase in activity. However, this was not constant over time, as there were peaks and troughs in activity at the intervening sampling points, and only D1 and E4 had constant and gradual increases in substrate utilisation over time.

Time appeared to have little effect on the batch type when examining the individual substrates, and only six of the carbon substrates (D1, A2, A3, D3, F4, and H4) were shown to have significant differences in relation to time ($p<0.05$).

Statistically significant differences were observed when assessing the effect of the different batch type on the individual carbon substrates ($p<0.05$). Of those showing differences, there were four from the carbohydrates group (G1, A2, B2 and C2), three from the polymers group (C1, D1 and E1), four from the carboxylic acids group (F2, A3, B3 and C3), one from the phosphorylated chemicals (G2), two from the amino acids group (E4 and F4), two from the amines group (G4 and H4) and one from the esters group (B1) that exhibited these differences.

The batches that were inoculated with glucose (batch 2), itaconic acid (batch 3) and Tween 80 (batch 4) all showed a mean relative increase in utilisation compared to the control batch, e.g. in substrates C1,D1, E1,G1, A2, B2, C2, F2, G2, A3, C3, E4 and G4.

From these 13 substrates, glucose had the highest relative utilisation in five of the substrates (E1, G1, B2, F2, and G2), and the mesocosms inoculated with itaconic acid displayed the highest utilisation in four of the substrates (C1, C2 and C3). Tween 80 exhibited the highest relative utilisation in the remaining three substrates (D1, A2 and A3). The inoculants appeared to have little effect on four of the substrates (B1, B3, F4 and H4), where the control batch showed higher relative utilisation compared to the three batches that had been inoculated. In the case of substrates C1, C3 and D1 the control batches had higher relative utilisation than the glucose batches, but lower relative utilisation when compared to those inoculated with itaconic acid and Tween 80 (see Table 6.1).

Table 6.1 Relative activity of the individual carbon substrates found within the Biolog EcoPlates™ platform that showed significant differences between the four batches. Results highlighted in bold text indicate those with higher relative activity than the control (n=144).

Key	Carbon Source	Batch 1 Control	Batch2 Glucose	Batch3 Itaconic Acid	Batch4 Tween 80
B1	Pyruvic Acid Methyl Ester	2.05%	1.45%	0.81%	1.95%
C1	Tween 40	3.11%	2.61%	**6.36%**	4.42%
D1	Tween 80	2.17%	0.76%	2.75%	**3.05%**
E1	Cyclodextrin	0.94%	**4.43%**	1.58%	2.88%
G1	D-cellobiose	2.08%	**4.96%**	0.98%	2.92%
A2	βMethly-D-Glucoside	2.40%	2.04%	1.82%	**2.96%**
B2	D-Xylose	0.80%	**2.43%**	2.35%	1.74%
C2	i-Erythritol	0.41%	1.37%	**1.65%**	0.96%
F2	D-Glucosaminic Acid	0.52%	**1.79%**	1.75%	1.32%
G2	Glucose-1-Phosphate	1.25%	**3.03%**	1.59%	2.09%
A3	D-Galactonic Acid γ-Lactone	2.09%	1.85%	0.70%	**2.28%**
B3	D-Galacturonic Acid	3.60%	2.26%	1.50%	2.57%
C3	2-Hydroxy Benzoic Acid	0.56%	0.45%	**4.09%**	1.14%
E4	L-Threonine	2.91%	2.04%	3.13%	1.87%
F4	Glycyl-L-Glutamic Acid	3.75%	2.24%	2.77%	0.95%
G4	Phenylethyl-amine	0.82%	1.46%	1.95%	1.06%
H4	Putrescine	4.32%	1.00%	0.74%	1.46%

These differences are highlighted in Figure 6.6, where reduced branches of clustering are visible in the control batch, whilst for the other three batches less clustering is in evidence. This patterning suggests that although higher substrate utilisation is observed in the inoculated batches, especially for glucose, the utilisation effect is not similar between the substrates.

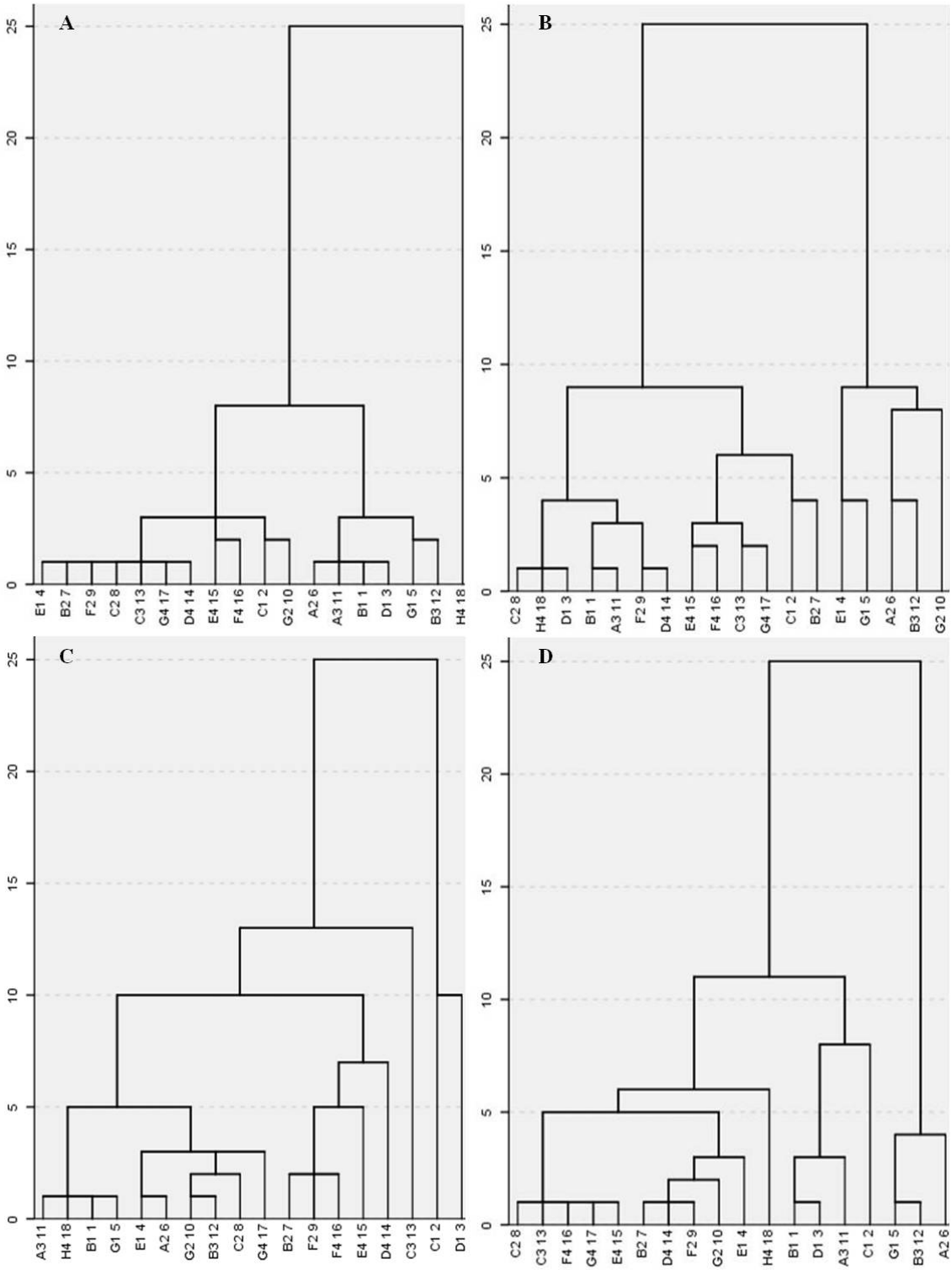

Figure 6.6 Hierarchical cluster analysis of the different carbon substrates that showed significant difference between batch types for (a) batch 1, no inoculation (b) batch 2, inoculated with glucose (c) batch 3, inoculated with itaconic acid (d) batch 4, inoculated with Tween 80 (n=144).

When comparing response of batch type against time, over half of the 31 carbon substrates showed significant differences ($p<0.05$); these were B1, C1, G1, A2, H2, A3, B3, C3, D3, E3, G3, B4, C4, F4, G4 and H4.

Temperature appeared to have a higher overall effect on the utilisation of individual carbon substrates, with 21 of the 31 substrates shown to have statistical differences between temperatures ($p<0.05$). Of those showing differences, five were from the carbohydrates group (H1, A2, C2, D2 and E2), two were polymers (C1 and D1), six were from the carboxylic acids group (F2, A3, B3, C3, E3 and G3), five from the amino acids group (B4, C4, D4, E4 and F4), two from the amines group (G4 and H4), and one from the Esters group (B1).

The majority of the substrates that demonstrated statistically significant differences displayed higher rates of utilisation at 25ºC when compared 5ºC and 16ºC. The substrates showing this higher utilisation were B1, D1, H1, A2, C2, D2, E2, F2, A3, B3, B4, C4, D4 and H4. Higher substrate utilisation was also observed at the lower temperatures: at the lowest temperature of 5ºC there were six observed substrates being utilised (C1, C3, G3, E4, F4 and G4), and one was utilised at 16ºC (E3).

The substrates that showed significant differences between the temperatures were further analysed using hierarchical cluster analysis, where visible differences in clustering was observed for each temperature. The lower temperatures of 5°C and 16°C had similar grouping comprising one large low cluster, however, this was not visible at the higher temperature of 25°C (see Figure 6.7).

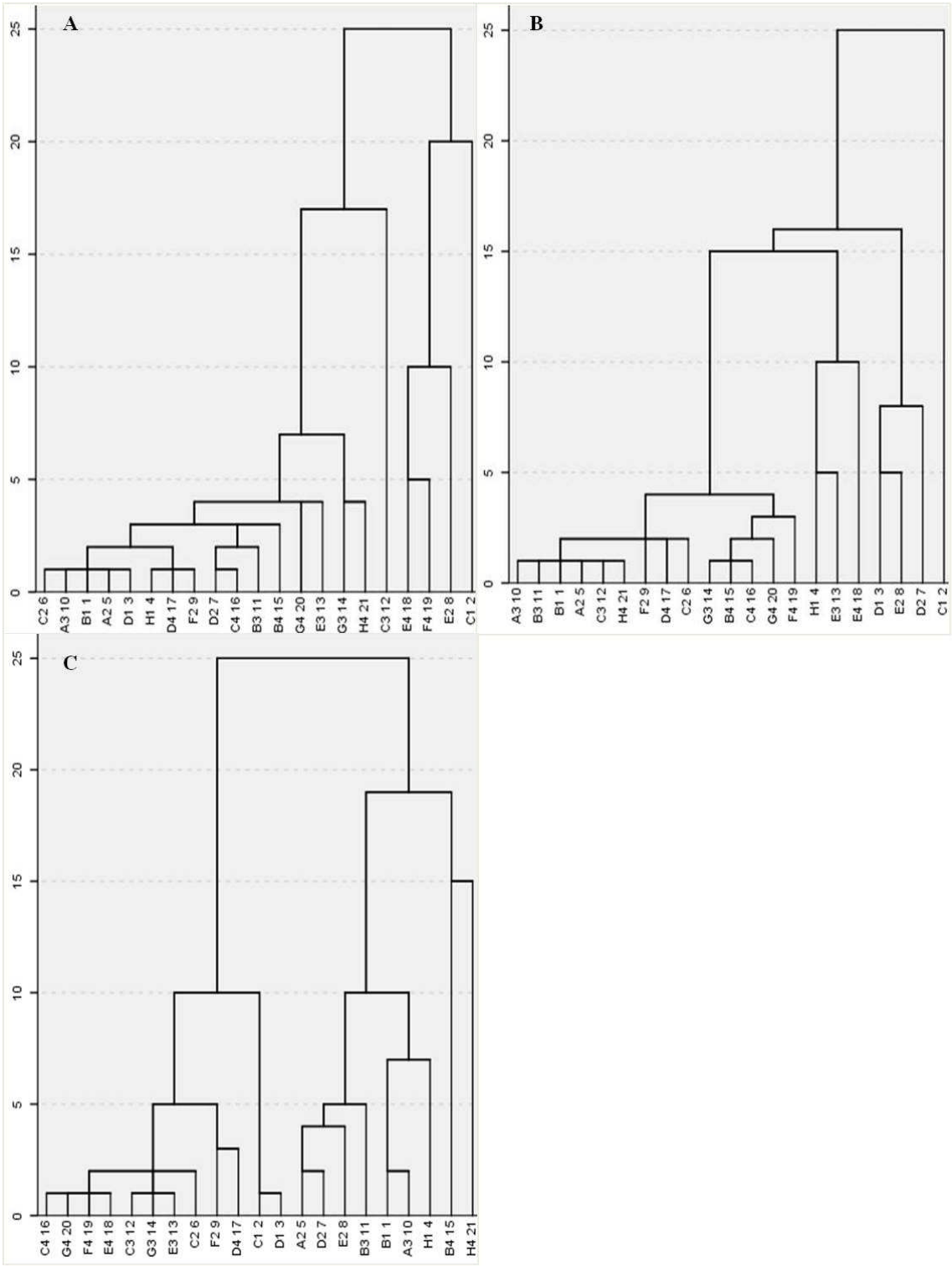

Figure 6.7 Hierarchical cluster analyses of the different carbon substrates that showed significant difference when incubated at different temperatures for (a) 5ºC (b) 16ºC and (c) 25ºC (n=144).

Time had very little effect on the different temperatures, with only three substrates (C1, B3 and E4) showing significant differences ($p<0.05$).

6.5 Discussion

This chapter has built on previous research (Chapter 5) further investigating the behaviour of the gully pots in a model *in situ* environment. Measuring the effects of an additive on the waste (identified from high utilisation during the experimental work that was reported on in Chapter 5) at different temperatures can provide an indication of the ideal conditions for degradation, should the waste be affected.

6.5.1 The effects of additives to the batches

The batches inoculated with glucose, itaconic acid and Tween 80 appeared to have an effect on the parameters measured. These three inoculants were selected for use after the results from the previous microbial community assay were assessed (see Chapter 5). This exercise indicated that three inoculants in particular, as outlined above, had increased rates of utilisation throughout the experiment.

There are a wide range of compost additives/accelerators available commercially, claiming the positive impacts of different mixtures of additives on the composting process (Himanen and Hänninen, 2009). However, there is little direct evidence in relation to the effectiveness of compost additives, especially in the scientific literature indicating that they have not been assessed through experimentation. As noted above, in general these compost additives are a mixture of different amounts of various

microorganisms, mineral nutrients or readily available forms of carbon, enzymes, and pH balancing compounds.

Whilst direct assessment of the efficacy of commercially available compost additives is scarce, there has been research undertaken which indicates the effectiveness of inoculating waste, soils and compost with additives (e.g. Gabhane *et al.,* 2012; Nakasaki *et al*., 1994; Olcay and Kocasoy, 2004). The addition of simple and complex organic substrates to soils has been termed the priming effect, which has been shown to result in short term changes in the turnover of organic matter, leading to either an increase (positive priming) or a decrease (negative priming) in organic matter decomposition (Brant *et al.,* 2006).

The pH can influence the microbial community, and therefore decomposition, with optimum values for microbial activity occurring between pH of 5.5 and 8.0 (de Badiane *et al*., 2001). The pH values for the current experiment were within this range, however, differences were observed between the batches used. The control batch had a mean pH of 7.4, which was similar to that of the Tween 80 batches (pH 7.25), whereas the remaining glucose and itaconic acid batches had mean values of 6.40 and 6.12 respectively. The control batch values were similar to those found during the previous experiments (see Chapter 5) which was to be expected given the samples that were used. It has previously been implied that the use of itaconic acid decreases soil pH (Magnuson and Lasure, 2004). The decrease in the pH observed in the glucose and Tween 80 batches did not appear to affect the waste, as the pH values appeared to remain constant throughout the experiment. Therefore these decreases appear to be a consequence of the composition of the sample as a result of substrate addition. By

contrast, in previous waste composting experiments (e.g. Liu *et al*., 2011; Shi *et al*., 2006) the inoculation of Tween 80 has been shown not to affect pH, with the samples following the same pattern across time as their control samples. This result has also been observed when soils have been inoculated with glucose, where no significant change was observed over time, and this was considered to be due to glucose being a neutral compound (Rukshana *et al.*, 2010).

Batch type had no significant effect on the levels of moisture, a factor which could be due to the mesocosm setup, where the flask lid was covered, preventing the loss of moisture from evaporation. This result was previously observed during the six month experiment (Section 5.5.4), and as such this was partially expected even though there had been substrates added to the mesocosms. For this reason the effect of moisture was not incorporated into the experiment as an independent variable. When using Tween 80 as an additive it has been shown that moisture levels will not generally decrease as the evaporation of water is significantly reduced when using this substrate. This is due to the prevention of surfactant molecule development on the water surface and trapping of water in the pores; this trapped water provides a better microenvironment for the microorganisms (Zeng *et al.*, 2006).

The addition of substrates to the waste significantly affected total dry matter values, and in turn the organic matter values of the samples. The control and the itaconic acid batches appeared to have the lowest level of loss of dry matter over time, when compared to the other two batches analysed. The low levels of dry matter and organic matter loss in the control samples was observed previously (Chapter 5), where a slow degradation process was identified in the controlled *in situ* experiment. It is commonly

believed that low quality organic matter limits the amount of available energy for soil microorganisms, and this in turn impacts upon the rate of organic matter degradation (Fontaine *et al.*, 2003). Previous experiments undertaken as part of the current study (Chapters 4 and 5) have demonstrated that the samples have low levels of organic matter content, which could also be of low quality due to the *in situ* conditions of the gully pots. Organic matter quality is controlled by nutrient availability, soil moisture, pH, temperature and oxidation – reduction potential. When microorganisms are not limited by these, the highest quality organic matter is decomposed most rapidly and the poorest quality organic matter is decomposed most slowly (White *et al.*, 2004). Therefore, it is entirely feasible that these factors could explain the low decomposition rates that have been observed.

Low levels of dry matter loss were recorded in the itaconic acid batches, alongside the control batches. However, the results obtained for the itaconic acid varied when assessing the organic matter content, as this substrate has the lowest overall organic matter losses when compared to the other three batches (including the control batch). The low levels of organic matter loss could be due to uncharacteristic fluctuations and decreases in the ash content within the sample, as similar fluctuations were previously observed in gully waste with no additives (see Chapter 5). As the organic matter loss in the itaconic acid batch was significantly less than that of the control, the effect of itaconic acid could be considered to have a negative priming effect. This is where decreased organic matter decomposition results from substrate addition, a phenomenon that has been observed in soils (Brant *et al*., 2009). Acidification of the environment, caused by the addition of itaconic acid to the waste, could also exert an effect on

organic matter decomposition, as the low observed pH will inhibit the growth of many microorganisms (Magnuson and Lasure, 2004).

Whilst a lower pH was also observed in glucose, the decomposition rate was significantly higher, a factor that could be directly due to the readily available energy in glucose (Fontaine *et al.*, 2003); a source that may not be as easily available from itaconic acid. Existing microorganisms can degrade a vast array of waste material, and some compounds are more easily degraded than others (Rittmann, 1993). It should also be noted that the use of itaconic acid as a decomposition accelerator has not been investigated within soils or composting environments and this substrate was used in the current experiment with the sole purpose of assessing another dimension within this study due to the high utilisation rates that were observed in the earlier experiments (Chapter 5).

Tween 80 showed slight, but significant, increases in degradation of organic and dry matter content when compared to the control. Similar accelerated degradation due to the inoculation of Tween 80 has been observed previously in composting studies where organic matter content decreased more rapidly with the addition of the surfactant (Liu *et al.*, 2011; Shi *et al*., 2006; Zeng, 2006). Tween 80 and related surfactants have been used for some time in bacterial cultures to assist in microbial community growth, where they have also been found to promote the entrance of compounds into cells (Reese and Maguire, 1969), and previous research has shown that surfactants, most commonly Tween 80, can promote the production and release of enzymes such as cellulose (e.g. Liu *et al.*, 2006; Pardo 1996; Reese and Maguire, 1969), and amylase (Goes and Sheppard, 1999; Reddy *et al*., 1999).

The enhancement of extracellular enzyme activity promotes the degradation of biomacromolecules, which will in turn speed up the composting process (Zeng *et al.*, 2006). Furthermore some surfactants play an essential role in the survival of the producing microorganisms, either through facilitating nutrient transport or by affecting microbe-host interactions (Goes and Sheppard, 1999). This activity could in turn prolong the enhanced levels of degradation that were observed. Due to the positive acceleration resulting from the use of Tween 80 in previous studies e.g. Shi *et al.* (2006), this particular carbon substrate has been observed to (potentially) be beneficial for composting processes.

Glucose appears to have promoted the highest rates of loss in relation both to the dry matter, and the total organic matter contents of the samples observed in the current experiment. This conforms to the results of previous studies where the addition of glucose to soils has been widely used as a soil primer and has been shown to increase rates of organic matter degradation (e.g. Brant *et al.*, 2006; Shen and Bartha, 1996), and is a substrate often used in biodegradation experiments (Degens and Sparling, 1996, Tuomela *et al.*, 2002). Glucose has been shown to occur in laboratory incubations with the addition of simple organic compounds in a range of soils (Brant *et al.*, 2006). The priming effect is thought to result from an increase in overall microbial activity due to the greater availability of energy and nutrients released from fresh organic matter (Fontaine *et al.*, 2003). However, it should be noted that, in several studies glucose addition has actually been shown to induce a negative priming effects (e.g. Degens and Sparling, 1996).

By contrast, glucose and sugars have also been shown to significantly increase the specific growth rates of the soil microorganisms that accelerate composting (Blagodatskaya *et al.*, 2009), and recent research has demonstrated that sugars are effective compost enhancers (Gabhane *et al.*, 2012). In addition to the elevated losses of both dry and organic matter, the decomposition process was enhanced through increased microbial biomass, in turn increasing the organic matter degradation (Gabhane *et al.*, 2012). The size of the priming effect is shown to increase with the amount of organic substances that are added to the samples studied (Muhammad *et al.*, 2007). This could be the reasoning behind the increased degradation within the glucose batches.

Due to the small organic matter content observed throughout the current trial, microbial community assays were used to back up the findings of the *in situ* experiments. The Biolog Ecoplate™ assay is more sensitive to the environment and therefore capable of identifying changes within the microbial community when there are only small amounts of organic matter available (Biolog, 2000).

Batch type was shown to exert a slight influence on the microbial community when the carbon substrates were analysed individually; however, they were not as influential as temperature. No significant difference was observed between the batches for 14 of the 31 carbon substrates, indicating that utilisation was similar across all batches for those 14 substrates. From the remaining substrates, 17 inoculated batches displayed an increase in utilisation compared to the control batch, whilst 11 of these substrates showed better utilisation when an inoculant had been added. Previous research has shown that inoculating soils and compost with carbon substrates such as glucose and

surfactants increases microbial activity (e.g. Fontaine *et al.*, 2003; Blagodatskaya *et al.*, 2009).

Glucose had the highest relative utilisation in five of the substrates (E1, G1, B2, F2, and G2), and the mesocosms inoculated with itaconic acid displayed the highest utilisation in four of the substrates (C1, C3, E4 and G4). Brant *et al.* (2006) have demonstrated changes in the microbial utilisation of carbon compounds by altering the input into soils, such as the addition of glucose, with this resulting in an alteration in the size and composition of the microbial community. The application of glucose to soils has been shown to increase soil microbial community activity for a short period of time (Martínez-Trinidad *et al.*, 2010; Mondini *et al.*, 2006). Martínez-Trinidad *et al.* (2010) have suggested that the addition of glucose to soils could be used as a way to modify short term microbial activity, as the effect of carbohydrates on microbial activity only lasts until the carbohydrates are metabolised by soil microorganisms.

Tween 80 showed the highest relative utilisation in the remaining three substrates (D1, A2 and A3). Tween 80 has been widely used as an amendment in the production of enzymes (Shi *et al.*, 2006), and has been used for some time in bacterial cultures to assist in growth (Reese and Maguire, 1969). Tween 80 has also been found to promote the entrance of compounds into cells (Reese and Maguire, 1969), and has been shown to have slight stimulatory effects on the microbial community, which exhibits increased activity compared to the control (Shi *et al.*, 2006).

However, the impact of Tween 80 on the type of waste used in the current study was small, with lower levels of utilisation than the control batches. The inoculants appeared

to have little effect on four of the substrates (B1, B3, F4 and H4), where the control had the highest utilisation. These differences are observed in Figure 6.8 where reduced branches of clustering were observed in the control batch, however, for the remaining three batches less clustering was observed. This suggests that although higher utilisation was observed in the inoculated batches (especially for glucose) the utilisation effect is not similar between the substrates. Furthermore the increased utilisation in the glucose batches compared to the control is also in proportion with a higher rate of organic matter decomposition, as indicated during the physical analysis. However, in the current study almost half of the carbon substrates showed no difference between the control and the inoculated batches, which raises questions as to why there was only a small change in the microbial community when there were proportionally greater losses in the physical parameters measured (e.g. dry matter decay over time).

In general, utilisation did not increase gradually over the course of the experiment in those substrates that showed a difference over time. Peaks and troughs in utilisation were observed, throughout the experiment, as previously noted (Chapter 5). This suggests that the addition of the substrate was not the controlling factor in microbial community functioning during this study, and the substrates do not increase this.

6.5.2 The effects of temperature

Temperature appeared to have a significantly larger effect on the physical parameters (pH, organic matter etc) and on the microbial community, compared to the batch type. The range of temperatures observed in the field (Chapter 4) provided an indication of the optimum temperatures that would need to be simulated in the experimental elements

of the current study, thereby allowing for a complete range of external temperatures to be considered.

The pH values did not appear to be effected by temperature, with these values exhibiting no significant differences between the three temperatures. Similarly, differences were not observed over time in relation to the separate temperatures. These results reinforce the results that were generated previously (Section 5.5.3) where no temperature effects were observed, despite the fact that three out of the four batches were inoculated with substrates. Temperature also had no significant affect on the levels of moisture. However, this was anticipated as, although there had been substrate additions, a similar situation was previously observed during the six month experiment (Section 5.5.4).

Temperature did have a positive effect on total dry matter content, which exhibited variability between the three temperature categories. Greater losses were observed at the highest temperature, i.e. at 25ºC, when compared to the two lower temperatures, both of which had similar losses in evidence (e.g. mean loss of 2.70g at 25ºC compared to the mean loss of 2.01g at 5ºC).

The results for total organic matter mirrored that of the total dry matter content, where overall greater losses were observed at higher temperatures, and with no significant differences occurring over time. This result was again anticipated as total organic matter is the organic portion of the total dry matter, and consequently it should decrease in the same way. When analysing the effect of individual temperatures over time on total dry matter content, a difference was observed, but this was not statistically significant.

Similarly, no significant difference was observed in the amount of organic matter lost between each sampling point, unlike previous observations where greater losses were observed at the initial sampling points. However, losses were observed between the start and end of the experiment, indicating an overall loss through the study.

Each batch was affected by temperature, exhibiting variability in the losses of dry and organic matter at different temperatures. The control and itaconic acid batches showed increased losses of dry- and organic matter at the higher temperature of 25ºC. An increase in the decomposition of the gully pot waste at the higher temperature was also observed in the previous *in situ* monitoring experiment (Chapter 5). This was determined to be a result of higher microbial activity occurring due to the 'better' conditions that are promoted by elevated temperatures. As there have been no previous studies investigating the effects of treatment of itaconic acid on decomposition, it would be misguided to state this as the reason for the increased dry matter losses observed in the current study. The itaconic acid batches showed similarly high organic matter losses at 5ºC. However, these losses were significantly lower than those observed in the control samples, which suggests that itaconic acid has a negative effect on degradation, and that there is no temperature specificity.

The Tween 80 batches showed higher losses at the lowest temperature of 5ºC. As Tween 80 has mainly been used to accelerate composting (Liu *et al.*, 2011; Shi *et al*., 2006; Zeng *et al.*, 2006) the effect it has at lower temperatures, such as 5°C, has not been investigated, and it is unclear why the waste is affected in this manner.

Glucose displayed its highest dry matter and organic matter loss at 16ºC and also showed the greatest loss of organic matter in the lower temperature of 5ºC, but not 25ºC. This result was surprising as the higher temperature can allow for higher microbial activity; therefore, it was assumed that with the addition of glucose, which can also promote greater microbial activity, the greatest loss would be observed at the higher temperature. The effect of glucose as an inoculant has not been assessed at the lower temperature levels. However, Vasconcellos (1998) compared soils from tropical and temperate regions that were amended with glucose, and found that the microbial biomass appeared to be more sensitive to higher temperatures. This result conflicts with those observed in the current experiment, however, a direct comparison cannot be made as the composition of the waste may differ from that of the soils.

Glucose appeared to have the overall highest loss of dry and organic matter throughout the study, showing a particularly high increase in rates of dry and organic matter loss at 16°C. These observations suggest that the variables of temperature and glucose as a substrate additive are optimum for dry and organic matter degradation. This result is interesting, as when assessing the effects of temperature on the microbial community those incubated at 16°C appeared to have the lowest substrate utilisation. This suggests that the effects of batch type and temperature may have conflicting results, especially when comparing the physical and microbial parameters. This issue has been observed in bioremediation of contaminated soils, where Rittmann, (1993) stated the importance of realising that no single set of characteristics will favour bioremediation of all contaminants, e.g. certain compounds can only degrade when oxygen is absent, but destruction of other contaminants requires that oxygen is present.

Temperature had a significantly greater overall influence on the microbial community during the Biolog assays when compared across the batches. Out of the 31 analysed substrates 21 were shown to have statistical differences between the temperatures, where the majority of these showed increased utilisation during the highest monitored temperature of 25ºC. Lower utilisation at lower temperatures was observed, which was perhaps unsurprising as it is known that lower temperatures can inhibit microbial growth. Time had very little effect on the different temperatures, with only three substrates showing significant differences, indicating little change in utilisation over the eight week experiment. The increased utilisation at higher temperatures and the small change in substrates over time coincides with the previous work on the gully waste undertaken during the current study (which was explored in Chapter 5).

Reduced branches of clustering, causing larger groups containing the same carbon substrates, were observed in the lower temperatures of 5°C and 16°C. This clustering indicates that the carbon substrates are being utilised in a similar manner, unlike the higher temperature of 25°C which showed smaller groups of clustering. The smaller clustering could be induced by the higher activity, which may not be due to the additives.

6.6 Conclusion

Temperature and carbon substrate inoculants appeared to significantly increase the decomposition rates of the waste when examining the physical parameters, such as organic matter. Glucose appeared to enhance the waste decomposition, especially when incubated at 16°C. However, this was not consistent with the findings from the

microbial community. Mesocosms incubated at 25°C showed the greatest loss, which was supported by the higher microbial activity at that temperature when analysing the carbon substrate utilisation pattern. In the mesocosms, inoculation with glucose and Tween 80 appeared to increase the decomposition rate. However, the microbial community did not appear to support these findings as there was only a slight increase or change when the waste was inoculated compared to the control. This low community increase could be due to short term effects from the inoculants.

Whilst there was no complete agreement between the physical parameters and the microbial community, as indicated by the Biolog EcoPlates^{TM}, increases in decomposition were observed. As such, a more detailed, i.e. lengthier, investigation would be needed to confirm, or resolve, these issues before it would be appropriate to suggest this as a suitable sustainable method of managing gully waste. The costs of using an additive would also need to be evaluated as although it may be a more sustainable method of management it may not be a cost effective approach. As a result it may be valuable to investigate further alternative methods for promoting degradation of gully pot waste, such as an *ex situ* method like composting.

7.0 Gully pot waste management via *ex situ* treatment

7.1 Introduction

This chapter builds upon the previous investigations into enhancing degradation in gully pots with an *ex situ* approach, applying similar techniques to those used in composting. As the additive trials undertaken in Chapter 6 have produced conflicting results showing little support for the viability of an effective/systematic approach to *in situ* management, it is important to assess alternative methods for the management of waste material within roadside gully pots. Preliminary experiments using mesophilic *in situ* approaches have demonstrated that degradation is occurring within the system (Chapter 5). Given that previous work has not explored the potential of enhancing the degradation processes within gully pot waste through the application of a positive control, such as a starch treatment, it may be possible to investigate this technique as a potential method for promoting degradation in these environments.

As elevated temperature profiles are a key feature of industrial organic waste processing activities (Finstein *et al.*, 1975) the assessment of thermophilic activity will clearly be an important area to assess in the development of sustainable management solutions. *Ex situ* treatments allow for thermophilic temperatures to develop due to the increased volumes treated, and thermophilic temperatures are thought to be important for composting processes. To date, a wide range of approaches using studies of extracellular enzyme activity, have been employed in order to investigate composting, and this technique has also been used repeatedly to monitor the progression of organic matter stability and maturity (e.g. Cayuela *et al*., 2008; Komilis *et al*., 2011; Mondini *et al*., 2004). However, by contrast there is relatively limited literature on detailed

controlled studies with regards to the effect of temperature on these dynamics, and reciprocal approaches aimed at investigating temperature effects on extracellular enzyme activity have only been reported upon twice in the literature (Adams *et al*., 2008; Adams and Umapathy, 2011). Therefore, understanding how the enzymes may adapt to different temperature regimes as a causal variable is an important variable to assess, if we are to confirm whether the effect observed is an acclimatisation effect, or if it is in fact a true temperature effect that is a result of the *ex situ* composting process.

7.2 Aim

The main aim of this chapter is to assess whether gully pot waste degradation processes, and enzyme substrates activity, occur under *ex situ* mesophilic and thermophilic conditions with the use of a positive control. Alongside this, the relative effects of the *in situ* composting temperature and assay temperature on the enzyme substrates are also assessed using a reciprocal experimental design to determine if there are differences between the two. This reciprocal design measures the effects of increased or decreased assay temperatures compared to the temperatures the waste is composted at.

7.3 Method

7.3.1 Sample collection

The samples were collected and stored as previously outlined (Section 3.1), and were then prepared for the trial which started the day after collection. As previously mentioned (Section 5.3.1), the waste was treated as a composite sample, as opposed to creating different mesocosms for different areas. As such, a variety of contents were

collected from gully pots from the four different geographical areas (as previously described in Section 3.1).

7.3.2 Experimental setup

In total, 40 model gully pot mesocosms were set up in the laboratory (as described in Section 3.2.2.4) to measure the effects of a substrate addition (starch) on a putatively linked enzyme substrate (α-glucosidase), under mesophilic and thermophilic conditions over a 6 week period. As a cost-effective starch amendment, food grade cornflour (aka cornstarch; the wet milled product from maize) was used. Each temperature and additive mesocosm was measured in duplicate for accuracy and all temperatures were maintained to +/- 1°C during the incubation period. The mesocosms were prepared in two batches of 20, where the first batch was prepared without an additive as it was to be used as a control. The starch amended mesocosms were prepared by adding 10g of cornflour (Tesco, U.K.) with 70ml of distilled sterile water, into a 250ml conical flask of known weight. The solution was mixed thoroughly before adding a further 30g of dried sieved gully waste. This was then re-mixed until a homogenous slurry had been created. Control mesocosms were prepared in a similar manner but without the starch addition.

Tin foil was placed on the top of all of the conical flasks and the final weights of the mesocosms were recorded before incubation. From each batch 10 mesocosms were incubated at temperatures typical of mesophilic (30^{o}C) and thermophilic environments (50^{o}C). Over a 6 week period two mesocosms from each temperature regime and starch treatment were sampled to destruction. The moisture content of all samples were

measured on a weekly basis and maintained to the original moisture content percentage. The first sampling period occurred after seven days and sampling continued on a weekly basis for a further 3 weeks, with the final sampling date occurring after 6 weeks.

7.3.3 Physical parameter analysis

During sampling, the total weight (including flask) of the mesocosms was recorded to determine any loss which may have occurred during incubation. Subsequently, 1g of waste material was removed for slurry preparation for the enzyme analysis. The pH (as described in Section 3.2.2.1), moisture content (see Section 3.2.2.2), total dry weight and subsequently organic matter content (see Section 3.2.2.3) were measured using the remaining material from the mesocosms.

7.3.4 Extracellular enzyme analysis

The activity of all eight selected enzymes (sulphatase, phosphatase, β-glucosidase, α-glucosidase, β-galactosidase, β-glucuronidase, butyrate esterase and β-xylosidase) were analysed as described in Section 3.3.1 for the first two weeks of the experiment. Due to the low levels of enzyme activity observed during the first 2 weeks of the experiment, only β-glucosidase and α-glucosidase were analysed for the remaining four weeks. Replicate samples were incubated for 1 hour at both 30°C and 50°C, under a reciprocal design (Adams *et al*., 2008) to investigate the effect of assay conditions on the enzyme activity.

7.3.4 Statistical analysis

For all of the data, an ANOVA model was used to assess the effects of the starch addition, compost temperature, assay temperature and time for each mesocosm, followed by a LSD test. All tests were performed using PASW statistics, version 18.

7.4 Results

7.4.1 Physical parameter analysis

7.4.1.1 pH

There was no observed statistical difference between the pH of the incubated mesocosms that contained the untreated waste, and the starch amended positive control ($p>0.05$). The mean pH for the untreated waste was slightly higher at 7.58 whereas the positive control samples with the starch added had a mean of 7.48. In addition, temperature did not have any significant effect upon the overall pH ($p>0.05$). However, time did ($p<0.05$), where the pH range appeared to converge over time (see Figure 7.1). Further assessment indicated that only week one and week two showed statistical differences when compared to weeks three, four and six ($p<0.05$). This statistical difference over time was not observed when investigating the difference between the separate temperatures of the untreated waste and the positive control ($p>0.05$).

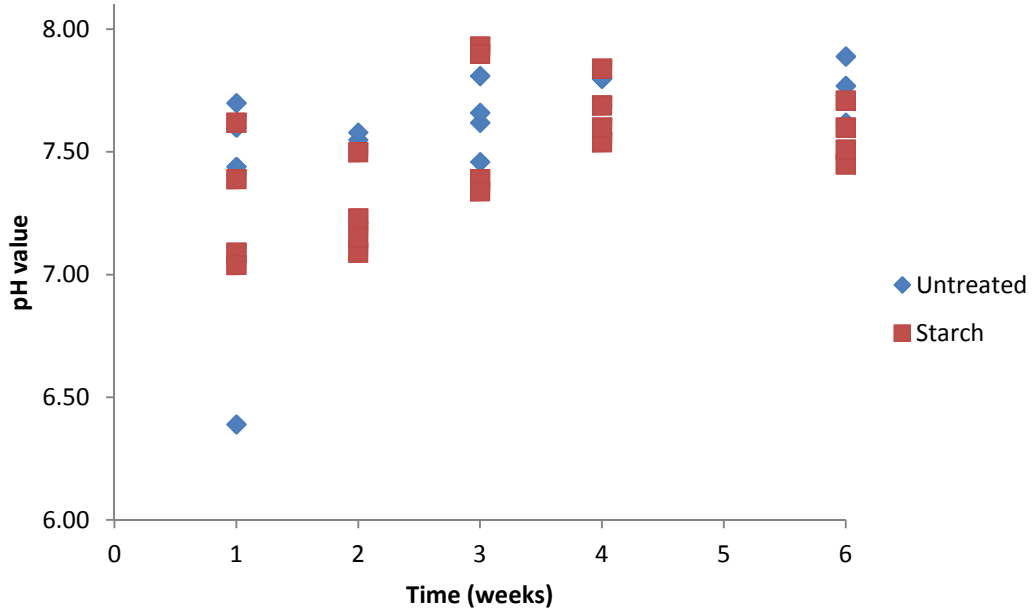

Figure 7.1 The overall observed pH throughout the six week experiment, displaying the values observed for the untreated samples and the positive control (starch amended) samples (n=40).

7.4.1.2 Moisture content

When assessing the effect of treatment (i.e. starch amended) ($p>0.05$) and time ($p>0.05$) no significant difference in the moisture content measured at the time of sampling was observed. Temperature did however, have a highly significant effect on the moisture content of the samples ($p<0.001$) even though moisture was maintained at their original level by topping up on a weekly basis throughout the duration of the experiment. The mesocosms incubated at 50°C had a 5% overall lower moisture loss compared to those incubated at 30°C for all samples. Although the greatest loss of moisture content was observed during the second week, there was no significant change observed over time at the different temperature levels ($p>0.05$).

7.4.1.3 Total dry matter

Temperature had no significant effect on the total dry matter content of the samples ($p>0.05$). Furthermore, similar results were observed when the total dry matter of the untreated samples and the positive control was assessed: temperature appeared to have no significant effect on the untreated samples ($p>0.05$) or the positive control ($p>0.05$). The addition of starch as a positive control appeared to be highly significant in relation to the total dry matter content when comparing it to the untreated waste ($p<0.001$). The positive control samples displayed a larger overall total dry matter loss, with a mean loss of 5.98g when compared to the untreated waste, with a mean loss of 1.28g over the 6 week period.

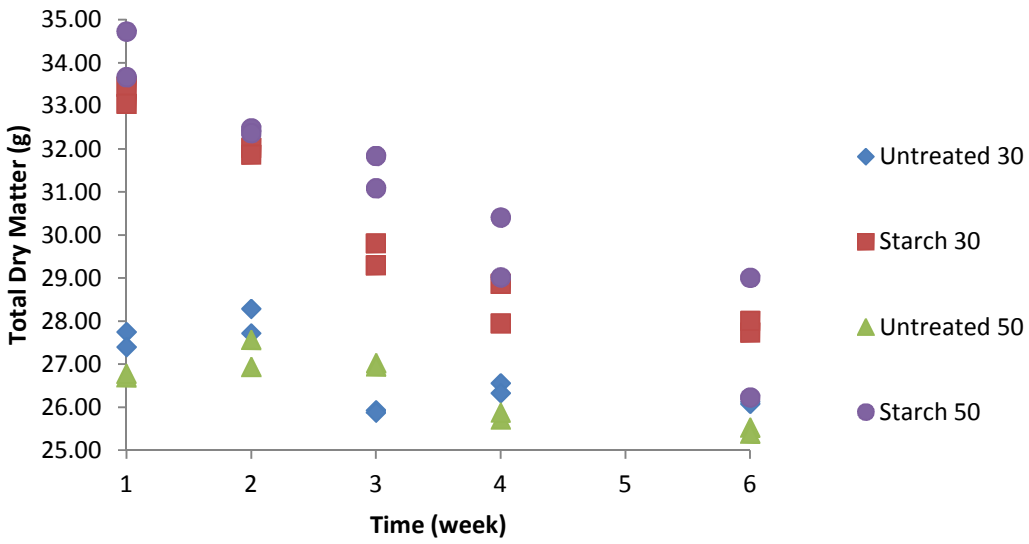

Figure 7.2 Total dry weights for the untreated samples and positive control (starch amended) samples incubated at 30°C and 50°C over the six week period (n=40).

Time appeared to have a highly significant effect on the overall dry matter losses ($p<0.001$) where significant differences occurred between each week ($p<0.05$); apart

from the initial two sampling point where there was no significant difference between the two ($p>0.05$). These significant differences in relation to time on the dry matter content were also observed when the two types of waste samples, untreated and positive control, were analysed ($P<0.001$). The effect of time was shown to be highly significant on the dry matter of the samples that were modified with the addition of starch ($p < 0.001$) and on the un-treated samples ($p<0.001$). The decreases in all samples are displayed in Figure 7.2. From visual assessment of Figure 7.2, there appears to be little difference between the dry matter losses at the two different temperatures in the untreated samples, unlike the positive control that showed a steeper initial decrease at 50°C.

7.4.1.4 Total organic content

Temperature appeared to have no significant effect ($p>0.05$) on total organic matter content. Furthermore, similar results were observed when each individual treatment type was analysed: temperature appeared to have no significant effect on either the untreated ($p>0.05$) or the positive control samples ($p>0.05$).

The addition of starch as a positive control appeared to have a highly significant effect in terms of the organic content compared to the untreated waste ($p<0.001$). The positive control samples had a greater mean loss of 6.53g when compared to those that were not amended with starch, which displayed a mean loss of 0.23g over the 6 week trial. The untreated samples had a lower mean loss compared, which could be due to anomalies in the dataset (as observed in Figure 7.3). A low organic content measurement of 7.38g was recorded in the control batch incubated at 30°C, which was due to a high ash

content measurement of 20.02g. This anomaly did not appear to have a great effect on the difference between the two types of waste; if this high ash anomaly had not been observed, there still would have been a statistical difference ($p<0.05$) between the wastes types as the mean loss would have been 1.88g. One further irregularity is visible in a positive control sample at week four (see Figure 7.3) and this was also a result of a high ash content within that sample.

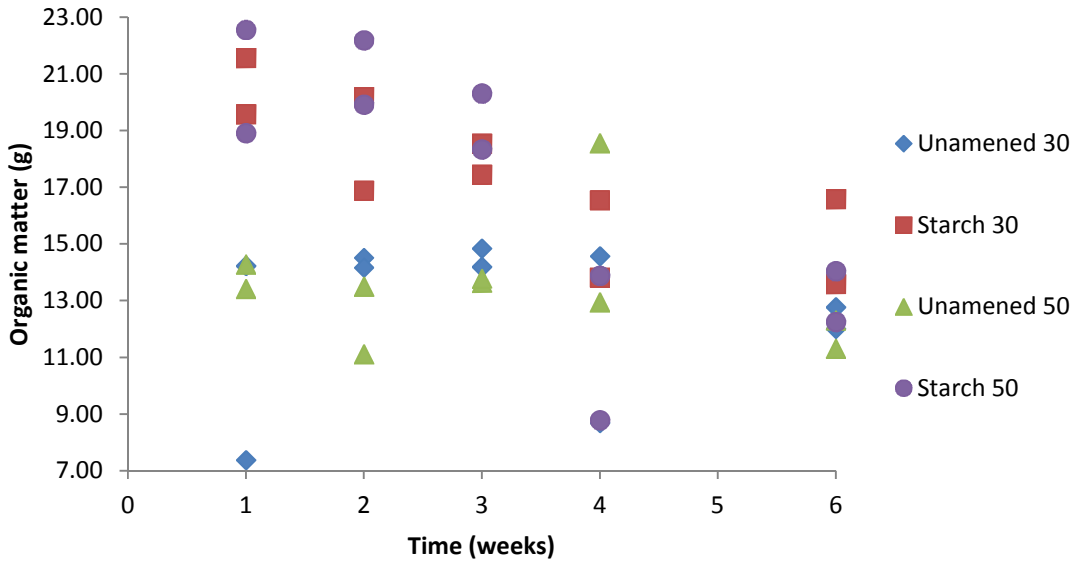

Figure 7.3 Total organic weights for the untreated samples and positive control (starch amended) samples incubated at 30°C and 50°C over the six week period (n=40).

The overall organic matter content was significantly affected by time ($p<0.05$), with differences observed between weeks. Further analysis showed significant differences occurred throughout the trial ($p<0.05$), apart from the initial three sampling points where there was no significant difference between them ($p>0.05$). In addition, time did not have a statistically significant effect on the untreated samples ($p>0.05$), however, it was shown to be highly significant in the positive control samples ($p<0.001$). The effect of time on organic matter is displayed in Figure 7.3. As can be seen in Figure 7.3

organic matter loss over time appears to exhibit very few similarities between the organic matter losses at the two temperatures with the different treatments.

7.4.1.5 Total Ash content

The total ash content was not significantly affected by time ($p>0.05$), waste type ($p>0.05$) or temperature ($p>0.05$). Furthermore, time did not appear to significantly affect the ash content of either of the sample types ($p>0.05$), nor did temperature ($p>0.05$). High ash contents were observed in two of the samples which were described in Section 7.4.1.4.

7.4.2 Extracellular enzyme analysis

7.4.2.1 Preliminary assessment of the full suite of eight enzymes

A full suite of the eight enzymes (sulphatase, phosphatase, β-glucosidase, α-glucosidase, β-galactosidase, β-glucuronidase, butyrate esterase and β-xylosidase) was initially assessed to measure enzyme activity. Temperature, waste type and time were shown to have no significant effect ($p>0.05$) on seven of the eight enzymes (sulphatase, phosphatase, β-glucosidase, β-galactosidase, β-glucuronidase, butyrate esterase and β-xylosidase). This however, was not the case for α-glucosidase which was shown to be highly affected by *in situ* temperature ($p<0.001$) and waste type ($p<0.001$). Due to the little observed effect of the variables, sulphatase, phosphatase, β-galactosidase, β-glucuronidase, butyrate esterase and β-xylosidase were not measured further in the assay. The enzyme α-glucosidase continued to be analysed due to the highly significant differences observed ($p<0.001$). In addition, β-glucosidase was also used for the remainder of the study as a comparable source because it displayed fewer fluctuations in

activity levels than the other enzymes studied, and it generally had higher activity when compared to the control. It is also a similar, but non-specific enzyme to α-glucosidase.

7.4.2.2 Full six week analysis

Neither *in situ* composting temperature or assay temperature appeared to significantly affect the α-glucosidase activity ($p>0.05$), nor did these variables affect the activity of β-glucosidase ($p>0.05$). Whilst there is no significant effect from temperature ($p>0.05$), the activity of β-glucosidase and α-glucosidase is on average increased with the higher temperatures. There was a weakly significant interactive effect of assay and composting temperature on α-glucosidase activity ($p<0.05$), but not with β-glucosidase ($p>0.05$).

The activity of α-glucosidase was shown to be significantly increased by the positive control compared to the untreated waste ($p<0.001$). This was the same result that was observed for the activity of β-glucosidase, where weakly significant increases were in evidence ($p<0.05$).

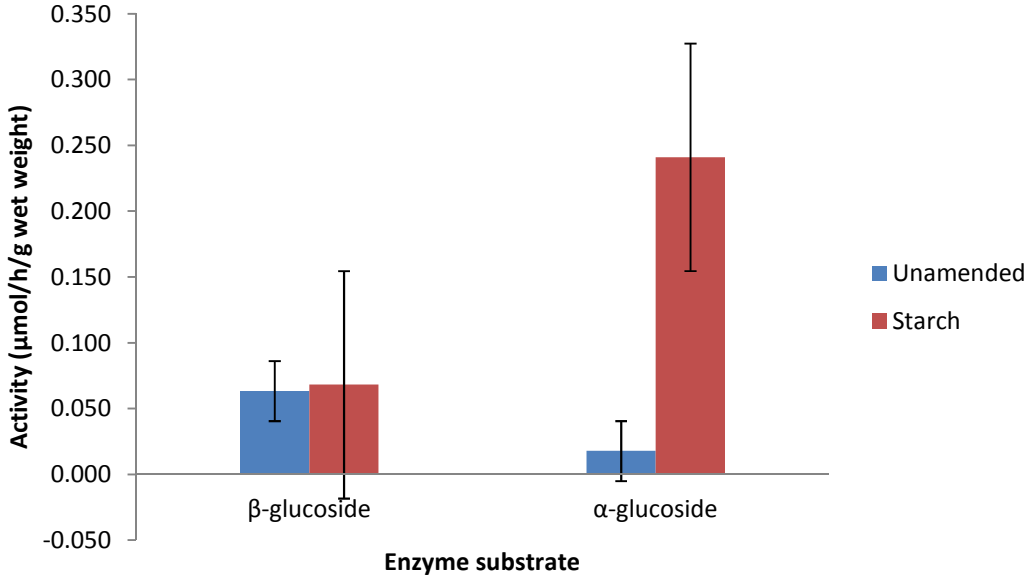

Figure 7.4 Mean β-glucosidase and α-glucosidase activity of all the starch amended and control (starch unamended) samples from the 30°C in situ and 30°C assay temperature combination fitted with error bars (n=20).

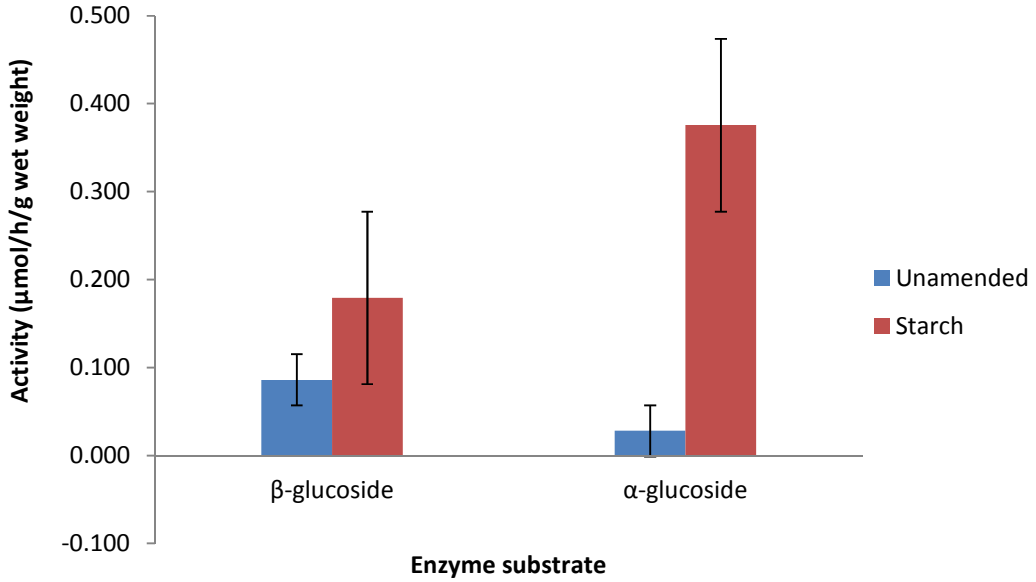

Figure 7.5 Mean β-glucosidase and α-glucosidase activity of all the starch amended and control (starch unamended) samples from the 50°C in situ and 50°C assay temperature combination fitted with error bars (n=20).

The mean activities of α-glucosidase and β-glucosidase for all samples from the 30°C /30°C (30°C *in situ* and 30°C assay temperatures) are shown in Figure 7.4. The mean activities for all samples from the 50°C /50°C (50°C *in situ* and 50°C assay temperatures) are shown in Figure 7.5. As can be seen in both Figures 7.4 and 7.5 the mean activity for α-glucosidase is lower, by at least one order of magnitude, in the untreated samples group when compared to the positive control samples during both temperature variations. The activity of β-glucosidase is also higher in the positive control samples when compared to the untreated group, but to a much lesser extent at 50°C /50°C. This higher activity was not observed in the 30°C /30°C samples.

The activity of both β-glucosidase and α-glucosidase was shown to be significantly affected over time ($p<0.001$ and $p<0.05$ respectively). Although a significant effect was observed, there appears to be no obvious distribution of the activity of both enzymes over time, except that α-glucosidase activity appears to peak in week 4 of sampling during both temperatures. The weekly mean activity of both α-glucosidase and β-glucosidase is shown in Figure 7.6 and 7.7 for the positive control samples at 30°C /30°C and 50°C /50°C respectively.

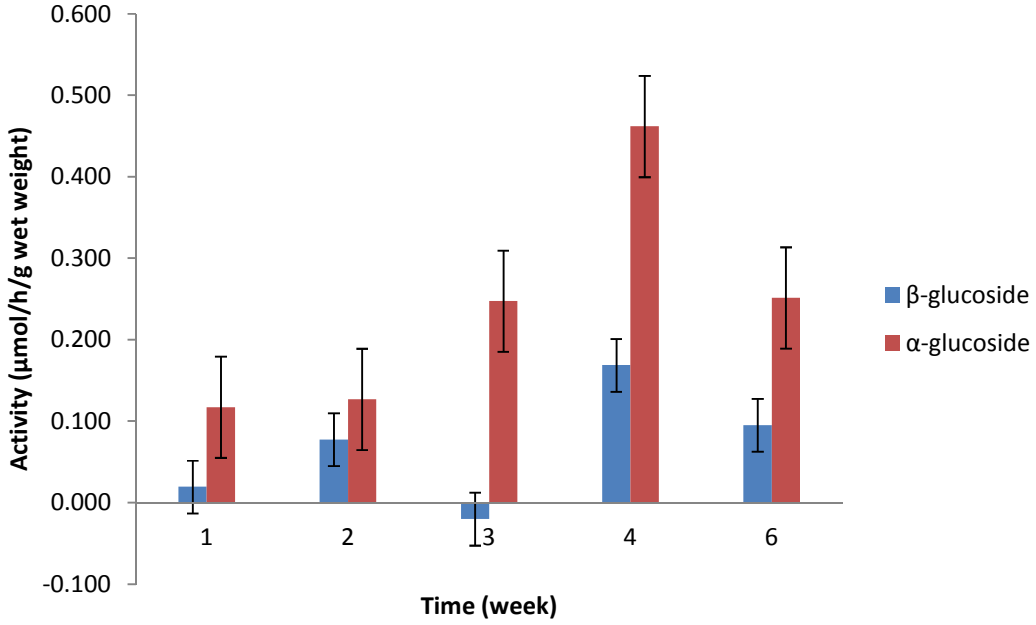

Figure 7.6 Mean β-glucosidase and α-glucosidase activity over six weeks for the positive control samples from the 30°C in situ and 30°C assay temperature combination fitted with error bars (n=20).

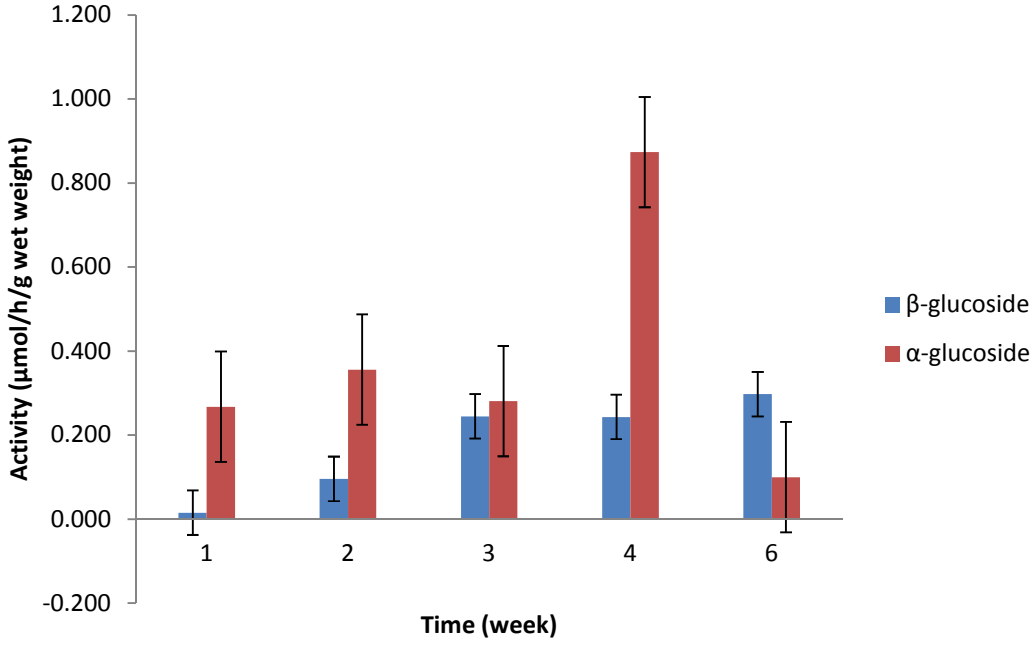

Figure 7.7 Mean β-glucosidase and α-glucosidase activity over six weeks for the positive control samples from the 50°C in situ 50°C assay temperature combination fitted with error bars (n=20).

The reciprocal design illustrated that neither *in situ* composting temperature nor assay temperature had a significant effect on either α-glucosidase ($p>0.05$) or β-glucosidase activity ($p>0.05$). However, a weak interactive effect was observed in the α-glucosidase activity, between the *in situ* composting temperature and assay temperature ($p<0.05$). This was not observed in the β-glucosidase activity ($p>0.05$).

The positive control continued to have a significantly higher influence on the activity of α-glucosidase when compared to the untreated waste ($p<0.05$), but not on β-glucosidase activity ($p>0.05$), during the reciprocal design. The positive control samples incubated at 50°C *in situ* displayed, on average, α-glucosidase activity that was twice as high at 50°C assay temperature when compared to the 30°C assay temperature (see Figures 7.5 for the 50°C/50°C and 7.9 for the 50°C/30°C). The positive control samples that were incubated at 30°C *in situ* only displayed 20% higher α-glucosidase activity at 30°C compared to the 50°C assayed samples (see Figures 7.4 for the 30°C/30°C and 7.8 for the 30°C/50°C). These observed differences of α-glucosidase activity in the positive control samples were not significant at either 30°C ($p>0.05$) or 50°C ($p>0.05$).

Only the activity of β-glucosidase was shown to be significantly affected over time ($p<0.05$) during this design. Time did not appear to have a significant effect on the activity of α-glucosidase ($p>0.05$).

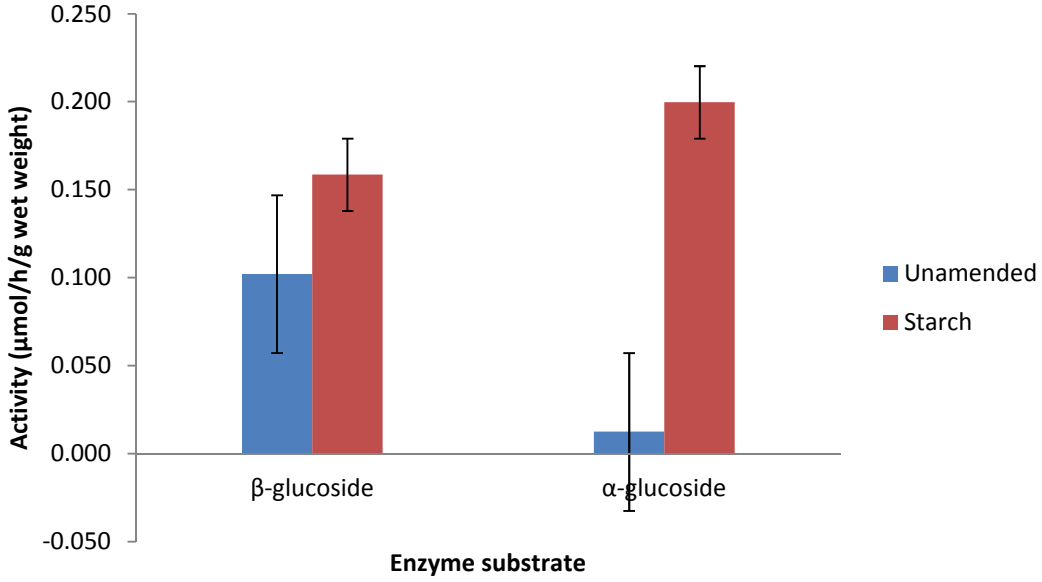

Figure 7.8 Mean β-glucosidase and α-glucosidase activity of all the untreated and positive control (starch amended) samples from the 30°C in situ and 50°C assay temperature combination fitted with error bars (n=20).

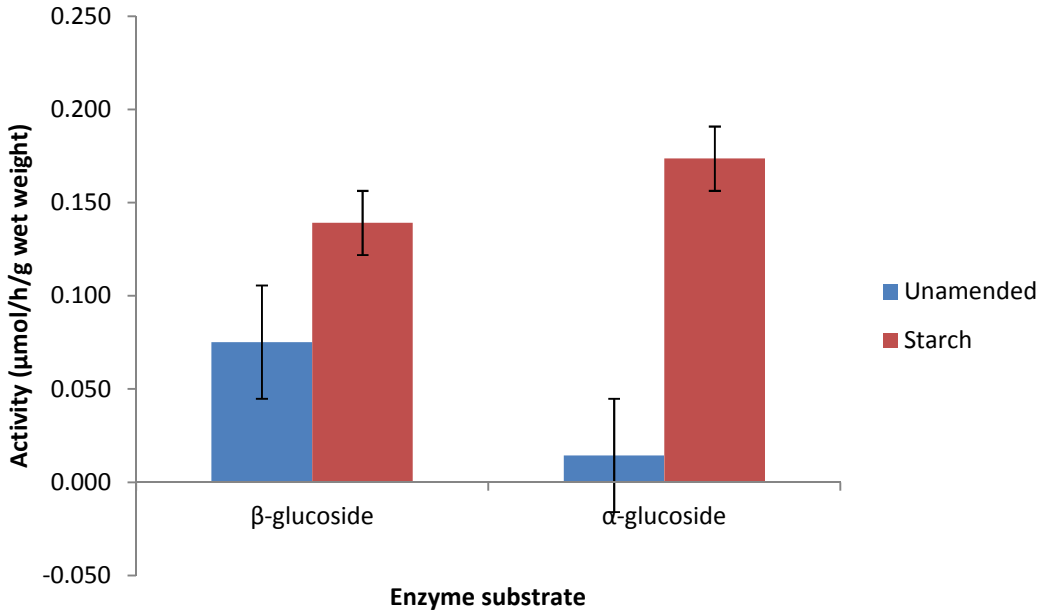

Figure 7.9 Mean β-glucosidase and α-glucosidase activity of all the untreated and positive control (starch amended) samples from the 50°C in situ and 30°C assay temperature combination fitted with error bars (n=20).

7.5 Discussion

This Section presents the discussion of the effects of mesophilic and thermophilic temperatures on the management of gully pot waste using an *ex situ* treatment.

7.5.1 The degradation dynamics of gully pot waste using starch addition as a positive control

The physical parameters were measured to assess the general decay of the organic matter *ex situ* and then compared with treated samples that were used as a positive control. This was carried out using a co-composting technique, alongside an extracellular enzyme activity approach. The extracellular enzyme activity was employed to assess the degradation dynamics that may result from the addition of starch to the samples. Co-composting approaches have been used previously to assess microbial processes, as they are partially dependent upon the additive used (Adams and Umapathy, 2011).

7.5.1.1 Physical parameter analysis

The addition of substrates to soils and composts has been observed to affect the pH, such as the use of lime to reduce acidity (e.g. Flower and Crabtree, 2011; Williams and Donald 1957). The addition of starch appeared to slightly reduce the mean pH value to 7.48 when compared to the untreated samples at 7.58, with lower pH values more prominent in the first two weeks of the experiment (see Figure 7.1). The corn starch itself had an original lower pH of 6.5 when compared to the mean gully waste of 7.56, therefore the addition of this slightly acidic substrate could presumably be responsible

for the lowering of the pH. Although a slight decrease in pH was observed, it was not a statistically significant shift in pH values.

A significant decrease of dry matter in the untreated waste was observed over time, showing a mean loss of 1.28g over a 6 week period. This observed loss is greater than that observed in the *in situ* experiments at both 60% and 80% moisture level, incubated at 30°C for eight weeks (Chapter 5), suggesting that greater losses might be anticipated in *ex situ* studies.

Total dry matter and organic matter was significantly affected by treatment type, indicating greater losses in the starch amended samples when compared to the untreated samples (Figure 7.2 and 7.3 respectively). It is clear that the addition of starch enhanced decomposition. This observation corresponds to previous research where starch has been shown to have a positive effect on degradation in clays (Mtambanengwe *et al.*, 2004).

As observed previously, in Chapters 5 and 6, there were fluctuations in ash content in both the untreated and positive control samples. In particular, two samples were shown to exhibit abnormally low organic contents, which was a result of high ash content. These two anomalous samples did not appear to affect the overall statistical significance of the organic matter results, and as such they were not considered to warrant amendment. The variability in ash contents could be due to fine heavy particles, a factor which has been considered above in Section 3.5.

7.5.1.2 Extracellular enzyme activity analysis

From the full suite of enzymes considered, only α-glucosidase was shown to be significantly affected by the treatment, with this enzyme exhibiting increased activity in the starch amended samples. The remaining seven enzymes were not significantly affected by treatment, and as a consequence, with the exception of β-glucosidase, were not used during the remainder of the assay. The activity of β-glucosidase was investigated as a reference, as this approach has been adopted in similar studies previously (Adams and Umapathy, 2011).

The full six week study indicated that the addition of starch had a demonstrable effect on the activity of α-glucosidase, by significantly increasing the enzyme activity, and a weak significant effect on β-glucosidase activity (Figures 7.4 and 7.5). Interestingly, the results obtained during this stage of the research show similar trends to lake sediments, where enzyme activity was strongly stimulated by starch addition, and where this addition also had a weakly significant stimulatory effect on β-glucosidase activity (Mallet and Debroas, 2001). However, contrastingly, Hernández and Hobbie (2010) have found that starch significantly stimulated β-glucosidase and not α-glucosidase in a soil environment. Allison and Vitousek (2005) also studied substrate additions in a soil environment, finding that the addition of a substrate alone did not significantly increase activity in its focal enzyme, and these researchers observed that enhancements in microbial activity required the addition of minerals (N and P) as a supplement. Across the six week study there was no observed pattern in β-glucosidase activity, however there was for α-glucosidase activity, which appeared to peak at week four then decrease in week six. The decrease in activity was much larger in the higher temperature and could be a result of oxygen depletion.

As there is very little previous research on the effect of substrate addition to extracellular enzyme activity within the composting area, studies from other environments were sourced (e.g. Zeng *et al*., 2010). These studies also demonstrated conflicting results, suggesting there is no consistent response of extracellular enzyme activity to substrate addition. These inconsistencies in the data may of course be due to differences in the methodological approaches used. For example, a comparative study of deep-sea sediments and Antarctic soils showed differing responses in enzyme activities with different substrates between the two environments (Zeng *et al*., 2010). Although extracellular enzyme activity has been used in a number of composting studies (e.g. Poulsen *et al*., 2008; Su *et al.,* 2009), there is little empirical evidence to demonstrate a direct association between extracellular enzyme activity substrates and their focal polymer. Poulsen *et al.* (2008) showed that two different types of composts amended with chitin increased chitinase activity; however, the magnitude of the effect was less than double the control group.

7.5.2 The effect of temperature

7.5.2.1 Physical parameter analysis

In previous studies, temperature has been shown to affect the physiochemical characteristics of the environment, including pH (Paul and Clark, 1996). However, despite this observation temperature did not appear to significantly affect pH values in the *ex situ* study. The negligible effect of temperature on gully pot waste has previously been observed during the current study (e.g. Section 5.0 and 6.0).

Moisture level was significantly affected by temperature; showing increased loses at higher temperatures, even though the study moisture levels were maintained to their original level on a weekly basis after sampling. Over the course of each week an average of 5% moisture was lost at 50°C. This was not observed at 30°C, and is likely to be due to higher temperatures increasing evaporation. This higher moisture loss did not dry out the sample, as it was only 5% on average, and therefore it had little effect upon the processes active within the waste environment.

Temperature did not appear to affect the overall total dry matter content of the samples. Samples with and without the starch amendment indicated that regardless of incubation temperature, be it mesophilic or thermophilic, they reacted in a similar way. This was also the case with organic matter, which did not appear to be significantly affected by temperature. These results appear to contradict the general literature, where temperature has been shown to have been a main controlling factor during composting (Joshua *et al.,* 1998; He *et al.,* 2010). However, many other studies (e.g. Jung *et al.,* 1999; Liang *et al.,* 2003; Tang *et al*., 2007; Tremier *et al*., 2005) have indicated that temperature is not necessarily the driving force in every situation, or that thermophilic temperatures are optimal for degradation.

The change in dry matter for each treatment is visible over time in Figure 7.2, where a larger loss of dry matter initially occurred, and that these rates then slowed down towards the end of the six weeks. This was not observed with the other three variables, indicating a relatively stable loss of dry matter throughout. There was very little observed difference between the two temperature types, as with the untreated samples.

Previous research into starch amended composts (Adams and Umapathy; 2011) has shown comparable results that demonstrate temperature independent losses of dry matter between mesophilic and thermophilic conditions. Decreases were observed in the organic matter content, but not in such a predictable manner as in the dry matter. This could be an effect caused by the removal of the ash content, where small fluctuations were observed from each sample. Total ash content was not affected by temperature, showing no statistical difference between the three different temperatures. There was also very little fluctuation in ash content over time for each individual temperature. These small fluctuations had no significant effect on the total ash content, but they may have slightly impeded the results of the organic matter contents by affecting the decomposition rates. These fluctuations have been previously observed throughout the study (see Chapters 5 and 6).

7.5.2.2 Extracellular enzyme analysis

The *in situ*, and assay temperature, did not have any effect on the activity of seven of the eight enzymes. Only the activity of α-glucosidase was observed to be statistically affected by the *in situ* temperature. This could indicate that the *in situ* temperature, the temperature that the samples were composted at, had a larger effect on the activity of α-glucosidase when compared to the temperature of the assay. However, this result was not observed when the assay was continued for the remaining four weeks.

Consistent with the dry and organic matter results, neither composting temperature nor assay temperature had a significant effect on either α-glucosidase or β-glucosidase activity. However, whilst this is statistically non-significant, the activity of both enzymes in the untreated samples and the positive control samples were on average

slightly higher with the higher incubation temperature. This effect of increased activity with increasing temperature was most apparent in β-glucosidase (see Figures 7.4 and 7.5) and is consistent with previous results (Adams *et al*., 2008; Adams and Umapathy, 2011). The results of the extracellular enzyme activity analyses complement those of the dry matter and organic matter degradation results, which have indicated that they function independent of temperature. This suggests, as previously discussed (Section 7.5.2.1), that temperature is not the driving force in degradation.

Interestingly, during the reciprocal design a weakly significant interactive effect of assay and composting temperature on α-glucosidase activity was observed. This result could be indicative of an adaptive effect of temperature on the enzymes responsible for this activity, and therefore presumably starch degradation. The data observed during the reciprocal design for α-glucosidase presents direct evidence for thermal adaption in this environmental system. Regardless of the *in situ* temperature, both α-glucosidase and β-glucosidase showed increased activity when the assay temperature was at its highest, albeit a statistically insignificant increase. Previous studies have produced results which are consistent with the observed β-glucosidase activity during the current study (e.g. Adams *et al*., 2008, Adams and Umapathy, 2001).

The increase in enzyme activity linked to increasing assay temperature has not only been observed in composting environments, but also in soil (German *et al*., 2012; Stone *et al*., 2012). This would suggest that enzyme activity is independent of the environmental temperature. Furthermore, the results observed during this study suggest that enzyme activity generally increases with temperature regardless of *in situ*

temperature, unless it is under selection, as with α-glucosidase (due to the direct link between the focal enzyme and starch).

Composting is generally regarded as being inhibited at excessive temperatures. However, previous studies show that a number of thermophilic α-glucosidase enzymes have been isolated, for example *Pyrococcus furiosus*, which has an optimum activity of over 100°C (Constantino *et al*., 1990) indicating that α-glucosidase activity has the potential to adapt to the temperature range used in the study. The results observed during this assay suggest that higher rates of activity are displayed at higher temperatures for non-adaptive enzymes. Ntougias *et al*. (2006) recommend performing all assays at their respective i*n situ* temperatures; however, it would be difficult to understand how adaptive an environment could be when performing the assay in this manner. Whereas the reciprocal design can help indicate this as it measures the activity of the waste at a different temperature during the assay compared to the *in situ* composting.

7. 6 Conclusion

This study has demonstrated the ability of the gully pot waste to degrade using *ex situ* management techniques when assessing the physical parameters. It was apparent that this degradation was independent of temperature between the mesophilic and thermophilic stages. These results were consistent with the microbial changes observed in both the untreated and positive control samples. Furthermore, the effect of temperature appeared to have a selective influence on enzyme activity during the reciprocal design. Higher activity was observed at the higher temperatures, particularly with the non-selected β-glucosidase, regardless of the *in situ* temperature. Examining

these temperature variables provided a better understanding of the processes occurring *ex situ* and displaying the wastes ability to decompose, to a degree, under conditions similar to those that it would be subjected to if using *ex situ* management systems such as composting. Through the support of the positive control the study shows that the waste is able to degrade within this environment, however, due to the rate of degradation it suggests that the gully pot environment appears to be recalcitrant.

Overall, these findings suggest that, although the waste degrades at a slow rate that is similar to that of the *in situ* study, *ex situ* treatment of the waste alone would not be the optimum way to manage the waste. Future work, incorporating longer term studies would be needed to confirm this, alongside exploring the potential of aerated *ex situ* management, instead of the anaerobic system used in the current study.

8.0 Conclusion

This chapter summarises the key findings from monitoring the gully pot waste in the field and in the laboratory under controlled conditions. These findings are the consequence of the original research questions which were created to understand and investigate sustainable management for gully pot waste. The controversial blaming of blocked gully pots for exacerbating the 2007 floods in Hull was the main rationale behind these questions, as discussed in the introductory chapter (3.0):

- What *in situ* decomposition and enzyme processes are occurring within the gully pot?
- Do seasonal factors and variations in geographical location have an impact upon these processes?
- Can methods be developed to assist with the speeding up of the decomposition of the gully waste *in situ* to assist in the remediation of blockages?
- Is management of the waste *ex situ* viable if *in situ* management is not possible?

The efficacy of this study to answer these questions will be assessed, the meaning of the results assessed and the scope for future work explored. There will also be a consideration of alternative methods for sustainable gully pot waste management.

8.1 Key findings relating to the research questions

8.1.1 What *in situ* decomposition and enzyme processes are occurring within the gully pot?

The analysis of samples from a one year repeated survey of 15 gully pots and a two year study analysing 180 individual pots, indicated that there was a wide range of organic content observed within the gully pots (from 2.17% - 71.76%), indicating there was large potential for the contents to decompose. The pH ranged from 7.26 – 13.22, with the majority of pots positioned in the lower alkaline range. These results were backed up by the extracellular enzyme activity, which indicated all eight enzymes were active within the gully pot waste. These results suggested a suitably active, albeit anaerobic, environment in which decomposition could take place.

Assessing the decomposition processes of the waste was a vital stage in the study. By doing so, it was possible to gain an understanding of two important issues. Firstly, if the waste could actually decompose; and secondly, if it was possible, what rate was it decomposing at? During this study the decomposition activity *in situ* was monitored using modelled gully pots under controlled conditions in the laboratory, in order to manipulate and mimic the external environment. The *in situ* assessment exhibited that decomposition rates that fluctuated under a range of temperatures, indicating a higher rate of decomposition at higher temperatures, which was confirmed by shifts in the microbial community. Decomposition at the lower temperature was observed at a slower rate and varying the moisture levels did not appear to have any impact upon the speed of the decomposition rate.

8.1.2 Do seasonal and geographical location variations have an impact upon these processes?

Assessing the waste over a set time period and from different areas allowed the waste to be analysed to identify whether seasonal (temperature and runoff temperature, pH, biological activity) and geographical variations (e.g. contributions from surrounding foliage inwash, detritus, rubbish from urban areas) had any impact upon such processes occurring. Investigating if there was any effect from geographical location was important for assessing if the waste could be viewed in the same manner, or if sustainable solutions would have to be considered on a location basis. Assessing if there was a seasonal effect was also important, since the fluctuating temperatures and wet/dry spells could directly affect the conditions within gully pots.

Chapter 4 indicated that seasonality did not play as big an effect on the physical processes and extracellular enzyme activity as geographical location. This was surprising as it was anticipated there would be large differences observed in selected parameters through seasonal variation, for example fluctuation of enzyme activity, during temperature change, and organic matter, due to leaf fall. The lack of effect could be due to the conditions in which the waste is constantly subjected to, such as high water levels, which could assist in the production of a microenvironment within the gully pot.

Although there were a greater number of observed differences between geographical areas, these differences did not appear to have a major effect on the physical parameters and activity. Areas that were typically industrial in nature displayed lower physical parameter values (e.g. organic matter) than others, and this can be attributed to the

general lack of foliage in these localities. Enzyme activity was not as high in these areas as was found in the other three location types, but nonetheless was still displayed to some degree.

8.1. 3 Can methods be developed to assist with the speeding up of the decomposition of the gully waste *in situ* to assist in the remediation of blockages?

As there has been no work in this area previously, an additive style treatment which has been used in composting and soils was assessed to see if it would be suitable for this kind of waste. Using results gained during the microbial community analysis of the long term *in situ* models it was possible to pick out three carbon substrates (glucose, Tween80 and itaconic acid) that displayed high utilisation. Utilising carbon substrates as an accelerator was decided upon because of the high influence carbon substrates have on soil microbial activity. The waste was also subjected to differing temperatures that have been observed in the field.

When the physical properties were examined, the results indicated that the application of glucose to the waste increased the decomposition rate, particularly at 16°C. However, these results differed to that of the microbial community, where only small changes were observed. Temperature appeared to be the main controlling factor in this study, indicating overall greater losses at 25°C, regardless of the substrate. Whilst there was not a complete agreement between the physical parameters and the microbial community, increases in decomposition were observed. A more detailed and lengthier investigation would be needed to confirm or resolve these issues.

8.1.4 Is management of the waste *ex situ* viable if *in situ* management is not possible?

During this study the decomposition activity was monitored in two defined separate stages - *in situ* and *ex situ.* The *in situ* activity was monitored using modelled gully pots under controlled conditions in the laboratory, in order to manipulate and mimic the external environment. The *ex situ* activity was also monitored under controlled conditions using small scale mesocosms, where the systems were essentially the same but with higher temperatures.

Both stages indicated that the waste decomposed under controlled environments. The *ex situ* analysis indicated the waste also decomposed at similar rates under mesophilic and thermophilic conditions. This decomposition was confirmed using extracellular enzyme activity, which showed higher activity at higher temperatures which was not visible in the physical parameter analysis. A positive control was created using a starch amendment to confirm this decomposition. This study indicated that the waste decomposed under conditions it would be subjected to in the field *in situ* and *ex situ,* however, both these results indicated similarly slow degradation rates. Further analysis would be needed to analyse the degradation potential of the waste *ex situ* under longer term conditions to assess how that could affect the process.

8.2 Outcome for present sustainable gully pot management

The results obtained during this study were inconclusive in finding a definitive sustainable method to manage the gully pot waste. The waste demonstrated a tendency

to decompose within the gully pots, but at a slow rate which was not much improved when the waste was inoculated with carbon substrates. This slow degradation rate was observed when the waste was assessed using *ex situ* composting techniques through the increase of temperature. These results suggest that the environment the waste is subjected to may not be ideal for an *in situ* management process as it stands. However, the study was useful in revealing potential solutions which could be investigated in the future.

8.2 Scope for future research

While researching this study, it became evident there was scope for further work which could prove beneficial for sustainable management of gully waste.

8.2.1 Littering within the gully pot

Throughout the study it was obvious that littering is a major problem within the gully pots. Litter can accumulate within the gully pots through a variety of methods, e.g. being transported by wind or purposely dumped. Litter found within the gully pots ranged from small confectionary packets, to car parts through to pots filled with sand and cement. This kind of excessive littering can result in surface flooding due to poor drainage, as the drainage pipe that leads to the sewers becomes blocked up with debris, but also impedes the decomposition rate due to the non-degradable nature of inorganic litter. Further assessment of the kind of littering that is occurring within the gully pots, as well as the frequency and the kind of catchment areas it occurs in can be important if trying to predict or prevent such littering. Furthermore, public awareness of the damage that can be caused by this type of littering is needed to assist in the reduction.

8.2.2 Further assessment of anaerobic processes

Assessing the microbial community under controlled conditions proved useful in understanding how the microbes act under anaerobic conditions and how they may adapt during changes in the seasons, e.g. high waste input with lower moisture values. However, not all aspects associated with anaerobic conditions were assessed, and could be extended by analysing reducing bacteria, such as sulphate, iron and phosphate. These bacteria could play an important role in the decomposition of the waste under the conditions exhibited in the gully pot. By analysing these reducers, it could be possible to evaluate the effects the lack of oxygen within these confined environments has on the microbial community. Understanding these effects it could be possible to gain further knowledge on factors that may impede or improve the degradation ability.

8.2.3 Further assessment into extracellular enzyme activity

The results shown throughout this study have indicated the importance of understanding extracellular enzyme activity, especially when analysing the effects of the putatively linked substrates on the enzymes. As the methods that were proposed, and the results received in this area of the study are novel and relatively pioneering, it could be of interest to investigate how they interact and/or behave in different environments. Understanding enzymes and how they behave naturally can explain a lot about a desired substrate element using a relatively simple method.

8.2.4 *Ex situ* composting of gully waste

Within this study the ability for the waste to compost was assessed for short periods. Future studies could utilise the same methods, adapting them for a larger, longer term analysis while also assessing the internal temperature of the waste. Analysing temperature within the composting waste can assist with verifying at what temperature the waste reaches its optimal degradation rate, and if this complies with previous research investigating the thermal degradation during composting. Examining the waste on a longer term study can be beneficial when assessing the amount of time the waste is able to compost for, identifying the optimum latency. It will also be possible by extending the time, for example to 12 weeks which is the general composting assessment time, to compare the reductions to better characterised environments. It would also be beneficial, if analysing this *ex situ* management technique further, to assess the effect of aeration on the samples. From this, it would then be possible to decide, if possible, the best approach to compost the waste on a much larger scale, making full use of the waste removed from the gullies in Hull.

8.2.5 Experimental design

An attempt was made to analyse the gully pots in real time via a data logger measuring the pH, conductivity, DO and temperature which took place over a six month period. However, after multiple issues with data collection, *in situ* conditions and technicalities (see Section 3.6.1) the data logger was removed and deemed ineffective in this environment. It is still believed the results that could have been obtained from this part of the study would have given insightful real time information into the processes occurring within the gully pot. Further investigation would need to take place to assess

what kind of equipment would be able to work in this semi-stagnant enclosed environment. Doing so it will be possible to gain further in-depth information on how the waste could potentially be affected by external environmental effects.

Model gully pots were setup in the laboratory under controlled anaerobic conditions with the longest experiment lasting six months, which proved to be insightful and beneficial for the analysis required throughout this study. As the methods during this study were developed under small-scale conditions, due to space restrictions, it was not possible to extend or upscale the setup. However, it would be interesting to analyse the gully pots on a larger scale, such as over a twelve-month period, where the effects of rain and litter within the gully pot can be measured. Assessing the effects of these conditions can give a further in-depth indication of how the wastes processes perform in real time

A dataset consisting of 180 individual gully pot wastes was initially collected to gain a basic understanding of the activity and how it is affected by season or geographical location affects it. Although this was a large dataset, giving a wide range of results, there are over 70,000 gullies pots within the city of Hull alone. Therefore it would be beneficial to the study to assess more. Doing so could indicate a stronger result, providing firmer evidence to back up what has already been found. Furthermore, if this study was to be repeated, a firmer assessment of geographical locations should be created. For example, areas with high foliage could be indicated by distance from different types of local flora such as trees, grass, bushes, etc., examining the catchment of the gully pot at a much more in-depth level.

While investigating methods to accelerate the decomposition of gully waste, positive results were received when assessing the physical parameters due to the addition of carbon compounds. However, the microbial community results contradicted these findings. Further work could be used to clarify the results, further assessing if these kinds of treatments would be beneficial for the management of waste. Different substrates appeared to have differing reactions under temporal conditions Tween 80 was also shown to slightly influence the decomposition, but it is unknown how the Tween 80 and glucose would have acted together which raises an interesting possibility for further exploration. It is possible that one kind of treatment is not enough for this kind of environment and a mixture of two or more would be helpful, and this has been observed in compost additives.

8.3 Suggestions for alternative gully pot waste management and blockage prevention

The study, although providing valuable results in the microbial activity and physical parameters which were previously unknown, did not find a suitable *in situ* solution to enhance the decomposition of the gully pot waste, indicating further research is needed. However, alternative sustainable solutions for gully pot management were identified during the assays and research. As previously mentioned (Chapter 2) this is already in practice. However, the waste from the gully pots is mixed with other wastes and sent on to external contractors as a compost additive. From the results presented in this study, it is evident that the waste could be composted on its own which may be a viable method for the Council to undertake. Obviously further research will need to be undertaken (as outlined in 8.3.4) to determine the finer details needed to set up a large scale waste composting system. Using a composting system will alleviate the need to landfill waste,

saving the authorities money, with even greater savings possible if the composting system is set up ‘in house’. Authorities within the UK and Europe have adopted such a system in which water is also treated, allowing around 98% reuse of the waste input to be achieved (as mentioned in Chapters 2 and 4).

There are currently novel techniques present in the literature that are aimed at gully waste blockage prevention. Though these techniques would not provide sustainable solutions for management of the waste, they could assist with blockage prevention, in turn saving money for the authorities and the public by reducing the risk of flooding. One method to consider would be the use of wireless sensors which had been piloted on residential gully pots (See *et al.,* 2012). This method is designed to alert the local authorities prior to a blockage or leakages occurring allowing time for maintenance on the pot before flooding occurs. This method is still in the prototype phase, nevertheless it appears to be a more effective and proactive approach to take as opposed to the current visual monitoring which takes place at the moment.

8.4 Conclusion

This study has allowed for the collection an analysis of gully waste under long term conditions within a controlled laboratory environment and in the field. The ability of the wastes to decompose *in situ* and *ex situ* was also assessed, with both thermophilic and mesophilic conditions compared to a positive control. Throughout the study the microbial community and enzyme activity has been assessed and used to clarify/confirm the results obtained during the physical parameters assessment. From the results obtained, it was possible to analyse the ability of the waste to degrade *in situ* and *ex situ*

assessing if an *in situ* treatment method could be used to assist with speeding up decomposition, and what alternative methods were available.

Although the results did not provide a solution to *in situ* management, it is hoped the novel findings will allow for further investigation into the area, expanding on research and methods adapted during this study. It was possible through this research to highlight a possible alternative sustainable management solution through extra *ex situ* analysis in the form of composting. Utilising the results obtained can be beneficial for forming a base line to refer to and build on in this matter. Further blockage prevention was also addressed referring to remote sensors and gully pot adaptations; although this does not affect decomposition, being, proactive regarding blockages can be cost effective in the long run.

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