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The preservation and degradation of wood in wetland archaeological and landfill sites

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Abstract

This study has investigated the patterns and processes responsible for oak wood degradation in different burial environments by characterizing the biological, chemical and physical nature of sediments from a variety of '*in situ*' wetland archaeological and landfill sites. It has used oak wood as the complimentary organic biomarker to help determine the main factors responsible for the unwanted decay of archaeo-organic materials in wetland archaeological sites and identify the useful deterioration of organic materials in landfill sites.

A laboratory-based simulation (lysimeter study) has been used to act as a representation of the conditions that exist within the burial environments chosen for this research. The main parameters which characterize all burial contexts were artificially manipulated within the lysimeters. These were subsequently measured by using a number of technological applications in order to produce a multi-disciplinary analysis of each environment studied. The interpretation of the data generated from these techniques highlighted patterns within the sediments which were responsible for the degradation of oak wood.

As a result of these findings, this study has not only illustrated the key environmental parameters that are responsible for oak wood degradation over a variety of time scales, i.e. the short- (lysimeter study), medium- (landfill sites) and long-term (wetland archaeological sites), but also determined the most appropriate conditions which are able to preserve wood in wetland archaeological sites and optimize organic waste degradation in landfill sites.

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Chapter 1 Introduction

1.1 Introduction

The decomposition of woody tissue is an important process which occurs naturally in soils over periods of decades to centuries and even millennia. The use of wood and artefacts made from wood by humans, dates back to the Hoxnian Interglacial (420,000-320,000 BC) where its incorporation into hunting practices was detected for the first time in Britain (Ashton 2004). Throughout time, humans have abandoned woody remains in the ground, either by the deliberate disposal of waste to land in the form of middens, the abandonment of archaeological settlements, or through other mechanisms of discard or loss.

Owing to the availability and tensile strength of wood, in particular oak wood, it is the primary material found on waterlogged/water saturated archaeological excavations (Cronyn 2001). Wood has been used in the construction of large structural objects for over 5,000 years in North-West Europe, e.g. the foundations for houses in the Lake Villages of Somerset (Coles & Coles 1986) and at Flag Fen, Cambridgeshire (Pryor 2001), as a means of fortification on the prehistoric landscape at Sutton Common, South Yorkshire (Parker-Pearson & Sydes 1997), numerous trackways which connect people and places together (Coles & Coles 1986, Rafferty 1990) and prehistoric water craft (Parfitt 1994, Wright 1990), to name but a few.

In more recent times discarded woody tissue has been buried purposely as part of municipal solid waste (refuse) within landfill sites. It has been estimated that refuse contains approximately 69 % by weight of paper, food waste and woody debris

(Tchobanoglous *et al.* 1993), all of which are biodegradable and can be biologically recycled (Das *et al.* 2002).

As such, and for different purposes, it is imperative to understand both the patterns and processes of wood decay, as the preservation of woody remains and the disposal of waste are vital to the management of wetland archaeological and landfill sites respectively.

1.2 Woody tissue decomposition in wetland archaeological and landfill sites

1.2.1 Wood as a 'biomarker'

This study will use oak wood as the complementary organic biomarker between wetland archaeological and landfill sites. The nature of oak wood enables it to be used as a representation of the biodegradable portion within refuse, whilst its availability from wetland archaeological sites enables samples to be obtained for further analysis. Consequently, it is possible to determine the respective patterns and processes responsible for the useful destruction of organic waste (degradation), as well as identifying the unwanted biological decay (deterioration) of artefacts (Allsopp & Seal 1986).

1.2.2 The interaction between wood and the burial environment

It is important to characterize the interaction between wood and the burial environment in order to determine the biological, chemical and physical patterns and processes responsible for degradation (Caple 2001). Although the majority of wood decay occurs at the interface between the wood and the environment, during the initial period of burial (Caple 2000), it will continue to penetrate towards the core of the

wood as interaction continues, until the object is completely transformed into minerals, carbon dioxide (CO_2) and methane (CH_4) (Caple & Dungworth 1995). This process is particularly important in the case of archaeological artefacts where the majority of the information about their formation and use is found at, or close to, the artefact's surface (Caple 2000).

To understand the patterns and processes responsible for oak wood degradation it is necessary to obtain samples with varying degrees of preservation, ranging from very well preserved samples showing only early signs of superficial degradation, to those with evidence for the near complete deterioration of the wood structure (Trofymow 1998), i.e. the loss of the different carbohydrate fractions within the wood (cellulose and hemicellulose) prior to the loss of the more resistant lignin fraction. Examples that best illustrate these processes can be found in burial environments of varying ages, i.e. in landfill sites where organic material can persist for a number of decades and wetland archaeological sites where organic material can survive for several thousand years. However, this is not to say that the older the material the greater the extent of degradation, as there are many other biological, chemical and physical factors within the burial environment which can significantly influence the speed of this process. These factors include temperature, pH, substrate availability, soil density and composition, moisture content, ion species, redox potential (Eh), concentration of organic molecules and microbiological activity (Caple 1994, Hobson 1988).

By using material obtained from wetland archaeological and landfill sites a pattern of degradation will be constructed over a known time span. The importance of the relationship between the length of time within a burial environment and the amount of degradation suffered by the wood will be assessed.

1.2.3 Wetland archaeological and landfill site management

The information contained within this thesis will not only be particularly significant to the disciplines of wetland archaeological science and landfill site management, but also it will be relevant to conservationists and environmentalists. The optimization of organic waste degradation is particularly important to landfill site managers, whilst the preservation of archaeological wood is essential for archaeological conservation and '*in situ*' management. Identifying the processes involved in wood degradation and the symbiotic interaction between wood in different burial environments can increase our understanding regarding the '*in situ*' stabilization of organic material and the bio-availability of the by-products of such processes.

1.3 Characterizing burial environments

Characterizing a burial environment is extremely complex and requires the measurement of a number of biological, chemical and physical parameters (as described in Section 1.2.2) (Caple 1996, 1994). It is essential to describe the relevant aspects of the burial environment in order to understand the processes responsible for wood degradation (Caple 1996). However, there is a lack of detailed knowledge concerning burial deposits, particularly those relating to both archaeological material and landfill waste. There has been little previous research on the specific characteristics of waterlogged sites, either in terms of the soil matrix and the composition of the groundwater, or the interaction between the decay of organic materials and the burial environment (Caple 1994, Caple & Dungworth 1995).

One of the primary aims of this thesis is to define the variability of both wetland archaeological and landfill sites by employing techniques that measure key biological,

chemical and physical parameters. In doing so, changes in the nature of a burial environment and its importance in the role of wood degradation can be determined.

1.4 Wetland archaeological sites

1.4.1 Spatial and temporal variability of wetland archaeological sites

Natural burial environments are spatially and temporally variable; with the existence of micro-environments within sediment producing patterns of small scale variation in environmental parameters and hence noisy data (see Section 1.2.2). Artefacts within such environments can be exposed to the atmosphere and surface weathering processes which can significantly affect both the nature and extent of degradation. In addition, artefacts may be buried in a variety of different environments which can be dry, occasionally or permanently saturated (Raiswell 2001). There are many differing natural burial environments rich in archaeological remains including salt marshes, marine sediments, paddy fields, mangrove swamps, estuarine sediments, freshwater sediments and peatlands (Caple 1996).

Finds from such contexts illustrate the rich complexity and diversity of the material culture of the past (Caple 2001), and can all contain artefacts made of wood, textile, skin and other organic materials. These objects provide an important opportunity for researchers to gain a greater understanding of the cultural, environmental and social aspects of past societies. Such remains are mainly preserved in frozen, desiccated or waterlogged anaerobic conditions where free oxygen is in short supply, the parameters responsible for decay are inhibited and organic materials survive in varying degrees of preservation (Caple 2001).

1.4.2 Preservation of archaeological remains

The preservation of organic materials such as wood is not only important in increasing our understanding of our cultural heritage (Caple 1994), but also it can help address a range of more specific archaeological questions, including the following (Cronyn 2001, 627):

> 'Can the absence or condition of material tell us something of its environment prior to and immediately after burial, or is this survival/loss more to do with the nature of the original material? What effect will excavation have on organic material? How can damage to the material be prevented whilst it is still being excavated, lifted, stored and examined? Can it be preserved '*in situ*'? How can it be preserved for long-term study and for meaningful display? Can close examination of this deteriorated material tell us something about its original appearance, use and manufacture?'

This study will provide information to help researchers address the above questions by characterizing both wetland archaeological and landfill burial environments in order to determine the patterns and processes responsible for wood degradation.

1.4.3 Patterns and processes responsible for wood degradation

The level of preservation of a wooden artefact controls the amount of information that can be inferred from it. Depending on the burial conditions acting upon the wood, deterioration can either occur within weeks of burial by the biological, chemical and physical factors responsible for the chemical and physical changes to the wood, or it can be delayed (Cronyn 2001).

The most common forms of wood decay are microbiological activity which is mediated by the reducing-oxidizing nature of the burial environment (redox potential [Eh]) and hydrolysis of the fibrous polymers inherent in organic materials which is associated with anaerobic conditions (Corfield forthcoming, Cronyn 2001).

Recent work has shown that the major cause in the deterioration of wood during burial is the activity of microorganisms (Blanchette *et al.* 1990). This is especially pertinent in the case of aerobic bacteria which can attack and consequently erode the cell walls even in low oxygen conditions (Blanchette *et al.* 1990, Blanchette & Hoffmann 1994, Nilsson 1999).

Upon excavation, wet archaeological wood can appear in good condition with little sign of deterioration (Blanchette *et al.* 1990, Cronyn 2001). However, even under highly preserving conditions, hydrolysis of the cellulose fibrils which compose the cell walls can occur, leaving only the original lignin remaining. This process reinforces and binds together the deteriorated skeletal walls whose dimensions are retained and bulked out by water from the burial environment (Cronyn 2001). Unfortunately, upon excavation, moisture loss often causes the cell walls to collapse and as a consequence the residual cellulose molecules shrink, destroying the original structure.

Redox potential is a major environmental discriminator in the survival of wet wood (Corfield forthcoming, Cronyn 2001). It is a measure of the electron availability in the soil (Corfield forthcoming), i.e. the oxidizing capacity (defined as the ability to accept electrons) or the reducing capacity (ability to donate electrons) of the soil (Raiswell 2001). The extent of abiotic deterioration which occurs in relation to redox potential is

still unclear (Hedges 1990). It is probable that there is hydrolysis of the cellulose and hemicellulose components of the cell walls which results in shortened polymers that may cause physical disruption to the structure of the wood (Hoffmann & Jones 1990).

1.4.4 Problems associated with the preservation of archaeological remains

The fundamental condition responsible for the preservation of organic remains is the existence of saturation, promoted by a high water table that serves to exclude atmospheric oxygen, thereby reducing the activity of the majority of microorganisms (Blanchette *et al.* 1990, Kenward & Hall 2000). Consequently, waterlogged organic material that is not normally preserved on dryland sites may account for between 75-90 % of all the material recorded from archaeological excavations (Coles 1984).

There is very little understanding of the way wetlands will react to human interference (Kenward & Hall 2000). In recent decades there has been a dramatic increase in drainage and water abstraction activities in and around wetland sites which has led to a drop in water levels in many areas (Hill 1976). A drop in water levels increases the aerobic nature of the once anaerobic environment. This promotes microbiological activity, bioturbation and consequently the degradation of the archaeological material (Brunning 1999). Water abstraction for agricultural, domestic or industrial consumption can lead to either a drop in water levels or changes in water chemistry. Many wetlands have been drained and converted into rich arable land, whilst other threats include mineral extraction, usually of underlying sand and gravel, industrial development and forestry (Caple 1996).

Since the 1970's there has been an increase in archaeological investigations aimed at preventing the degradation of waterlogged sites through drainage and water abstraction. However, this has led to an associated increase in the volume of archaeo-organic material recovered from waterlogged sites in Britain and has consequently created a need to identify the most appropriate way in which to deal with the vast quantity of material being produced through rescue excavations (Caple 1994).

1.4.5 Archaeological strategies and policies

To circumvent the problems associated with a drop in water levels, government guidelines prior to 1990, concerning the preservation of excavated archaeological material, highlighted the need for either conservation, storage in tanks and frozen storage or reburial of waterlogged archaeological remains (Caple 1994). However, as outlined below, each technique has major limitations:

1) Conservation - The shortage and expense of conserving the artefacts and the high costs of treatment either in terms of equipment, i.e. capital for freeze-drying, or in consumables, i.e. long-term polyethylene glycol (PEG) immersion treatments, result in only a limited amount of waterlogged wood being preserved (Caple 1994).
2) Storage in tanks - Although storing waterlogged wood in tanks above the ground has proved to be a satisfactory means of short-term storage, it has been suggested that long-term storage can be detrimental to the condition of the wood (Dawson *et al.* 1981). This method is also expensive in terms of the time and space needed in which to conserve the artefacts (Caple 1994).
3) Frozen storage - There remains uncertainty over the risk of disruption to the cell structures in wood from ice crystal formation (Cronyn 1990). This method is also expensive and requires a large capacity for storage (Caple 1994).

4) Reburial - This involves the excavated material being reburied in a new location in an anaerobic waterlogged environment similar to the one from which it was recovered (Caple 1994). Several experiments have been undertaken, but these have met with only limited success (Jeperson 1985, Lucas 1982).
5) Discard - Although the disposal of material after recording it fully or partially is the norm (Nayling 1989), this method deliberately destroys our national heritage (Caple 1994).

This consideration of the options currently available highlights the need for a new strategy to be implemented in order better to preserve our finite waterlogged archaeological wood archive/record. In response to this, in 1990, the Department of the Environment issued guidelines regarding the most appropriate approach to the management and preservation of Britain's buried archaeological remains by promoting preservation '*in situ*' rather than excavation (Caple 1994, Chapman & Cheetham 2002). These guidelines were implemented partly in response to the Department's inability to finance excavations of waterlogged sites owing to the high costs involved and also in response to the subsequent costs of conservation and storage of the objects and the vast data generated from such remains (Caple 1994).

Although these guidelines have been instrumental in the '*in situ*' preservation of high profile sites such as The Globe and Rose theatres, there is virtually no accurate data on the rate and degree of deterioration of archaeo-organic materials over a specific time period after remediation measures have been put in place (Kenward & Hall 2000). On the basis of this information, it is important to research the waterlogged anaerobic

soils in which organic materials are found in order to determine the optimum conditions responsible for preservation (Caple 1994).

Recent studies have focussed on the burial of fresh artefacts (primarily wood) into natural sites (Hogan *et al.* 2001, Lawson *et al.* 2000), combined with a period of post-environmental monitoring. This research is broadening our understanding of the burial environment and highlighting the conditions necessary for preservation '*in situ*' by comparing fresh wood samples to those from a variety of burial environments.

Among the primary aims of this thesis, in addition to the assessment of the rate of wood degradation over a specific time period under different environmental conditions, is an experimental study to artificially manipulate certain key environmental determinants in the burial environment in order to establish the optimum conditions necessary for preservation '*in situ*'. Particular attention will be given to the assessment of the two main factors responsible for wood decay; the oxidizing-reducing nature of the burial environment and microbiological activity.

1.5 Landfill sites

1.5.1 Spatial and temporal variability of landfill waste

Landfill sites are the main artificial burial environments in the world, providing the dominant disposal route for municipal solid waste in most developed countries (Barlaz 1997). Such sites frequently require large tracts of land, on or immediately beyond, the urban growth boundary of metropolitan areas (Zeiss & Atwater 1989). However, owing to differential settlement, leachate generation and landfill gas emissions which can continue for approximately thirty years after landfill completion, it is not possible

for the land to be developed for agricultural, domestic, or industrial use during this period (Wall & Zeiss 1995). As a result, such areas of land are left undeveloped.

The environmental, health and safety issues associated with landfill sites have intensified in recent years owing to the urban and suburban sprawl which has consumed vast tracts of land around metropolitan areas, consequently making it difficult to locate new landfill sites (Stessel & Murphy 1992).

1.5.2 Patterns and processes responsible for refuse decomposition

Although humans have for centuries deliberately disposed of waste to land in the form of middens, it is only in the last twenty to thirty years that research has been undertaken into the various aspects of the patterns and processes responsible for refuse decomposition (Fletcher 1991). This research has been achieved by using both experimental laboratory-scale (Barlaz *et al.* 1989a, 1989b, Delbrès *et al.* 1998, Stessel & Murphy 1992, Zumstein *et al.* 2000) and field study lysimeters (Collins & Spillmann 1982, Ham & Bookter 1982, Read & Hudgins 2000) to replicate the burial conditions within '*in situ*' landfill sites (as described in Section 1.6).

A great deal of early research considered the waste in landfills as a 'black box', in which changes made to the burial conditions and the subsequent effects, in terms of rate of degradation, gas production and leachate quality, were observed without considering the mechanisms involved in the degradation process (Fletcher 1991).

More recent research undertaken on a variety of scales, from small laboratory experiments (Barlaz *et al.* 1989a, 1989b, Delbrès *et al.* 1998, Ding *et al.* 2001), to large experimental lysimeters containing many tons of waste (Collins & Spillmann

1982, Ham & Bookter 1982), have begun to investigate this degradation process. This research usually provides far more rapid results than manipulation of landfill sites alone (Barlaz 1997). These studies have examined the effects of moisture content, temperature, pH, nutrient and air addition, and concentration of various organic and heavy metal contaminants upon the rate of degradation.

More targeted research has focussed on the actual patterns and processes of degradation, in particular what happens at the microbiological level (Hale Boothe *et al.* 2001). By understanding microbiological physiology and ecology it is possible to influence methane production, increase decomposition rates and achieve faster stabilization of the organic matrix (Barlaz *et al.* 1989b, Cummings & Stewart 1994).

1.5.3 Problems associated with the decomposition of refuse

The disposal of organic-rich waste to land and its subsequent degradation has progressed over the centuries from open, indiscriminate dumping to what is now carefully controlled, engineered, sanitary landfilling. Nevertheless, despite the technical advances in landfill design, there are still fundamental problems associated with containing decomposition within a hole in the ground.

The earliest types of landfills were designed to overcome health risks associated with problems of fly and rat infestations and were constructed through the consecutive open dumping of waste materials to create a large deposit (Fletcher 1991). Heavier and more inert materials were placed upon the deposit at the end of each working day to minimize contamination. However, concerns were raised regarding environmental contamination by leachate migration off-site and gas generation (Fletcher 1991, Ham & Bookter 1982).

Although the generation of methane from landfills is a major atmospheric contaminant, leachate is considered to be the main pollutant to the burial environment from a landfill as it has much higher concentrations of organic matter and toxic substances such as metals, than domestic wastewater (Ding *et al.* 2001). In response to such problems, landfill companies have adopted a 'dry entombment' system, which minimizes the volume of water entering the waste that is deposited, consequently reducing the volume of the leachate produced and limiting the potential for groundwater pollution (Read *et al.* 2001).

Unfortunately, the 'dry entombment' of landfill waste adversely increases the potential risks to human health and the environment (Onay & Pohland 1998, Read *et al.* 2001), by increasing the toxicity of leachate (Loizidou & Kapetanios 1993), lengthening the amount of time required to stabilize the materials (Rathje & Murphy 1992) and increasing the potential for fugitive methane contributing to the global greenhouse effect (Das *et al.* 2002). Although this landfilling technique is used as a standard practice in today's waste management, it can only be a temporary solution to the problems regarding the reduction of toxic contaminants into the environment, as both liner and leachate collection systems ultimately fail over time owing to natural degradation (Das *et al.* 2002). To exacerbate the situation further, this technique does not promote waste decomposition, and as a result landfill owners find themselves using a design that will incur increasing costs and long-term environmental liabilities (Read *et al.* 2001).

1.5.4 Landfill processes and engineering policies

In response to the problems associated with refuse decomposition new techniques are currently being utilized to address environmental and health concerns through the

acceleration of degradation in landfills. The potential benefits of these new techniques are as follows (Stessel & Murphy 1992, 486):

> 'Land could be perpetually re-used, with little need to acquire new land; a humic material could be recovered for soil improvement, as with composting; considerable volume reduction could be achieved; recyclable materials not otherwise recovered or recoverable could be extracted; the lining system could be repeatedly inspected after mining to ensure integrity; and the coincident treatment of leachate before discharge'.

Accelerated degradation is achieved by running the landfill aerobically, recycling leachate and controlling moisture. Previous investigations using aerobic leachate recirculation applications have demonstrated that the aerobic degradation of landfill waste can provide significant advantages over anaerobic approaches (Heyer *et al.* 1999, Leikam & Heyer 1997). The recirculation of leachate combined with the injection of air through the waste mass has not only been shown to increase the rate of waste decomposition (Das *et al.* 2002, Stessel & Murphy 1992), but also to reduce levels of certain organic toxins in the leachate (Read *et al.* 2001).

The aerobic landfill approach could not only lead to significant long-term cost savings in post-closure maintenance costs (Yuen *et al.* 1999) and liability reduction for many landfills (Read & Hudgins 2000), but also reduce potential environmental problems by stabilizing and re-utilizing the volume of the waste mass (Das *et al.* 2002). One would also expect a significant reduction in methane production within the waste mass of the landfill (Yuen *et al.* 1999). Landfills with leachate re-circulation could, therefore, be

operated as municipal solid waste bioreactor treatment systems rather than as conventional waste dumping sites (San & Onay 2001).

Although we may be some way from completely understanding all the subtle interactions between the different biological, chemical and physical processes that are involved in the degradation of waste, we are now developing a clearer picture concerning many of the factors that affect the rate of degradation (Fletcher 1991). Degradation in landfills is a four-stage process by which solid organic particles are solubilized by polymer hydrolysis and converted through acidogenesis, acetogenesis and methanogenesis to carbon dioxide (CO_2) and methane (CH_4) (AFRC Institute of Food Research 1988, Barlaz *et al.* 1989a, 1989b, Wall & Zeiss 1995). As the majority of the carbon sources in landfill waste (cellulose and hemicellulose) are insoluble, the main decomposition process is governed by hydrolysis (Halvadakis *et al.* 1983). By using oak wood as the organic biomarker to determine the patterns and processes of degradation within landfill sites our understanding regarding the optimum conditions necessary for waste decomposition will improve.

Determining the biological, chemical and physical factors responsible for waste decomposition in a laboratory-scale simulation may lead to future research on full-scale landfill sites. In doing so it may be possible to minimize environmental contamination of the land and groundwater supplies, whilst rewarding the landfill owners financially by post-productive use of the land sooner than was previously envisaged.

1.6 Lysimeter studies

1.6.1 Degradation and preservation of wood

A major aim of this thesis is to develop a laboratory-scale simulation, i.e. lysimeter study of natural wood degradation over a period of two years to determine the patterns and processes affecting wood decay during early diagenesis within wetland archaeological and landfill sites. It is essential that these experiments are undertaken in order to enhance our understanding of two pairs of related processes: the relationship between decay rates of organic materials and the pathways they follow *en route* to the burial environment (AFRC Institute of Food Research 1988); and the link between burial conditions and subsequent in-ground degradation (Kenward & Hall 2000).

The lysimeter study acts as a bridge between two different disciplines, wetland archaeological science and landfill waste management. Although both fields of study have contrasting agendas, i.e. the stabilization of conditions responsible for wood preservation in wetland archaeological sites (Caple 2001, 1996, Corfield 1996) and the optimization of organic waste degradation in landfill sites (Stessel & Murphy 1992), the information gained from the lysimeters can, by their very nature, be mutually beneficial.

1.6.2 Manipulation of parameters for characterizing the nature of burial environments

A number of different parameters which define all burial environments will be artificially manipulated within the lysimeters in order to determine the effect that they have on the patterns and processes of wood degradation. Particular consideration is given to the influence of redox potential and microbiological activity. A change in the

redox potential of a soil will directly alter microbiological activity (and vice-versa) (Cronyn 2001), which in turn will determine the potential rate of organic degradation (Embley & Widdick 1991).

There are two major types of bacteria, aerobes that require oxygen for their metabolism, and anaerobes which do not. Anaerobes can be sub-divided into facultative and obligate anaerobes. Facultative anaerobes can grow either in the presence of oxygen or without it, while obligate anaerobes can only grow where there is an absence of oxygen (Corfield forthcoming). Positive redox values (which are indicative of oxidizing conditions) will cause an increase in aerobic bacterial activity and promote greater potential for degradation, whilst negative values (indicative of reducing conditions) will increase obligate anaerobic activity, but inhibit degradation.

1.6.3 Changes in diagenesis over time

The lysimeter study will help determine the patterns and processes responsible for the initial stages of wood decay in the short-term (over a two-year period), particularly any primary loss of the main carbohydrate fractions (cellulose and hemicellulose). This information can then be used in conjunction with wood and soil samples taken from artificial and natural environments of known ages to elucidate a profile of wood degradation in the short- (lysimeters), medium- (landfill sites) and long-term (wetland archaeological sites). The lysimeter study can therefore highlight whether the main phase of wood decay occurs within a short period after deposition (Kenward & Hall 2000, Trofymow *et al.* 1995). The significance placed upon the particular conditions within the burial environment can also be better understood by characterizing differing environmental media and establishing how fast organic materials decay in different burial environments (Kenward & Hall 2000).

1.7 Aims and Objectives

In relation to the key issues outlined throughout this chapter, the aims and objectives of this thesis are as follows:

1) To characterize wetland archaeological and landfill sites by describing their main biological, chemical and physical characteristics
2) To manipulate key parameters of wetland archaeological and landfill sites under the controlled conditions within the lysimeter study
3) To assess the extent of oak wood degradation in both the lysimeter study and wetland archaeological and landfill sites
4) To elucidate a profile of wood degradation in the short-, medium- and long-term by using the information gained from the lysimeter study and wetland archaeological and landfill sites
5) To determine the patterns and processes responsible for oak wood degradation in different environments
6) To determine the most appropriate environmental conditions that will help promote the optimum conditions responsible for wood preservation in wetland archaeological sites and that will optimize organic waste degradation in landfill sites

1.8 Thesis structure

The thesis contains 8 chapters, with this chapter forming the first. Chapter 2 outlines the methodologies which will be used to characterize the differing burial environments from a biological, chemical and physical perspective. Chapter 3 outlines the methodology which will be used to determine the extent of oak wood degradation.

Chapters 4, 5 and 6 will present the results of the study. Chapter 4 presents the chemical and physical results obtained during the monitoring of the lysimeters. Chapter 5 presents the biological results obtained from both the lysimeter study and additional '*in situ*' wetland archaeological and landfill sites. Chapter 6 presents the results obtained from the assessment of oak wood degradation in samples from the lysimeter study and the wetland archaeological and landfill sites.

The penultimate chapter (7) considers the main observations drawn from the results chapters. The significant conclusions which have been identified during the study are presented in Chapter 8.

1.9 Summary

This chapter has outlined the rationale behind the utilization of oak wood as the complementary organic biomarker used to assess the deterioration of wooden artefacts/structures in wetland archaeological sites and the decomposition of organic refuse in landfill sites. It not only highlights the importance of defining the interaction between the rate of wood decay and its relationship with the biological, chemical and physical characteristics of burial environments, but also its significance in relation to different time scales in the short-, medium- and long-term.

The lysimeter study will act as an artificial representation aimed at optimizing organic waste decomposition in landfill sites and assessing the parameters needed to stabilize the burial environment in order to promote wood preservation in wetland archaeological sites. The study can be used to manipulate the key biological, chemical and physical parameters which define all burial environments and highlight the effect that these have on the patterns and processes of wood degradation.

The oxidizing/reducing nature of the lysimeters is of fundamental importance when assessing the '*in situ*' burial environments chosen for this study. Wetland archaeological policies necessitate the identification of the optimal conditions for the stabilization and preservation of archaeological remains (English Heritage 1991), in particular wood, whilst landfill engineering policies require the optimization of organic putrefaction, with minimal environmental contamination (Environment Agency 2000).

This thesis will define contrasting '*in situ*' burial environments by employing techniques that measure key biological, chemical and physical parameters and use lysimeters to manipulate these parameters, thereby enabling the optimum environmental conditions to be determined for both the preservation and the degradation of oak wood.

Chapter 2 Methods I

2.1 Introduction

The aim of this chapter is to outline the multi-disciplinary approach used to assess the main biological, chemical and physical processes which characterize the different types of burial environments investigated during this study. Using this approach enables a greater appreciation of the conditions necessary for the preservation of organic materials (Cheetham 2004). This has previously been demonstrated in studies by Brunning and co-workers (2000), Caple (2000, 1996, 1993), Caple & Dungworth (1995) and Cheetham (2004) who used multi-disciplinary approaches to characterize the nature of sediments in wetland archaeological sites in order to determine the conditions responsible for wood preservation. In addition to these investigations, other research has also employed similar approaches to optimize organic waste degradation in landfill environments (e.g. Barlaz 1997, Barlaz *et al.* 1989a, 1989b, Ding *et al.* 2001, Ham & Bookter 1982, McKinley & Vestal 1985, Stessel & Murphy 1992).

The location and context of the differing wetland archaeological and landfill sites chosen for analysis during this study are presented in Section 2.2. The construction, development and monitoring of the twelve lysimeters used in the lysimeter study, are outlined in Section 2.3.

The penultimate sections (2.4 and 2.5) discuss the biological, chemical and physical methods developed in the lysimeter study which are designed to replicate and ultimately characterize the patterns and processes occurring within '*in situ*' wetland archaeological and landfill sites. A critical assessment of the methods used during this study has also been undertaken in order to determine how improvements can be made

to enhance further the accuracy of the monitoring of the sediments contained in the lysimeters.

2.2 Selected wetland archaeological and landfill sites chosen for research

Although the financial and time constraints associated with the monitoring of '*in situ*' burial environments prevented the chemical and physical assessment of the wetland archaeological and landfill sites chosen during this study, it was still possible to obtain sediment and wood samples for biological assessment.

2.2.1 Wetland archaeological sites

Sediment and wood samples were excavated from a variety of wetland archaeological sites where deposition had occurred during different periods in the past. The oldest sites chosen for sampling included the Bronze Age wooden platform located in clays and peat at Flag Fen, Cambridgeshire and an Iron Age enclosure in peat, with some alluvial and sand outcrops, at Sutton Common, South Yorkshire. More recent sites included a Medieval log boat in alluvial sands and silts from Nigtevecht, the Netherlands and an inter-tidal Saxon wooden fish trap in alluvial sands and clays, recovered from Rolls Farm, Essex.

2.2.2 Landfill sites

Unfortunately, owing to the stringent regulations that exist for landfill site development and post-closure, the only site where sampling was permitted was the Woldgate Former Landfill Site at Bridlington, East Yorkshire. This site is a former landfill that covers an area of approximately 12 ha. The depth of the waste material within this landfill is 12-13 m (Johnson & Jackson 1999).

2.3 Lysimeter studies

2.3.1 Philosophy behind combining field and laboratory experiments

Lysimeters are self-contained cells that allow modelling of various environments through the control of a number of different biological, chemical and physical parameters (Stessel & Murphy 1992). Generally, lysimeters act as smaller representations of conditions that occur naturally in various burial environments. Because of their size, it is more convenient to undertake extensive monitoring of the different parameters in lysimeters instead of monitoring conditions previously determined '*in situ*' (Das *et al.* 2002, Hale Boothe *et al.* 2001). In addition, it is also possible to conduct a range of duplicate lysimeter studies over various time scales and replicating a range of burial environments (Barlaz *et al.* 1989a, 1989b, Westlake *et al.* 1992), a procedure that is not feasible with '*in situ*' environments (Stessel & Murphy 1992).

Complementary to the use of laboratory-scale lysimeters, data obtained from wetland archaeological and landfill sites can add a further dimension to the investigation of the patterns and processes responsible for wood degradation in differing burial contexts. This can be achieved through the assessment of sediment and wood samples of different ages. By combining the results from the lysimeter study with data collected from the '*in situ*' sites, it is not only possible to determine the effectiveness of using laboratory experiments to act as indicators of '*in situ*' burial environments, but also to provide greater insights into the diagenetic factors responsible for wood decay in the short-, medium- and long-term.

2.3.2 Spatial and temporal variability of lysimeters

The patterns and processes responsible for the decomposition of organic matter have been investigated by other authors using lysimeters of varying shapes and sizes. Large-scale studies of waste decomposition have been undertaken in 'test cells' which are almost 'mini-landfill' sites in their own right, owing to their sheer size (Read *et al.* 2001), whilst other smaller test cells (which are capable of containing 100-200 tons of waste) have also been developed and studied (Collins & Spillmann 1982, Ham & Bookter 1982).

Most lysimeter studies tend to be on a far smaller scale than the above examples. Several replicates are generally constructed, where numerous environmental conditions are artificially manipulated in order to promote the optimum conditions necessary for the microbiological decomposition of the organic portion of the waste (Barlaz *et al.* 1989a, Delbrès *et al.* 1998, Ding *et al.* 2001, Stessel & Murphy 1992). The most commonly manipulated conditions in lysimeter studies are aeration (Stessel & Murphy 1992), leachate circulation (San & Onay 2001) and length of monitoring (Barlaz *et al.* 1989a, 1989b, Collins & Spillmann 1982, Ham & Bookter 1982, Stessel & Murphy 1992, Zumstein *et al.* 2000). Lysimeters of this scale are far easier to develop, monitor and manipulate. However, because the lysimeters represent scaled-down versions of full-scale sites, the information gained cannot be used as a direct comparison, even when similar conditions are imposed. Nevertheless, lysimeters can still provide useful baseline information concerning the patterns and processes responsible for organic decomposition.

2.3.3 Key criteria in lysimeter research

On the basis of previous investigations it is apparent that a lysimeter study must take into account a number of key criteria which are determined by the aims and objectives of the research being undertaken. The main parameters that need to be addressed during the design stage of the study are as follows:

1) The size and shape of the lysimeter
2) The length of time required for operation and monitoring
3) The type and frequency of monitoring
4) The environmental parameters which need to be assessed in order to determine the primary objective of the study, i.e. the optimization of wood decomposition
5) The number of replicate lysimeters needed to produce a reliable data set

2.3.4 Monitoring and design of lysimeters

The development of the lysimeters used in the current study encompasses the key criteria outlined above. The aims and objectives of the lysimeter study are as follows:

1) To replicate the conditions within '*in situ*' wetland archaeological and landfill sites
2) To manipulate water/leachate levels and the anoxic/aerobic nature of the burial environment
3) To determine the optimum conditions responsible for the preservation and degradation of oak wood over a two-year period

With the aim of fulfilling the objectives highlighted above, twelve replicate lysimeters were constructed (as shown in Figures 2.1 and 2.2). The structure of each lysimeter consisted of a white polyvinylchloride (PVC) home fermenting container (approximate dimensions 0.52 m height x 0.30 m diameter). Graduations at 0.12 m

intervals from the base towards the top provide three distinct levels. These graduations act as the visible boundaries between the three zones of saturation present within the container. The three zones are based on the degree of saturation of the sediment and are as follows: zone of aeration (1), zone of fluctuation (2) and zone of permanent saturation (3) (*cf.* Chapman & Cheetham 2002). Each zone is characterized by the degree of deterioration of wood structures within a burial environment and is designed to replicate the height of the water table within '*in situ*' environments. The zones range from almost total deterioration of the wood structure (1), to fully saturated conditions exhibiting good preservation (3).

Three white PVC taps were attached to the outside of the container at 0.12 m intervals to coincide with the boundaries between the different zones. Epoxy resin was used to seal the taps onto the container. Each tap contained a plastic mesh fitted within it to prevent any sediment escaping during a drop in the water/leachate levels. A washed gravel base, to a height of 0.03 m, aided the flow of water/leachate through the system.

A length of clear, flexible, PVC tubing (0.50 m length x 0.015 m diameter) which was fitted onto the outside of each lysimeter by the attachment and subsequent inversion of a white PVC tap, acted as the manometer. The manometer was used to monitor changes in water/leachate levels. Epoxy resin was again used to seal the tap onto the container. A metal bracket held the tubing in place at the top of the container.

The recirculation of water/leachate back into the lysimeter by means of a sprinkler increased the height of the water/leachate level every six months. This ensured an even distribution of water percolation and absorption through the sediment. The drop

in water/leachate levels defined a specific zone of saturation, i.e. water levels remained high in the zone of aeration (1) for a period of 1 week, high in the zone of fluctuation (2) for a period of 18 weeks and high in the zone of saturation (3) throughout the duration of the experiment.

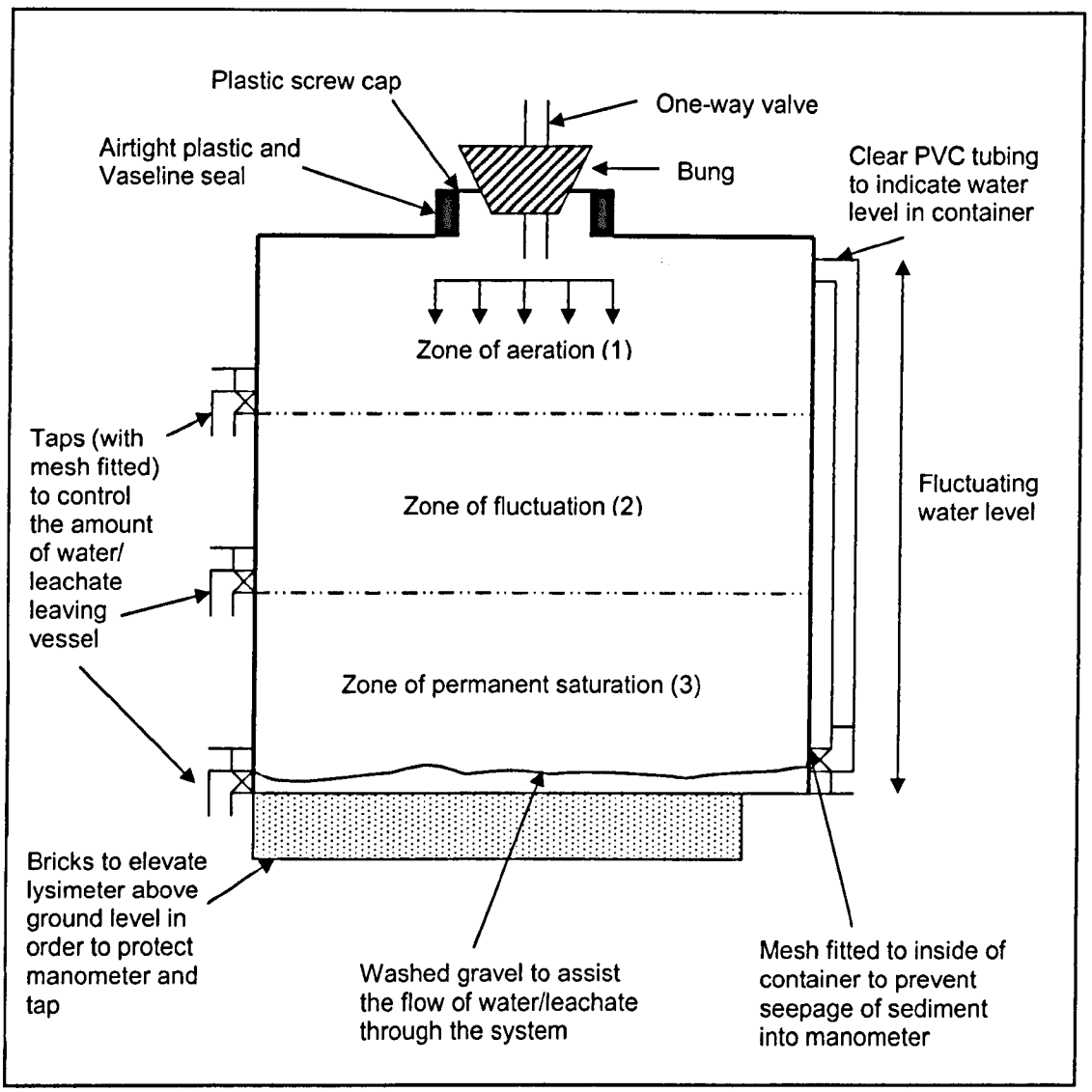

Figure 2.1: Diagram of a lysimeter (as used in the present study).

Each lysimeter contained a combination of peat and water, peat and leachate, and/or waste and leachate, depending upon the aims and objectives of the study. Table 2.1 shows the sediment type and environmental conditions imposed within each lysimeter.

Figure 2.2: Example of a lysimeter. The dashed lines on the side of the container show the level of the wood samples in the sediment.

Table 2.1: Sediment type and environmental conditions imposed within each lysimeter.

Lysimeter	Sediment type	Liquid medium	Liquid levels	Duration
1	Peat	Water	Saturated	2 years
2	Peat	Water	Fluctuating	1 year
3	Peat	Water	Fluctuating	2 years
4	Peat	Leachate	Fluctuating	2 years
5	Waste	Leachate	Fluctuating	6 months
6	Waste	Leachate	Fluctuating	1 year
7	Waste	Leachate	Fluctuating	1.5 years
8	Waste	Leachate	Fluctuating	2 years
9	Waste	Leachate	Saturated	6 months
10	Waste	Leachate	Saturated	1 year
11	Waste	Leachate	Saturated	1.5 years
12	Waste	Leachate	Saturated	2 years

Peat sampling took place from an area of Thorne and Hatfield Moors, South Yorkshire where peat cutting was currently in progress. Household waste sampling took place

from recently deposited material at the Roxby Landfill Site, East Yorkshire. Within each lysimeter, several freshly cut oak sapwood blocks from the same tree (dimensions 2 cm^3) were placed into the sediment and were located in the centre of each zone of saturation. A screw-top lid, airtight plastic seal, rubber bung and one-way valve prevented excessive oxygen diffusion into the sediment within the permanently saturated lysimeters. The remaining aerobic lysimeters which were manipulated by water/leachate levels did not require sealing.

2.4 Characterizing wetland archaeological and landfill sites by physico-chemical measurements

Differing burial environments can be characterized by using a number of biological, chemical and physical measurements (Caple 1996); these include soil hydrology (which relates to water table dynamics), soil chemistry (which relates to the measurement of redox potentials and pH within the soil profile) and microbiological assessment of the soil profile (Caple 1994, Cheetham 2004, Hobson 1988).

This section will present the chemical and physical measurements used to characterize the sediments in the lysimeter study. It includes the field methods, as well as the laboratory techniques and analysis.

2.4.1 pH

The value of pH is as a measure of the hydrogen ion concentration of a substance or system and it can provide an indication of the acidity or alkalinity of a soil (Corfield forthcoming). It is dependent upon a number of parameters which include soil composition, water levels, redox potential, microbiological activity and the level of organic matter within the soil (Caple 1994).

The value of pH measurement has been determined in a variety of natural burial environments containing organic material, including peat litter (Bergman *et al.* 2000), *Sphagnum* from peat bogs (Dedysh *et al.* 1998) and a variety of soils containing organic archaeological remains (Caple 1996, 1992, Caple *et al.* 1997). The latter environments have established a clear correlation between pH and redox potential values which can act as indicators of organic preservation (Caple 1993, Caple *et al.* 1997, Corfield forthcoming). Consequently, by characterizing archaeological sites, more information can be generated to assist in the preservation of archaeological remains.

The value of pH has also been determined in lysimeter studies containing landfill waste, with the results confirming the close correlation that exists between changes in pH values and organic material degradation (Barlaz 1997, Barlaz *et al.* 1989a, Ham & Bookter 1982, McKinley & Vestal 1985, San & Onay 2001, Stessel & Murphy 1992).

2.4.1.1 Data acquisition

The pH measurement of the differing sediments and water/leachate was undertaken independently. Sediment pH was measured by using a temperature compensated Sentix 21 combination electrode TFK 325/HC temperature sensor attached to a pH/mV-meter (Wissenschaftlich-Technische Werkstatten GmbH). Water/leachate pH was analyzed by a Watercheck™ portable pH and conductivity meter (Hanna Instruments). Water/leachate pH measurements were undertaken owing to concerns surrounding the potential poisoning of the Sentix 21 combination electrode by metal ions in the waste/leachate, which subsequently necessitated the use of a more durable and cheaper alternative pH measurement. Both sets of equipment were used in accordance with the manufacturers' instructions.

Sediment pH was measured fortnightly and in conjunction with redox monitoring, as the pH of the sediment has a direct influence upon the redox system (Cheetham 2004). The pH was obtained by taking a small peat sample from the surface of each lysimeter and agitating the sediment in deionised water. The pH electrode was placed into the mixture and wired to the pH/mV-meter. Readings were taken when equilibrium had been attained. Water/leachate pH was measured prior to a manipulation in water/leachate levels.

2.4.2 Water levels

Previous studies have shown that the degree of saturation within the burial environment is the primary factor responsible for the preservation of organic material (Brunning *et al.* 2000, Chapman & Cheetham 2002, Cheetham 2004, Hogan *et al.* 2001, Powell *et al.* 2001).

The most efficient means of measuring the degree of saturation in natural environments is by using piezometers. These are purpose-built polyvinylchloride (PVC) tubes with tips which are installed into holes that have been augered down to the required depth in order to assess the level of the water table (and as a consequence the saturation of the soil) (Brunning *et al.* 2000, Chapman & Cheetham 2002, Hogan *et al.* 2001).

Although there is currently no information to the author's knowledge regarding the use of piezometers for measuring the level of leachate saturation in landfill sites, the moisture content of the waste in a number of lysimeter studies has been assessed in order to investigate the relationship between the various decomposing processes inside

landfills (Barlaz *et al.* 1989a, 1989b, Collins & Spillmann 1982, McKinley & Vestal 1985, Stessel & Murphy 1992).

2.4.2.1 Data acquisition

In order to prevent any potential disturbance of the sediment which may be created by inserting piezometers into a small-scale lysimeter study, the water/leachate levels in all the aerobic lysimeters were confirmed using a manometer attached to the outside of each lysimeter. The levels were measured at fortnightly intervals. Measurements were also obtained from the lysimeters which were permanently saturated with water/leachate, over a similar time scale. This was undertaken in order to determine any drop in level which may result from the pH sampling using the taps.

2.4.3 Redox potential

The redox potential of sediment is a semi-quantitative measure of the oxidation-reduction status of the burial environment (Cheetham 2004) and is probably the single most important variable influencing the rate of organic degradation (Caple & Dungworth 1995). As a corollary, it can identify burial environments where organic materials may be preserved (Caple & Dungworth 1995).

Owing to the continuing policies aimed at the preservation of archaeological sites '*in situ*' it became necessary to devise a means with which to monitor the chemical balance of such environments. Consequently, over the last decade, there have been extensive investigations into the chemical and physical limits of natural burial environments in which archaeological organic remains are preserved, in order to determine the optimum reducing conditions necessary for preservation (between –110

mV and –400 mV) (Brunning *et al.* 2000, Caple 1996, 1993, Caple & Dungworth 1995, Caple *et al.* 1997, Cheetham 2004).

In contrast to the many investigations which have been undertaken to measure redox potential in natural burial environments, the measurement of redox potential in landfill sites is extremely limited (Ding *et al.* 2001, San & Onay 2001). However, as an alternative to this method, research has been carried out on the dissolved oxygen of leachate produced from landfill waste in order to determine the optimum conditions required for organic waste degradation (as described in Section 2.4.4) (Barlaz 1997, Barlaz *et al.* 1989a, 1989b, Stessel & Murphy 1992).

Redox potential is a measure of the electron availability in the sediment, and therefore its oxidation or reduction characteristics (Corfield forthcoming). It can determine the oxidizing-reducing nature of the environment over a number of years without a reduction in accuracy (Veneman & Pickering 1983). The concentrations of electrons present in solutions control the redox potential reactions in the burial environment. The measurement of these electrons in a sample can determine the electrode potential that develops when incorporated into an electrochemical cell (Howard 1998). The most common measure of redox activity is Eh, the electrode potential measured against the hydrogen electrode. The unit of Eh is the volt, which is measured at specific points using permanently installed platinum electrodes and a portable voltmeter.

The standard classification used to define the redox status of soils originated from the research by Patrick & Mahapatra (1968) for well-drained and waterlogged soils during studies of rice production. Table 2.2 presents the categories of redox potential.

Table 2.2: Categories of redox potential (derived from Patrick & Mahapatra 1968).

Redox potential (mV)	Category
>+400	Oxidized
+100 to +400	Moderately reduced
–100 to +100	Reduced
–300 to –100	Highly reduced

Numerous subsequent studies have used these standard categories in order to measure the redox potential of burial environments (Brunning *et al.* 2000, Caple 1996, 1993, Caple & Dungworth 1995, Cheetham 2004, Hogan *et al.* 2001). It is on this basis that the same scheme will be employed to describe the redox results obtained from the lysimeter study.

It is acknowledged that the categories of redox potential shown in Table 2.2 can only serve as a generic indicator of the type of conditions occurring within a variety of different soils, owing to the use of Eh creating unavoidable inaccuracies associated with the measurement of mixed potentials within the burial environment. The inherent complexities concerning the low concentration of redox couples within oxidized environments (Bohn 1971) and the measurement of mixed potentials in water, makes thermodynamic interpretation difficult (Stumm & Morgan 1981). Nevertheless, redox potentials do provide boundary conditions, i.e. the natural limits of redox in water (Bohn 1971) and are suitable as a semi-quantitative measure of soil reduction (Ponnamperuma 1972) when taken from anoxic and aerobic environments (Caple & Dungworth 1995).

2.4.3.1 Probe construction

Construction of the redox probes used during this study follows the design presented by Faulkner and co-workers (1989) for the 'welded' type of probe. Each probe

comprised a tip of 0.5 mm gauge platinum fused to a copper terminal wire; with the connection waterproofed by a sheath of shrink fit sleeving. At the opposite end of the wire, a short length of copper was exposed, where an additional band of sleeving was applied to prevent water penetrating the inside of the wiring sheath.

Hunter's Dale, Berkshire constructed the redox probes used during this study to a 5 % tolerance. The construction was based upon previous research undertaken at Royal Holloway Institute for Environmental Research (Hogan *et al.* 2001). The tolerance of a redox probe is dependent upon the reliability of the readings obtained when immersed in a redox buffer solution (10.211 g of potassium hydrogen phthalate in 1 litre of deionised water [previously saturated with quinhydrone] at a potential of +218 mV) (Cheetham 2004). Any probes showing readings that were ≥5 % of +218 mV were rejected.

2.4.3.2 Probe installation

A small diameter electric drill was employed to puncture the top of each lysimeter to enable the insertion of redox probes into the sediment. An auger was inserted through this hole in order to remove the sediment in each lysimeter down to the required depth in preparation for the insertion of each probe. The probes were pushed down into position within the sediment until a good contact was made between the platinum tip and the undisturbed matrix. The probes were left in place for the duration of the lysimeter study. Approximately 0.10 m of wire was left protruding above the sediment surface to enable readings to be taken from the outside of the lysimeter. The drill holes in the top of the lysimeter were sealed with white waterproof sealant in order to secure the probes in place (hence ensuring a good contact with the sediment) and to prevent exposure of the sediment to the elements. Probe clusters were installed at three depths

(0.08 m, 0.20 m and 0.32 m) in order to measure the zones of saturation within each lysimeter. Three to four replicates were installed at each depth depending upon the availability of spare probes. Figure 2.3 shows the implementation of the redox equipment within a lysimeter.

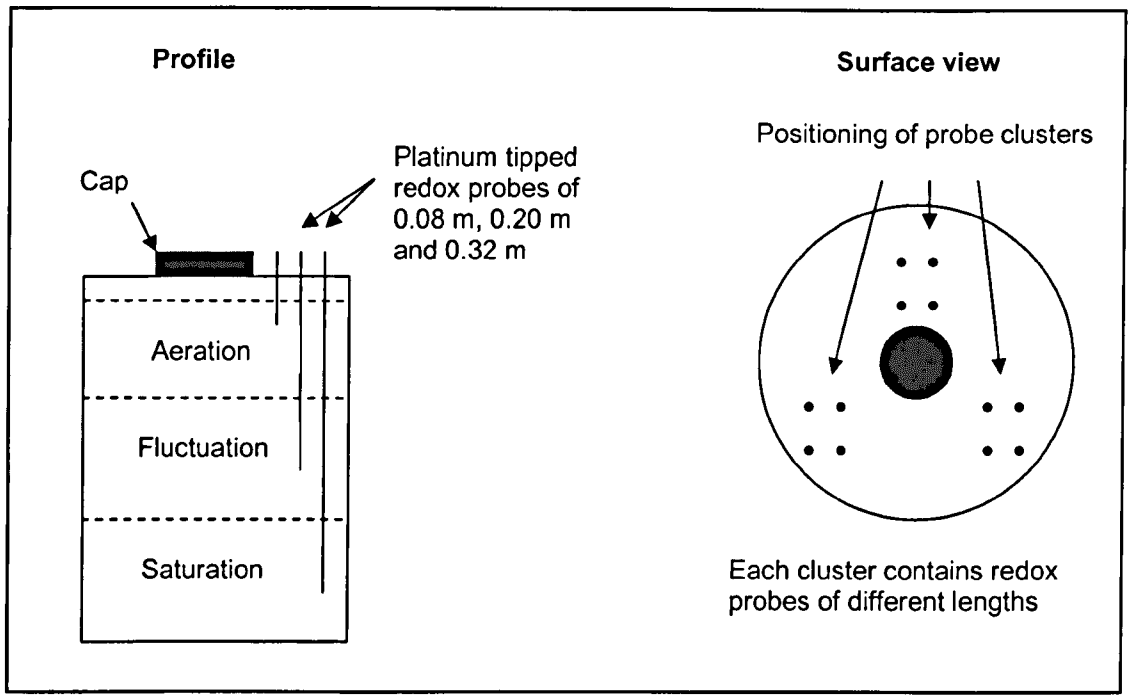

Figure 2.3: Diagram showing the implementation of redox equipment within a lysimeter.

Only Lysimeters 1, 2 and 3 had probes inserted into them for the duration of the experiment. This was primarily due to concerns regarding the potential contamination of the reference electrode when inserting it into the lysimeters which contained landfill and/or leachate, consequently increasing the potential for erroneous readings. However, the construction of five salt bridges following the method of Veneman & Pickering (1983), six months after the project was initiated, enabled redox probes to be installed in all of the remaining lysimeters without risk of contamination to the reference electrode.

2.4.3.3 Salt bridge construction

The salt bridges were constructed using 1" diameter polyvinylchloride (PVC) pipe, agar, potassium chloride (KCl) and deionised water. Two holes were drilled into the PVC pipe approximately 0.05 m from the tip to allow the agar to come into contact with the sediment. The bottom of the PVC pipe was sealed using a rubber bung and epoxy resin. 25 g agar and 250 ml KCl solution were mixed with 1 litre of boiling deionised water. The mixture was allowed to cool until a viscous gel formed. The gel was poured into the PVC tubes until they were full and then allowed to cool completely.

The salt bridges were installed in the sediment of the lysimeters using a 1" auger to a depth of 0.25 m from the sediment surface. Four salt bridges were installed in Lysimeters 3, 6, 7 and 8 for the duration of the experiment. In order to maintain the anaerobic status in Lysimeters 10-12, the remaining salt bridge was only inserted to obtain redox readings.

Redox readings in Lysimeter 3 were taken twice; one set of readings was taken with the salt bridge in place, whilst the other set of readings was taken by the standard procedure described below. Both sets of readings were compared to assess the accuracy of the salt bridge over the duration of the experiment. The results are shown in Appendix 1.

2.4.3.4 Data acquisition

The redox probes were left for a period of two weeks in order to establish equilibrium within the burial environment before monitoring began (Caple 1996, Cheetham *pers comm.* 2001). Redox readings were taken fortnightly using a portable pH/mV-meter

(Wissenschaftlich-Technische Werkstatten GmbH) connected to a Silver Chloride (AgCl) double junction reference probe (BDH Gelpas) and to the '*in situ*' probes by means of a clip (as shown in Figure 2.4). The reference electrode was inserted into a shallow hole previously made in the sediment surface which was in close proximity to the redox probes. If the sediment surface was dry, a small amount of deionised water was poured into the hole prior to the insertion of the reference electrode to ensure a good electrical contact. The monitoring of the lysimeters containing salt bridges required contact between the reference electrode and the agar at the top of each bridge.

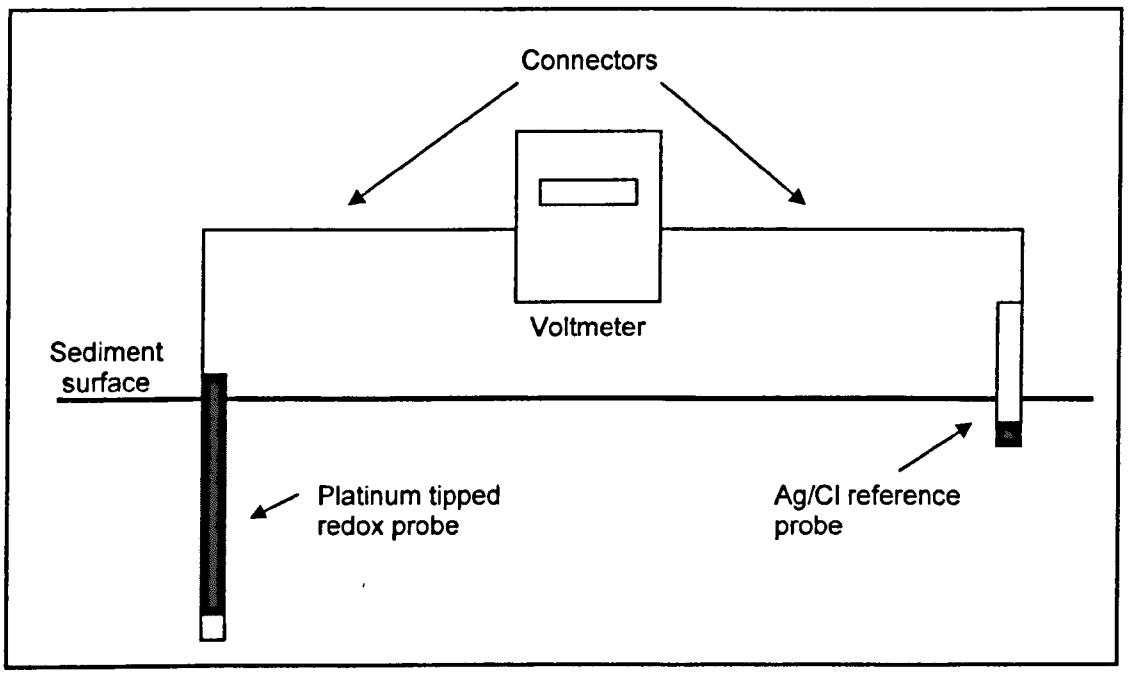

Figure 2.4: Diagram showing the set-up of the equipment used for redox monitoring.

2.4.3.5 Data processing

The data from all redox probes were recorded on a pre-prepared sheet. One reading from each cluster of probes at a particular depth was disregarded from subsequent analyses, as observation of the recorded values occasionally showed the existence of an extreme value (or outlier) (Cheetham 2004). This outlier, when incorporated into

the resulting analyses, would influence the mean value obtained from each cluster of probes. However, to ensure continuity throughout the results, the main outlier was always disregarded even if it was considered to be within an acceptable limit. The rejection of such a value, which was closely associated with the mean value from a cluster, would not adversely affect the overall outcome, whereas the rejection of a true outlier would ensure greater accuracy. It is recognized that this technique may produce biased values in an instance where there are two pairs of divergent values, but this situation should not occur under normal circumstances (Cheetham 2004).

The mean value obtained from each depth was adjusted to the Standard Hydrogen Electrode (SHE) (British Standards Institute 1990) in order to measure the redox potential (Howard 1998). This enabled comparisons to be made between values from different lysimeters. Owing to the Silver Chloride (AgCl) reference electrode having a potential value of +222 mV, the equivalent numbers of mV were therefore added to each redox value obtained. In addition, each meter reading was also adjusted to pH 7 to remove pH variability between sediments. This was achieved by adding a correction factor of –59 per unit of pH for values above pH 7, or subtracting the same correction factor below pH 7 (Bohn 1971, British Standards Institute 1990). Intermediate pH unit corrections were made proportionately, i.e. for pH 6.2 a factor of 0.8 x 59 would be adjusted from the redox value.

2.4.4 Dissolved oxygen

The measurement of dissolved oxygen is one of the most frequently used and the most important of all methods available for the investigation of the aquatic environment (Wetzel & Likens 1991). It gives indications of likely biological and biochemical reactions taking place within each environment (Wetzel & Likens 1991) and is useful

in distinguishing anoxic from aerobic conditions (Caple 1996). The level of dissolved oxygen in water is indicative of the concentration of nutrients and decaying organic matter in the water (Kegley & Andrews 1998).

As previously stated in Section 2.4.3, redox potential is the primary method of choice for distinguishing anoxic deposits from those with oxygen present (Caple & Dungworth 1995). However, dissolved oxygen can still be used as a supplementary technique to study the anoxic/aerobic nature of both natural (Caple & Dungworth 1995, Takatert *et al.* 1999) and landfill environments (Stessel & Murphy 1992) which are saturated with water.

2.4.4.1 Data acquisition

Dissolved oxygen was primarily used to assess the oxygen concentration of the lysimeters which contained leachate owing to the initial problems associated with the potential contamination of the redox reference electrode (as described in Section 2.4.3.2).

The dissolved oxygen of the water/leachate in each lysimeter was analyzed using a Photometer and Photometer Vial Adaptor (Palintest), and a Vacu-Vial Reagent Set DO/20 (CHEMetrics), in accordance with the manufacturers' instructions. Samples were not taken for the first two weeks of the study so that equilibrium could be established in the sediments prior to testing. Water/leachate samples were obtained by opening the taps on the side of each lysimeter prior to a change in water level.

2.5 Characterizing wetland archaeological and landfill sites by biological measurements

Microbiological assessment was a fundamental part of this research, as it is an essential factor when discussing the patterns and processes responsible for organic degradation (Corfield forthcoming, Hobson 1988). Several previous investigations have identified a correlation between organic material degradation and the presence or absence of microbiological activity within the burial environment (Lawson *et al.* 2000, Powell *et al.* 2001). However, little work has been carried out on the 'microbial' status of burial environments in relation to organic degradation and how it varies with changes in burial conditions.

This section will present the microbiological measurements used to characterize the sediment and wood samples obtained from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites. It includes the field methods, the laboratory techniques and analysis, and the statistical analysis of the data.

Until recently studies of the microbial communities present in natural environments relied on conventional optical microscopic observation and culturing-based approaches (Bruce *et al.* 1999). Although these traditional approaches can still provide useful information concerning microbial biomass and activity (Bogner *et al.* 1995, Tsui *et al.* 2001), they have a number of limitations. The most commonly cited limitation stems from the assumption that culturing techniques recover the majority of all microorganisms in a sample. However, research over the past fifteen years, from a number of differing burial environments, suggests that the true extent of microbial diversity far exceeds previous calculations. In fact <1 % of the organisms seen

microscopically can be cultured using conventional isolation techniques (Amann *et al.* 1995).

To circumvent the problems associated with microscopic observation and culturing-based approaches, various molecular techniques which involve the use of microbial nucleic acid (DNA and RNA) extraction, but do not require culturing, have been developed (Pickup 1991, Rondon *et al.* 1999, Santegoeds *et al.* 1998, Tiedje *et al.* 1999). These techniques have made it possible to sample entire microbial populations, and thus provide a more representative picture of the total microbial community (Bruce *et al.* 1999).

The molecular biological technique used for the analysis of the sediment and wood samples obtained during this study comprises of denaturing gradient gel electrophoresis (DGGE). DGGE is a relatively quick and easy way of analyzing genetic diversity within a microbial community (Hastings 1999) and it can be used to monitor changes in the microbial population by daily and seasonal fluctuations, or after environmental perturbations (Muyzer 1999).

Many studies have been undertaken to identify microbial community structure in a diverse array of natural environments, including differing soil types (Gelsomino *et al.* 1999), humus samples from forest floors (Pennanen *et al.* 2001) and decomposing wood (Helms & Kilstrup 2001, Pennanen *et al.* 2001, Vainio & Hantula 2000). Although there has only been limited research to date concerning microbial community diversity in landfill environments (Wise *et al.* 1999), the monitoring of microbial communities in lysimeter studies which contain waste is more routine (Ainsworth & Goulder 2001, Silvey & Blackall 1995).

The procedure for DGGE is based on the separation of polymerase chain reaction (PCR) amplified gene fragments by variation in the targeted nucleotide sequences during electrophoresis, through a polyacrylamide gel that contains an increasing denaturant gradient (Hastings 1999, Muyzer & Ramsing 1995, Muyzer *et al.* 1993).

Prior to DGGE analysis, it is necessary to extract and subsequently amplify microbial DNA by using the polymerase chain reaction (PCR). This is an automated technique of '*in vitro*' enzymatic amplification of a specific microbial sequence using a thermostable DNA polymerase (Saiki *et al.* 1988). During each cycle of PCR, any DNA that is present in the reaction is copied. Thus, during each cycle, the amount of DNA theoretically doubles. In practice, 25 cycles of PCR result in approximately a one-million-fold increase in the amount of DNA present. This amplification results in the production of a large amount of a specific microbial DNA sequence which can be purified via gel electrophoresis and visualized under ultra-violet (UV) light after ethidium bromide staining (Pepper 1997).

The PCR comprises three stages: stage 1 requires the melting of the double-stranded DNA to single-stranded DNA; stage 2 anneals the primers to the target DNA; and stage 3 involves the action of DNA polymerase which extends the DNA by nucleotide addition from the primers (Steffan & Atlas 1991). Figure 2.5 shows the three stages of PCR.

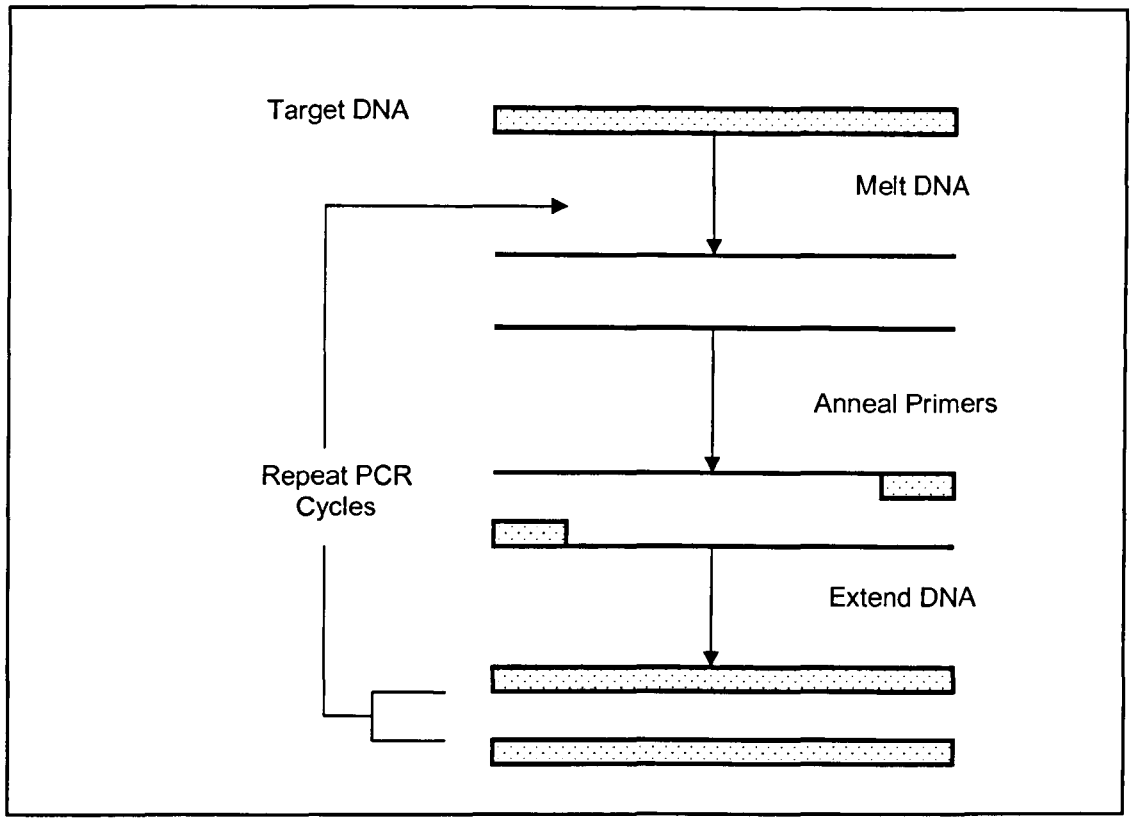

Figure 2.5: Diagram showing the three stages of the polymerase chain reaction (Steffen & Atlas 1991).

The primers bind to the conserved region of the desired target gene sequence. They then extend across the target sequence using DNA polymerase in the presence of free deoxynucleotide triphosphates, resulting in a duplication of the starting material. The continual melting of the PCR product can result in near exponential increases in target DNA over a number of cycles.

The PCR amplified gene fragments are then run on a polyacrylamide gel by DGGE (as shown in Figure 2.6). During electrophoresis, the melting domain within each DNA fragment losses its helical symmetry as it migrates through the gel and denatures according to its sequence composition when it reaches its melting temperature. Discrete bands form within the gel where the migration ends (Dean & Milligan 1998).

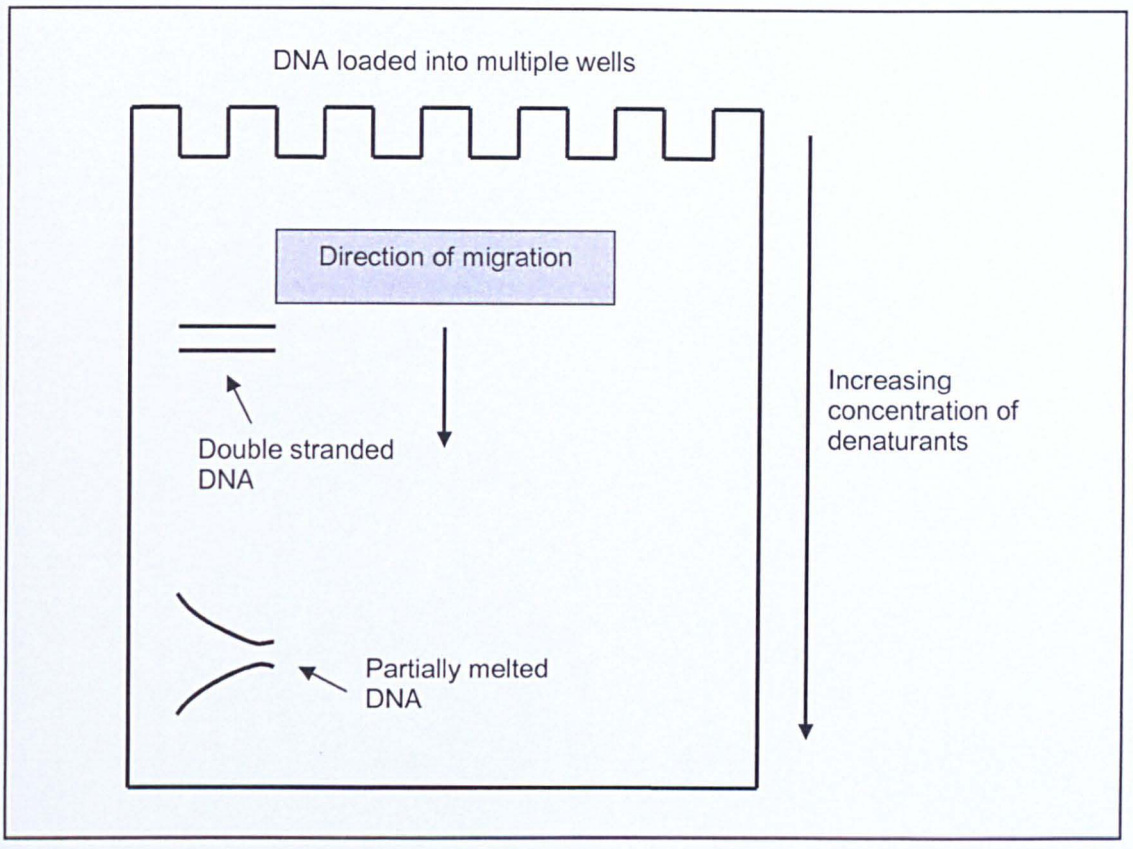

Figure 2.6: Diagram showing the principle of parallel denaturing gradient gel electrophoresis. DNA is loaded at the lower denaturant concentration and migrates parallel with the increasing gradient of denaturant. It denatures according to its sequence composition when it reaches its melting temperature.

The simultaneous analysis of multiple samples allows the construction of a community profile of fragments with differing base compositions. This data can subsequently be used to follow community changes over time (Muyzer *et al.* 1993).

2.5.1 Field sampling

The sampling strategy that has been used to remove the sediment and wood samples from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites for future analysis using DGGE is outlined below.

2.5.1.1 Sampling from lysimeters

It was necessary to excavate the surface of the sediment several centimetres at a time so as not to disturb the wood samples or their surrounding micro-environment. When the wood samples were located, sediment (which was approximately 10-20 mm thickness) was recovered immediately adjacent to the wood. The sediment samples were placed into plastic bags (Poly-Grips 3" x 3.25") and sealed. The wood samples were subsequently removed and also sealed in plastic bags.

In order to stabilize the conditions that existed within each lysimeter prior to excavation, all samples were immediately stored at –20 °C for future investigation. All analyses were undertaken within a month.

2.5.1.2 Sampling from wetland archaeological and landfill sites

'*In situ*' wood samples obtained from the wetland archaeological and landfill sites were subjected to the same excavation procedure as above. However, for the purpose of analysis, only small sub-samples were extracted from the surface 5 mm of the whole wood. Further samples were also obtained from the centre of the woods that were excavated from the Rolls Farm archaeological site. The sediment samples were again taken adjacent to the wood surface. All samples were subsequently placed into plastic bags (Poly-Grips 3" x 3.25"), sealed and stored at –20 °C.

2.5.2 Sediment preparation for microbiological assessment

1 g of waste from each sediment sample was broken down using a pestle and mortar in order to enable it to be placed within a 2 ml microcentrifuge tube for subsequent microbiological assessment. All large pieces of glass, metal, plastic and stone were

removed. When the waste resembled the texture of a paste it was placed into plastic bags (Poly-Grips 3" x 3.25"), sealed and stored at –20 °C.

2.5.3 Wood sectioning for microbiological assessment

The size of the microcentrifuge tube prevented the use of the complete wood blocks recovered from the lysimeter study for further microbiological assessment. To circumvent this problem, thin sections were taken from the surface of the wood samples using a microtome knife which was set at a thickness of 15 µm. This thickness ensured that the same amount of material was sectioned from each wood surface upon consecutive turns of the handle. 30 sections (which weighed 0.2 g) were taken from each wood surface. In total, this provided 1.2 g of wood for further analysis.

Identical weights of all the wood samples obtained from both the lysimeter study and the wetland archaeological and landfill sites were subsequently homogenized, weighed and halved. One half of the material was stored at –20 °C for microbial assessment, whilst the other was oven dried at 85 °C for 24 hours to ensure the removal of water from the cellular structure. The latter samples were ground down using a pestle and mortar until the powder would pass through a 213 µm sieve. They were subsequently stored in a desiccator to prevent any moisture adhesion.

2.5.4 Microbiological assessment

2.5.4.1 Centrifugation

All centrifuging was carried out at 13,000 RCF in an IEC Micromax Model 230 centrifuge (International Equipment Company).

2.5.4.2 Sterilization

All autoclaving was carried out at 121 °C at 15 psi for 20 minutes. Solutions were sterilized by filtration using a sterile 0.2 µm filter (Millipore) where necessary.

2.5.4.3 Cell culturing

Escherichia coli were used as a positive control for all bacteria throughout this study and were resuscitated from a glycerol stock stored at –20 °C. A loopful of culture was placed into sterile nutrient broth (0.1 % [w/v] Lab-Lemco powder, 0.2 % [w/v] yeast extract, 0.5 % [w/v] peptone, 0.5 % [w/v] sodium chloride [NaCl] in distilled water, Sigma) and incubated overnight at 37 °C. The cells were plated onto nutrient agar (which is similar to the nutrient broth above, but with the addition of 1.5 % [w/v] agar, Sigma) and incubated overnight at 37 °C. The *E. coli* colonies were then sub-cultured onto nutrient agar and incubated overnight at 37 °C. This organism was sub-cultured onto fresh nutrient agar each month and the plate stored at 4 °C.

2.5.4.4 Horizontal agarose gel electrophoresis

The DNA fragments were analyzed by horizontal agarose gel electrophoresis and ultra-violet (UV) photography. In order to make the gel, 0.5 g of agarose (Pharmacia, New Jersey, USA) was added to 50 ml of 1 x Tris acetate-EDTA (TAE) buffer (0.04 M Tris acetate [Tris (hydroxymethyl) aminomethane acetate], 0.001 M EDTA [ethylenediaminetetraacetic acid], pH 8.0). The mixture was then boiled and left to cool. Once cooled the gel was poured into a gel casting tray where it was left to set at room temperature for 15 minutes. 10 µl of DNA mixed with 1 µl of 6 x blue/orange gel loading dye (Promega, Madison, USA) (40 % [w/v] sucrose, 0.25 % [w/v] bromophenol blue in molecular biology grade water, Sambrook *et al.* 1989) were added into each well. 5-7 µl of a purple ladder size marker (*Lambda Hind III* DNA

digest, Sigma) were added into the first well to act as a reference to the size of the DNA fragments present during the electrophoresis. Electrophoresis proceeded at 5-20 V/cm for 45 minutes. On completion, the gel was stained in ethidium bromide solution for approximately 15 minutes. Gels were visualized under a UV transiluminator and recorded using a digital camera (Gene Genius Gel Documentation and Analysis System, Syngene).

2.5.5.5 DNA extraction from cell cultures

DNA samples were extracted using a Nucleon Phytopure DNA Extraction Kit according to the manufacturer's instructions. 4.6 ml of Nucleon Phytopure Reagent 1 were added to the *E. coli* culture and thoroughly mixed. 1.5 ml of Nucleon Phytopure Reagent 2 were then added to the mixture. This was again thoroughly mixed and incubated in a water bath at 65 °C for 10 minutes. The samples were subsequently placed on ice for 20 minutes. 2 ml of chloroform (stored at –20 °C) and 300 µl of Nucleon Phytopure Silica Resin were added to the mixture and agitated using a whirlimixer (Fisons Scientific Apparatus) for 3 minutes. Centrifugation proceeded for 5 minutes. The aqueous layer was then transferred to a 1.5 ml microcentrifuge tube. To precipitate the nucleic acid an equal volume of isopropanol (stored at –20 °C) was added to the mixture. The sample was mixed by inversion and centrifuged for 2 minutes. The supernatant was removed and the DNA pellet washed by adding 500 µl of 80 % ethanol, centrifuging the sample for 2 minutes and removing the ethanol. The pellet was left to air dry for 1 hour and then re-suspended in 100 µl of molecular biology grade water. Using 10 µl of the DNA solution, horizontal agarose gel electrophoresis was undertaken to check for DNA product. The extracted DNA was stored at –20 °C.

2.5.4.6 DNA extraction from sediment and wood

Disruption of microbial cells and extraction of genomic DNA were achieved using the bead-beating method (Kuske *et al.* 1998). 0.5 g of glass beads (0.17-0.18 mm diameter, BioSpec Products Inc., Oklahoma, USA) was placed into a 2 ml plastic screw top microcentrifuge tube along with either 1 g of sediment or 0.6 g of wood. 750 µl of 0.12 M sodium phosphate buffer (0.03 M Na_2HPO_4, 0.09 M NaH_2PO_4: adjusted to pH 8.0 with NaOH; autoclaved sterile) and 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v, Sigma) were then added. The sample was placed into a shaking vial and fixed into a dismembrator (BioSpec Products Inc., Oklahoma, USA). The sample was homogenized for 1 minute at 2,500 beats per minute. Any greater length of time and the DNA in the sample can be subjected to shearing.

The sample was centrifuged for 5 minutes. After centrifugation the aqueous layer was removed and stored on ice in a fresh 2 ml microcentrifuge tube. A further 500 µl of sodium phosphate buffer and 500 µl of phenol:chloroform:isoamyl alcohol were added to the residue and the homogenizing procedure was repeated. The sample was centrifuged for 2 minutes. The supernatants were then pooled, and the pellet discarded. The pooled supernatants were then extracted with a further 500 µl of phenol:chloroform:isoamyl alcohol until no protein interface was observed (Muyzer *et al.* 1993). The supernatants were shaken thoroughly in order to mix the layers and then centrifuged for 5 minutes.

To precipitate the DNA, 900 µl of the supernatant (aqueous layer) were transferred to a fresh 2 ml microcentrifuge tube. One-tenth volume 5 M sodium chloride (NaCl) (typically 100 µl) plus an equal volume of 30 % (w/v) polyethylene glycol 8000

(PEG) were added, whereupon it was left at room temperature for 2 hours. The sample was then centrifuged for 10 minutes in order to pellet the DNA. The supernatant was removed and the DNA pellet washed with 100 µl of 80 % ethanol and centrifuged for 2 minutes. It was then left to air-dry (usually overnight). The pellet was re-suspended in 100 µl of molecular biology grade water and placed at –20 °C until required. Using 10 µl of the DNA solution, horizontal agarose gel electrophoresis was undertaken to check for DNA product. The extracted DNA was subsequently stored at –20 °C prior to use.

Upon successful extraction of DNA, a portion (10 µl) of sample was visualized by horizontal agarose gel electrophoresis and subsequently stained in ethidium bromide. This gave a preliminary estimation of the quantity of the material extracted (Bruce *et al.* 1999).

2.5.4.7 Amplification of polymerase chain reaction products

For each reaction, 50 µl of polymerase chain reaction (PCR) master mixture (as shown in Table 2.3) were aliquoted into a 0.5 ml microcentrifuge tube. 1 µl of template DNA was added to each tube. Negative controls using sterile distilled water were used to monitor for contamination. Positive controls for the effectiveness of the reagents and conditions using DNA extracted from *E. coli* were also undertaken. 50 µl of mineral oil overlaid each reaction mixture.

A Hybaid Omnigene Thermal cycler was used to perform the thermal cycling. The 'touchdown' PCR procedure was applied in order to increase the specificity of the amplification and to reduce the formation of spurious by-products (Teske *et al.* 1996). The first cycle consisted of denaturation for 5 minutes at 94 °C, annealing at 65 °C for

1 minute and primer extension at 72 °C for 1 minute. In the following cycles, the annealing temperature was set at 65 °C and lowered by 1 °C every second cycle down to 55 °C, at which temperature twelve additional cycles were carried out. Denaturation was at 94 °C for 1 minute. Extension was at 72 °C for 1 minute. To check for DNA product, 5 µl of the PCR reaction product were analyzed using horizontal agarose gel electrophoresis.

Table 2.3: PCR reaction master mixture per reaction (MWG Biotech AG, Munich, Germany supplied all reagents [with the exception of molecular biology grade water and sample]).

Reagent	Volume
10 x PCR reaction buffer (100 mM Tris-HCl, pH 8.8, 15 mM magnesium chloride [$MgCl_2$], 500 mM potassium chloride [KCl], 1 % [v/v] Triton X-100)	7 µl
Forward primer at 20 pmol/µl	1 µl
Reverse primer at 20 pmol/µl	1 µl
Deoxyribonuclease triphosphate (dNTP) solution (125 µM of dATP, dCTP, dGTP, dTTP)	0.5 µl
Taq DNA polymerase	0.2 µl
Sample DNA	1 µl
Molecular biology grade water	39.3 µl
Total volume per reaction	50 µl

Oligonucleotide primers amplified the eubacterial component of the microbial community present in the sediment and wood samples. A 550-bp fragment of the 16S rRNA was amplified with the universal eubacterial primer combination GM5 F-GC clamp and DS907 R (Brinkhoff *et al.* 1998, Santegoeds *et al.* 1999, 1998, Teske *et al.* 1996). Table 2.4 shows the primer sequences and the target regions within the 16S rRNA gene. Primers to the 16S rRNA gene are numbered in accordance with the *E. coli* numbering system (Brosius *et al.* 1978) and indicate the base position where the primer binds. Forward primers denoted by F, are complimentary to the coding strand

of the 16S rRNA gene, whereas reverse primers denoted by R, are complementary to the non-coding strand.

Table 2.4: Primer sequences and target sites.

Primer [1]	Sequence (5' to 3')	Target site
GM5 F	CCT ACG GGA GGC AGC AG	341-357
DS907 R	CCC CGT CAA TTC CTT TGA GTT T	907-928
GC-clamp[2]	CGC CCC GCC GCG CGC GGC GGG CGG GGC GGG GGC ACG GGG GG	

[1] - F, forward; R, reverse

[2] - GC clamp attached to the 5' end of primer GM5 F

2.5.4.8 Purification of polymerase chain reaction products

The extracted DNA from the sediment and wood samples contained a proportion of organic matter. Dissolved organic matter resulting from the breakdown of both samples is composed primarily of organic macromolecules, such as carbohydrates and phenolic groups (Steeling & Petsom 1987). These components can form complexes with DNA upon contact, rendering the DNA unavailable for further molecular analysis.

In order to circumvent this problem, the polymerase chain reaction (PCR) products were purified (Sambrook *et al.* 1989). The overlaying mineral oil was removed by placing the PCR product at –20 °C until the mixture had frozen and the oil could be pipetted off. An equal volume of chloroform was added to the product and the sample mixed thoroughly. The sample was then centrifuged for 2 minutes and the aqueous layer pipetted into a fresh 0.5 ml microcentrifuge tube. One-tenth volume (5 µl) 10 M ammonium acetate and 55 µl of isopropanol were added to each sample in order to precipitate the DNA. The tube was inverted several times to ensure mixing and stored at –20 °C for 1 hour. The mixture was then centrifuged for 10 minutes to pellet the

DNA. The aqueous solution was removed and the DNA washed with 50 µl of 80 % ethanol. The pellet was left to air dry overnight. It was then re-suspended in 25 µl of molecular biology grade water. A horizontal agarose check gel was run with 5 µl of the sample in order to establish the presence and purification of the DNA product. Samples were subsequently stored at –20 °C for future use.

2.5.4.9 Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was performed with a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad Laboratories Ltd.) according to the manufacturer's instructions. Figure 2.7 shows the main components of the system. DGGE was used to generate bacterial community profiles in both sediment and wood by separating DNA fragments of the same size on the basis of differing base composition (Muyzer *et al.* 1993). To prevent the DNA bands from completely disassociating a 30′ to 50′ base guanine/cytosine rich sequence (referred to as a GC clamp) was added to the 5′ of one of the PCR primers (Sheffield *et al.* 1989). The primers used were GM5 F-GC and DS907 R.

Plate preparation for denaturing gradient gel electrophoresis

Plates, spacers and combs were all washed in distilled water and cleaned in 80 % ethanol. Spacers were fitted between the glass plates. When the bottom of the two plates and the spacers were aligned correctly the right and left clamps were secured in place. The plates were then placed into the alignment slot of the casting stand with the larger plate at the back.

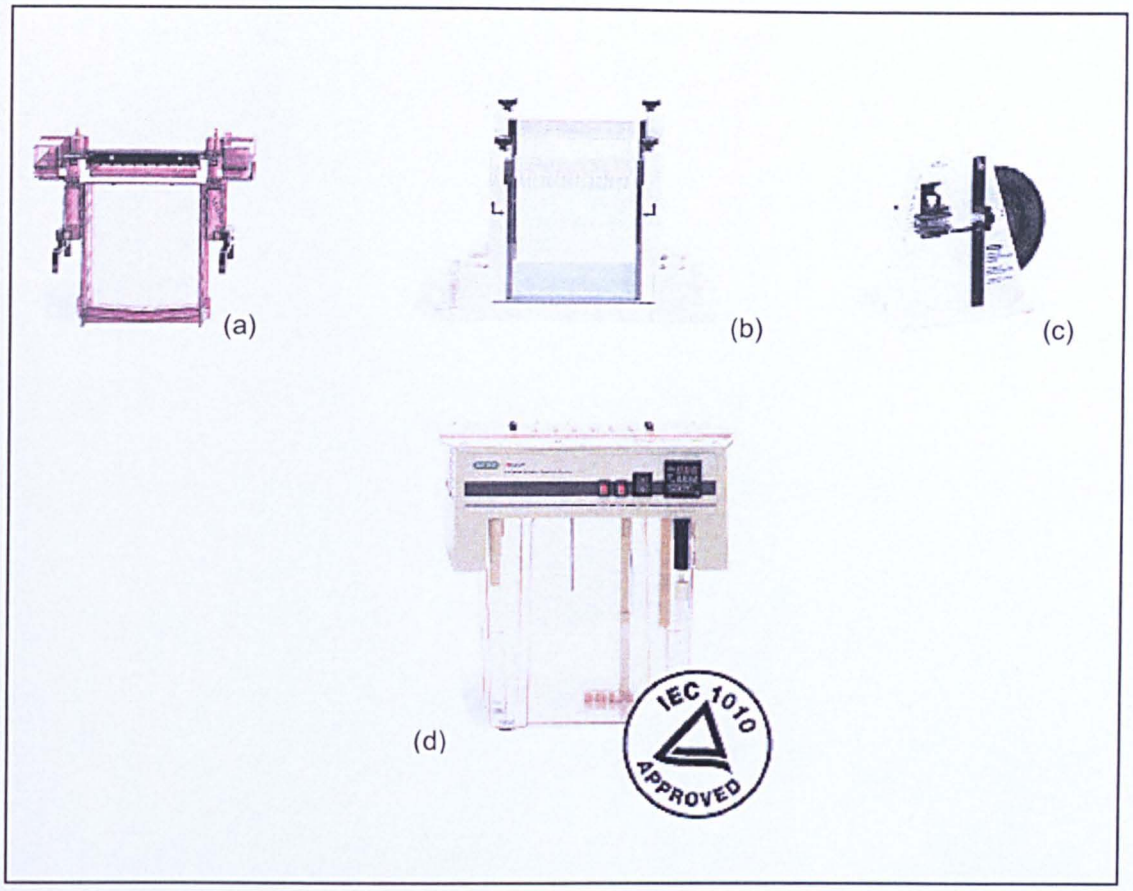

Figure 2.7: Diagram of the DCode System used for DGGE; a) sandwich core, b) gel caster with gel sandwiches, c) cam-operated manual gradient former, d) electrophoresis temperature control module (Bio-Rad Laboratories 2004).

Gel pouring for DGGE

Gel pouring was undertaken using a Bio-Rad Model 475 Gradient Detection System (Bio-Rad Laboratories Ltd.) according to the manufacturer's instructions. Solutions of 7.5 % polyacrylamide (urea and formamide) with the appropriate denaturant were prepared (as shown in Tables 2.5 and 2.6). Two syringes of the same volume were connected via luer fitting to Tygon tubing. In order to polymerize the polyacrylamide 20 µl of TEMED (N,N,N',N'-tetramethlethylene-diamine, Sigma) and 200 µl of 10 % (w/v) ammonium persulphate solution (APS) (Bio-Rad Laboratories Ltd.) were added to 20 ml of each denaturing solution contained in a sterile universal tube. Each tube was then agitated to mix the solutions. The solutions were then drawn up into high and low density syringes. The syringes were screwed into place in the Gradient Delivery

System and any air present was expelled. A further length of tubing was connected to a Y-fitting which was attached to the syringe tubes. The other end was connected to a 19′ gauge needle. The needle was placed in the centre of the plates and the gel solutions poured. When the gel reached towards the top of the plates the comb was added. The gel was left for 1 hour to set, after which point the comb was taken out and the gel used immediately.

Table 2.5: Composition of a 7.5 % polyacrylamide, 0 % denaturant solution.

Reagent	Quantity
40 % Acrylamide/Bisacrylamide	18.8 ml
50 x TAE buffer	2.0 ml
Formamide	0 ml
Urea	0 g
Molecular biology grade water	To a volume of 100 ml

Table 2.6: Variation of urea and formamide concentrations in denaturing solutions.

Reagent	Quantity for Low (30 %) Denaturant	Quantity for High (60 %) Denaturant
40 % Acrylamide/Bisacrylamide	15 ml	15 ml
50 X TAE buffer	2 ml	2 ml
Formamide	12 ml	24 ml
Urea	12.6 g	25.2 g
Molecular biology grade water	To a volume of 100 ml	To a volume of 100 ml

Gel loading and running

Prior to electrophoresis, 6.86 litres of 1 x Tris acetate-EDTA (TAE) buffer were poured into the buffer tank and pre-heated to 60 °C. The glass plates containing the gel were lowered into the buffer along with another set of glass plates. 1 x TAE was poured over the plates to form an upper buffer reservoir. A 19′ gauge needle and syringe were then used to incorporate the 1 x TAE buffer into the wells in the gel prior to loading. 5 µl of loading buffer were added to 30 µl of each sample to be run. The

mixture was loaded into the wells. When all the samples had been loaded into the wells, the lid was replaced and electrophoresis was performed at 200 V for 270 minutes. After electrophoresis, the gel was removed from between the plates and stained for 30 minutes in ethidium bromide at a concentration 0.5 μg/ml. It was subsequently rinsed in distilled water, visualized under an ultra-violet (UV) transiluminator and recorded using a digital camera (Gene Genius Gel Documentation and Analysis System, Syngene). Image analysis software (Gene Genius Gene Tools, Syngene) was used to determine the presence or absence of bands in the lanes of the DGGE gels.

2.5.5 Statistical analyses

Each particular band within the lane of a denaturing gradient gel electrophoresis (DGGE) gel can potentially represent different bacterial taxa (Ainsworth & Goulder 2001). By comparing individual bands within lanes in the same gel, differences in microbial community composition can be determined. A number of biological (as described in the current section), chemical and physical (as described in Section 2.4) factors that characterize the burial environment may account for these differences. By employing a number of statistical tests to the banding patterns, identification of relationships between differing bacterial taxon and the different environmental parameters influencing their diversity can be achieved.

2.5.5.1 Sorensen's pairwise similarity coefficient

A Sorensen's pairwise similarity coefficient was calculated for each pair of lanes within a DGGE gel as follows (Lindström 1998):

$$C_s = 2j / (a+b)$$

Here, j is the number of bands shared by both lanes, a is the number of bands in lane A and b is the number of bands in lane B. C_s x 100 gave the percentage similarity.

On the basis of this, similarity coefficients obtained from different lanes in the same DGGE gel can indicate the percentage similarity between lanes in a variety of burial environments.

In order to represent several variables in a reduced dimensional space, ordination techniques were applied to microbial variable data sets (previously identified by using Sorensen's pairwise similarity coefficient). Classification and data reduction methods such as hierarchical cluster analysis and principal-component analysis are techniques widely used in ecology, taxonomy and other fields (Peterson 2002) and are the ordination methods of choice for the microbial data in the current study.

2.5.5.2 Hierarchical cluster analysis

Hierarchical cluster analysis is an exploratory multivariate statistical method for identifying 'natural' groupings of objects in an analysis. It categorizes cases or variables into groups or 'clusters' and seeks to identify a set of groups which both minimize within-group variation and maximize between-group variation, based on attribute information about the objects (Peterson 2002). Hierarchical cluster analysis was performed using SPSS 11.5 for Windows (SPSS Inc. 2004). Sorensen's pairwise similarity coefficients were converted to distance values, E_s, where $E_s = 1 - (C_s / 100)$ to produce a matrix of E_s values (Sait *et al.* 2003). The use of Ward's methods and Euclidean distance proximity measurements in hierarchical cluster analysis clustered the data sets. Dendrograms displayed the results of the similarities between the DGGE

band profiles from the matrix of E_s values. The lines produced indicate the degree of similarity or dissimilarity between cases.

2.5.5.3 Principal-component analysis

Principal-component analysis is a method designed to transfer a set of interrelated variables to a new set of uncorrelated components that account for all the variance in the original variables (Peterson 2002). The analysis was again performed using SPSS 11.5 for Windows (SPSS Inc. 2004). The use of Varimax orthogonal rotation in principal-component analysis enabled the main components from the matrix of E_s values to be calculated. In order to determine the significance of the components, the Kaiser-Guttman criterion was applied to the matrix of E_s values (Howitt & Cramer 1997). The Kaiser-Guttman criterion states that the interpretation of the principal component should only occur if the corresponding eigenvalue is larger than the mean of the eigenvalues. In the case of the edited data set, the mean of the eigenvalues is one, therefore only the components whose eigenvalues are larger are interpretational. The percentage of variance for each of the orthogonally rotated components was calculated as follows (Howitt & Cramer 1997):

$$V = (v^2 + C) / N \text{ x } 100$$

Here V is the percentage of variance, v^2 is the loading of the variables on each factor, C is the sum of the squared loadings and N is the number of variables.

2.6 Critical assessment of the techniques used

The biological, chemical and physical techniques used in the present study can provide a wealth of information about the dynamics of differing sediments and the patterns and processes that influence oak wood degradation. The results will be

discussed in subsequent chapters. However, during the generation of the results, several difficulties were experienced, both in the fieldwork component and the laboratory-based assessments. This section will provide a critical assessment of the issues regarding the techniques used and identify possible sources of error which may affect the validity of the results.

2.6.1 Physico-chemical analyses

2.6.1.1 pH values

During the lysimeter study, the Watercheck™ portable pH and conductivity meter was re-calibrated prior to each use and tested for signs of poisoning from the leachate by comparing pH values obtained from the Sentix 21 combination electrode. However, this study recognises that pH measurements of sediment and water may differ and it has not been possible to take this factor into account during the analysis. Nevertheless, while this area of research requires further investigation, it is anticipated that this factor alone will not significantly influence the integrity of the results.

2.6.1.2 Water/leachate level management

During the manipulation of water/leachate levels in the lysimeter study, the slow draw-down of interstitial water in the lysimeters which contained peat prevented the loss of all the water from the required level in the time available (within a period of 1 week). Although this did not affect the levels of saturation around the wood samples, it does suggest that similar studies in the future may benefit from a greater understanding of the hydraulic conductivity of different sediments.

2.6.1.3 Redox potential

Several issues have been identified which may impact upon the effectiveness of the redox potential values presented from the lysimeter study. These issues will be discussed in relation to the generation, manipulation and presentation of the data, and the interpretations based upon the findings.

The redox probes used during this study were inserted into all twelve lysimeters, where they remained '*in situ*' for a maximum of two years. Research by Bohn (1971) has shown that platinum-tipped redox probes and reference probes are susceptible to poisoning from the sediment due to the absorption of organic substances on the platinum surfaces, or to platinum sulphide reactions. To assess the potential poisoning of the probes used in the lysimeter study, all were removed during excavation and laboratory-tested. The results from this testing confirmed the integrity of all the probes.

The problem of reference probe contamination was avoided by the development and installation of salt bridges using the method of Veneman & Pickering (1983) (as described in Section 2.4.3.3). The accuracy of the salt bridges was demonstrated by the redox values obtained from Lysimeter 3 (as shown in Appendix 1). The results from both sets of data display similar values, indicating a <30 mV difference over the duration of the experiment. The integrity of the reference probe was also assessed by using a redox standard solution (200 mV) (Speck Analytical Ltd., Clackmannanshire) and an unused redox probe.

Research by Bohn (1971) and Cheetham (2004) has identified the irreproducibility of redox potentials in dry, oxidized conditions, owing to the low concentration of redox

couples. On the basis of this, the development and installation of the salt bridges avoided the generation of erratic and inconsistent readings associated with the low levels of saturation which were produced by a drop in the level of water/leachate. However, as there was only a slight difference between the two sets of redox values obtained from Lysimeter 3 during the experiment, the dry conditions associated with a drop in water/leachate levels did not adversely affect the data generated without the salt bridge in place.

The presentation of redox data requires the adjustment of the raw values to the Standard Hydrogen Electrode (SHE) in order to account for variation of pH. However, for purposes of direct comparison, the pH values used in subsequent analyses were obtained from the sediment surface in the lysimeters which contained water and from the taps nearest to the sediment surface in the lysimeters which contained leachate. This method of redox monitoring and pH adjustment did not account for the changes in pH in relation to depth, a factor which might influence the accuracy of the results.

2.6.1.4 Dissolved oxygen

The main reason for the use of dissolved oxygen in this study was to give a preliminary indication of the anoxic/aerobic nature of the water/leachate. The measurement of dissolved oxygen was initiated owing to the problems associated with the potential contamination of the reference probe in the lysimeters containing waste/leachate during redox monitoring, which was not resolved until after the lysimeter study had begun. The validity of the redox data was judged to exceed that of dissolved oxygen. This was primarily due to the high levels of metal ions present within the waste/leachate which produced erroneous dissolved oxygen values that could not be used in any further interpretations (CHEMetrics *pers comm.* 2004).

erroneous dissolved oxygen values that could not be used in any further interpretations (CHEMetrics *pers comm.* 2004).

2.6.2 Microbiological analyses

Denaturing gradient gel electrophoresis (DGGE) has been used in the current study to display temporal and spatial variations in the genetic diversity of complex bacterial populations (Muyzer *et al.* 1993). These were present in both the sediment and wood samples obtained from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites. However, several sources of error have been identified which might impact upon the validity of the findings. These issues will be discussed below in relation to the sampling methods, the laboratory analysis and the interpretation of the results.

2.6.2.1 Sampling

During the removal of the sediment and wood samples from the differing burial environments, care was taken not to disturb the wood samples or their surrounding micro-environment. However, owing to the difficulty in removing sediment samples immediately adjacent to the wood surface, it is likely that a portion of the sediment remained on the wood. Although the wood samples were rinsed with sterile water in order to remove the sediment particles in preparation for wood sectioning, some of the sediment may still have adhered to the surfaces. Under such circumstances, the results highlighting similarities between the bacterial communities present in the wood and sediment samples immediately adjacent to the wood may indicate a closer relationship than exists in reality. Nevertheless, every effort was taken to ensure that this possible source of error did not occur.

It should also be noted that the characteristics of the samples may alter between the time of excavation and storage at –20 °C. Therefore, the removal and subsequent storage of the sediment and wood samples was undertaken as quickly as possible. However, the slight potential for unavoidable changes occurring within the samples remains.

2.6.2.2 Laboratory analysis

The greatest potential source of contamination in the laboratory environment was the amplification of polymerase chain reaction (PCR) products produced from the sediment and wood samples prior to DGGE. During the addition of 1 µl of template DNA to 50 µl of PCR master mixture, atmospheric contamination may have occurred. This can influence the effectiveness of the PCR amplification. However, during each PCR procedure, negative controls which contained sterile water were monitored for contamination, whilst positive controls using DNA extracted from *E. coli* were used to assess the effectiveness of the reagents and conditions. No results were obtained which indicated potential sources of contamination or which inhibited the effectiveness of the reagents.

2.6.2.3 Interpretation

Although the use of DGGE is an absolute measure of microbial community diversity, it must be treated with caution. Previous investigations by Ferris & Ward (1997) and Liesack and co-workers (1991) have shown that the number of bands on DGGE gels may overestimate the diversity of a sediment sample owing to the formation of heteroduplex or chimeric molecules, respectively. Conversely, research by Muyzer and co-workers (1993) highlighted that DGGE gels may underestimate the bacterial diversity of a sediment sample. Two possible reasons may account for this

discrepancy; firstly, the PCR may selectively amplify DNA from specific bacterial taxa, resulting in a qualitatively distorted representation of the natural community; and secondly, DNA fragments with different sequences may have the same melting behaviour, a factor which cannot be separated by DGGE.

2.7 Summary

It is possible to measure the biological, chemical and physical patterns and processes that define wetland archaeological and landfill sites by using a number of different techniques. In doing so, the techniques used to define '*in situ*' sites can also help to characterize the environmental matrix contained within the lysimeter study. The primary techniques include pH, water level, redox potential, dissolved oxygen and denaturing gradient gel electrophoresis (DGGE).

No chemical or physical definitions concerning the '*in situ*' wetland archaeological and landfill sites described in Section 2.2 were possible, owing to the time and financial constraints associated with monitoring. However, it was possible to assess bacterial diversity and activity within the wood and sediment excavated from each site for use in combination with the biological, chemical and physical data generated from the lysimeter study. This information can further define the patterns and processes that characterize these particular types of burial environments. As a consequence, it is possible to determine their influence on wood degradation.

In order to assess the extent of wood degradation in different environments, it is fundamental to find the most effective technique to measure oak wood decay. Chapter 3 will assess a number of different techniques used to determine wood degradation and highlight the most viable technique to use within the remit of this thesis.

Chapter 3 Methods II

3.1 Introduction

The aim of this chapter is to evaluate the main technological applications that can be used to measure oak wood degradation in the samples obtained from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites, in order to determine the most appropriate method for use within the remit of this thesis. Although there are several other techniques available for studying wood degradation, the approaches discussed in this chapter represent the most widely used methods of wood analysis (*cf.* Hedges 1990).

Section 3.2 will outline the main techniques selected for the current study to assess wood degradation. The techniques include microscopy, wet chemical analysis, Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and gas chromatography (GC).

Although there is some information available on wood degradation in natural burial environments, there has been little research published on the decomposition of organic materials in landfill or lysimeter environments. The only research that has been undertaken relates to the measurement of the drop in the height of the waste mass (Ham & Bookter 1982, Stessel & Murphy 1992). Consequently, as a result of this lacuna, it is important to improve our understanding of organic material degradation, particularly regarding artificial burial environments. However, owing to the paucity of literature available, the following discussion focuses on the research which has been undertaken on wood degradation in natural contexts.

Section 3.3 presents the method selected to analyze the wood samples, followed by a critical assessment of the technique demonstrating what improvements can be made to enhance the accuracy of the method (as described in Section 3.4).

3.2 Methods used to measure wood degradation

The most appropriate method to assess oak wood degradation must take into account five key criteria which are fundamental to the research in question. The main issues that need to be addressed during this study are as follows:

1) Quantification of cellulose and hemicellulose present in a sample
2) Assessment of degradation using a 'stand alone' technique, without the need for further analysis
3) High technological reproducibility and resolution
4) Level of expertise required to obtain satisfactory results
5) Length of time taken for sample analysis

The differing techniques discussed below (with the exception of wet chemical analysis) have either been applied during preliminary investigations undertaken as part of the background research to this study, or were applied during research at Masters level (Smith 1999). Therefore, the following discussion will use the results from both the preliminary investigations and research by Smith (1999), in conjunction with previous literature on the subject, in order to determine the most appropriate method to fulfill the key criteria outlined above.

3.2.1 Microscopy

The structure of different species of wood and their chemical components of cellulose, hemicellulose and lignin has been studied in great detail using a variety of

microscopic techniques (Hoffmann 1981). Light-, electron- and scanning-electron microscopy can all be used to highlight the patterns and processes responsible for wood degradation from a physico-chemical (Gregory 1998, Gregory *et al.* 2001, Hoffmann & Jones 1990) and biological (Björdal *et al.* 1999, Blanchette *et al.* 1990, Hogan *et al.* 2001, Jagels 1981, Kim 1990) perspective. However, microscopy can be extremely subjective when assessing the amount of cellulose and hemicellulose lost from a sample. This observation is particularly pertinent in heavily degraded samples, where degraded areas within the wood can be located immediately adjacent to more well-preserved areas (-termed degradation fronts) (Hoffmann & Jones 1990, Paajanen & Viitanen 1988), potentially resulting in bias in the interpretation of the data generated.

Preparation of the wood samples for use with scanning- and electron-microscopy can also affect the accuracy of the data generated (Smith 1999). The main limitation on effective preparation of wood samples for microscopic analysis is the conversion of a normally wet sample to a dry state, while still preserving its three-dimensional shape (-termed fixation and dehydration) (Falk 1980). The most appropriate method of fixation uses immersion of the wood into liquid nitrogen, which rapidly turns all the water present into a solid. Owing to the speed of transformation of water from a liquid into a solid, the physical structure of the sample remains stable. However, slower freezing of samples (especially those with minimal degradation) creates larger ice crystals and can cause gross ultra-structural damage (Falk 1980).

The removal of water from the wood by sublimation from a solid state into a gas (-termed dehydration), can also produce inaccurate results. Although the dimensional changes in the wood structure are relatively low during this process (approximately 15

%), some spatially unequal dimensional changes can still hinder the interpretation of the results.

The qualitative nature of microscopy, and the problems associated with sample fixation and dehydration, show that it cannot be used as a 'stand alone' technique accurately to assess the cellulose and hemicellulose lost from the wood samples in the current study. As a consequence, it is essential to identify a different methodological approach which fulfills all the key criteria outlined above.

3.2.2 Wet chemical analysis

Wet chemical analysis can be used to quantify wood degradation by measuring cellulose, hemicellulose and lignin (Hedges 1990), against standard values associated with a corresponding fresh wood sample of the same species (Hoffmann 1981).

Although the complete fractional breakdown of wood by wet chemical analysis involves a multi-step procedure which can be time consuming and labour intensive (Pettersen 1984), numerous studies have previously used wet chemical methods to detect degradation in wood samples (Iiyama *et al.* 1988, Meshitsuka & Nakano 1985, Pavlikova *et al.* 1993, Zech *et al.* 1986).

The standard methods used for the chemical analysis of wood were originally developed by the Technical Association of the Pulp and Paper Industry (TAPPI) and the American Society for Testing Materials (ASTM), both of which are very similar in character and are given below:

1) Determination of the maximum water content and actual water content of each sample

2) Extraction of cellulose and hemicellulose from the wood by the use of 1 % sodium hydroxide (NaOH) (ASTM 1995)
3) Removal of water-soluble extractives (tannins, gums, sugars, starches and colouring matter) by hot water extraction (ASTM 1996)
4) Removal of non-polar extractives (waxes, fats, some resins, and portions of wood gums) by ethanol-benzene extraction (ASTM 1996)
5) Dichloromethane (DCM) extraction of phytosterols and nonvolatile hydrocarbons (ASTM 1996)
6) Removal of any remaining acid insoluble lignin fractions with 72 % NaOH (ASTM 1996)
7) Determination of the wood's mineral content and any other inorganic matter within by 'ashing' (ASTM 1995)

By using the multi-step procedure shown above it is possible to quantify the breakdown of a wood sample and determine the remaining proportions of cellulose, hemicellulose and lignin. However, there are several limitations with these methods, including the potential need for replication owing to the complex nature of the techniques employed (Fengel & Wegener 1979, Hedges 1990, Hoffmann 1981, Pettersen 1984), the low reproducibility associated with the extraction techniques (Fengel & Wegener 1984, Hedges 1990, Pettersen 1984), and the need for a high degree of technical skill and application to extract successfully the different wood fractions. As a consequence, these analyses are usually undertaken by specialized personnel with a background in chemistry (Gailbraith *pers comm.* 2001).

In order to circumvent these problems, wet chemical analysis is generally used in combination with other techniques (Iiyama *et al.* 1988, Meshitsuka & Nakano 1985,

Meshitsuka *et al.* 1982, Pavlikova *et al.* 1993, Saiz-Jimenez *et al.* 1987, Zech *et al.* 1986). However, owing to the limitations highlighted above and the requirement of a further complimentary technique to ensure accurate data generation, this particular technique was considered unsuitable for the current study.

3.2.3 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy can give a quick, qualitative assessment of the cellulose, hemicellulose and lignin present in decayed wood (Gilardi *et al.* 1995, Wilson *et al.* 1993). The wood fractions are measured by pressurizing a pellet of powdered wood mixed with potassium bromide (KBr), which is subsequently analyzed in a Fourier spectrometer to produce absorption band spectra over a known wavelength. The peaks in these wavelengths correspond to the wood fractions.

Although this method has been used on both recently degraded (Pandey & Pitman 2003) and archaeological wood (Wilson *et al.* 1993), the current study has found that it is difficult to obtain reproducible results owing to inconsistencies associated with passing ultra-violet (UV) light through a powdered wood/KBr pellet. This problem is further exacerbated by the lack of sensitivity of some equipment when dealing with highly preserved samples. The small differences in the chemical composition of these woods are not accurately reflected in the corresponding peaks of the wavelengths produced.

Although Backa & Brolin (1991) circumvented this problem to some degree by minimizing the direct reflectance from the sample surface, through the use of a blocker, the accuracy of the technique still remained low owing to the lack of unique

spectral points that differentiated cellulose and hemicellulose respectively (Schultz & Burns 1990).

In order quantitatively to determine the amount of cellulose and hemicellulose present in a wood sample, FTIR spectroscopy must be used in combination with another technique (usually wet chemical analysis). The data produced can then be interpreted using various statistical analyses, i.e. partial least squares (Backa & Brolin 1991, Lopes *et al.* 2000), multivariate analysis (Ferraz *et al.* 2000) and stepwise regression (Schultz & Burns 1990, Schultz *et al.* 1985). However, it is not possible accurately to replicate the regression equations which correspond to the different wood fractions produced from the various forms of statistical analyses.

On the basis of these problems and findings, this technique was considered unsuitable for the current study.

3.2.4 Nuclear magnetic resonance spectroscopy

Solution- and solid state nuclear magnetic resonance (NMR) spectroscopy has been used as a means of qualitatively determining wood degradation by analyzing the structure of organic molecules in a sample (Davis *et al.* 1994, Hatfield *et al.* 1987, Lorenz *et al.* 2000, Maciel *et al.* 1980, Pan *et al.* 1990, Smith 1999, Wilson *et al.* 1993). It is able to show changes at a molecular level which cellulose and hemicellulose undergo during biological, chemical and/or physical attack (Pan *et al.* 1990), in both fresh (Davis *et al.* 1994, Lorenz *et al.* 2000) and archaeological wood (Smith 1999, Wilson *et al.* 1993).

Although the preparation of samples for NMR spectroscopy is minimal, the technique inherently suffers from complications associated with ^{13}C spin-lattice relaxation times, nuclear Overhauser effects and spectral peak overlaps (Maciel *et al.* 1980). In addition to these problems, and in order to gain a quantitative assessment of cellulose and hemicellulose, NMR must always be used in conjunction with another technique (Newman *et al.* 1993). On the basis of these observations, it is clearly necessary to identify an alternative technique which can determine individual cellulose and hemicellulose degradation.

3.2.5 High performance liquid chromatography and gas chromatography

By hydrolyzing the polysaccharides within wood using either sulphuric acid (H_2SO_4) or trifluoroacetic acid (TFA), it is possible to separate cellulose and hemicellulose from lignin in solution. Whilst previous research has demonstrated that TFA has important advantages over H_2SO_4, i.e. a shorter retention time (Albersheim *et al.* 1967) with no need for conventional neutralization (Fengel & Wegener 1979), the TFA method is more suited to the hydrolysis of the hemicelluloses than the hydrolysis of cellulose, as it would take a considerable length of time for TFA to breakdown completely the crystalline cellulose into glucose (Jones *pers comm.* 2001). Both high performance liquid chromatography (HPLC) and gas chromatography (GC) can subsequently analyze the sugars that are produced.

There are seven major sugars present in oak wood; these include glucose (which corresponds to cellulose), xylose, mannose, galactose, arabinose, rhamnose and ribose (which correspond to hemicellulose). HPLC and GC are able to detect and separate each individual sugar from the wood. By calculating the area of each sugar peak, quantifiable data can be determined from the analyses with no loss in accuracy

(Albersheim *et al.* 1967, Barlaz *et al.* 1989a, Fengel & Wegener 1979, Karr *et al.* 1991, Kelly *et al.* 1991, Kim 1990, Pettersen 1984, Wentz *et al.* 1982).

Several investigations have used either HPLC or GC in conjunction with other analytical techniques in order to determine wood degradation (Budgell *et al.* 1987, Helleur *et al.* 1987, Wilson *et al.* 1993). In addition, some of the investigations have highlighted the fact that the reproducibility and quantifiable nature of these techniques enable them to be used for individual measures of assessment (Albersheim *et al.* 1967, Barlaz *et al.* 1989a, Fengel & Wegener 1979, Karr *et al.* 1991, Kelly *et al.* 1991, Kim 1990, Pettersen 1984, Wentz *et al.* 1982).

On the basis of these findings, and owing to the lack of availability of HPLC at the University of Hull, H_2SO_4 hydrolysis was used in conjunction with GC analysis during this study. None of the techniques evaluated above (with the obvious exception of HPLC) can be used alone to quantify accurately the amount of cellulose and hemicellulose present within wood. The reproducibility of GC is proven and the resolution obtained is high. Although H_2SO_4 hydrolysis is quite time consuming, it can be mastered reasonably quickly and can act as a viable alternative to the laborious wet chemical approaches outlined previously.

3.3 Gas chromatographic analysis of oak wood

This section will present the method used for the gas chromatographic (GC) analysis of the wood samples. There are three distinct phases involved in the complete analysis of cellulose and hemicellulose; these are hydrolysis, reduction and acetylation. Each distinct phase will be presented below.

3.3.1 Standards

Prior to hydrolysis of the polysaccharides, it is essential to analyze the corresponding sugars in their pure form in order to establish the retention times of the acetates of the individual sugars and to display the peak areas for the sugar standards of previously determined weights (Albersheim 1967, Gailbraith *pers comm.* 2001).

0.02 mg of each pure sugar (Sigma Aldrich Ltd.) was subjected to the same procedure as the wood samples shown below (with the exception of vortexing in 72 % sulphuric acid [H_2SO_4] for 20 minutes). The data produced from the sugars was then used as a baseline to help determine the cellulose and hemicellulose present in the wood (Albersheim 1967).

3.3.2 Hydrolysis

The glycosidic bonds between the high molecular weight linear polysaccharides (cellulose) and the matrix polysaccharides (hemicellulose) were split into their components (mixed acetals) from the lignin matrix by hydrolysis (Lehmann 1998).

0.15 mg of oven dried wood powder and 0.125 ml 72 % H_2SO_4 were placed into a round-bottomed flask (Fengel & Wegener 1979, Leskovesek 1994). The mixture was vortexed for 45 minutes at 20 °C. 1.35 ml of deionised water were then added to the flask and the mixture hydrolyzed at 105 °C for 150 minutes (Jones *pers comm.* 2001). The flask was then cooled in an ice bath. 0.32 ml 15 M ammonia was added to neutralize the acid (Blakeney *et al.* 1983).

3.3.3 Reduction

The hemicelluloses were reduced by adding 0.1 ml of the mixture to 1 ml 2 % sodium borohydride (NaBH4) in dimethyl sulphoxide (DMSO) solution and kept at 40 °C for 90 minutes. Any excess NaBH4 was then destroyed by the addition of 0.1 ml 18 M acetic acid. 0.1 ml inositol was added to the flask to act as the internal standard (Blakeney *et al.* 1983).

3.3.4 Acetylation

0.2 ml 1-Methylimidazole and 2 ml acetic acid ($C_2H_4O_2$) were added to the mixture and stirred for 3 minutes. The mixture was left for 10 minutes at room temperature, whereupon 5 ml deionised water were added to decompose the excess of acetic anhydride ($[CH_3CO]_2O$) (Jones *pers comm.* 2001). The liquid was cooled in an ice bath. Once cool, 1 ml of dichloromethane (CH_2Cl_2) was added and the mixture was vortexed for 8 minutes. The aqueous phase was allowed to separate and the CH_2Cl_2 layer was transferred into gas chromatographic (GC) vials, where it was sealed with a 1 ml septum cap and stored at –20 °C (Blakeney *et al.* 1983, Jones *pers comm.* 2001). The samples were run on a GC Series 610 using a 30M BPX70 capillary column and auto sampler (Unicam) for 30 minutes. Figure 3.1 is a typical gas chromatogram of a fresh wood sample, showing the peaks corresponding to the seven major wood sugars.

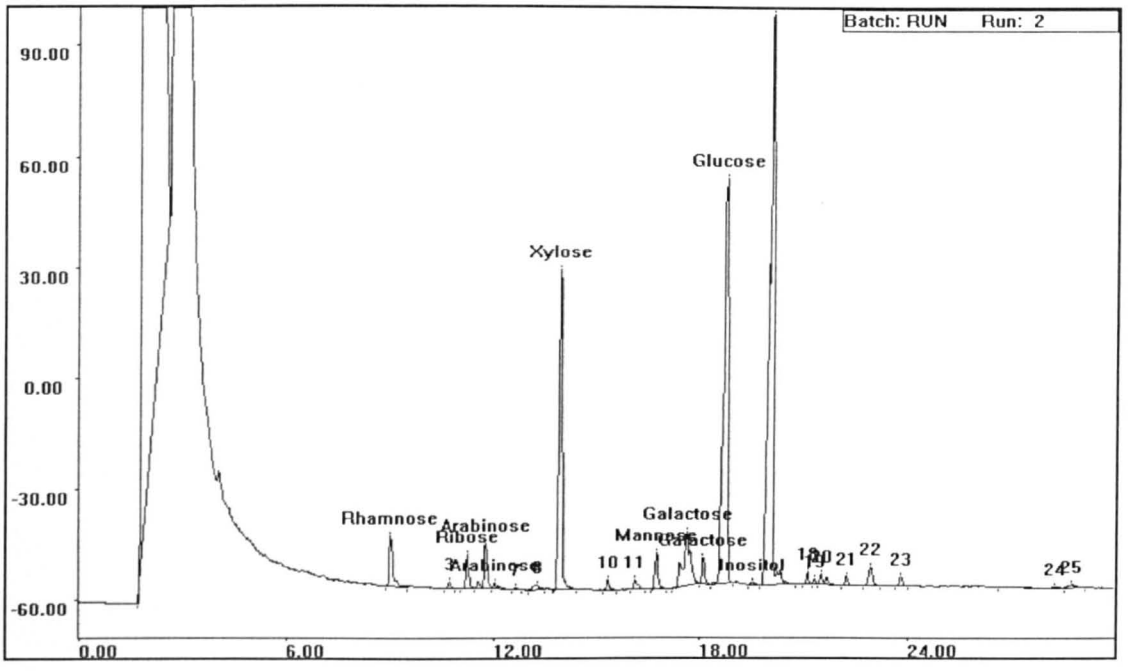

Figure 3.1: Gas chromatogram showing the seven major sugars in a fresh wood sample. Inositol acts as the internal standard.

3.3.5 Statistical analyses

In order quantitatively to determine the amount of cellulose and hemicellulose in a wood sample the following calculations were performed:

1) Area of sugar in test x (area of inositol [standard] / area of inositol [test]) = relative area (test)
2) Relative area (test) / area of sugar standard x weight of sugar standard = weight of sugar in test
3) Weight of sugar in test / weight of test sample x 100 = percentage of sugar in test

Unfortunately, the inclusion of sediment particles on the surface of the wood during field sampling (as described in Sections 2.5.1.1 and 2.5.1.2) and within the wood during the preparation stages prior to GC analysis (as described in Section 2.5.3), prevented the quantitative determination of the individual sugars. As an alternative, the proportions of the individual sugars in a degraded sample were compared with the

proportions of the corresponding fresh wood sugars, in order to show changes in cellulose and hemicellulose. The quantitative determinations of the total sugar losses from the wood samples were not affected by this contamination.

3.4 Critical assessment of the techniques used

The gas chromatographic (GC) analysis of the oak wood samples has provided a wealth of information regarding the inter-relationship between the various wood sugars and their associations with the total wood sugars. This has enabled the elucidation of different patterns of decay (as discussed in Chapter 6).

Throughout the course of this study several issues have arisen that could impact upon the validity of the results obtained from the GC analysis of the wood samples. These issues will be discussed in relation to the sampling of the wood and the interpretation of the data generated.

3.4.1 Sampling

The adhesive nature of the sediment particles prevented their complete removal from the wood during field sampling (as described in Sections 2.5.1.1 and 2.5.1.2) and wood sectioning (as described in Section 2.5.3). As a consequence, sediment contamination occurred during GC analysis, making the results more difficult to interpret.

It is suggested that any future work undertaken on wood samples recovered from burial environments should employ the use of a microscopic technique to identify and remove all of the remaining sediment particles prior to GC analysis. Although this

procedure would be time consuming and laborious, it would ensure that adhering sediment would not influence the resolution of the GC equipment.

3.4.2 Interpretation

Although sediment contamination prevented the quantitative determination of the individual sugars, the results that were generated still enabled the quantitative and qualitative determination of the total sugar and individual sugar losses from each sample respectively.

3.5 Summary

This chapter has evaluated the most applicable techniques for measuring cellulose and hemicellulose degradation in oak wood. Gas chromatography (GC) was chosen as the most appropriate method to use within the remit of this thesis as it enables the key criteria outlined in Section 3.2 to be fulfilled.

The following three chapters will present the physico-chemical results obtained during the monitoring of the sediment within the lysimeter study (Chapter 4), the microbiological results taken from the sediment in the lysimeter study and from the '*in situ*' wetland archaeological and landfill sites (Chapter 5), and the wood degradation results from the lysimeter study and the '*in situ*' burial environments (Chapter 6).

Chapter 4 Physico-chemical results

4.1 Introduction

This chapter presents the physico-chemical results used to characterize the sediments contained within the lysimeter study. Previous studies by Caple (1994), Cheetham (2004) and Hobson (1988) have shown that the main parameters which define sediments in burial environments are moisture content (which is controlled by the manipulation of water/leachate levels in the present study), pH, redox potential (Eh), dissolved oxygen and concentration of organic molecules (Caple 1994, Hobson 1988) (as described in Section 2.4). On the basis of these findings, therefore, the lysimeter study will measure the parameters highlighted above. The sediment type and environmental conditions imposed upon each lysimeter during the study are outlined in Table 2.1.

Although several previous studies have used these parameters to define wetland archaeological sites by identifying the conditions necessary for optimum preservation (Caple 1993, Caple *et al.* 1997, Hogan *et al.* 2001, Smit 2004), little research has been undertaken using these parameters to characterize sediments under controlled conditions. However, with the exception of redox potential, investigations using the techniques outlined above have for a number of years been undertaken in order to characterize landfill environments through laboratory-scale replicates (e.g. Barlaz 1997, Barlaz *et al.* 1989a, 1989b, San & Onay 2001, Stessel & Murphy 1992).

The data obtained using the techniques outlined above will be presented in the form of linear graphs over time. This type of presentation of data has primarily been used to identify the effects that the manipulation of water/leachate levels and other

environmental parameters have upon the sediments and to identify any variations over the duration of the lysimeter study.

4.2 Water level management

Water/leachate levels were controlled using a series of three polyvinylchloride (PVC) taps attached to the outside of each lysimeter (as described in Section 2.3.4). The taps were situated at the base of the lysimeter and at 0.12 m intervals to a height of 0.24 m. The opening of the taps enabled the water/leachate levels to be manipulated, which in turn created the three zones of saturation (aerobic, fluctuating and saturated). Although taps were also inserted into the anaerobic lysimeters, their only function was to enable pH readings to be obtained, and not for the manipulation of water/leachate levels. Water/leachate levels were confirmed using a manometer.

Figure 4.1 shows the changes in water height during managed water level fluctuation in Lysimeter 3 over a two-year period. It demonstrates how water/leachate levels were manipulated within a typical lysimeter. The positioning of the wood samples within the three zones of saturation is also shown. (See Appendix 2 for the heights of the water/leachate levels in all the lysimeters collected during the course of the study).

To ensure continuity of sampling, water levels were lowered on two separate occasions within each six-month period; commencing with the zone of aeration (which represents the top zone in Figure 4.1 [0.24-0.42 m]) and ending with the zone of fluctuation (which represents the middle zone in Figure 4.1 [0.12-0.24 m]). As a consequence, the aerobic nature of the sediment was increased by the introduction of air into each lysimeter. The zone of saturation at the base of the lysimeter is never compromised.

The primary draw-down of the water in the zone of aeration in Lysimeter 3 took place on four occasions within the two-year monitoring period (10/01, 03/02, 09/02 and 02/03), during which the highest tap (Tap 1) was opened for a period of 1 week to allow the water to flow from the lysimeter. The draw-down of the water in the zone of fluctuation also took place on four occasions within the two-year monitoring period (02/02, 08/02, 12/02, 07/03), during which the middle tap (Tap 2) was also opened for a period of 1 week to allow the water to flow out of the lysimeter. All drops in water level over the duration of the experiment are a direct result of water level manipulation.

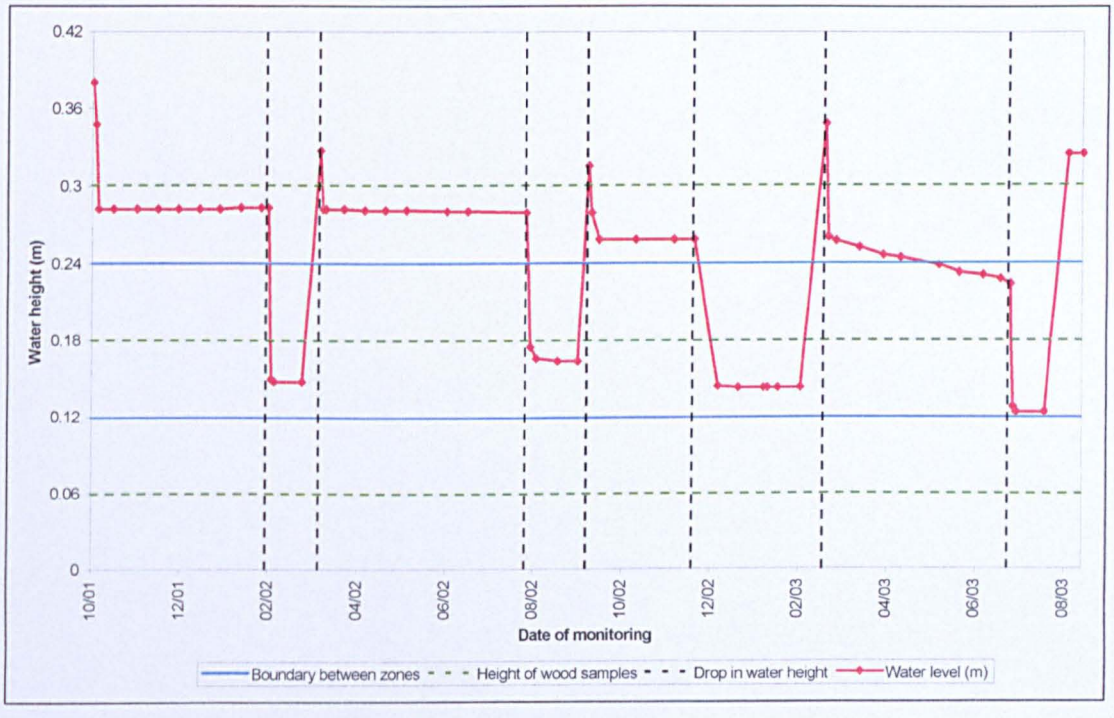

Figure 4.1: Linear plot of water level management in Lysimeter 3.

The wood samples were situated in the middle of each zone of saturation in order to ensure that oxygen diffusion and water flux associated with the boundary conditions between zones did not influence any of the samples. As water levels dropped, they fell below the height of the uppermost wood samples situated in the zone of aeration. As a

consequence, the saturated conditions surrounding the wood samples were replaced by increasingly aerated conditions, thereby promoting oxidation.

A further drop in water levels to below the wood samples situated in the zone of fluctuation again promoted oxidizing conditions around them. However, unlike the zone of aeration where oxidizing conditions existed for 18 weeks in any given six-month period, the second drop in water levels was for a shorter period, namely 4 weeks, before saturated conditions were reinstated. Water levels did not drop below 0.06 m, where saturated conditions persisted throughout the duration of the experiment.

During the period of water level manipulation, three water level measurements were obtained in order to determine the rate of draw-down of interstitial water. Although Figure 4.1 highlights rapid decreases in water level within the zone of aeration and fluctuation on four separate occasions, only during the last six months of the experiment did they drop sufficiently to reach the level of the open taps. This finding indicates that the high water retention characteristic of the peat used in this study prevented further loss of water from the system. However, it should be noted that the hydraulic conductivity of peat is extremely complex and the rate of water loss from the peat used in the current study may be different from that within '*in situ*' peat deposits (Baird *et al.* 2004, Baird & Waldron 2003). On the basis of this finding, it is suggested that the water levels may have dropped further if the tap had been left open for a longer period. Nevertheless, this situation did not prevent an increase in oxidizing conditions surrounding the wood samples.

The above conditions were not found to occur in the lysimeters manipulated by leachate levels, presumably because the larger air spaces within the waste matrix allowed water to flow from the system more efficiently in order to reach the level of the open taps.

The majority of the lysimeters shows changes in water/leachate levels that are similar to the general trend highlighted in Figure 4.1. However, Figure 4.2 shows specific changes in leachate levels in Lysimeters 4 and 8 that are not representative of this pattern. On three separate occasions (10/01-02/02, 03/02-08/02 and 09/02-01/03) the draw-down capacity of the peat in Lysimeter 4 lowered leachate levels below Tap 1; and on two occasions (08/02-09/02 and 08/03) the draw-down capacity of the peat in Lysimeter 4 lowered leachate levels below Tap 2. On one occasion (03/03-08/03) the leachate levels in Lysimeter 8 dropped in association with the decline in the height of the waste below Tap 1.

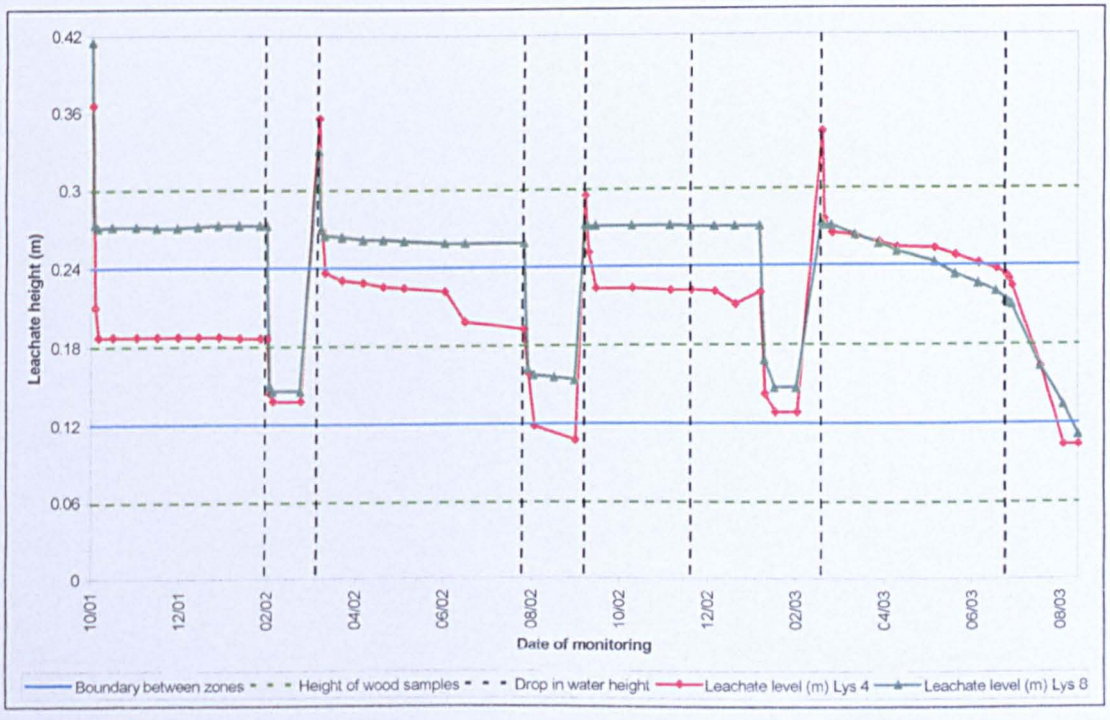

Figure 4.2: Linear plots of water level management in Lysimeters 4 and 8.

4.3 Redox potential

This section presents the results from the redox monitoring of the lysimeters. However, it should be noted that only Lysimeters 1, 2 and 3 had probes inserted into them for the full duration of the monitoring period owing to concerns regarding the potential contamination of the reference electrode by the waste/leachate (as highlighted in Section 2.4.3.2).

Figure 4.3 shows redox plotted against time and water level for Lysimeter 3 at 0.08 m, 0.20 m and 0.32 m depths from the surface of the sediment. This lysimeter contained peat and was manipulated by water levels over the duration of the study. It is representative of the patterns obtained from the majority of lysimeters where water/leachate levels were manipulated. Consequently, similar redox potential values obtained from other lysimeters will not be presented here. (See Appendix 3 which shows the length of time that the redox potentials of the sediments were measured in each lysimeter).

Over the duration of the experiment several lysimeters displayed differing patterns of redox values to the general trends described. These will be discussed and contrasted against the main trends identified.

Redox potential readings taken at 0.08 m and 0.20 m depths display trends associated with the manipulation in water levels. At 0.08 m depth, values of around +400 mV were recorded after a drop in water level. This clearly indicates the presence of oxidizing conditions. Lower readings recorded at 0.20 m depth are in the range of 0 to –200 mV, indicating reduced to highly reduced conditions. Readings at 0.32 m depth show consistently highly reduced conditions throughout the duration of the

experiment, with readings of –100 to –200 mV in evidence. (See Table 2.2 for the different categories of redox potential).

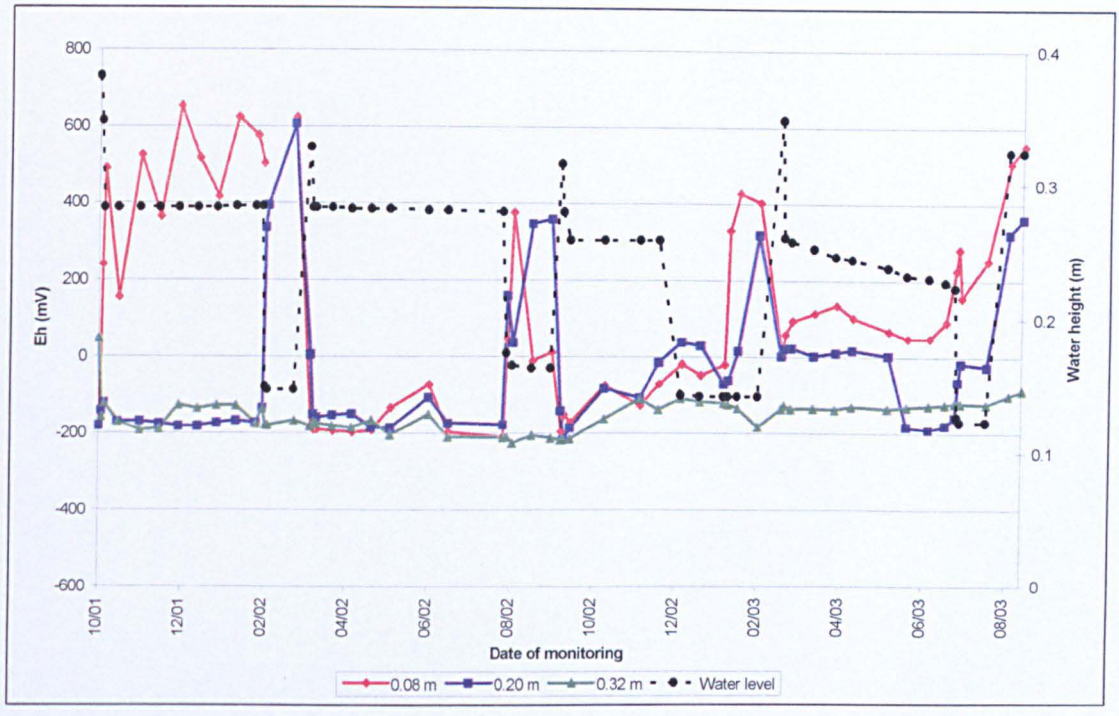

Figure 4.3: Linear plots of peat redox potentials and water levels for monitoring Lysimeter 3. Redox values are adjusted to pH 7.

The situation above suggests that there is a level of conformity within the sediments over the monitoring period which has produced stratification of redox potentials, with the presence of highly oxidized conditions forming at 0.08 m depth and reduced conditions at 0.32 m depth, reflecting the manipulation of water/leachate levels. In such conditions, the sediment at 0.08 m depth would exhibit oxidized conditions where the soil was aerated, consequently promoting aerobic microbial activity, and at 0.32 m depth the sediment is reducing, promoting anaerobic microbial activity. A continuation of these stable reducing conditions would progressively result in a decline of sediment thickness at 0.32 m depth owing to sequential reduction.

Sequential reduction relates to the series of chemical reactions that take place in sediment after saturation. The greater the duration of saturation the more pronounced the reducing conditions become (Ponnamperuma 1972). This sequence of reduction is heavily influenced by soil factors such as nitrate levels, organic matter content and manganese and iron concentrations (Cheetham 2004), all of which vary between different types of sediment.

Several redox readings do not conform to the general trends associated with the manipulation of water levels. Owing to peat shrinkage between 10/01 and 12/01, all 0.08 m redox probes were inserted a further 0.03 m into the peat. This appeared to influence values at 0.20 m depth, possibly as a consequence of disturbance to the surrounding environment. The insertion of the 0.08 m probes into the peat also produced erroneous values on subsequent samplings at this depth (03/02, 04/02, 05/02, 06/02, 07/02 and 08/02). This may be due to water flux and air diffusion associated with the boundary conditions between the zone of aeration and fluctuation, changes associated within the zone of fluctuation itself or the specific micro-environment into which the probes were newly introduced.

In light of these results, the 0.08 m redox probes were raised by 0.02 m at the start of the second year of monitoring. Subsequent redox values recorded in this period appeared to show trends more intimately associated with a manipulation in water levels, with the results indicating the presence of moderately reduced conditions. This observation suggests that the redox potentials may still be influenced by the disturbances resulting from sediment shrinkage.

Several lysimeters display differing patterns of redox values associated with the manipulation of water/leachate levels when compared with the trends discussed above. Figure 4.4 shows redox plotted against time for Lysimeters 3, 7 and 8 at 0.20 m depths. Comparisons between the redox values recorded from Lysimeter 3 and the values recorded from Lysimeters 7 and 8, show highly variable environmental conditions throughout the duration of the experiment.

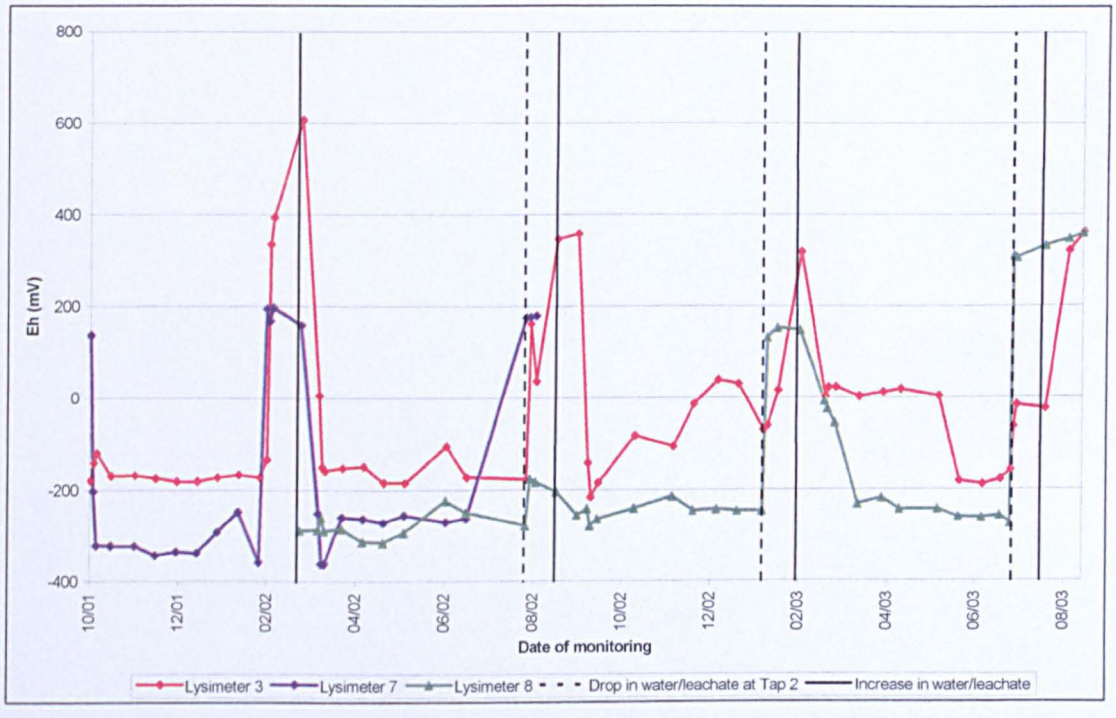

Figure 4.4: Linear plots of sediment redox potentials from 0.20 m depth for monitoring Lysimeters 3, 7 and 8. Redox values are adjusted to pH 7.

The discrepancies in the data set for Lysimeters 7 and 8 are identified as being due to settlement and the associated decline in the height of the waste. In the first six months of monitoring, the waste height in Lysimeters 7 and 8 decreased from 0.435 m, to 0.285 m and 0.268 m respectively. As a consequence, all 0.20 m redox probes were inserted a further 0.03 m depth into the waste in order to take into account this decline. However, measurements made at this new depth reflect more reducing conditions.

This may be due to either water flux associated with the boundary conditions between zones or the micro-environment into which the probes were introduced.

Lysimeter 4 also displays differing patterns of redox values associated with the manipulation of water/leachate levels when compared to the general trend. Figure 4.5 shows redox plotted against time for Lysimeters 3 and 4 at 0.20 m and 0.32 m depths. A drop in leachate levels at Tap 2 in Lysimeter 4 produced a change in redox values at 0.32 m depth; a pattern that is not repeated in the other lysimeters. This may indicate that the redox probes were not fully inserted into the zone of saturation at the start of the monitoring programme. As a consequence, during a drop in leachate levels, water flux and oxygen diffusion associated with the boundary conditions between the zone of fluctuation and permanent saturation appear to have affected the redox potential measurements obtained.

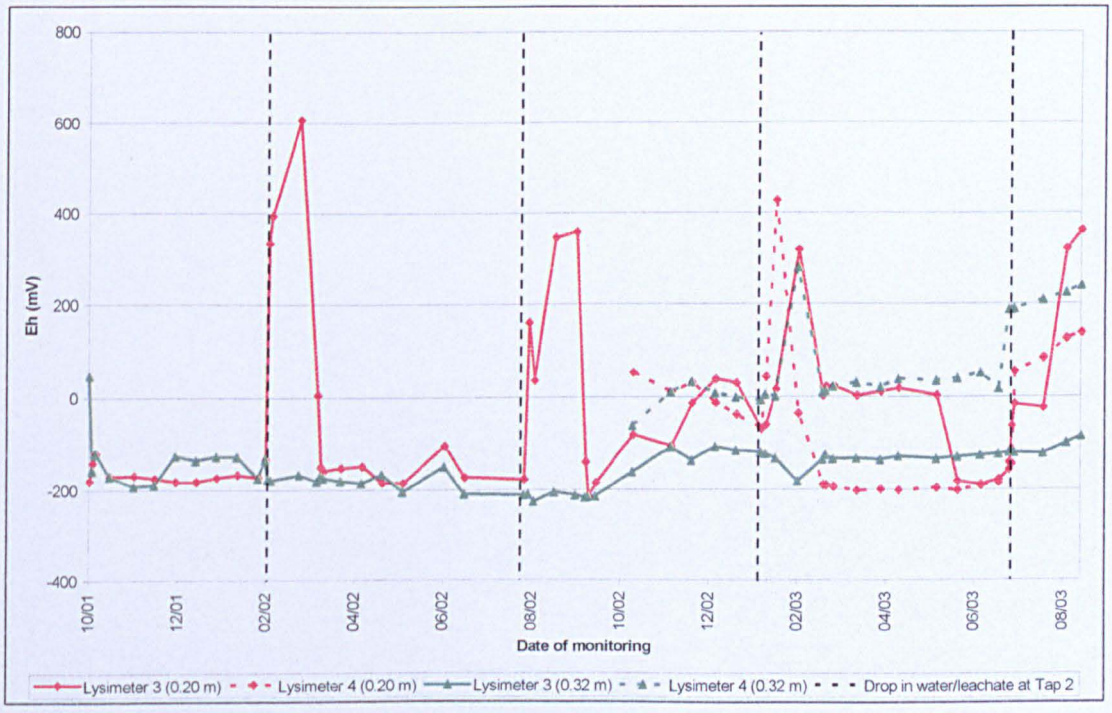

Figure 4.5: Linear plots of peat redox potentials from 0.20 m and 0.32 m depths for monitoring Lysimeters 3 and 4. Redox values are adjusted to pH 7.

Figure 4.6 shows redox plotted against time for Lysimeter 12 at 0.08 m, 0.20 m and 0.32 m depths. This lysimeter contained waste and was permanently saturated with leachate during the study. It is representative of the patterns obtained from all the lysimeters which contained waste and which were permanently saturated with leachate. Consequently, the similar redox potential values obtained from other lysimeters will not be presented here (but are presented in Appendix 3).

Redox potential readings obtained from all three depths in Lysimeter 12 during the first three months of the experiment exhibited similar values of around 0 to –200 mV, indicating the presence of reduced to highly reduced conditions. Readings obtained from 01/03 to the end of the experiment indicated the presence of highly reduced conditions (–150 mV to –250 mV) throughout the profile of the lysimeter.

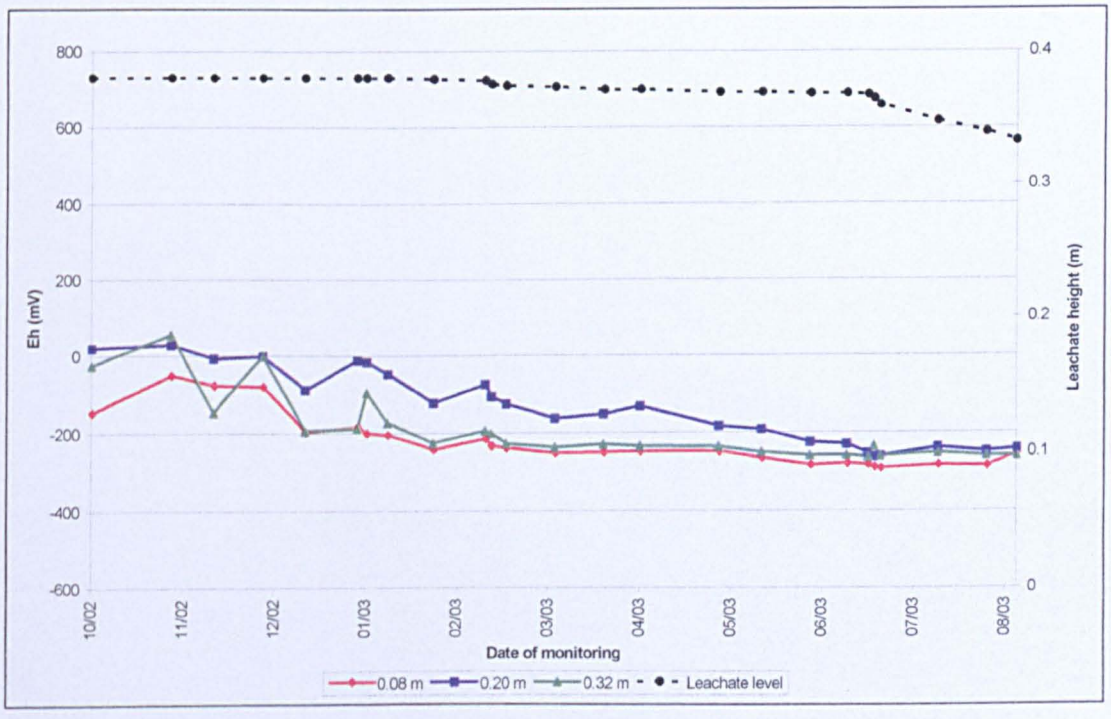

Figure 4.6: Linear plots of landfill waste redox potentials and leachate levels for monitoring Lysimeter 12. Redox values are adjusted to pH 7.

In contrast to the variable redox data generated from Lysimeter 3, which contained peat and which was manipulated by water levels, the redox potential readings from Lysimeter 12 suggest that the permanently high leachate level was responsible for the gradual decrease in redox values over the duration of the experiment (-termed sequential reduction).

This hypothesis is corroborated by Figure 4.7 which shows linear trend lines for redox plotted against time for Lysimeter 12 at 0.08 m, 0.20 m and 0.32 m depths. The results show that there is a continual decrease in redox values over the duration of the experiment, as would be anticipated in the process of sequential reduction.

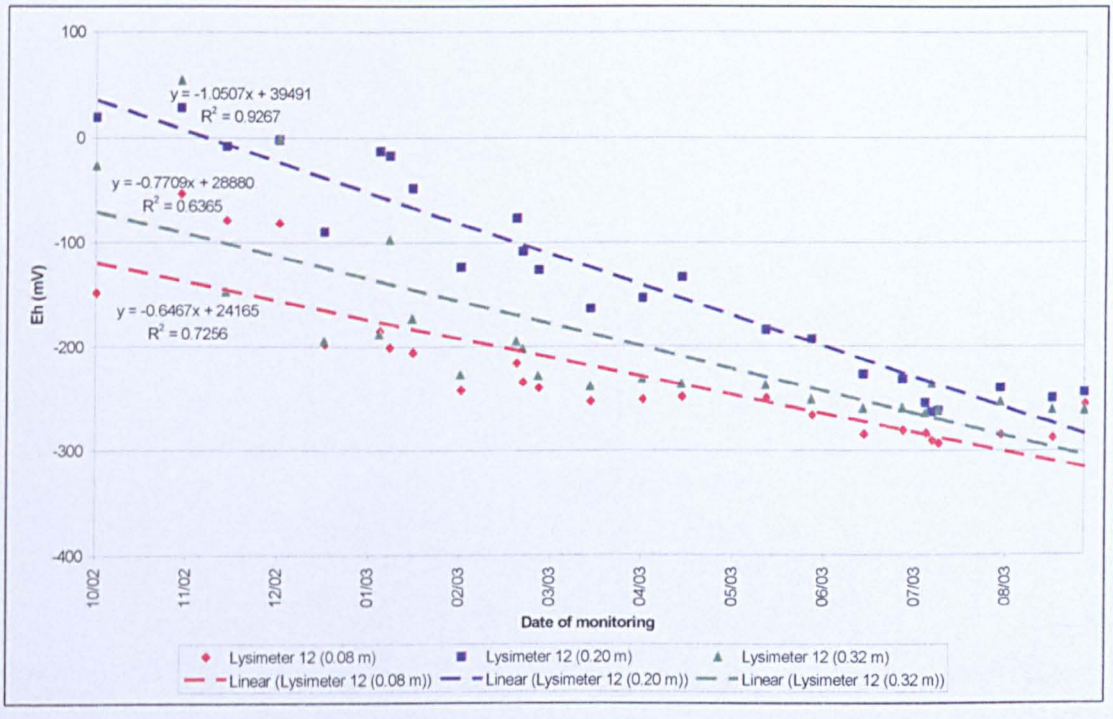

Figure 4.7: Linear plots of landfill waste redox potentials for monitoring Lysimeter 12. Redox values are adjusted to pH 7.

Lysimeter 1 which contained peat and which was permanently saturated with water displays differing patterns of redox values when compared to the trend described

above. Figure 4.8 shows redox plotted against time for Lysimeter 1 at 0.08 m, 0.20 m and 0.32 m depths.

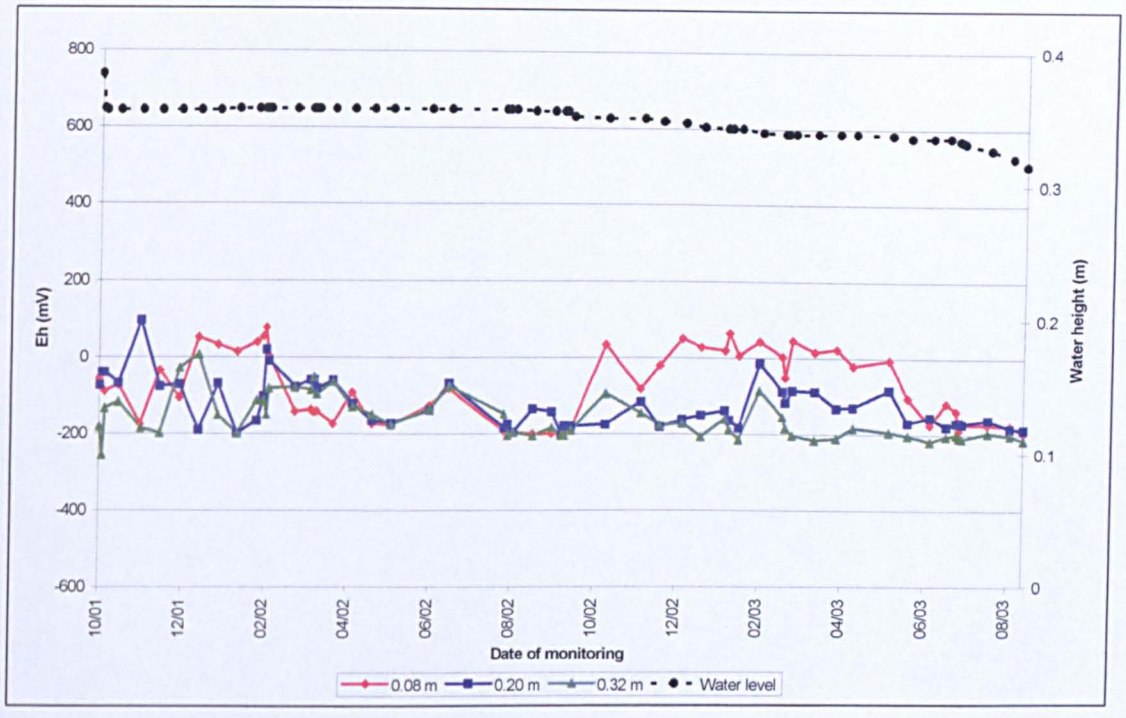

Figure 4.8: Linear plots of peat redox potentials and water levels for monitoring Lysimeter 1. Redox values are adjusted to pH 7.

The majority of the redox potential readings taken from 0.08m depth in Lysimeter 1 shows the presence of reduced conditions, though these appear to be dependent upon the time of year. In the colder months (October to March) the majority of the redox values were between –100 to +100 mV, indicating the presence of reduced conditions; whilst in the warmer summer months (April to September) redox values were generally between –50 to –200 mV, indicating the presence of highly reduced conditions. This is consistent with the fact that an increase in temperature during the summer months promotes microbial activity which consequently depletes the oxygen content of the sediment. The seasonality shown at 0.08 m depth impacts upon the optimum reducing conditions necessary for the preservation of organic material,

which is between –110 to –400 mV (Caple 1996), and implicates temperature in the processes of preservation.

The increase in temperature associated with seasonal fluctuations is not as pronounced at 0.20 m and 0.32 m depths where the majority of the redox values is between –100 to –200 mV for the duration of the experiment, indicating the presence of highly reduced conditions.

The seasonal fluctuations occurring at 0.08 m depth in the saturated peat of Lysimeter 1 were not encountered in the lysimeters which contained waste. This suggests that the temperature inherent within the waste mass, as produced by anaerobic degradation, is more influential than any external increases in temperature associated with seasonal changes.

4.4 Dissolved oxygen

This area of the investigation was undertaken in order to measure the dissolved oxygen of the water/leachate in all of the lysimeters over the duration of the experiment, in an attempt to determine the oxidizing potential of the lysimeters. However, during the monitoring of the lysimeters containing leachate, inconsistent readings were obtained throughout the period of study, when compared with the readings taken from the lysimeters containing water. As a consequence, it is suggested that the high levels of metal ions present in the leachate contaminated the equipment, consequently producing erroneous values (CHEMetrics *pers comm.* 2004). On the basis of this observation, the dissolved oxygen values from those lysimeters containing leachate are excluded from any future analyses. (See Appendix 4 for the dissolved oxygen values for all the lysimeters in this study).

Figure 4.9 shows dissolved oxygen values and water levels plotted against time for Lysimeter 3. This lysimeter contained peat and was manipulated by water level fluctuation over the duration of the study. The low values at Tap 3 are similar to the patterns obtained from Lysimeter 1, which contained peat and which was permanently saturated with water, whilst the general trends produced by all three taps are equivalent to the patterns obtained from Lysimeter 2, which contained peat and which had the water levels manipulated over time.

By its very nature, the monitoring of the dissolved oxygen of water dictates that it is only possible to obtain values prior to a decrease in water levels during the lysimeter study. As a corollary, the dissolved oxygen values shown for Lysimeter 3 were taken when the water levels were high. In those lysimeters where saturated conditions were maintained throughout their profile no special conditions for dissolved oxygen monitoring were necessary.

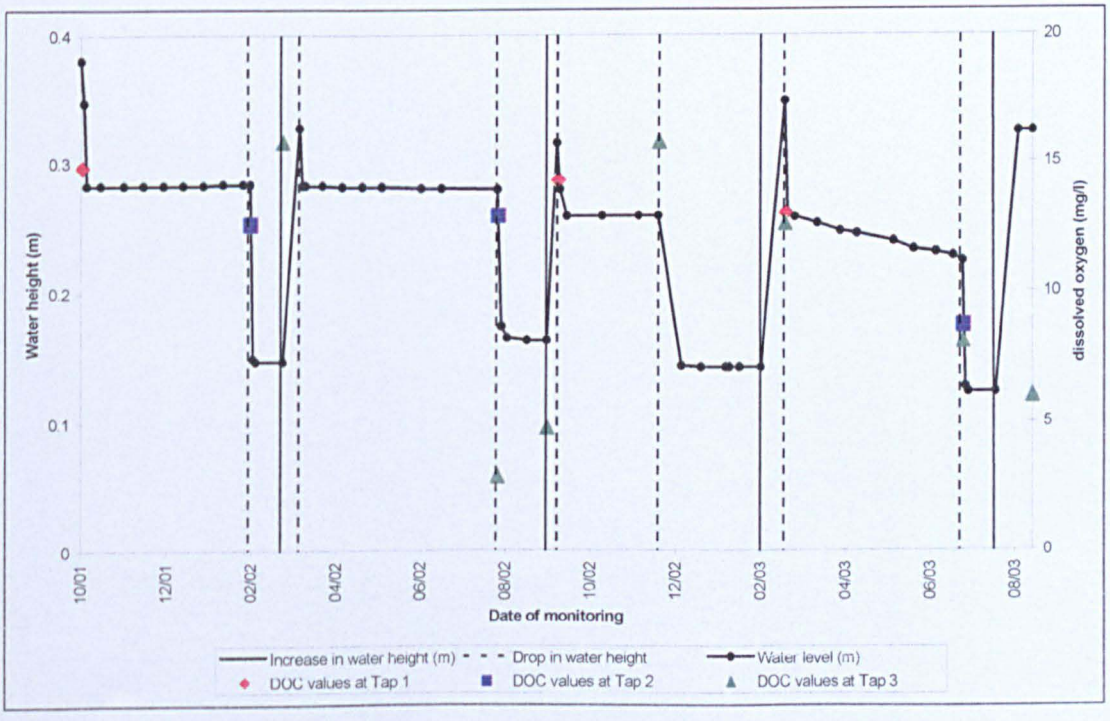

Figure 4.9: Linear plots of dissolved oxygen values and water levels for monitoring Lysimeter 3.

The majority of the dissolved oxygen values obtained at Taps 1 and 2, which were taken prior to a reduction in water levels, shows similar readings of between 13-15 mg/l. This indicates that immediately prior to a lowering of water levels, there was a high occurrence of dissolved oxygen in the water (Behar 1996), which persisted throughout the time that the water levels remained in the zones of aeration and fluctuation. Although a high water level only continued for 4 weeks in the zone of aeration, when compared to 18 weeks in the zone of fluctuation, in every six-month period, the dissolved oxygen values indicated similar conditions. Therefore, the length of time in which saturated conditions were prevalent within these two zones did not produce a drop in the dissolved oxygen of the water.

In contrast to Taps 1 and 2, the dissolved oxygen values at Tap 3 varied greatly over the duration of the experiment, ranging from over 13 mg/l on 03/02, 12/02 and 03/03, to below 8 mg/l on 08/02, 09/02, 07/03 and 08/03. This pattern suggests that the dissolved oxygen of the water in the zone of saturation is influenced by flux and oxygen diffusion through the profile, which is associated with the lowering of water levels.

4.5 pH values

The pH values of the sediment and water/leachate in the lysimeters displayed different patterns associated with sediment composition and level of saturation (*cf.* Caple 1993, Caple & Dungworth 1995). The patterns in evidence are presented in Figures 4.10-4.12.

Figure 4.10 shows sediment pH values plotted against time for Lysimeters 1-3 which contained peat and which had their water levels manipulated during the study (as

displayed in Appendix 5). Three pH values were obtained from the surface of the sediment in the lysimeters at fortnightly intervals (as highlighted in Section 2.4.1.1). An average of the three readings was used for subsequent interpretations. The pH of the peat was measured in conjunction with the redox potential readings.

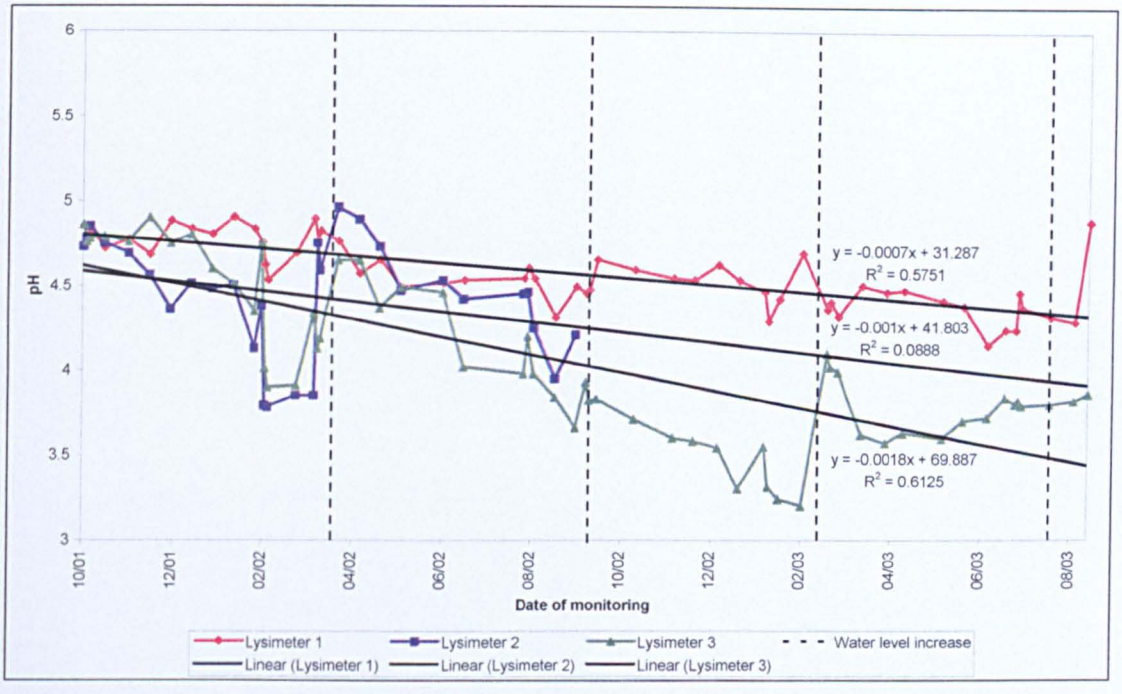

Figure 4.10: Linear plots of pH values for monitoring Lysimeters 1-3.

The linear trend lines of the pH values of each lysimeter indicate a general trend towards more acidic conditions over the duration of the experiment. Although an increase in water levels in Lysimeters 2 and 3 produces a sharp move towards neutral conditions, pH values subsequently decline to re-establish the earlier trend. This indicates that the acidic nature of the peat influences the pH value of the water over the duration of monitoring.

Lysimeters 4, 8 and 12 display differing trends to those described for Lysimeters 1-3. Figure 4.11 shows leachate pH values obtained at Tap 1 plotted against time for Lysimeters 4, 8 and 12. Lysimeter 8 is representative of the patterns obtained from the

lysimeters which contained waste and which had their leachate levels manipulated over time. Lysimeter 12 is representative of the patterns obtained from the lysimeters which contained waste and which were permanently saturated with leachate. Lysimeter 4 is the only lysimeter which contained peat and which had leachate levels manipulated during the study. (Appendix 6 shows the water/leachate pH values obtained from all the lysimeters in the current study).

Leachate pH readings were obtained during the drop in leachate levels (as highlighted in Section 2.4.2.1) and were taken in conjunction with the dissolved oxygen measurements. As a consequence, the low number of leachate pH readings obtained from Lysimeters 4, 8 and 12 was owing to the limited number of Vacu-Vials available to measure the dissolved oxygen of the leachate.

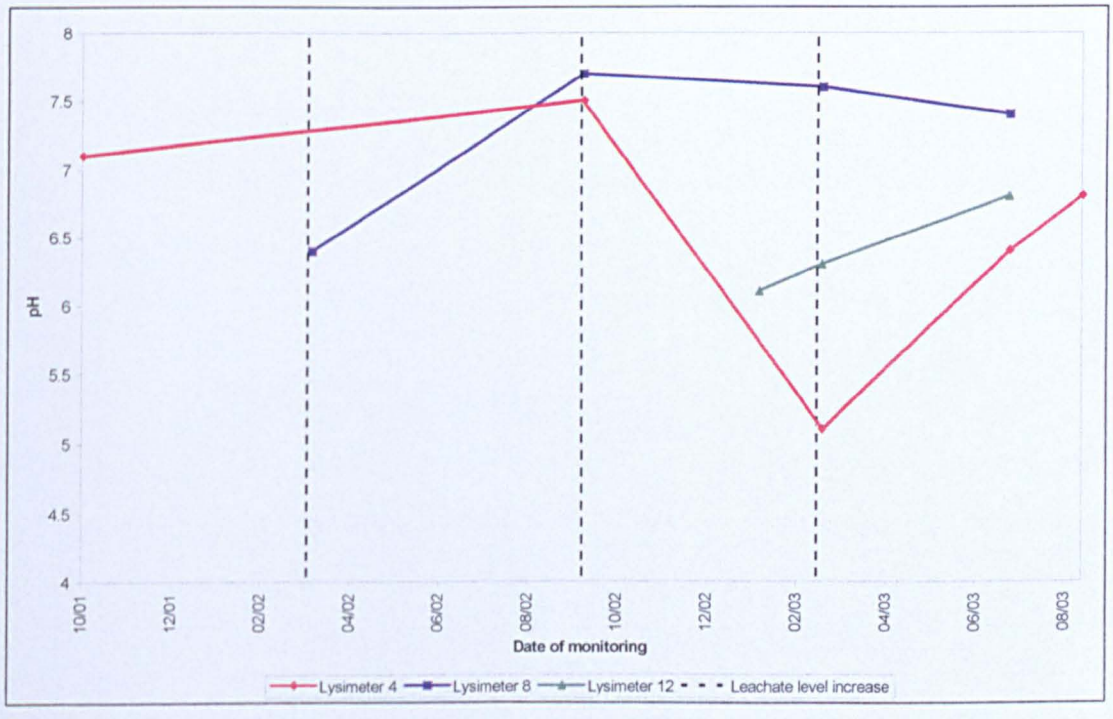

Figure 4.11: Linear plots of monitoring pH values from Taps 1 for Lysimeters 4, 8 and 12.

The results from Lysimeters 4, 8 and 12 exhibited different leachate pH values in contrast to one another over the duration of the experiment. The pH values in Lysimeter 4 indicate a shift from neutral conditions at the start of the experiment to more acidic conditions during the last increase in leachate level. By the end of the experiment pH values are again more neutral. This pattern suggests that the lysimeter was entering the third phase in the cycle of decomposition in landfill waste.

There are four main phases in the cycle of decomposition: the aerobic phase (1), the anaerobic acid phase (2), the accelerated methane phase (3) and the final phase of methanogenesis (decelerated methane phase-4) (Barlaz *et al.* 1989a). Each phase is characterized by differences in the physico-chemical composition of the waste mass and in the development of the microbial population (as described in Table 4.1). During the accelerated methane phase (3) there is an increase in the pH of the waste, little hydrolysis of solids, and an increase in the populations of cellulolytic, acetogenic and methanogenic bacteria (Barlaz 1997, Barlaz *et al.* 1989a).

Lysimeter 12 also showed a similar pattern to Lysimeter 4, as in the last eight months of the experiment pH values moved towards more neutral conditions. This suggests that the lysimeter was entering the accelerated methane phase (3). The acidic nature of the leachate in Lysimeter 12 at 01/03 is typical of the anaerobic conditions found in the majority of landfill sites (*cf.* Barlaz *et al.* 1989a).

Table 4.1: Four main phases in the cycle of landfill waste decomposition (derived from Barlaz 1997, Barlaz *et al.* 1989a).

Phase	Physico-chemical and microbiological composition of waste
(1) Aerobic	Consumption of oxygen and nitrate. There is little change in the populations of cellulolytic, acetogenic and methanogenic bacteria.
(2) Anaerobic Acid	Accumulation of carboxylic acids, decrease in pH, some cellulose and hemicellulose decomposition. The methanogen population increases.
(3) Accelerated Methane	Decrease in carboxylic acid concentrations, increase in pH, and little hydrolysis of solids. There is an increase in the populations of cellulolytic, acetogenic and methanogenic bacteria.
(4) Decelerated Methane	The pH, and cellulolytic and methanogenic populations remain similar to those levels in phase 3. The acetogen population increases, carboxylic acids are depleted, and there is an increase in the rate of cellulose and hemicellulose hydrolysis.

The pH values of the leachate in Lysimeter 8 indicate a shift from acidic conditions during the first increase in leachate level to more neutral conditions over the remainder of the experiment. This pattern is consistent with the final phase of methanogenesis (4), where there is an increase in the rate of cellulose and hemicellulose hydrolysis. The cellulolytic and methanogenic bacterial populations remain similar to those in the accelerated methane production phase (3), whilst the acetogen population increases (Barlaz 1997, Barlaz *et al.* 1989a).

Figure 4.12 shows pH values obtained at Taps 1 and 3 plotted against time for Lysimeters 7, 8 and 12. Lysimeters 7 and 8 contained waste and are representative of the patterns that follow the manipulation in leachate levels, whilst Lysimeter 12 exhibits trends associated with leachate saturation. Unfortunately, the low number of

pH values obtained at Tap 2 for all the lysimeters prevented their inclusion in Figure 4.12.

The pH values at Tap 3 for Lysimeters 7 and 8 are consistently lower than the pH values at Tap 1. However, in the last six months of the monitoring of Lysimeter 8 pH values at Tap 3 became increasingly alkaline, whilst the pH values at Tap 1 moved towards neutrality. The last pH values obtained were similar at both taps. This pattern suggests that the drop in the leachate levels influenced the cycle of decomposition. However, the pattern is not shown in the pH values obtained at Taps 1 and 3 for Lysimeter 12 where similar pH values exist throughout the profile of the lysimeter.

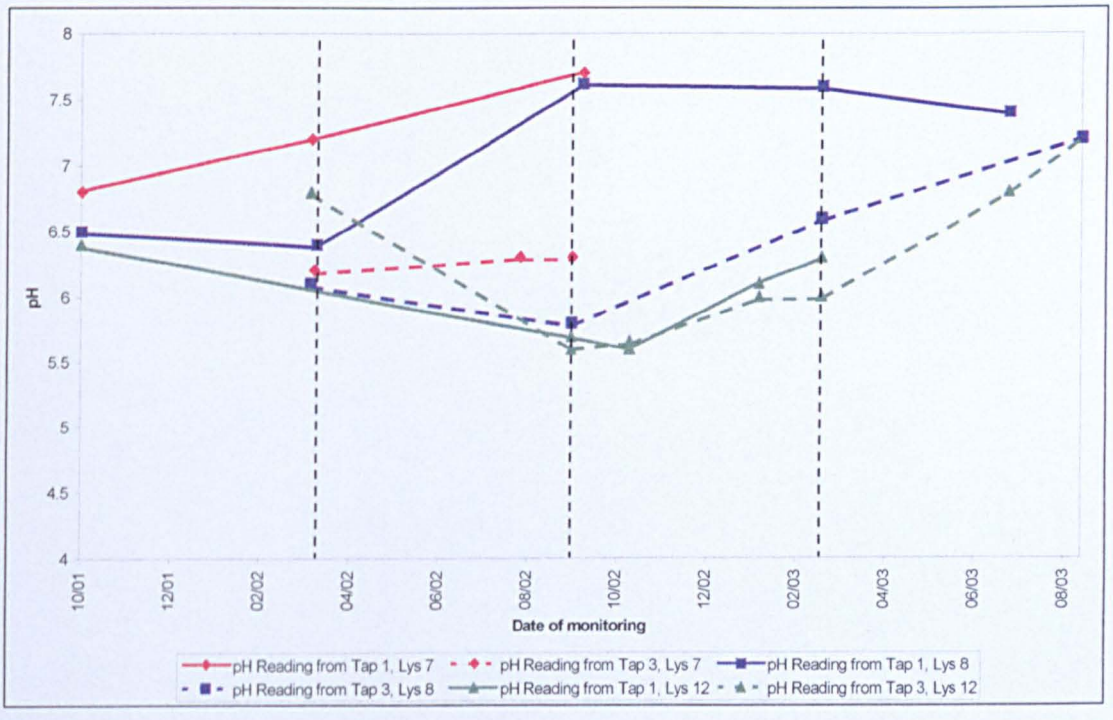

Figure 4.12: Linear plots of monitoring pH values from Taps 1 and 3 for Lysimeters 7, 8 and 12.

4.6 Organic material decomposition

In order to determine the rate of organic decomposition two sets of lysimeters are compared. The first set comprises Lysimeters 5-8 which contained waste and which

had their leachate levels manipulated, and the second set consists of Lysimeters 9-12 which contained waste and which were permanently saturated with leachate.

Figures 4.13 and 4.14 show the percentage settlement drop in the height of the waste plotted against the duration of the experiment for Lysimeters 5-8 and Lysimeters 9-12 respectively. (See Appendix 7 for individual values). The percentage of settlement within the first month of the experiment (-termed primary compression) is noticeably larger in Lysimeters 5-8 than in Lysimeters 9-12. This is due to the greater potential for air dissipation from void spaces in the waste mass associated with the manipulation of leachate levels, in contrast to liquid dissipation from void spaces associated with permanently saturated conditions (Wall & Zeiss 1995).

There are three stages to waste settlement; initial compression, primary compression and secondary compression (Morris & Woods 1990). Initial compression is settlement that occurs directly when an external load is applied to a landfill. This results in the immediate compaction of void spaces and particles in the waste (Tuma & Abdel-Hady 1973). Primary compression is compaction owing to the dissipation of water and gas from void spaces. This usually takes place within the first thirty days after load application (Wall & Zeiss 1995). Secondary compression is generally due to creep of the refuse skeleton and microbiological decay. This can account for a major portion of the total waste settlement and usually takes place over many years (Wall & Zeiss 1995).

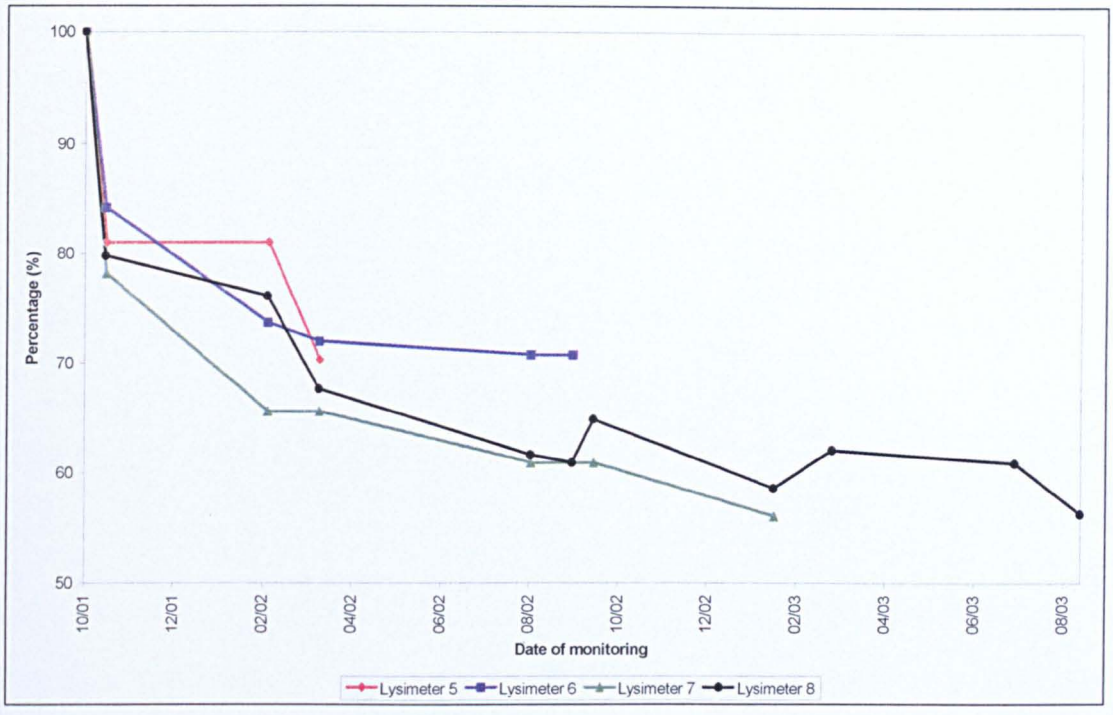

Figure 4.13: Linear plots of monitoring settlement drop in waste height for Lysimeters 5-8.

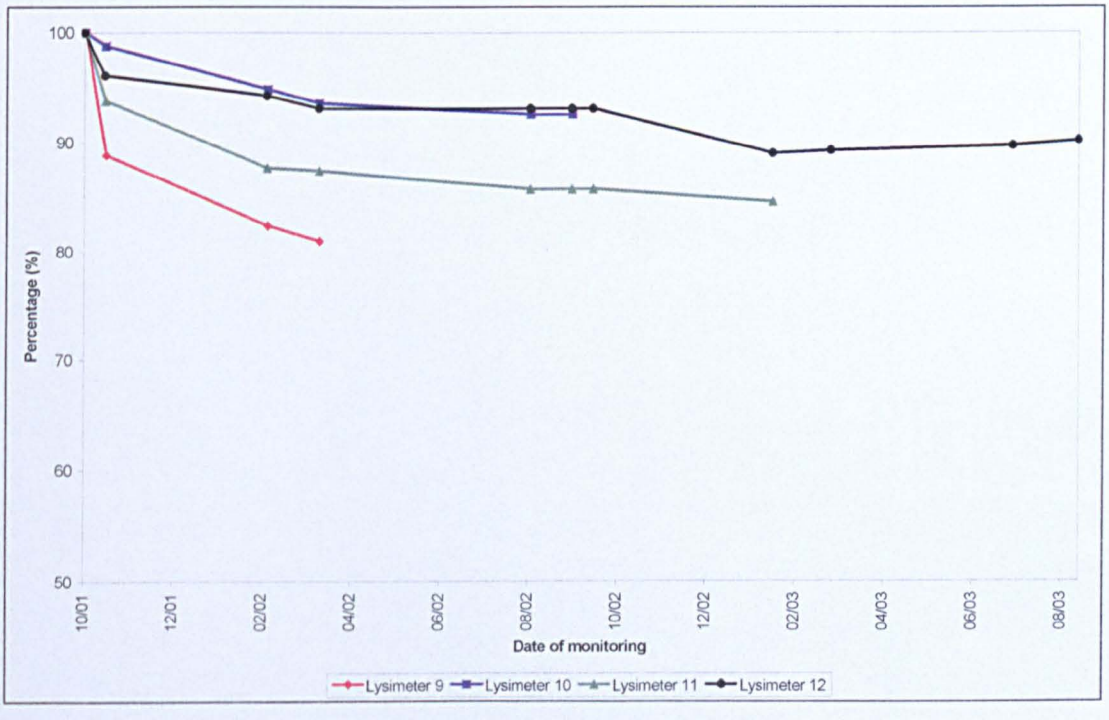

Figure 4.14: Linear plots of monitoring settlement drop in waste height for Lysimeters 9-12.

The rate of secondary compression follows a similar pattern to the trend described above, with Lysimeters 5-8 indicating a significantly greater decline in waste height than Lysimeters 9-12. The manipulation of leachate levels in Lysimeters 5-8 increases the aerobic nature of the waste by introducing air into each lysimeter. This promotes the biological degradation of organic materials and reduces the height of the waste.

When observing the trend lines of the percentage settlement drop in the height of the waste for Lysimeters 8 and 12 over the duration of the experiment, the waste height in the saturated lysimeter (12) stabilizes, whilst that of the fluctuating lysimeter (8) continues to decline. This shows that the settlement rates continued to be higher in Lysimeters 5-8 where conditions were favourable for decomposition than in Lysimeters 9-12 where conditions were unfavourable (*cf.* Yen & Scanlon 1975), reflecting the fact that decomposition promotes more rapid settlement.

Figures 4.15 and 4.16 show predicted plots of the percentage settlement drop in the height of the waste for a period of two years after the end of the experiment. With the exception of Lysimeters 5 and 9 the predicted values of the remaining lysimeters which had their leachate levels manipulated over time, show a greater potential decline in settlement when compared to the lysimeters which were permanently saturated by leachate. This suggests that the conditions in Lysimeters 5-8 will continue to be favourable for biological decomposition two years after the end of the experiment. However, as the duration of the experiment increases, the rate of settlement proceeds at a slower rate owing to a decrease in the microbial decomposition of the waste. As a consequence of this, it should be anticipated that the predicted rate of sediment compaction will be more pronounced during the initial stages of any monitoring exercise.

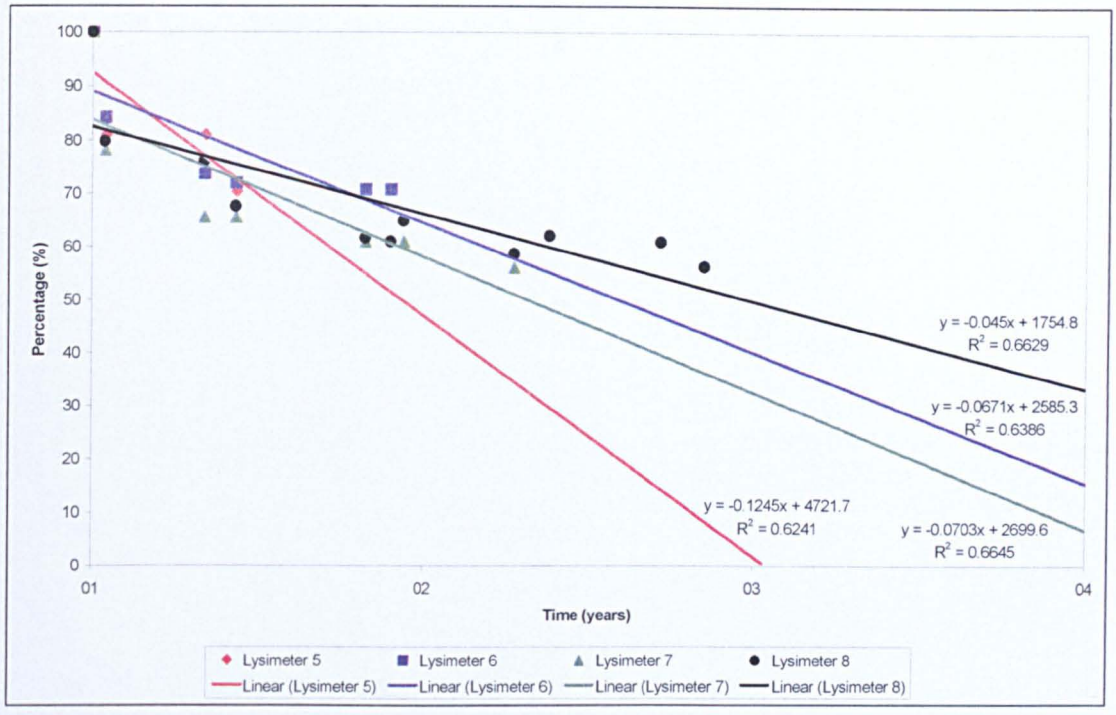

Figure 4.15: Linear plots of monitoring settlement drop in waste height for Lysimeters 5-8, two years after the end of the experiment.

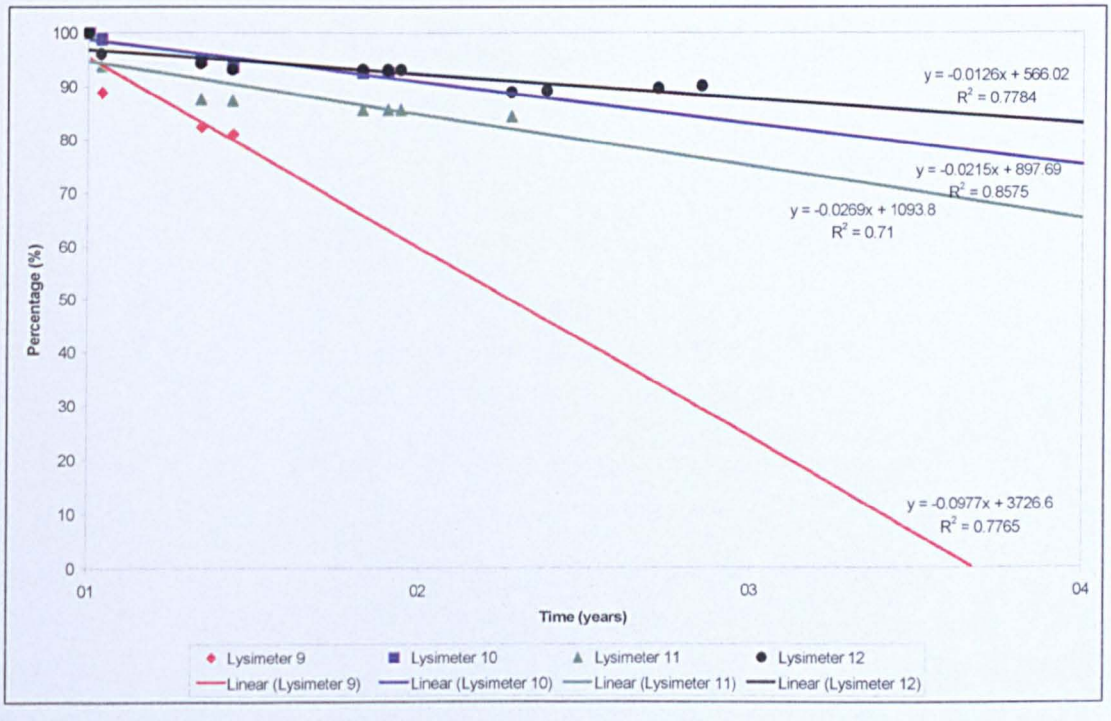

Figure 4.16: Linear plots of monitoring settlement drop in waste height for Lysimeters 9-12, two years after the end of the experiment.

4.7 Discussion of physico-chemical results

The techniques used to measure the physico-chemical patterns and processes which characterize the environmental matrix within the lysimeters as outlined above, have highlighted a number of trends in the data. These can be divided into two themes; the first relates to the results obtained from the application of the techniques that define the nature of the sediments in the lysimeters (as described in Section 4.7.1), whilst the second highlights the effects that these parameters have upon the sediments over the duration of the experiment (as described in Section 4.7.2).

4.7.1 Characterizing the sediments in the lysimeter study

Figure 4.1 highlights the nature of the manipulation of the water/leachate levels in Lysimeter 3, which is indicative of the general trends observed in the aerobic lysimeters. This manipulation determined the three zones of saturation (aeration, fluctuation and saturation). The only discrepancies within this general pattern relate to the draw-down capacity of the peat in Lysimeter 4, which depressed leachate levels below Tap 1, and in Lysimeters 7 and 8 there is a decline in the height of the waste over the duration of the experiment, which produced a subsequent lowering of leachate levels (as shown in Figure 4.2).

A comparison of redox potentials and associated water/leachate level data has shown that the chemical status of the burial environment is influenced by the degree of saturation (Gregory *et al.* 2001). Redox potential readings taken at 0.08 m and 0.20 m depths from the majority of the lysimeters displayed trends that reflect the deliberate manipulation of the water/leachate levels. At 0.08 m depth oxidizing conditions were usually recorded after a drop in water/leachate levels, which promoted aerobic microbial activity. At 0.32 m depth highly reduced conditions were recorded

throughout the duration of the experiment, a situation which promoted anaerobic microbial activity. The conditions at 0.32 m depth in the lysimeters controlled by the manipulation of water/leachate levels were comparable with those throughout the profiles of the lysimeters where full saturation was maintained.

The results from the analysis of the dissolved oxygen values and water levels plotted against time for Lysimeter 3 (as shown in Figure 4.9) indicate that there was a high level of dissolved oxygen in the water prior to a drop in water level. This suggests that an increase in water level may undermine the preservation of the wood samples.

The pH values of the water/leachate in the lysimeters display differing patterns associated with sediment composition and level of saturation. The linear trend lines of the pH values for each lysimeter containing water and peat (as shown in Figure 4.10) indicate a general trend towards more acidic conditions over the duration of the experiment. This suggests that the acidic nature of the peat influences the pH value of the water. The linear trend lines of the pH values for the lysimeters containing leachate (as shown in Figure 4.11) indicate the occurrence of variable conditions associated with the cycle of decomposition in landfill waste. The presence of oxygen in Lysimeters 5-8 enhanced the rate at which the cycle of decomposition occurred, whilst the low levels of oxygen in Lysimeters 9-12 impeded this process.

4.7.2 Assessing the patterns and processes in the lysimeter study

The redox values plotted against time for Lysimeter 12 (as shown in Figure 4.6) are representative of the patterns obtained from all the lysimeters which contained waste and which were permanently saturated with leachate. This graph illustrates the presence of a degree of stability throughout their profiles. The results suggest that the

greater the length of time that the waste is submerged in the leachate, the more prevalent reducing conditions become (-termed sequential reduction). The process of sequential reduction is also demonstrated in the redox values plotted against time for Lysimeter 3, which is shown in Figure 4.3. This lysimeter is representative of the patterns obtained from all the lysimeters where the water/leachate levels were manipulated. Redox readings recorded at 0.32 m depth showed a progressive reduction in the sediment over the duration of the experiment.

Lysimeter 1 displays patterns of redox values at 0.08 m depth which are intimately associated with seasonality. An increase in temperature during the summer months promotes microbial activity, which depletes the oxygen content of the sediment. As a consequence, this will impact upon the optimum reducing conditions necessary for the preservation of organic material, which is between –110 to –400 mV (Caple 1996).

The combined redox potentials and associated water/leachate level measurements undertaken over the duration of the lysimeter study have highlighted specific zones within the lysimeters where there is a potential for either the preservation or degradation of organic material. In the zones of permanent saturation within all of the lysimeters in this study, the recorded redox values indicated good conditions for preservation over the duration of the experiment. However, in the zones of aeration and fluctuation within the lysimeters where the water/leachate levels were manipulated, the redox values indicated poor and moderate conditions for preservation respectively.

The sediment pH values plotted against time (as shown in Figure 4.10) are representative of the patterns obtained from all of the lysimeters which contained peat

and water. This graph indicates a shift towards more acidic conditions over the duration of the experiment. The low pH values of the peat matrix (below pH 5) are important in determining the preservation potential of organic material. pH determines the intensity of the attack on the material by the acid in the soil (Smit 2004). Where acidic conditions prevail (around pH 4) the preservation of organic material is good. However, where pH values are closer to neutral fewer organics remain.

The leachate pH values plotted against time (as shown in Figures 4.11 and 4.12) are representative of the patterns obtained from all lysimeters which contained waste and leachate. These graphs highlight trends associated with different phases of waste decomposition. The value of pH fluctuates between acidic and alkaline conditions, and is dependent upon the cycle of decomposition prevalent within the lysimeter at a given point in time. The phases in the cycle of decomposition occurred over a longer time scale in the zones of permanent saturation and in a shorter time scale in the zones of aeration. As a consequence, the pH values at Tap 1 indicate that the waste in the zones of aeration was entering the final phase of methanogenesis (4), whilst the pH values at Tap 3 suggest that the waste in the zones of saturation was entering the accelerated methane production phase (3). These results suggest that the pH values of leachate are therefore able to give an indication of the potential extent of organic degradation within the lysimeter study.

A comparable trend also exists between the pH values for Lysimeter 12, which contained waste and which was permanently saturated with leachate, and the pH values at Tap 3 for Lysimeters 7 and 8, which contained waste and which had their leachate levels manipulated over time (as described in Figure 4.12). This indicates that

Lysimeter 12 was also entering the accelerated methane production phase (3) in the cycle of decomposition.

Organic material decomposition in all lysimeters which contained waste and leachate was primarily identified by the decline in the height of the waste mass during the three stages of waste settlement (as shown in Figures 4.13 and 4.14). Both primary and secondary compression was noticeably greater in Lysimeters 5-8, than in Lysimeters 9-12. This suggests that the manipulation of leachate levels within Lysimeters 5-8 increases the acrobic nature of the waste by introducing air into the system, which subsequently promotes biological degradation of the organic materials within the waste. As a consequence, microbial biosynthesis influences the rate at which the cycle of decomposition progressed.

The predicted plots of the percentage settlement drop in the height of the waste for a period of two years after the end of the experiment (as shown in Figures 4.15 and 4.16) suggest that although the rate of decline continues, it occurs at a slower rate as the organic component of the matrix is depleted.

4.8 Summary

This chapter has presented the physico-chemical results used to characterize the nature of the sediments contained in the lysimeters used in this study. The results obtained using the different techniques outlined in Sections 4.2-4.6 have been presented in the form of linear graphs against time in order to identify the effects that a manipulation in water/leachate levels and other environmental parameters have upon the sediments within the lysimeters. This approach is adopted in order to provide a greater understanding of the variability occurring in the burial environments studied.

By using other environmental parameters in conjunction with water/leachate management in order to define the character of the sediments, certain parameters have been identified which highlight the potential rate of preservation and degradation of organic material in different types of sediments. These are outlined below:

1) Comparison of redox data and associated water/leachate level data has shown that the chemical status of the sediments in the lysimeters is highly influenced by the degree of saturation.
2) The level of saturation directly corresponds to the three zones that are present within all aerobic lysimeters, and is controlled by the manipulation of water/ leachate levels. These particular zones (aerobic, fluctuating and saturated) indicate where there is the greatest potential for the preservation and degradation of organic material throughout the sediment profile.
3) The greater the duration of saturation the more pronounced the reducing conditions become (-termed sequential reduction).
4) A rise in water levels may not produce a significant lowering of the dissolved oxygen of the sediments in the lysimeters, which may undermine the preservation of the wood samples.
5) Temperature can directly influence aerobic bacterial activity, with cold seasonal temperatures suppressing activity and warmer temperatures promoting activity.
6) The value of pH can indicate the preservation potential of organic material and can highlight trends associated with different phases of waste decomposition.
7) Measurement of the drop in the height of the waste can be used to identify the rate of organic material decomposition during the three stages of waste settlement.

The following chapter will present the results obtained from the microbiological techniques applied to the sediment and wood samples excavated from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites.

Chapter 5 Microbiological results

5.1 Introduction

This chapter presents the microbiological results from the denaturing gradient gel electrophoresis (DGGE) of the sediment and wood samples that were recovered from the burial environments within the lysimeter study and the '*in situ*' wetland archaeological and landfill sites.

The use of DGGE has previously been identified in Section 2.5 as a molecular biological technique that can determine the genetic diversity of total bacterial communities, or particular populations, without further characterization of the individual species (Hastings 1999). DGGE is particularly useful in monitoring any shifts in microbial communities that result from daily and/or seasonal fluctuations (Ainsworth & Goulder 2001, Ferris & Ward 1997), or after environmental perturbations (Muyzer 1999, Muyzer *et al.* 1993).

Although many studies have been undertaken using DGGE successfully to determine the microbial ecology of natural environments, which can serve as useful data for this study, only limited research has been undertaken in wetland archaeological sites (as highlighted in Section 2.5). Previous studies include a variety of soil types (Gelsomino *et al.* 1999), humus samples from forest floors (Pennanen *et al.* 2001), decomposing wood (Pennanen *et al.* 2001, Vainio & Hantula 2000) and even extreme environments such as hot spring microbial mats (Ferris & Ward 1997, Muyzer *et al.* 1993, Santegoeds *et al.* 1996).

DGGE techniques have also been used to study microbial community diversity in artificial environments, for example in cover soils (Wise *et al.* 1999), leachate from landfill sites (Ainsworth & Goulder 2001) and laboratory-scale bioreactors (Silvey & Blackall 1995).

Prior to DGGE analysis, it is necessary to detect and polymerase chain reaction (PCR) amplify the DNA extracted from the microbial communities within the sediment and wood samples (as described in Section 5.2). The PCR amplified DNA fragments are subsequently separated during electrophoresis through a polyacrylamide gel that contains an increasing denaturant gradient (Hastings 1999).

The results from analysis of the sediment and wood samples in each DGGE gel are described in Section 5.3. The first DGGE gel includes sediment and wood samples obtained from Lysimeters 5-8, which contained waste and which had their leachate levels manipulated; the second DGGE gel includes sediment and wood samples from Lysimeters 9-12, which contained waste and which were permanently saturated with leachate; and the third gel includes sediment and wood samples from Lysimeters 3 (which contained peat and which was manipulated by water/leachate levels), 10 and 12, and the '*in situ*' wetland archaeological and landfill sites.

The results presented in Section 5.3 will be analyzed in two ways - qualitative descriptive analyses based on comparisons between DGGE band profiles for the sediment and wood samples, and semi-quantitative analyses based on the application of several statistical techniques (as described in Section 2.5.5). The presentation of DGGE band profiles by descriptive and statistical analyses has been widely used in ecology, taxonomy and other fields prior to this study (Peterson 2002).

5.2 Detection of bacterial communities

DNA samples extracted from microbial communities within sediment and wood (as described in Section 2.5.4.6) were amplified using the universal eubacterial primer combination DS907 R and GM F-GC clamp in order to assess the complete bacterial community in each sample. The universal primers target a 550-bp fragment of the V3 region of the bacterial 16S rRNA gene (Muyzer *et al.* 1993).

Figure 5.1 shows a typical image of the PCR amplified wood fragments on a 2 % agarose gel from Lysimeters 2, 6 and 9 and several '*in situ*' wetland archaeological sites. The fragments confirm the presence of bacterial DNA. The size of each fragment was confirmed by comparison with a size marker (consisting of 500 bp repeats from 0.5 to 3 kb, 1 kb repeats from 3 to 6 kb and 2 kb repeats from 6 to 10 kb, Sigma).

5.3 Results of denaturing gradient gel electrophoresis

5.3.1 Lysimeters 5-8

Polymerase chain reaction (PCR) amplified 16S rDNA fragments were separated by denaturing gradient gel electrophoresis (DGGE) in order to profile the bacterial communities in Lysimeters 5-8. These lysimeters contained waste and had their leachate levels manipulated during this study. Figure 5.2 shows the DGGE band profiles for Lysimeters 5-8 over the duration of the experiment. Image analysis software (Gene Genius Gene Tools, Syngene) clarified these bands.

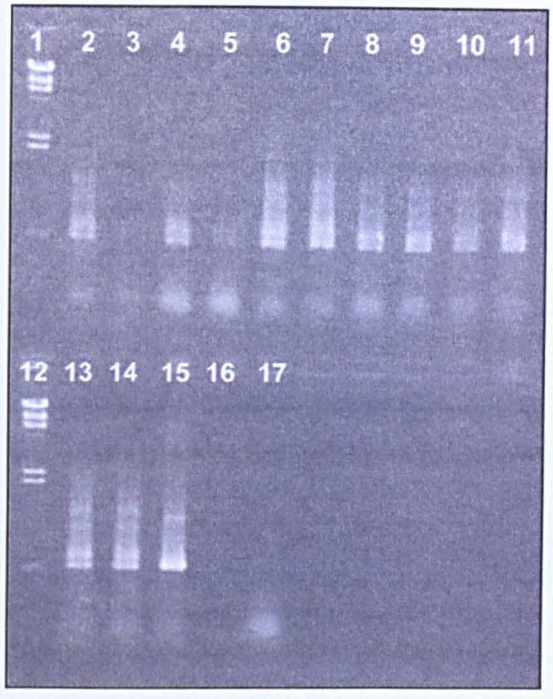

Figure 5.1: Agarose gel (2 %) showing the 550-bp fragment of the 16S rRNA, amplified with the universal eubacterial primers GM5 F-GC and DS907 R. Lanes 1 and 12 - PCR ladder (*Lambda Hind III* DNA digest) (consisting of 500 bp repeats from 0.5 to 3 kb, 1 kb repeats from 3 to 6 kb and 2 kb repeats from 6 to 10 kb); **Lane 2** - Lysimeter 2 at 0.08 m depth (wood); **Lane 3** - Lysimeter 2 at 0.20 m depth (wood); **Lane 4** - Lysimeter 2 at 0.32 m depth (wood); **Lane 5** - Lysimeter 6 at 0.08 m depth (wood); **Lane 6** - Lysimeter 6 at 0.20 m depth (wood); **Lane 7** - Lysimeter 6 at 0.32 m depth (wood); **Lane 8** - Lysimeter 9 at 0.08 m depth (wood); **Lane 9** - Lysimeter 9 at 0.20 m depth (wood); **Lane 10** - Lysimeter 9 at 0.32 m depth (wood); **Lane 11** - Rolls Farm 1 (inner wood); **Lane 13** - Rolls Farm 1 (outer wood); **Lane 14** - Nigtevecht (wood); **Lane 15** - Flag Fen Row 1 (wood); **Lane 16** - positive control (*E. coli*); **Lane 17** - negative control (molecular biology grade water).

A simplified version of Figure 5.2 is shown in Figure 5.3 in order to highlight the bands produced, with each of these potentially representing a distinct bacterial taxon (Ainsworth & Goulder 2001). Each DGGE band profile corresponds to a particular depth within the lysimeter from which the sediment/wood sample was removed. The band profiles represent the level of saturation present, i.e. samples removed from 0.08 m depths are representative of the zone of aeration, samples removed from 0.20 m depths are representative of the zone of fluctuation and samples removed from 0.32 m depths are representative of the zone of permanent saturation.

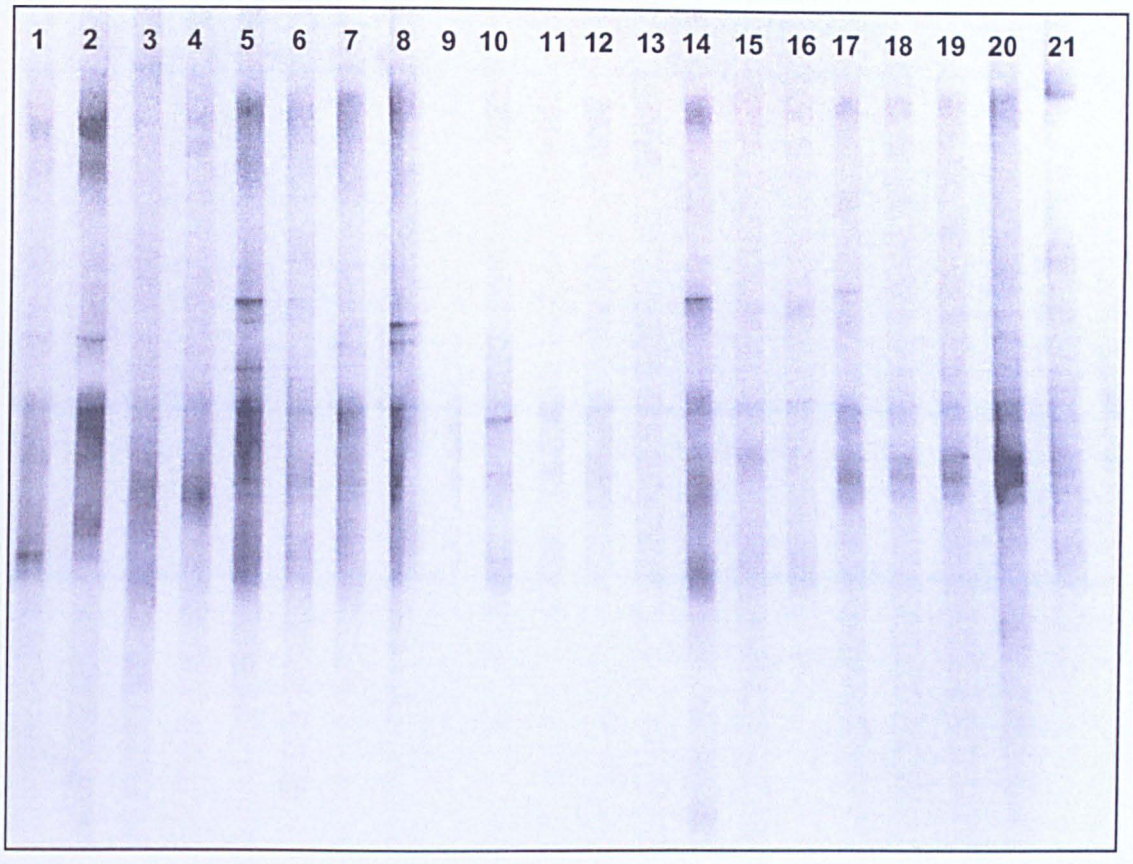

Figure 5.2: DGGE analysis of the bacterial community in Lysimeters 5-8. **Lane 1** - Lysimeter 5 at 0.08 m depth (wood); **Lane 2** - Lysimeter 5 at 0.08 m depth (sediment); **Lane 3** - Lysimeter 5 at 0.20 m depth (wood); **Lane 4** - Lysimeter 5 at 0.20 m depth (sediment); **Lane 5** - Lysimeter 5 at 0.32 m depth (wood); **Lane 6** - Lysimeter 5 at 0.32 m depth (sediment); **Lane 7** - Lysimeter 6 at 0.08 m depth (wood); **Lane 8** - Lysimeter 6 at 0.08 m depth (sediment); **Lane 9** - Lysimeter 6 at 0.20 m depth (wood); **Lane 10** - Lysimeter 6 at 0.20 m depth (sediment); **Lane 11** - Lysimeter 6 at 0.32 m depth (wood); **Lane 12** - Lysimeter 6 at 0.32 m depth (sediment); **Lane 13** - Lysimeter 7 at 0.08 m depth (wood); **Lane 14** - Lysimeter 7 at 0.08 m depth (sediment); **Lane 15** - Lysimeter 7 at 0.20 m depth (wood); **Lane 16** - Lysimeter 7 at 0.20 m depth (sediment); **Lane 17** - Lysimeter 7 at 0.32 m depth (wood); **Lane 18** - Lysimeter 7 at 0.32 m depth (sediment); **Lane 19** - Lysimeter 8 at 0.08 m depth (wood); **Lane 20** - Lysimeter 8 at 0.08 m depth (sediment); **Lane 21** - Lysimeter 8 at 0.20 m depth (wood).

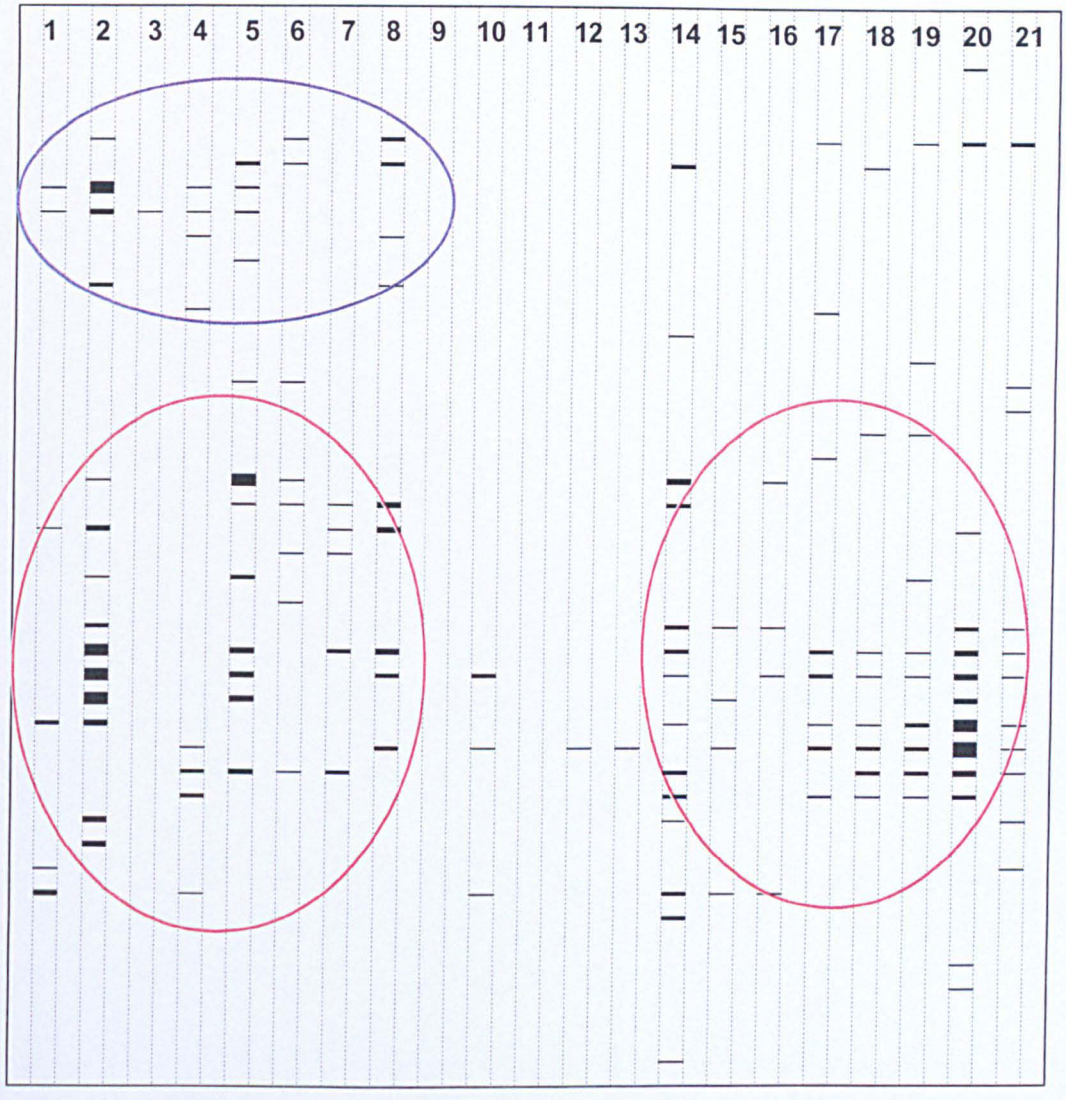

Figure 5.3: Banding patterns from DGGE analysis of the bacterial community in Lysimeters 5-8. Lane 1 - Lysimeter 5 at 0.08 m depth (wood); **Lane 2** - Lysimeter 5 at 0.08 m depth (sediment); **Lane 3** - Lysimeter 5 at 0.20 m depth (wood); **Lane 4** - Lysimeter 5 at 0.20 m depth (sediment); **Lane 5** - Lysimeter 5 at 0.32 m depth (wood); **Lane 6** - Lysimeter 5 at 0.32 m depth (sediment); **Lane 7** - Lysimeter 6 at 0.08 m depth (wood); **Lane 8** - Lysimeter 6 at 0.08 m depth (sediment); **Lane 9** - Lysimeter 6 at 0.20 m depth (wood); **Lane 10** - Lysimeter 6 at 0.20 m depth (sediment); **Lane 11** - Lysimeter 6 at 0.32 m depth (wood); **Lane 12** - Lysimeter 6 at 0.32 m depth (sediment); **Lane 13** - Lysimeter 7 at 0.08 m depth (wood); **Lane 14** - Lysimeter 7 at 0.08 m depth (sediment); **Lane 15** - Lysimeter 7 at 0.20 m depth (wood); **Lane 16** - Lysimeter 7 at 0.20 m depth (sediment); **Lane 17** - Lysimeter 7 at 0.32 m depth (wood); **Lane 18** - Lysimeter 7 at 0.32 m depth (sediment); **Lane 19** - Lysimeter 8 at 0.08 m depth (wood); **Lane 20** - Lysimeter 8 at 0.08 m depth (sediment); **Lane 21** - Lysimeter 8 at 0.20 m depth (wood).

Through the simultaneous analysis of multiple samples on the same gel it is possible to construct a community profile of fragments with differing base compositions and consequently follow any changes in community structure over time (Muyzer *et al.* 1993). However, it must be emphasized that owing to the inherent differences associated with gel pouring and polymerization, the denaturant concentration in one gel will not be identical to the denaturant concentration within another gel (Adams *pers comm.* 2001). As a consequence, it is only possible to compare DGGE band profiles within the same gel.

A total of 35 bands, each potentially representing a distinct bacterial taxon (Ainsworth & Goulder 2001), appeared in different positions in the gel. The highest species diversity, as indicated by the number of bands (Oliveira 2003), was found in the sediment samples from Lysimeters 5 and 7 at 0.08 m depth, both of which contained 14 bands. The sediment sample from Lysimeter 8 at 0.08 m depth contained 13 bands. This suggests (with the exception of the sediment sample from Lysimeter 6 at 0.08 m depth) that the aerobic nature of the sediment at 0.08 m depth produces the highest species diversity over the duration of the experiment.

The lowest species diversity was found in the wood samples from Lysimeter 6 at 0.20 m and 0.32 m depths where no visible bands were detected. The sediment sample from Lysimeter 6 at 0.32 m depth, and the wood samples from Lysimeter 5 at 0.20 m depth and Lysimeter 7 at 0.08 m depth also contained low species diversity, with 1 band present in each sample. The low number of bands associated with these lanes (lanes 9-13) could be associated with a less than adequate separation of the PCR amplified fragments, producing a poor result. It is also possible that a more subtle

change in the microbial population was not detected or that the changes occurred among undetected, possibly subdominant, populations.

In order to compare the lanes containing a low number of bands with the remaining lanes on the gel, it would be necessary to re-amplify the poorly amplified fragments and consequently re-run all the samples using DGGE again. However, owing to time constraints and the low number of samples involved in the poor separation, the current DGGE band profiles have been included for analysis. On the basis of these findings, the sediment and wood samples which correspond to lanes 9-13 of the DGGE gel will not be used for statistical analyses.

The DGGE profile of Lysimeters 5-8 can be separated into three distinct groups, based on the similarities between band clustering; the first group contains all the bands in lanes 1-8, the second group contains all the bands in lanes 9-13 and the third group contains all the bands in lanes 14-21. The similarity between the group containing lanes 9-13 has been discussed above.

Although the positioning of the majority of the bands present in lanes 1-8 and lanes 14-21 are similar (as highlighted by the red ovals in Figure 5.3), the positioning of certain bands within lanes 1-8, with the exception of lane 3, is different (as highlighted by the blue oval in Figure 5.3). Lanes 1-8 show sediment and wood samples obtained from Lysimeters 5 and 6, whilst lanes 14-21 show sediment and wood samples recovered from Lysimeters 7 and 8. The patterning suggests that, although the diversity of the microbial community remains the same, the length of time in the burial environment produces changes in population structure as waste degradation proceeds (Ainsworth & Goulder 2001).

5.3.1.1 Analysis of DGGE profiles

Table 5.1 shows the percentage similarity among lanes within the DGGE gel for the sediment and wood samples obtained from Lysimeters 5-8 using Sorensen's pairwise similarity coefficient (as described in Section 2.5.5.1). The table is formatted into three smaller tables for ease of incorporation in this section (Tables 5.1a-c). The importance of the percentage similarity values is derived from Sait *et al.* (2003) who determined that a value of 100 % indicates that all the bands in the corresponding two lanes are shared, whilst a value of 0 % shows that no bands are shared. Values over 50 % indicate a baseline level of significance.

Table 5.1 (a, b and c): Percentage similarity between gel patterns for Lysimeters 5-8 expressed by Sorensen's Index, C_S.

a) Percentage similarity between gel patterns in lanes 1-10.

Sample	1	2	3	4	5	6	7	8	9	10
1	100[a]	40	28.6	28.6	22.2	0	18.2	13.3	0	22.2
2			6.7	18.2	53.8	18.2	36.4	66.7	0	11.1
3				22.2	15.4	0	0	0	0	0
4					31.6	12.5	15.4	23.5	0	36.4
5						50	35.3	38.1	0	13.3
6							46.2	35.3	0	0
7								42.9	0	0
8									0	25
9										0

[a] **Similarity 100 % by definition**

b) Percentage similarity between gel patterns in lanes 1-9 and 11-21.

Sample	11	12	13	14	15	16	17	18	19	20	21
1	0	0	0	0.2	0.2	0.2	0	14.3	12.5	21.1	23.5
2	0	0	0	35.7	22.2	44.4	36.4	18.2	25	44.4	20
3	0	0	0	0	0	0	0	0	0	0	0
4	0	22.2	22.2	27.3	50	16.7	25	37.5	33.3	28.6	21.1
5	0	0	0	46.2	12.5	25	10	40	36.4	32	34.8
6	0	0	0	36.4	0	16.7	12.5	25	22.2	19	31.6
7	0	0	0	31.2	0	0	15.4	30.8	26.7	31.6	26.7
8	0	20	20	34.8	15.4	15.4	35.3	47.1	42.1	43.5	40
9	0	0	0	0	0	0	0	0	0	0	0

c) Percentage similarity between gel patterns in lanes 10-21.

Sample	11	12	13	14	15	16	17	18	19	20	21
10	0	50	50	23.5	57.1	57.1	36.4	36.4	30.8	25	28.6
11		0	0	0	0	0	0	0	0	0	0
12			100	0	40	0	22.2	22.2	18.2	14.3	16.7
13				0	40	0	22.2	22.2	18.2	14.3	16.7
14					22.2	44.4	36.4	54.5	41.7	44.4	48
15						25	16.7	16.7	14.3	35.3	26.7
16							8.3	8.3	14.2	23.5	26.7
17								62.5	66.7	57.1	52.6
18									77.8	57.1	52.6
19										60.9	57.1
20											58.3
21											100[a]

[a] **Similarity 100 % by definition**

A total of 20 shared DGGE profiles indicates a baseline level of significance between the bacterial communities present in the samples. However, three of these shared profiles relate to lanes 10 and 12, 10 and 13, and 12 and 13, all of which may be associated with the poor separation of PCR amplified fragments. The remaining shared profiles mainly cluster in lanes 17-21 which correspond to the sediment and wood samples in Lysimeters 7 and 8. This finding suggests that the sediment and wood samples display greater percentage similarities the longer they are in the

lysimeters, which may be due to the gradual penetration of bacteria into the wood from the sediment matrix.

The sediment samples obtained at different depths from lanes 18 and 20 also display a significant similarity. Owing to a reduction in the height of the waste in Lysimeters 5-8 during the experiment, it was not possible to obtain sediment samples from their original burial depths. As a consequence, the sediment samples were obtained from lower depths where greater similarities existed in the level of saturation, redox potential and pH values.

Significant similarities also exist between lanes 2, 8 and 20, which confirm that the aerobic nature of the sediment at 0.08 m depth not only produces the highest species diversity but also highlights the presence of certain common groups of bacteria. The presence of these bacterial groups suggests that a few dominant bacterial types are concentrated in the sediment at 0.08 m depth. The differences present within the bands of lower intensity represent the numerically less dominant bacterial populations (Gelsomino *et al.* 1999).

Figure 5.4 shows a dendrogram of the sediment and wood samples obtained from Lysimeters 5-8 using hierarchical cluster analysis (as described in Section 2.5.5.2). The samples are grouped together according to their similarities along the vertical axis. Arbitrary distance values along the horizontal axis show how closely related each sample is to its nearest equivalent, i.e. the longer the distance value the less the similarity that exists.

Hierarchical cluster analysis shows the presence of three main groups within the DGGE gel. The first group at the top of the dendrogram, which corresponds to the majority of the sediment and wood samples from Lysimeters 7 and 8, shows the closest similarities, whilst the remaining two groups show fewer similarities. This finding suggests that the groups tend to cluster in relation to the length of time of burial, with a noticeable shift in microbial community structure between the first and second year of the experiment. This may be influenced by physico-chemical changes in the burial environment.

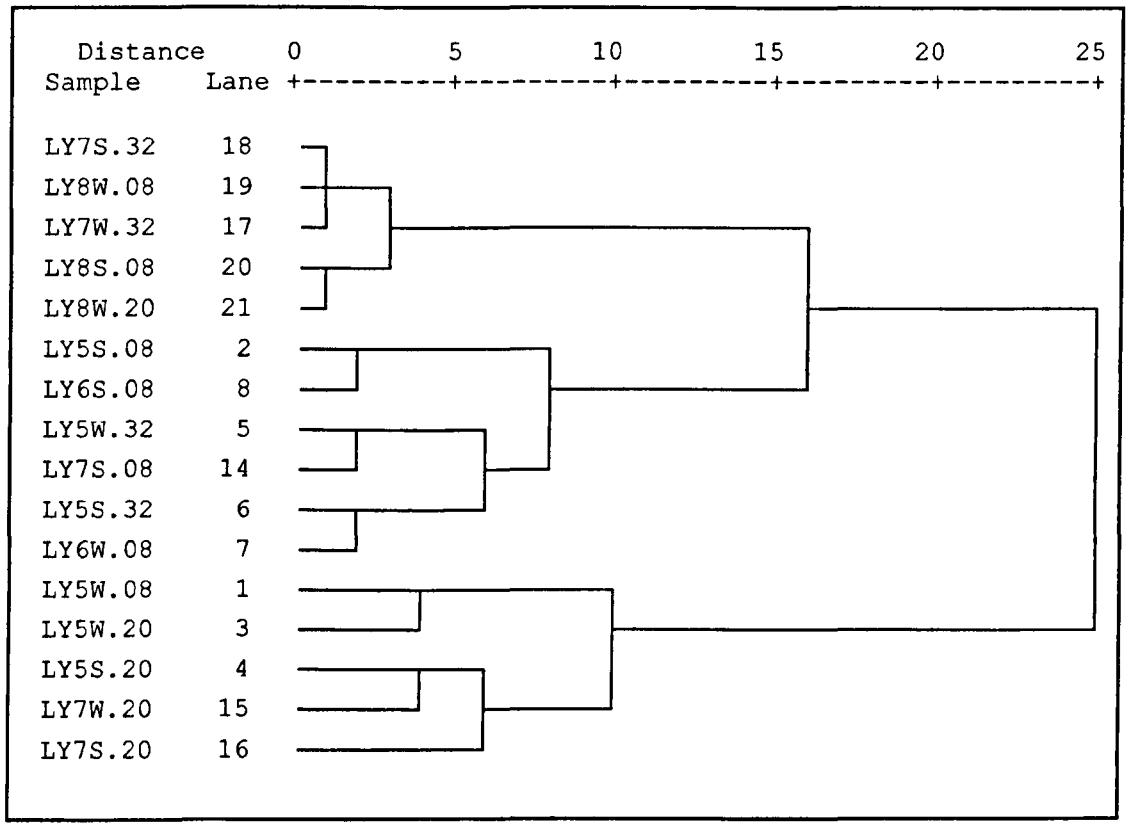

Figure 5.4: Hierarchical cluster analysis using the bands from the DGGE gel for Lysimeters 5-8. LY = lysimeter; **5-8** = number of lysimeter; **S** = sediment; **W** = wood; **.08, .20, .32** = depth.

Principal-component analysis was conducted using Varimax orthogonal rotation to calculate the main components from the matrix of E_s values (as described in Section 2.5.5.3). Five components were extracted with eigenvalues equal to or greater than

1.00. Table 5.2 shows the structure of the rotated component matrix, which is a matrix of the component loading for each variable onto each component. The significant component value for each sample was determined by taking the highest number from the list of five component values. The highest component value produced by the analysis for each sample can identify patterns of significance within the data set and help identify what external factors may be responsible for this value (Field 2000).

Table 5.2: Orthogonal factor loading matrix for Lysimeters 5-8. LY = lysimeter; **5-8** = number of lysimeter; **S** = sediment; **W** = wood; **.08, .20, .32** = depth.

Sample	Component				
	1	2	3	4	5
LY5W.08	.074	-.061	.128	.866	.072
LY5S.08	.548	.359	.449	.411	-.375
LY5W.20	-.373	-.019	-.327	.677	.226
LY5S.20	.243	-.033	.080	.292	.756
LY5W.32	.191	.858	.258	.184	.148
LY5S.32	.062	.898	-.028	-.228	-.036
LY6W.08	.249	.758	-.143	.020	-.291
LY6S.08	.616	.430	.181	.091	-.362
LY7S.08	.463	.642	.328	-.169	.316
LY7W.20	.111	-.498	.573	-.104	.328
LY7S.20	.036	.140	.897	.035	-.009
LY7W.32	.955	-.030	.016	.058	.067
LY7S.32	.861	.129	-.082	-.159	.380
LY8W.08	.944	.137	-.067	-.109	.069
LY8S.08	.896	.175	.191	.029	.019
LY8W.20	.826	.269	.222	-.005	.021
% variance	**40.3**	**26**	**16.7**	**11.8**	**8.3**

There are two components that account for over 10 % of the variance (components 3 and 4), one component that accounts for 26 % of the variance (component 2) and one component that accounts for 40 % of the variance (component 1). The majority of the sediment and wood samples from Lysimeters 7 and 8 displayed the highest values in

the first component which accounted for 40 % of the variance. This finding corroborates the results from the dendrogram which suggests that the similarities between the microbial communities increased during the experiment. This may be due to an increase in the number of specific groups of bacteria responsible for the decomposition of the waste mass, irrespective of the physico-chemical conditions within the lysimeters.

The sediment samples from Lysimeters 5 and 7 at 0.32 m and 0.08 m depths respectively, and the wood samples from Lysimeters 5 and 6 at 0.32 m and 0.08 m depths respectively, displayed the highest values in the second component which accounted for 26 % of the variance. This finding suggests that the size of the bacterial community increased during the experiment. The low numbers of samples included in components 3-5 make it difficult to identify relationships.

5.3.2 Lysimeters 9-11

Figure 5.5 shows the DGGE band profiles for Lysimeters 9-11. These lysimeters contained waste and were permanently saturated with leachate for the duration of the experiment. A simplified version of Figure 5.5 is presented in Figure 5.6 in order to clarify the bands produced.

A total of 34 bands appeared in different positions in the gel. The greatest bacterial species diversity was found in the sediment samples from Lysimeter 9 at 0.08 and 0.32 m depths, both of which contained 21 bands.

The wood sample from Lysimeter 10 at 0.20 m depth was the least diverse, with the gel containing no visible banding. It is possible that this sample has experienced poor

separation of the PCR amplified fragments. As a consequence, the wood sample from Lysimeter 10 which corresponds to lane 9 in Figures 5.5 and 5.6 is not used for further statistical analyses.

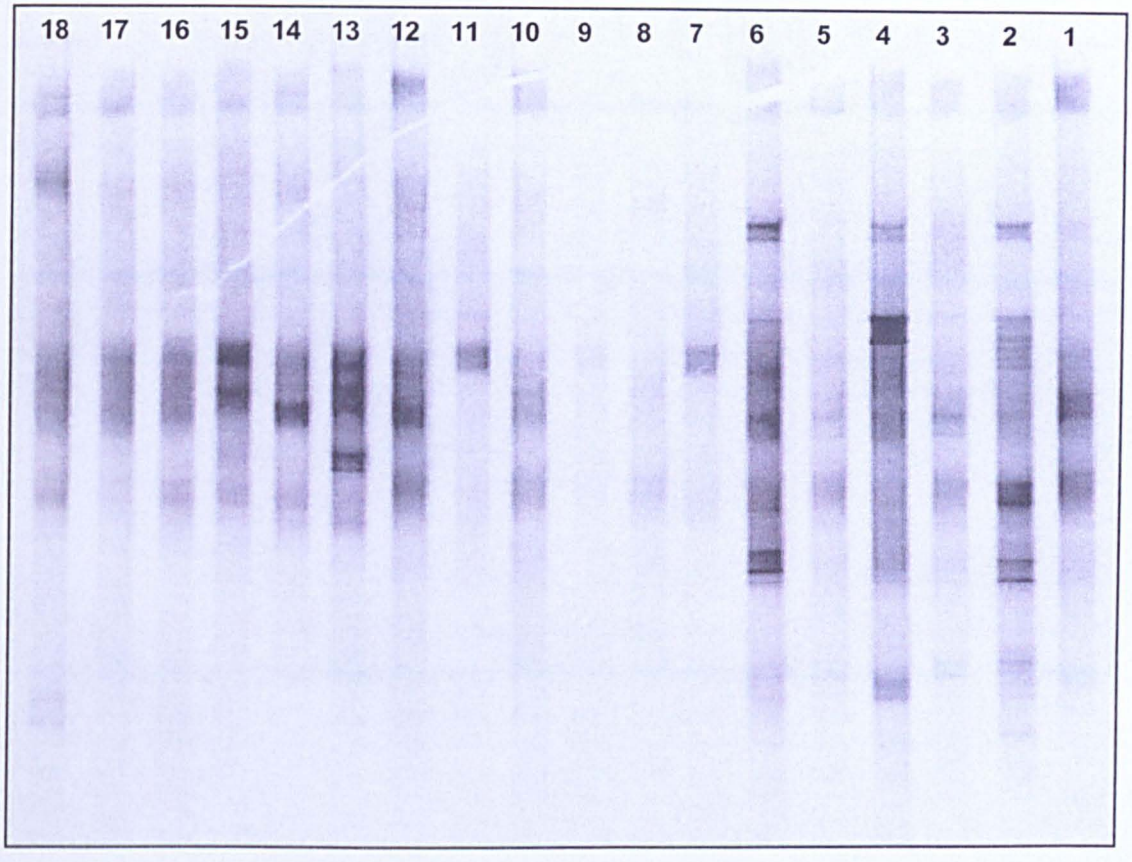

Figure 5.5: DGGE analysis of the bacterial community in Lysimeters 9-11. Lane 1 - Lysimeter 9 at 0.08 m depth (wood); **Lane 2** - Lysimeter 9 at 0.08 m depth (sediment); **Lane 3** - Lysimeter 9 at 0.20 m depth (wood); **Lane 4** - Lysimeter 9 at 0.20 m depth (sediment); **Lane 5** - Lysimeter 9 at 0.32 m depth (wood); **Lane 6** - Lysimeter 9 at 0.32 m depth (sediment); **Lane 7** - Lysimeter 10 at 0.08 m depth (wood); **Lane 8** - Lysimeter 10 at 0.08 m depth (sediment); **Lane 9** - Lysimeter 10 at 0.20 m depth (wood); **Lane 10** - Lysimeter 10 at 0.20 m depth (sediment); **Lane 11** - Lysimeter 10 at 0.32 m depth (wood); **Lane 12** - Lysimeter 10 at 0.32 m depth (sediment); **Lane 13** - Lysimeter 11 at 0.08 m depth (wood); **Lane 14** - Lysimeter 11 at 0.08 m depth (sediment); **Lane 15** - Lysimeter 11 at 0.20 m depth (wood); **Lane 16** - Lysimeter 11 at 0.20 m depth (sediment); **Lane 17** - Lysimeter 11 at 0.32 m depth (wood); **Lane 18** - Lysimeter 11 at 0.32 m depth (sediment).

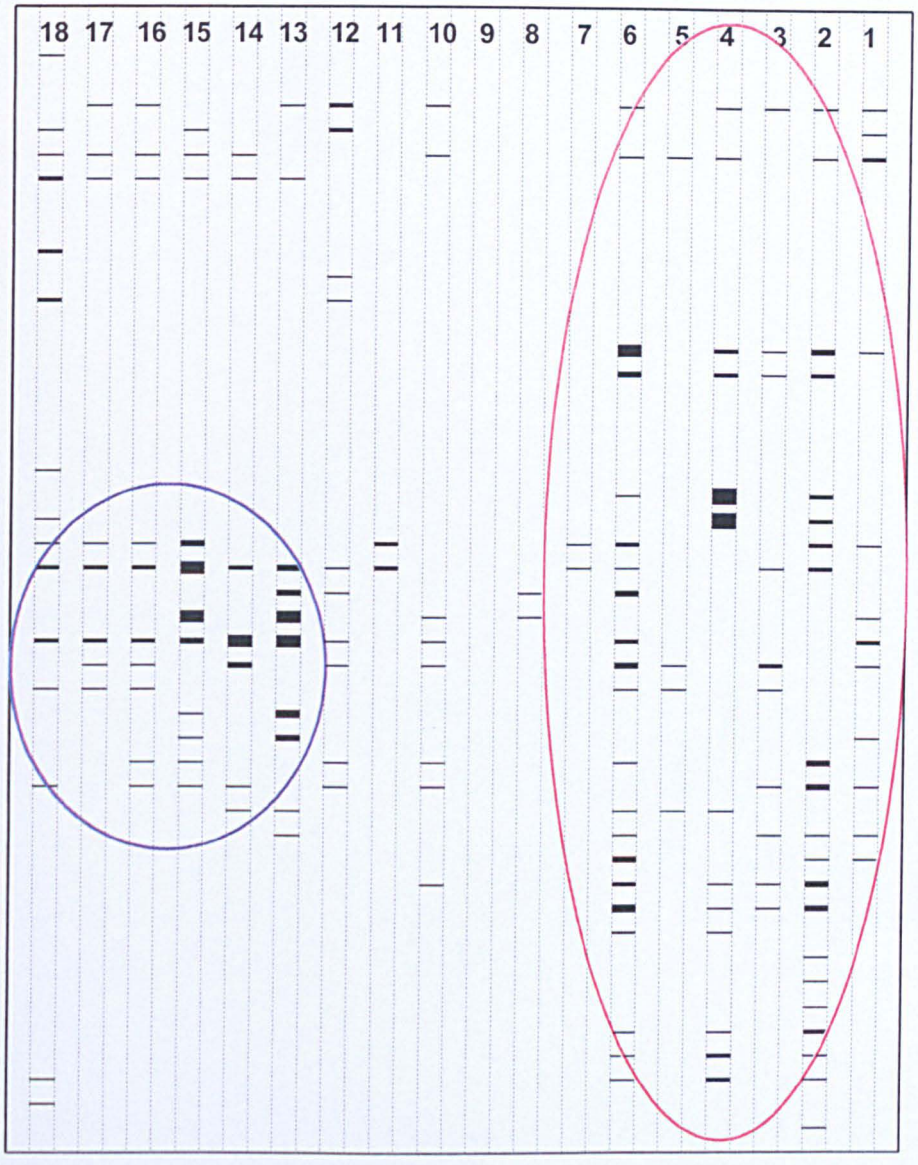

Figure 5.6: Banding patterns from DGGE analysis of the bacterial community in Lysimeters 9-11. Lane 1 - Lysimeter 9 at 0.08 m depth (wood); **Lane 2** - Lysimeter 9 at 0.08 m depth (sediment); **Lane 3** - Lysimeter 9 at 0.20 m depth (wood); **Lane 4** - Lysimeter 9 at 0.20 m depth (sediment); **Lane 5** - Lysimeter 9 at 0.32 m depth (wood); **Lane 6** - Lysimeter 9 at 0.32 m depth (sediment); **Lane 7** - Lysimeter 10 at 0.08 m depth (wood); **Lane 8** - Lysimeter 10 at 0.08 m depth (sediment); **Lane 9** - Lysimeter 10 at 0.20 m depth (wood); **Lane 10** - Lysimeter 10 at 0.20 m depth (sediment); **Lane 11** - Lysimeter 10 at 0.32 m depth (wood); **Lane 12** - Lysimeter 10 at 0.32 m depth (sediment); **Lane 13** - Lysimeter 11 at 0.08 m depth (wood); **Lane 14** - Lysimeter 11 at 0.08 m depth (sediment); **Lane 15** - Lysimeter 11 at 0.20 m depth (wood); **Lane 16** - Lysimeter 11 at 0.20 m depth (sediment); **Lane 17** - Lysimeter 11 at 0.32 m depth (wood); **Lane 18** - Lysimeter 11 at 0.32 m depth (sediment).

Bands indicating low bacterial species diversity were also found in the sediment and wood samples from Lysimeter 10 at 0.08 m depth and the wood sample from 0.32 m depth, all of which showed only 2 bands. Although these samples contain a small number of bands, some bacterial diversity is still present. As a consequence, they will be used in future statistical analyses but will be treated with caution during the interpretation.

The DGGE profile of Lysimeters 9-11 can be separated into three groups based on the positioning of their respective bands. The first group contains all of the bands in lanes 1-6, the second group contains all of the bands in lanes 7-12 and the third group contains all of the bands in lanes 13-18. The bands in lanes 1-6 are distributed throughout the length of the gel (as highlighted by the red oval in Figure 5.6), whilst the majority of the bands in lanes 13-18 cluster in the middle of the gel (as highlighted by the blue oval in Figure 5.6).

There are fewer bands in the sediment and wood samples from Lysimeter 10 than in the corresponding samples from Lysimeters 9 and 11. This patterning reflects a similar situation to that in the DGGE band profiles for Lysimeters 5-8 where it was suggested that the length of time in the burial environment exerts a control on the microbial population. This is possibly related to changes in the landfill environment as waste degradation proceeds. However, the banding patterns associated with Lysimeter 10 indicate that after one year microbial diversity starts to decrease, a situation that is not mirrored in the DGGE band profiles for Lysimeters 5-8. This may suggest that the main phases of landfill waste decomposition operate at different rates, depending on the dominant physico-chemical factors present in the lysimeters.

5.3.2.1 Analysis of DGGE profiles

Table 5.3 shows the percentage similarity between each lane within the DGGE gel of the samples obtained from Lysimeters 9-11 using Sorensen's pairwise similarity coefficient. The table is formatted into three smaller tables for ease of incorporation in this section (Table 5.3a-c). A total of 27 shared bands shows a level of significance. The majority of shared profiles clusters between lanes 1-5. This suggests that during the first six months of the experiment microbial community diversity remains similar throughout the profile of Lysimeter 9. These results are similar to those obtained from Lysimeter 8 where the height of the landfill waste declined owing to organic decomposition. This decrease in height subsequently increased the anaerobic conditions present within the remaining waste mass.

Table 5.3 (a, b and c): Percentage similarity between gel patterns for Lysimeters 9-11 expressed by Sorensen's Index, C_s.

a) Percentage similarity between gel patterns in lanes 10-18.

Sample	18	17	16	15	14	13	12	11	10
18	100[a]	42.4	36.4	27.3	25	48.5	14.3	14.3	0
17			51.6	71	12.5	90.9	28.6	0	0
16				25	28.6	58.1	14.3	0	0
15					28.6	38.7	0	0	0
14						32	0	0	0
13							17.4	8.7	0
12								0	0
11									0

[a] Similarity 100 % by definition

b) Percentage similarity between gel patterns in lanes 1-9 and 11-18.

Sample	9	8	7	6	5	4	3	2	1
18	0	0	23.5	37.8	21.1	21.4	24	33.3	37
17	0	0	20	48.3	50	19	44.4	27.6	50
16	0	0	33.3	45.2	42.9	17.4	50	38.7	54.5
15	0	15.4	30.8	31.3	13.3	8.6	19	15.6	43.5
14	0	0	22.2	35.7	54.5	20	35.3	21.4	42.1
13	0	33.3	16.7	38.7	14.3	17.4	30	19.4	45.5
12	0	16.7	16.7	38.7	14.3	8.7	40	25.8	36.4
11	0	0	100	17.4	0	0	16.7	17.4	14.3

c) Percentage similarity between gel patterns in lanes 1-10.

Sample	9	8	7	6	5	4	3	2	1
10	0	0	0	0	0	0	0	0	0
9		0	55.6	33.3	66.7	52.6	66.7	50	26.1
8			16.7	16.7	22.2	30.8	33.3	40	23.5
7				20	47.1	47.6	60	44.4	32
6					47.1	57.1	30	44.4	24
5						55.6	58.8	66.7	38.5
4							66.7	52.6	53.8
3								88.9	56
2									52.2
1									100[a]

[a] **Similarity 100 % by definition**

The relationship between microbial community diversity and depth changes after one year. The sediment and wood samples from Lysimeters 10 and 11 show differences amongst microbial populations at different depths. This finding suggests that the heterogeneous nature of landfill waste may influence microbial diversity throughout the profile of the lysimeters during certain phases of waste degradation. The consequences of the heterogeneity of the landfill waste may also be exacerbated due to the particle size of the refuse affecting microbial activity, i.e. the smaller the particle size, the greater the surface area for biosynthesis (AFRC Institute of Food Research 1988).

The band profiles of lanes 1-5 also demonstrate a relationship between the microbial community within the wood samples and the sediment samples immediately adjacent to them. This situation only occurs in Lysimeter 5 at 0.32 m depth during the first six months of the experiment, suggesting that the anaerobic nature of the sediment promotes the penetration of bacteria into the wood.

Hierarchical cluster analysis based on the DGGE profiles for Lysimeters 9-11 shows the presence of three main groups and one smaller group within the DGGE gel. The smaller group at the top of the dendrogram consists of two sediment samples and one wood sample from Lysimeter 10. These samples contain the lowest number of band profiles shown in Figure 5.6. It is not possible to determine whether such a low number of bands in each of the samples represent an accurate depiction of microbial diversity, or whether it is due to the difficulties associated with the amplification and separation of PCR fragments. With this in mind the results are included in the dendrogram but should be treated with caution.

The results suggest that the three main groups are related to the duration of monitoring, thereby producing a similar pattern to that displayed in Figure 5.5. The bottom two groups on the dendrogram, which correspond to the majority of the sediment and wood samples from Lysimeters 9 and 10, display the closest similarities, whilst the samples from the top group, which correspond to the sediment and wood samples from Lysimeter 11, display fewer similarities. These findings suggest that there is a noticeable shift in microbial community structure between 1 year and 1.5 years of monitoring, which may be due to the different physico-chemical processes associated with waste decomposition in an anaerobic environment.

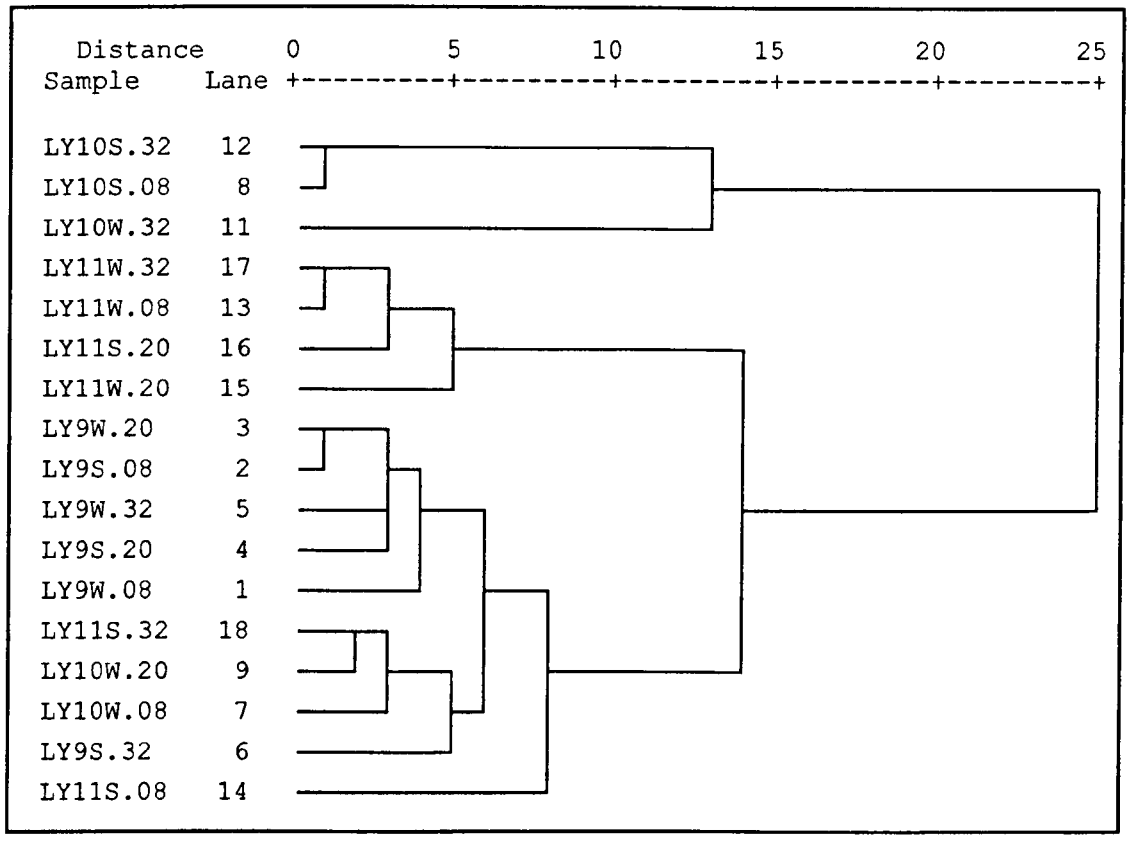

Figure 5.7: Hierarchical cluster analysis using the bands from the DGGE gel for Lysimeters 9-11. LY = lysimeter; **9-11** = number of lysimeter; **S** = sediment; **W** = wood; **.08, 0.20, .32** = depth.

All the groups in the dendrogram (with the exception of the smaller group at the top thereof) contain sediment and wood samples from lysimeters which correspond to the different sampling depths. This finding suggests the presence of similarities within the microbial communities throughout the profile of the lysimeters.

Principal-component analysis was conducted on the matrix of E_s values. Five components were extracted with eigenvalues equal to or greater than 1.00. Varimax orthogonal rotation of the components yielded the component structure shown in Table 5.4. There are three components that account for over 20 % of the variance (components 2, 4 and 5) and one component that accounts for over 30 % of the variance (component 1). The samples displaying the highest values in the first component were from a variety of depths and included at least one sample from each

lysimeter. This finding corroborates the results from the dendrogram which suggests the existence of similarities between microbial populations throughout the profile of the lysimeters.

Table 5.4: Orthogonal factor loading matrix for Lysimeters 9-11. LY = lysimeter; **9-11** = number of lysimeter; **S** = sediment; **W** = wood; **.08, .20, .32** = depth.

Sample	Component				
	1	2	3	4	5
LY11S.32	.437	.288	-.054	.485	.505
LY11W.32	.154	.952	.066	.041	.096
LY11S.20	.069	.683	-.009	.530	-.052
LY11W.20	.120	.782	-.380	-.300	-.087
LY11S.08	.596	.008	-.437	.200	-.433
LY11W.08	.116	.853	-.093	.308	.120
LY10S.32	.089	-.090	.963	-.044	-.017
LY10W.32	-.567	-.334	-.268	.039	.489
LY10W.20	.545	.355	-.282	.506	.251
LY10S.08	.080	-.089	.966	-.063	-.059
LY10W.08	.307	.062	-.096	.787	.166
LY9S.32	.122	.077	-.074	.101	.801
LY9W.32	.858	-.041	-.184	.238	.157
LY9S.20	.613	.005	.185	.237	.681
LY9W.20	.803	.219	.133	.457	.111
LY9S.08	.851	.144	.194	.312	.073
LY9W.08	.734	.234	.193	-.220	.242
% variance	**34.9**	**24.2**	**4.8**	**21.3**	**23**

The samples displaying the highest values in the second component accounted for 24.2 % of the variance. These samples were all from Lysimeter 11. This suggests that the longer the duration of the experiment, the closer the relationship between the microbial communities. Although Sorensen's pairwise similarity coefficient showed that the sediment and wood samples from Lysimeter 9 were closely correlated, the main loading given to these values by principal-component analysis highlights the relationship between different depths. The determination of this relationship was

influenced by the inclusion of samples from Lysimeters 10 and 11 in the first component.

It is difficult to interpret a relationship between the samples included in components 3-5; because component 4 only contains one sediment sample of significant value and components 3 and 5 only contain two sediment samples of significant value.

5.3.3 Lysimeters 3, 10 and 12, and wetland archaeological and landfill sites

Figure 5.8 represents the DGGE band profiles for Lysimeters 3, 10 and 12, and the '*in situ*' wetland archaeological and landfill sites. Lysimeter 3 contained peat and had the water levels manipulated during the study. Lysimeters 10 and 12 contained waste and were permanently saturated with leachate. A simplified version of Figure 5.8 is shown in Figure 5.9 in order to clarify the main bands produced.

A total of 44 bands appeared in different positions in the gel. The highest species diversity was found in the outer wood sample from Rolls Farm 3 which contained 15 bands. The wood sample from Lysimeter 3 at 0.32 m depth contained 14 bands. The lowest species diversity was found in the sediment sample from Roxby 2 at 3.00 m depth and the wood sample from Lysimeter 10 at 0.32 m depth, which contained 2 bands each.

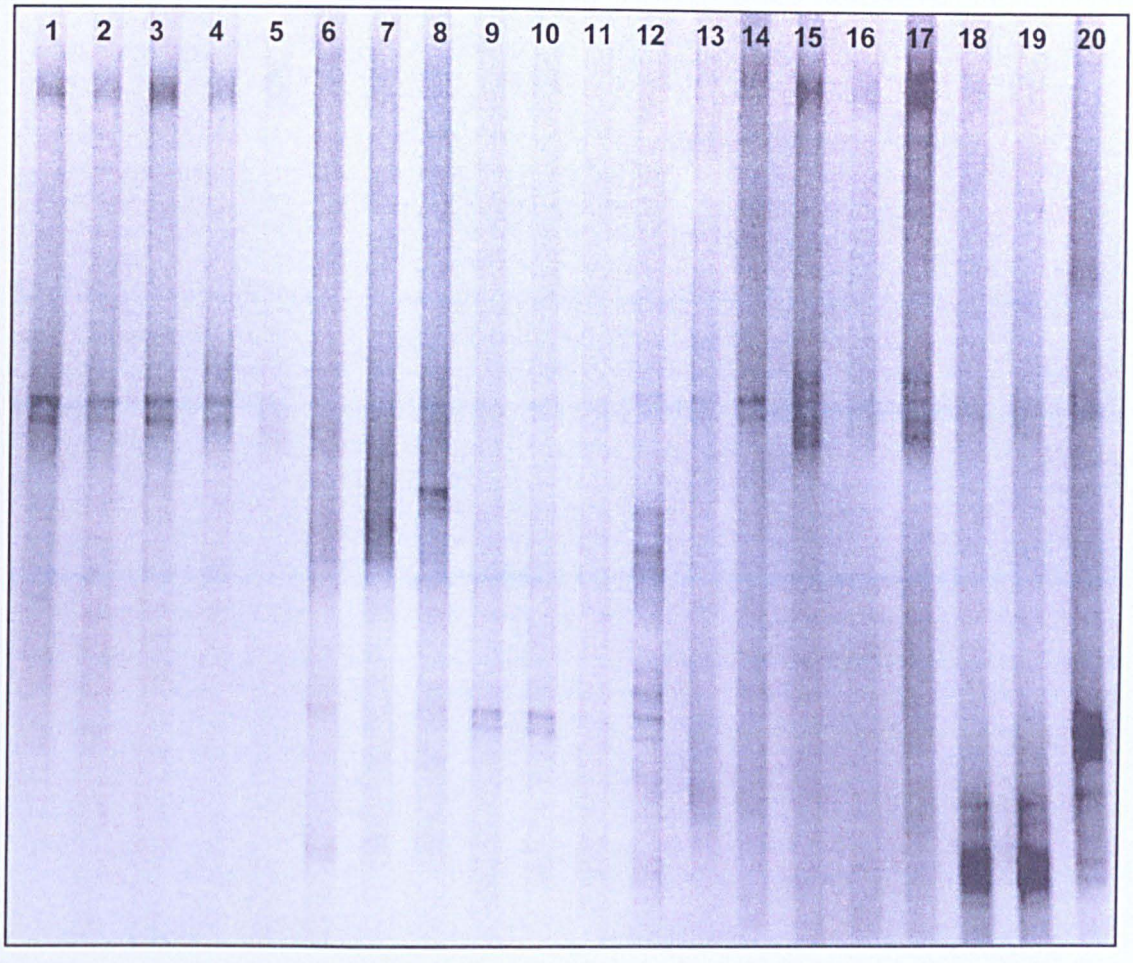

Figure 5.8: DGGE analysis of the bacterial community in Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites. Lane 1 - Roxby 1 at 2.20 m depth (wood); **Lane 2** - Roxby 1 at 2.20 m depth (sediment); **Lane 3** - Roxby 2 at 1.00 m depth (sediment); **Lane 4** - Roxby 2 at 2.00 m depth (sediment); **Lane 5** - Roxby 2 at 3.00 m depth (sediment); **Lane 6** - Lysimeter 12 at 0.08 m depth (sediment); **Lane 7** - Lysimeter 12 at 0.20 m depth (sediment); **Lane 8** - Lysimeter 12 at 0.32 m depth (sediment); **Lane 9** - Lysimeter 10 at 0.08 m depth (wood); **Lane 10** - Lysimeter 10 at 0.20 m depth (wood); **Lane 11** - Lysimeter 10 at 0.32 m depth (wood); **Lane 12** - Rolls Farm 3 (outer wood); **Lane 13** - Rolls Farm 1 (sediment); **Lane 14** - Rolls Farm 3 (sediment); **Lane 15** - Flag Fen Row 1 (wood); **Lane 16** - Flag Fen Row 2 (wood); **Lane 17** - Nigtevecht (wood); **Lane 18** - Lysimeter 3 at 0.08 m depth (wood); **Lane 19** - Lysimeter 3 at 0.20 m depth (wood); **Lane 20** - Lysimeter 3 at 0.32 m depth (wood).

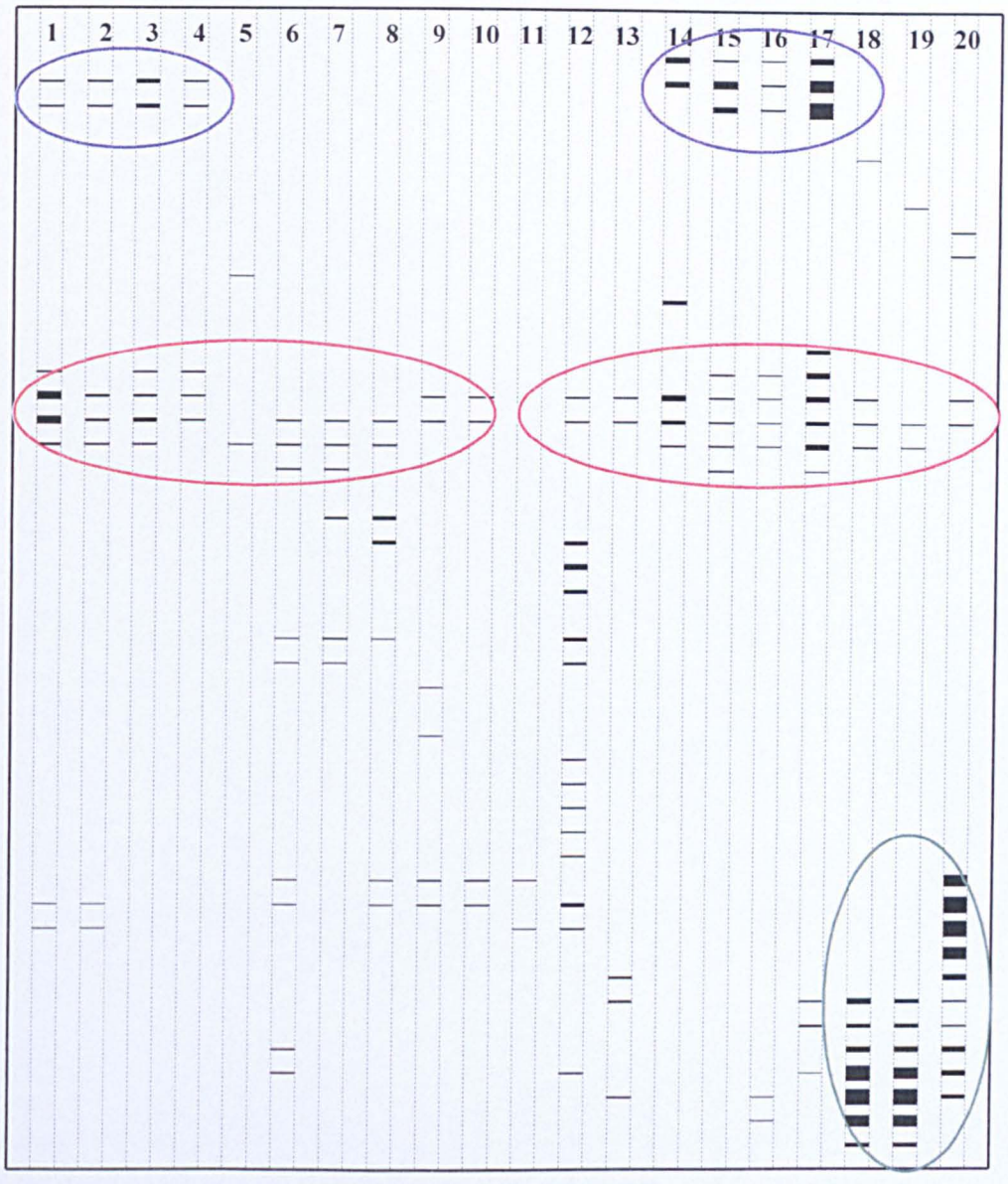

Figure 5.9: Banding patterns from DGGE analysis of the bacterial community in Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites. Lane 1 - Roxby 1 at 2.20 m depth (wood); **Lane 2** - Roxby 1 at 2.20 m depth (sediment); **Lane 3** - Roxby 2 at 1.00 m depth (sediment); **Lane 4** - Roxby 2 at 2.00 m depth (sediment); **Lane 5** - Roxby 2 at 3.00 m depth (sediment); **Lane 6 -** Lysimeter 12 at 0.08 m depth (sediment); **Lane 7** - Lysimeter 12 at 0.20 m depth (sediment); **Lane 8** - Lysimeter 12 at 0.32 m depth (sediment); **Lane 9** - Lysimeter 10 at 0.08 m depth (wood); **Lane 10** - Lysimeter 10 at 0.20 m depth (wood); **Lane 11** - Lysimeter 10 at 0.32 m depth (wood); **Lane 12** - Roll Farm 3 (outer wood); **Lane 13** - Rolls Farm 1 (sediment); **Lane 14** - Rolls Farm 3 (sediment); **Lane 15** - Flag Fen Row 1 (wood); **Lane 16** - Flag Fen Row 2 (wood); **Lane 17** - Nigtevecht (wood); **Lane 18** - Lysimeter 3 at 0.08 m depth (wood); **Lane 19** - Lysimeter 3 at 0.20 m depth (wood); **Lane 20** - Lysimeter 3 at 0.32 m depth (wood).

The banding patterns show several similarities between lanes. There is a common general pattern of banding throughout the majority of the lanes which is shown by the presence of two groups of bands that are situated slightly above the centre of the gel (as highlighted by the red ovals in Figure 5.9). The only exceptions to this are the samples that show the lowest species diversity. A similar banding pattern at the top of the gel can also be seen between lanes 1-4 and lanes 14-17 (as highlighted by the blue ovals in Figure 5.9). These correspond to the sediment and wood samples from Roxby 1 and 2 and the sediment and wood samples from three wetland archaeological sites (Rolls Farm, Flag Fen and Nigtevecht). These patterns suggest that despite the changes in the populations, there are stable communities of dominant bacterial types which are common to the majority of the burial environments chosen for this study.

Several bands of high intensity within the wood samples from Lysimeter 3 are also present in Lysimeters 10 and 12 (lanes 6-12) which suggest that there are similarities in the microbial populations among lysimeters containing different sediments.

5.3.3.1 Analysis of DGGE profiles

Table 5.5 shows the percentage similarity between each lane within the DGGE gel of samples obtained from Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites using Sorensen's pairwise similarity coefficient. The table is formatted into three smaller tables for ease of incorporation in this section (Table 5.5a-c).

A total of 38 shared DGGE profiles indicates a level of significance. The majority of the profiles shows similarities within three separate sets of lanes (lanes 1-4, lanes 14 - 17 and lanes 18-20) and between two sets of lanes (lanes 1-4 and lanes 14-17). These findings confirm the results from the qualitative descriptive analyses based on

comparisons between DGGE band profiles highlighted in the previous section, i.e. there are similarities in the microbial populations between most of the burial environments used in this study.

Table 5.5 (a, b and c): Percentage similarity between gel patterns for Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites expressed by Sorensen's Index, C_s.

a) Percentage similarity between gel patterns in lanes 1-10.

Sample	1	2	3	4	5	6	7	8	9	10
1	100[a]	93	85.7	77	20	35.2	42.9	40	42.9	50
2			76.9	61.5	20	35.2	33.3	40	42.9	54.5
3				54.5	12.5	26.7	33.3	30.8	33.3	40
4					0	14.3	36.4	16.7	36.4	44.4
5						18.2	25	22.2	0	0
6							66.7	62.5	40	46.2
7								46.2	16.7	20
8									46.2	54.5
9										80

b) Percentage similarity between gel patterns in lanes 1-9 and 11-20.

Sample	11	12	13	14	15	16	17	18	19	20
1	0	34.8	30.7	71.4	66.7	70.6	60	31.6	22.2	36.4
2	22.2	36.4	30.8	61.5	66.7	62.5	52.6	33.3	23.5	38.1
3	0	19	36.4	66.7	85.7	80	66.7	35.3	25	20
4	0	10	40	54.5	76.9	71.4	58.9	25	13.3	21
5	0	0	0	25	20	18.2	14.3	15.4	16.7	12.5
6	18.2	41.7	14.3	26.7	35.3	22.2	38.1	40	42.1	26.1
7	0	28.6	18.2	33.3	42.9	26.7	33.3	23.5	25	10
8	22.2	27.3	16.7	30.8	26.7	25	21.1	22.2	23.5	28.6
9	25	28.6	36.4	33.3	28.6	26.7	22.2	23.5	12.5	40

c) Percentage similarity between gel patterns in lanes 10-20.

Sample	11	12	13	14	15	16	17	18	19	20
10	33.3	30	50	40	33.3	30.8	23.5	28.6	11.1	44.4
11		11.1	0	0	0	0	0	0	0	25
12			20	18.2	16.7	24	20.7	30.8	20	40
13				40	33.3	30.8	35.3	42.9	22.2	44.4
14					71.4	33.3	52.6	37.5	20	20
15						82.4	66.7	33.3	18.2	18.2
16							63.6	52.3	34.8	26.1
17								60.9	44.4	44.4
18									75	58.3
19										42.9
20										**100**[a]

[a] **Similarity 100 % by definition**

Hierarchical cluster analysis based on the DGGE profiles for Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites showed the presence of two main groups of bands within the DGGE gel (as shown in Figure 5.10).

The first group, shown at the top of the dendrogram, consists of the majority of the sediment and wood samples from the wetland archaeological and landfill sites. Distance values depicting the relationship between each sample indicate that all the samples within the group are closely related to one another, confirming the results from Sorensen's pairwise similarity coefficient.

The second group consists of the remaining sediment and wood samples from the wetland archaeological and landfill sites and all the sediment and wood samples from Lysimeters 3, 10 and 12. Although this group can be sub-divided into four smaller groups, there appears to be no obvious pattern of similarity between the sub-groups. However, the distance values between samples within each of the smaller groups and the distance values connecting each of the smaller groups together, show that all the

samples are similar to one another. This finding corroborates the results from the previous analyses of the samples, i.e. that there are many bacterial species present in both the lysimeters and the '*in situ*' burial environments used in this study.

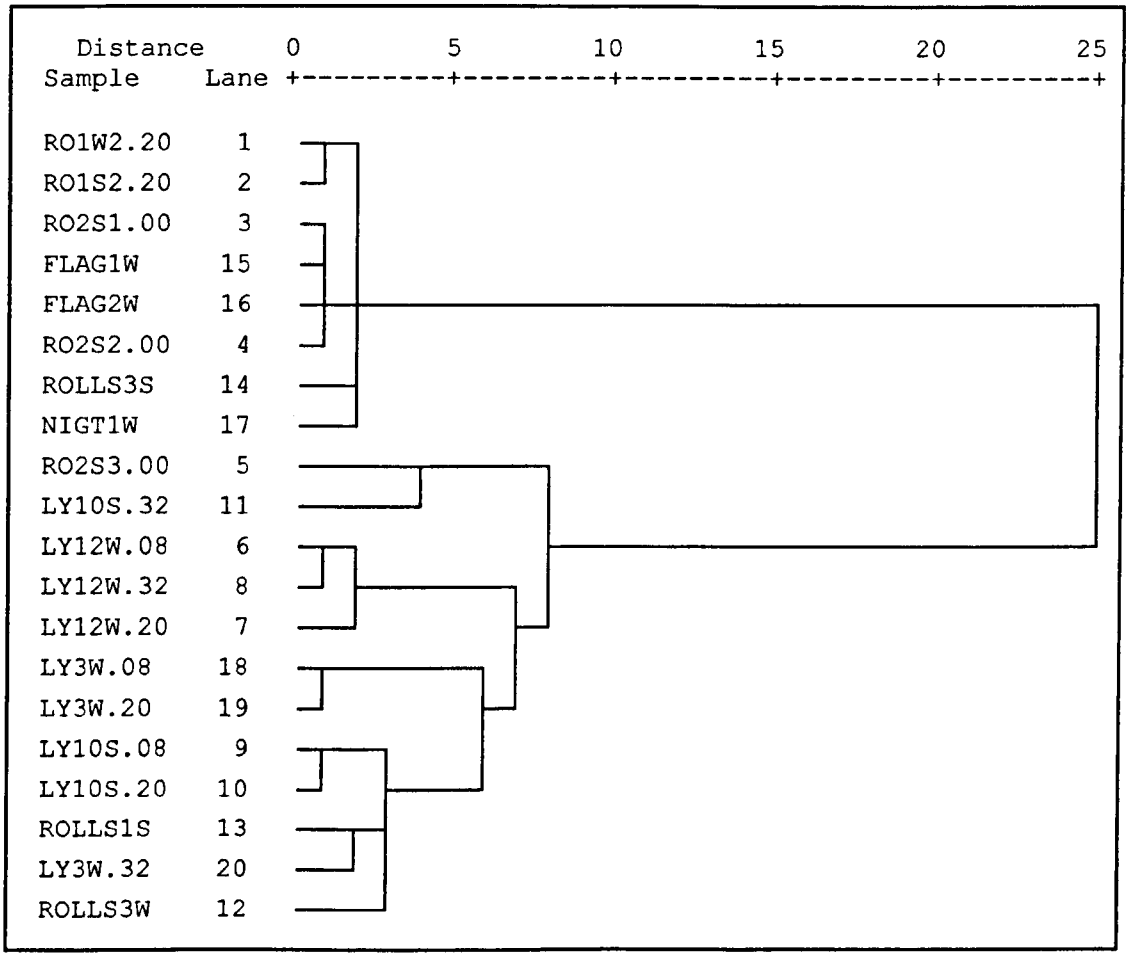

Figure 5.10: Hierarchical cluster analysis using the bands from the DGGE gel for Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites. LY = lysimeter; **3, 10 and 12** = number of lysimeter; **RO1 and RO2** = Roxby Landfill; **Flag 1 and 2** = Flag Fen; **ROLLS1 and ROLLS2** = Rolls Farm; **NIGT1** = Nigtevecht; **S** = sediment; **W** = wood; **.08, 0.20, .32, 1.00, 2.00, 2.20, 3.00** = depth.

Principal-component analysis was conducted on the matrix of E_s values. Four components were extracted with eigenvalues equal to or greater than 1.00. Varimax orthogonal rotation of the components yielded the component structure shown in Table 5.6. Two components accounted for approximately 10 % of the variance

(components 3 and 4), one component accounted for 16 % of the variance (component 2), whilst the main component accounted for 31.4 % of the variance (component 1).

Table 5.6: Orthogonal factor loading matrix for Lysimeters 3, 10 and 12, and the wetland archaeological and landfill sites. LY = lysimeter; **3, 10 and 12** = number of lysimeter; **RO1 and RO2** = Roxby Landfill; **Flag 1 and 2** = Flag Fen; **ROLLS1 and ROLLS2** = Rolls Farm; **NIGT1** = Nigtevecht; **S** = sediment; **W** = wood; **.08, 0.20, .32, 1.00, 2.00, 2.20, 3.00** = depth.

Sample	Component			
	1	2	3	4
RO1W2.20	.915	.120	-.220	.074
RO1S2.20	.862	.258	-.174	-.040
RO2S1.00	.971	.015	.026	.005
RO2S2.00	.919	.127	-.123	-.110
RO2S3.00	-.092	-.745	-.089	.124
LY12W.08	-.162	.140	.120	.917
LY12S.08	.203	-.273	-.037	.836
LY12W.20	-.188	.391	-.205	.767
LY12S.20	.009	.894	-.249	.109
LY12W.32	.156	.892	-.209	.077
LY12S.32	-.479	.298	-.553	-.194
ROLLS3W	-.189	.530	.119	.208
ROLLS1S	.358	.486	.291	-.337
ROLLS3S	.842	-.131	.048	-.068
FLAG1W	.965	-.133	.007	.035
FLAG2W	.883	-.055	.213	-.082
NIGT1W	.750	-.023	.534	-.134
LY3W.08	.043	.085	.957	-.039
LY3W.20	-.155	-.141	.912	-.006
LY3W.32	-.331	.510	.559	-.353
% variance	31.4	16.1	9.6	8.9

The majority of the sediment and wood samples obtained from the wetland archaeological and landfill sites displayed the highest values in component 1. Although not all the samples from these sites are included in the first component, the numbers confirm the results from the previous statistical analyses. On the basis of this

observation, the most probable relationship between these samples is the similarity between the microbial communities in all the '*in situ*' burial environments. The second component consists of the sediment and wood samples obtained from Lysimeter 12 and Rolls Farm, which suggests a similarity between the microbial community present in landfill waste and a natural burial environment.

The source of the relationships highlighted between the samples in components 3 and 4 is more difficult to assess owing to the lower number of significant values. The three highest sample values in component 3 are the wood samples from Lysimeter 3 at 0.08 m, 0.20 m and 0.32 m depths. This suggests that there is a strong similarity between the microbial communities in the different zones of saturation. The three highest sample values in component 4 are the sediment and wood samples from Lysimeter 12. This finding suggests similarities between the microbial communities in the sediment and wood samples at the end of the experiment.

5.4 Discussion of microbial analysis

The results from the DGGE band profiles for all the sediment and wood samples from the lysimeter study and the wetland archaeological and landfill sites have identified a number of distinct patterns. The first two patterns are discussed in relation to the effect that they have upon the microbial community, including the length of time in the burial environment and the different levels of saturation present in the lysimeter study (as described in Sections 5.4.1-5.4.2). The second two patterns explore the microbial populations present in the sediment and wood samples and between different types of sediment (as described in Sections 5.4.3-5.4.4).

5.4.1 Length of time in the burial environment

The duration of the lysimeter study produced a pattern of change in the microbial populations. This was evident from the DGGE band profiles and principal-component analysis of the majority of the sediment samples. The results from Lysimeters 5-8 and 9-11 showed that the greater the length of burial, the closer the similarity between the microbial populations in samples obtained from similar depths.

On several occasions during the monitoring period there were changes in the microbial community. Hierarchical cluster analysis of the sediment and wood samples from Lysimeters 5-8 and 9-11 showed shifts in the microbial communities between 1.5-2 years, and 1-1.5 years respectively. The results suggest that several environmental factors are responsible for these changes. The constantly saturated conditions within Lysimeters 9-11 indicate that the shift in the microbial communities between 1-1.5 years may be associated with changes in the composition of the waste mass as waste degradation proceeds. Barlaz (1997) and Barlaz and co-workers (1989a & 1989b) presented a four-phase characterization of landfill waste decomposition in laboratory-scale landfills (see Table 4.1). This research showed that the changing microbial populations were responsible for compositional changes within the waste mass.

The manipulation of leachate levels in Lysimeters 5-8 promoted similar changes in the microbial communities previously seen in Lysimeters 9-11, leading to a decline in the height of the waste over the duration of the experiment. However, although changes in the composition and microbial diversity of the waste mass may still have occurred, the fundamental environmental factor responsible for the shifts in certain microbial populations between 1.5-2 years was a change in the level of saturation.

5.4.2 Level of saturation

The level of saturation, which was a product of the manipulation in water/leachate levels, produced several patterns in the data. Hierarchical cluster analysis and principal-component analysis of the sediment and wood samples obtained throughout the profiles of Lysimeters 9-11 indicated the existence of a relationship between the microbial populations at all depths. These observations demonstrate the presence of stable communities of dominating bacterial types over the duration of the experiment.

Analysis of the DGGE band profiles and principal-component analysis of the sediment and wood samples from Lysimeters 3, 10 and 12 not only showed similarities between the microbial communities in permanently saturated conditions but also the communities subjected to different levels of saturation. This is confirmed by the principal-component analysis of the sediment and wood samples from Lysimeters 5-8 which indicate similarities between microbial communities at 0.08 m and 0.32 m depths over the duration of the experiment. The results from Sorensen's pairwise similarity coefficient confirm this observation. Although these analyses did not show a similarity between microbes at 0.20 m depths, and those at 0.08 m and 0.32 m depths, the difference may be attributable to the heterogeneity of the landfill waste which contains variable concentrations of biodegradable material (Biostrategy Associates Ltd. 1992).

The microbial populations at 0.08 m and 0.32 m depths in Lysimeter 8 display significant similarities to the microbial populations in Lysimeters 9-11. Therefore, the results from both sets of lysimeters suggest that the decline in the height of the waste in Lysimeters 5-8 promotes a greater level of saturation, which is similar to those prevalent within Lysimeters 9-11.

5.4.3 Relationship between sediment and wood samples

The relationship between the sediment and wood samples from all the lysimeters shows a distinct pattern. Sorensen's pairwise similarity coefficient sample values from Lysimeters 5-8 and 10-12 indicate a gradual penetration of bacteria into the wood, from the sediment, during the experiment. The greatest similarity between the sediment and wood samples occurs during the last six months of the experiment. However, the samples from Lysimeter 9 show that the greatest penetration of bacteria into the wood from the sediment occurs within the first six months of the experiment. This discrepancy may be attributable to the exposure of pore spaces and fissures within the wood during the cutting of the samples, which would render the newly exposed outer surfaces particularly vulnerable to microbial attack.

The penetration of bacteria into the wood is more pronounced in Lysimeter 8 than the other lysimeters in the same set. This is not necessarily due to the length of time in the burial environment but instead may be due to a decline in the height of the waste mass, which produced a change in saturation. This increase in saturation promoted the growth of bacterial types that had previously been inactive in the zone of aeration.

5.4.4 Relationship between different types of sediment

Sediment sample values produced using Sorensen's pairwise similarity coefficient and hierarchical cluster analysis from Lysimeters 3, 10 and 12 show similarities between the microbial populations of peat and landfill waste. Principal-component analysis of the sediments from the wetland archaeological and landfill sites also indicate the same relationship. These findings suggest that there are several common bacterial types in the majority of the '*in situ*' burial environments used in this study.

There are further similarities between the microbial communities present within the landfill waste contained in Lysimeter 12 and the sediment from Rolls Farm archaeological site. This relationship not only confirms the existence of certain types of bacteria common to very different burial environments but also highlights the representative nature of the lysimeter study in determining the microbial communities present in the '*in situ*' wetland archaeological and landfill sites.

5.5 Summary

This chapter has presented the microbiological results from denaturing gradient gel electrophoresis (DGGE) of the sediment and wood samples from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites. The results were presented in two forms; qualitative descriptive analyses based on comparisons between DGGE band profiles, and semi-quantitative analyses based on the application of several statistical techniques. The interpretation of these analyses highlights several distinct patterns:

1) Sediment samples obtained from similar depths in the lysimeter study indicate that similarities between microbial communities within the various lysimeters increased over the duration of the experiment.
2) There is similarity in the microbial communities present in the sediments from all three zones of saturation in the lysimeter study.
3) Irrespective of the burial environment, bacteria gradually penetrate the wood samples from the sediment over the duration of the experiment.
4) There are several bacterial types common to the majority of the '*in situ*' wetland archaeological and landfill sites used in this study.

The following chapter will outline the extent of degradation that exists within the oak wood samples obtained from the lysimeter study and the wetland archaeological and landfill sites.

Chapter 6 Wood degradation results

6.1 Introduction

This chapter presents the results of the gas chromatographic (GC) analysis of the oak wood samples which were recovered from the burial environments within the lysimeter study and the '*in situ*' wetland archaeological sites.

The use of GC has been identified in Chapter 3 as the most appropriate technique for measuring the amount of degradation within the oak wood samples, as it can be performed with both precision and accuracy (Albersheim *et al.* 1967, Kim 1990). GC is particularly useful in determining the amount of holocellulose present within the wood samples (Jones *pers comm.* 2002, Kelly *et al.* 1990) through the separation of the major wood sugars produced by the hydrolysis of the polysaccharides within the wood (Fengel & Wegener 1979, Jones *pers comm.* 2002).

In terms of structure, this chapter is divided into three main parts; the first part (Section 6.2) highlights the inter-relationship between the various wood sugars and their associations with the total wood sugars; the second part (Sections 6.3-6.5) incorporates the two methods outlined in Section 3.3.5 in order to assess the biological, chemical and physical factors responsible for the degradation of the wood samples from the lysimeters and the '*in situ*' wetland archaeological sites; and the final part (Section 6.6) discusses the findings from the wood degradation studies.

6.2 The sugar composition of oak wood

Oak wood is composed of seven major sugars; these include glucose (which corresponds to the cellulose fraction), xylose, galactose and mannose (which

correspond to the primary hemicellulose fractions), and arabinose, ribose and rhamnose (which correspond to the secondary hemicellulose fractions) (Jones *pers comm.* 2002).

Analysis of the wood samples has highlighted several trends between the seven different wood sugars and the total wood sugars, as decay proceeds. Figure 6.1 shows the relationship between the total weights of wood sugars and the proportional weights of glucose, xylose and galactose. (See Appendix 8 for the proportional weights of all the individual sugars and the corresponding total weights of wood sugars used during this study).

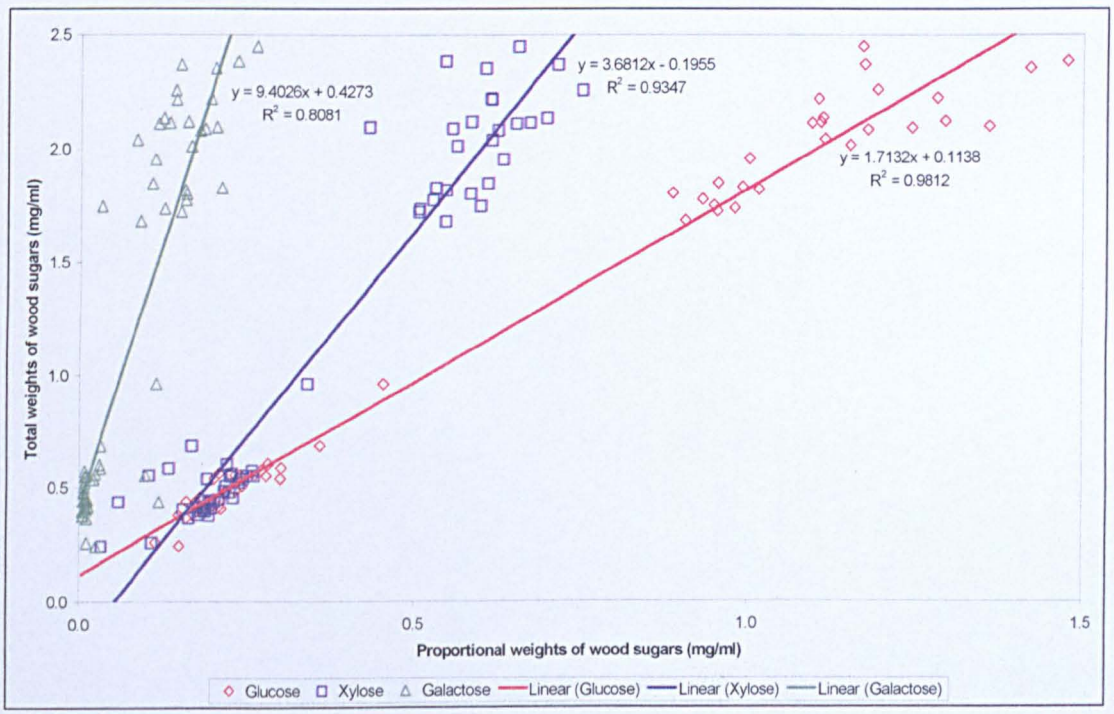

Figure 6.1: Relationship between the total weights of wood sugars and the proportional weights of glucose, xylose and galactose.

The R-squared values, which are produced from the amount of the variance in the total weight of each wood sugar attributable to the variance in their proportional weight, show that there is a significant correlation between the wood samples, i.e.

between 0.80-1.00. The relationship between two variables is most reliable when its R-squared value is at or near 1. As a consequence, this indicates that the weight of glucose (which corresponds to the cellulose fraction of wood), and xylose and galactose (which correspond to the primary hemicellulose fractions of wood), decays at a constant rate, irrespective of the degraded nature of the sample.

Although the remaining primary hemicellulose (mannose) and secondary hemicellulose fractions of wood (arabinose, ribose and rhamnose) produce a level of correlation, the R-squared values produced do not indicate a level of significance. This is demonstrated in Table 6.1 which shows the Pearson correlation coefficients between the proportional weights of each of these sugars and their corresponding total weights.

Table 6.1: Relationship between an individual wood sugar and the total weights of the wood sugars.

Sugar	Equation	R-squared value
Mannose	$y = 11.595x + 0.5393$	0.5858
Arabinose	$y = 28.635x + 0.8216$	0.2978
Ribose	$y = 25.264x + 0.544$	0.347
Rhamnose	$y = 30.771x + 0.657$	0.3937

It is suggested that the biological, chemical and physical factors responsible for hemicellulose degradation, such as chemical hydrolysis or hemicellulose biosynthesis (Björdal *et al.* 1999), may effectively decrease the weights of the sugars, and/or that the molecular structure and nature of bonding (Goodwin & Mercer 1983) may prevent separate linear degradation.

Figure 6.2 shows the relationship between the proportional weights of glucose and xylose obtained from all the wood samples used in the current study. The R-squared

value produced shows that there is a significant correlation between the weights of these sugars. This indicates that the weights of both sugars decrease at a similar rate, which is proportional to the total weight. Therefore, the cellulose (glucose) and primary hemicellulose (xylose) fractions of the wood degrade steadily, irrespective of the different biological, chemical and physical processes impacting upon them.

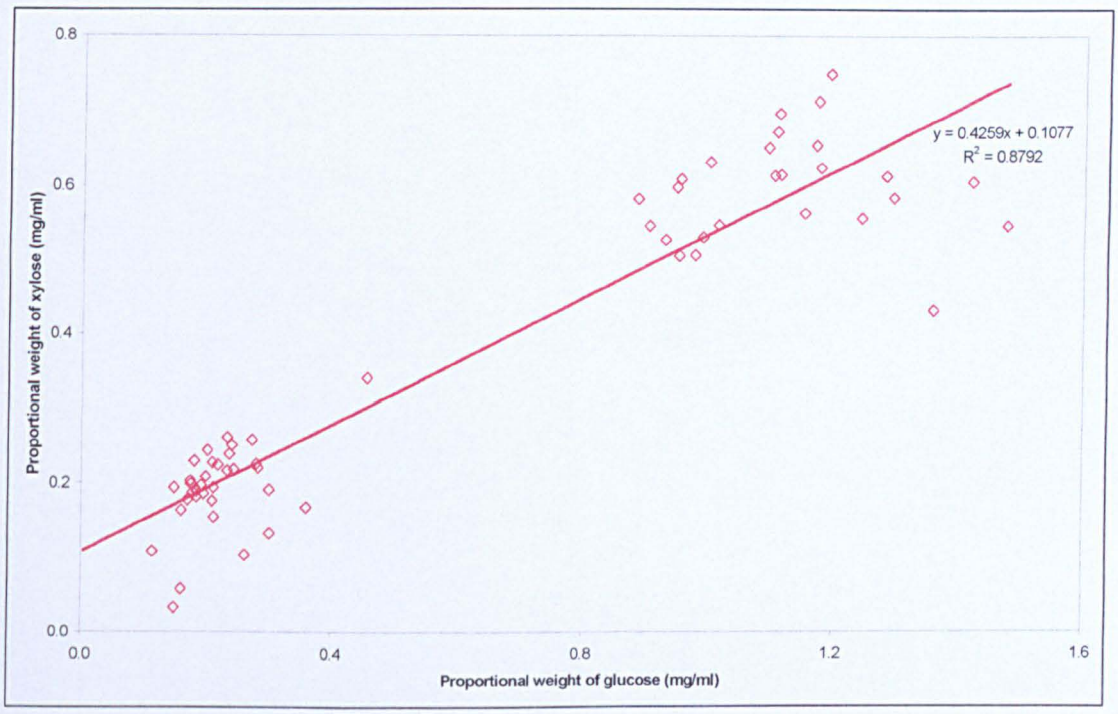

Figure 6.2: Relationship between the proportional weights of glucose and xylose.

Table 6.2 shows the Pearson correlation coefficients between the proportional weights of the secondary wood sugars. The relationship between the proportional weights of glucose and xylose to these sugars is not included in Table 6.2 owing to the poor level of correlation. The majority of the R-squared values shown below is between 0.50-0.70 which indicates a lowered level of significance.

Table 6.2: Relationship between the proportional weights of secondary wood sugars.

	Galactose	Mannose	Arabinose	Ribose	Rhamnose
Galactose	1[a]	0.521	0.508	0.654	0.674
Mannose		1	0.618	0.429	0.576
Arabinose			1	0.365	0.839
Ribose				1	0.652
Rhamnose					1[a]

[a] **Similarity 1 by definition**

In contrast to this, the R-squared value of the weights of arabinose and rhamnose is 0.839 which indicates a far more significant relationship. This suggests that the biological, chemical and physical processes responsible for wood degradation decrease the weights of both sugars, and/or that the structural association and bonding between the sugars promotes decay at a constant rate.

6.3 Water/leachate level management

Several biological, chemical and physical factors are responsible for the degradation of the wood samples from both the lysimeter study and the '*in situ*' wetland archaeological sites. The current section and Sections 6.4-6.5 will highlight the main factors responsible for this decay.

Figure 6.3 shows the total sugar losses for Lysimeters 3, 4, 7 and 8 at 0.08 m, 0.20 m and 0.32 m depths. This figure is representative of the patterns obtained from the majority of the lysimeters during manipulation of their water/leachate levels.

All the wood samples from the lysimeters (with the exception of Lysimeter 4 at 0.32 m depth) show similar total sugar losses throughout their profiles. This suggests that the aerobic conditions established at 0.08 m depth did not increase the total sugar losses of the samples when compared to the permanently saturated conditions at 0.32

m depth. On the basis of this finding, the results show that the manipulation of water/leachate levels in the lysimeters did not influence the degree of wood degradation.

The percentage total sugar loss in Lysimeter 4 at 0.32 m depth does not conform to the previous trend. At this depth, total sugar loss was approximately 25 % lower in comparison to the sugar losses at 0.08 m and 0.20 m depths. These data suggest that the different ecological and physiological requirements of the bacteria responsible for the degradation of wood within the zone of saturation (Eriksson *et al.* 1990) in the leachate and peat of Lysimeter 4 may result in different rates and patterns of wood decay which are not encountered in Lysimeters 3, 7 and 8.

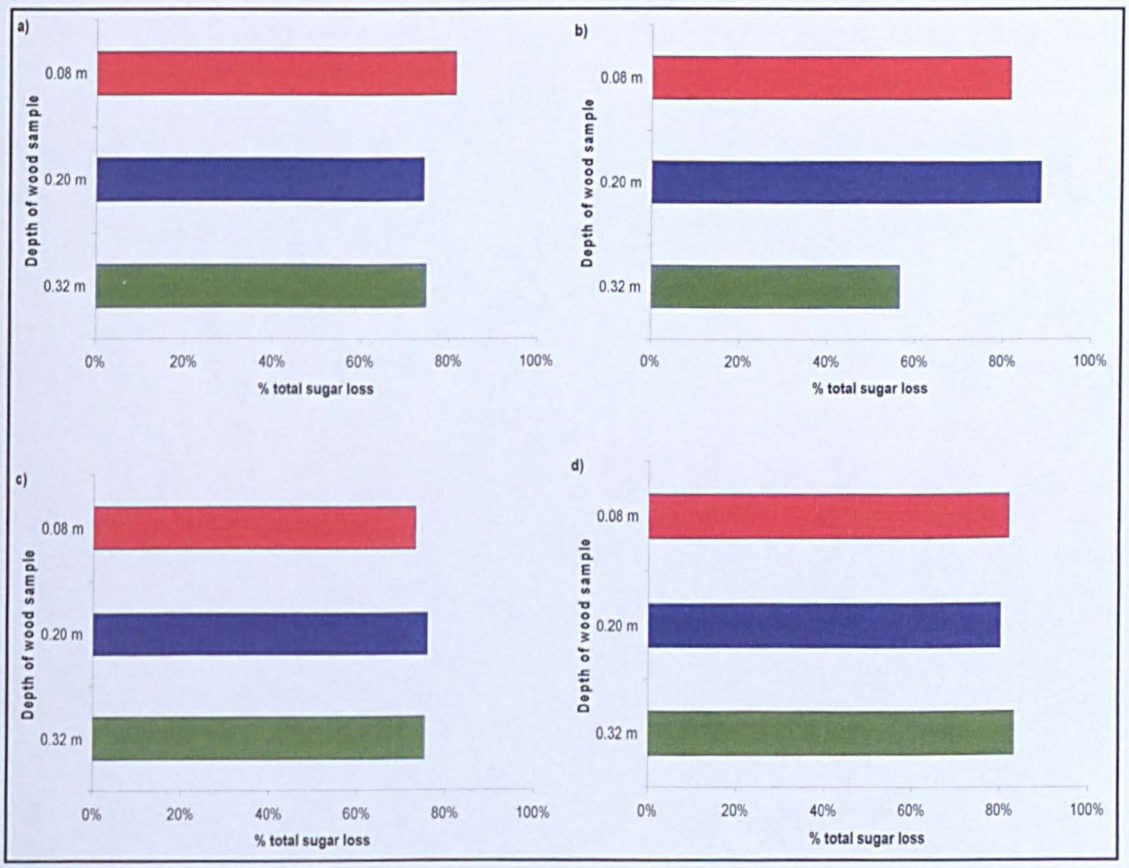

Figure 6.3: Total sugar losses for Lysimeters 3(a), 4(b), 7(c) and 8(d) at 0.08 m, 0.20 m and 0.32 m depths.

Figure 6.4 shows the proportional weights of the primary sugars (glucose, xylose, galactose and mannose) for Lysimeters 7 and 8 at 0.08 m, 0.20 m and 0.32 m depths, compared with a fresh wood sample. All four sugars indicate similar significant decreases in their weights throughout the profile of the lysimeters when compared with the weights of the sugars in the fresh wood. These results suggest that the main cellulose and hemicellulose fractions of the wood decrease at similar rates within the three zones of saturation in the lysimeters.

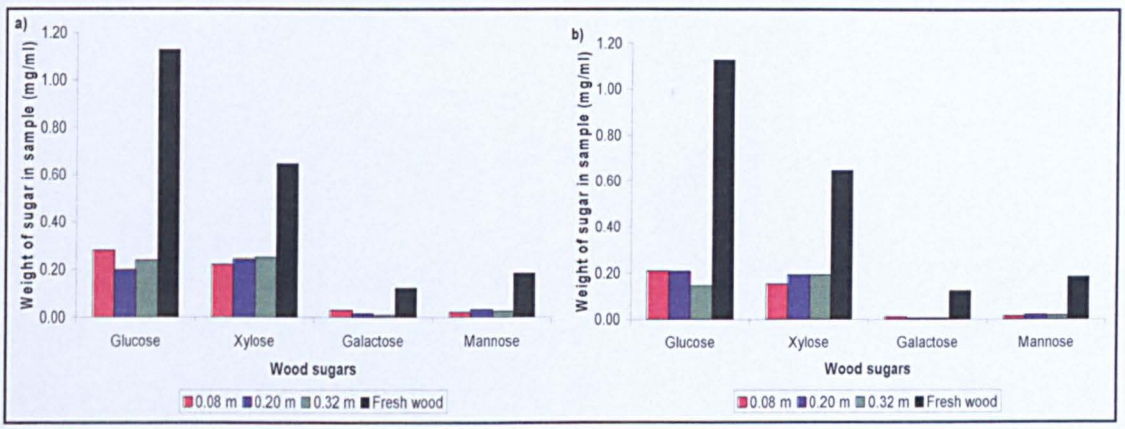

Figure 6.4: Proportional weights of primary sugars for Lysimeters 7(a) and 8(b) at 0.08m, 0.20 m and 0.32 m depths.

Certain lysimeters show different trends to those described above. Figure 6.5 shows the proportional weights of the primary sugars for Lysimeters 3 and 4 at 0.08 m, 0.20 m and 0.32 m depths. The weights of all the sugars in Lysimeter 4 at 0.32 m depth are larger than those in Lysimeter 3 at 0.32 m depth. This variation may be due to two factors; firstly, the permanently saturated conditions in Lysimeter 3 at 0.32 m depth contained peat and water, which may promote greater chemical hydrolysis than the saturated conditions at the same depth in Lysimeter 4 which contained peat and leachate; and secondly, the optimum ecological and physiological requirements of certain microbial species responsible for the degradation of wood may only exist in the peat and water of Lysimeter 3 and not in the peat and leachate of Lysimeter 4. In

addition, variations in the temperature and pH values associated with different sediments and liquid media are parameters which can affect the production, activity and stability of a number of microbial species (AFRC Institute of Food Research 1988, McCarthy 1987).

Recent studies of archaeological wood from waterlogged environments have led to new information on the long-term degradation processes in natural burial contexts (Blanchette 1995, Singh & Kim 2000). Research by Björdal and co-workers (1999) has verified that the chemical and morphological changes in waterlogged archaeological woods result from microbial decay and not from chemical hydrolysis, as previously assumed. By presupposing the existence of similar changes in fresh wood samples, the most likely explanation for the variation between the total sugar losses in Lysimeters 3 and 4 at 0.32 m depths may relate to the differences in the microbial populations responsible for wood degradation.

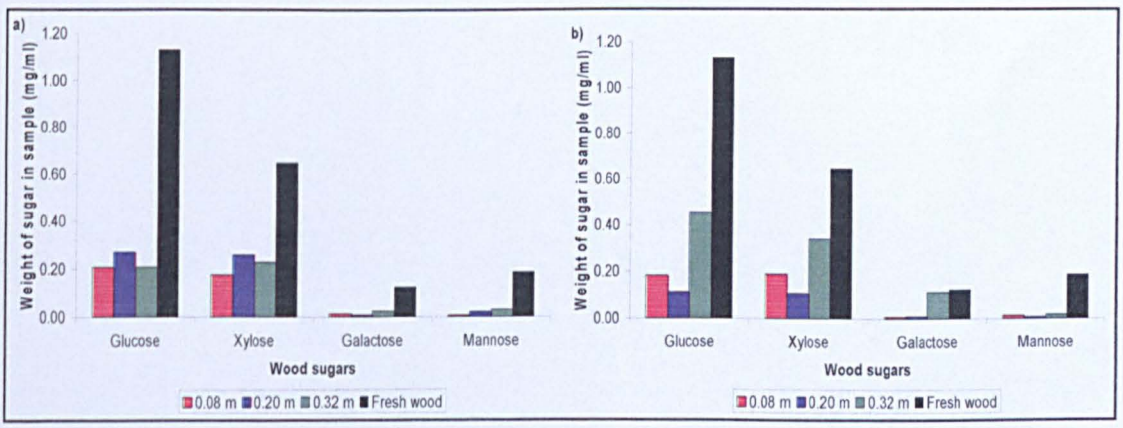

Figure 6.5: Proportional weights of primary sugars for Lysimeters 3(a) and 4(b) at 0.08 m, 0.20 m and 0.32 m depths.

The relationship between the proportional weights of the secondary sugars from the lysimeters manipulated by water/leachate levels is shown in Table 6.3. The low weights of these sugars, when compared with the primary sugars, prevented their

inclusion on the graphs shown in Figures 6.4 and 6.5. As a corollary to the findings shown above, the Pearson correlation coefficient values between the weights of these sugars suggest that the majority of the secondary hemicellulose fractions of the wood decreased at similar rates within the three zones of saturation in the aforementioned lysimeters.

Table 6.3: Relationship between the proportional weights of secondary wood sugars in the lysimeters affected by water/leachate level management.

		arabinose	ribose	rhamnose
Pearson Correlation	**arabinose**	1[a]	0.429	0.814
	ribose		1	0.824
	rhamnose			1[a]

[a] **Similarity 1 by definition**

Table 6.4 shows the percentage total sugar losses of the wood samples recovered from the lysimeters which were permanently saturated with water/leachate. The total sugar losses at all three depths from each of Lysimeters 1, 11 and 12 are between 75-83 %. These significant losses indicate that all of the wood samples have been subjected to high levels of degradation associated with chemical hydrolysis and/or holocellulose biosynthesis. There is also a <5 % difference in the sugar losses throughout the profile of each of the lysimeters suggesting the existence of ubiquitously saturated conditions.

The wood samples obtained from Lysimeters 9 and 10 indicated small sugar losses at all three depths. It is suggested that as the lysimeter study entered its second year, changes in the microbial populations associated with the cycle of decomposition influenced the rate of wood degradation in the lysimeters which contained waste and leachate (as described in Section 4.5).

Table 6.4: Total sugar losses of samples taken from the lysimeters saturated with water/leachate.

	Depth of wood sample (m)	% total sugar loss
Lysimeter 1	0.08	83
	0.20	82
	0.32	82
Lysimeter 11	0.08	80
	0.20	80
	0.32	76
Lysimeter 12	0.08	75
	0.20	76
	0.32	77
Lysimeter 9	0.08	0
	0.20	0
	0.32	16
Lysimeter 10	0.08	3
	0.20	9
	0.32	17

6.4 Sediment composition

Replicate wood samples were taken from Lysimeters 1, 3, 4, 6 and 10 at 0.08 m, 0.20 m and 0.32 m depths, in order to assess whether the rate of wood decay remained constant within similar sediments and at similar depths in the lysimeter study. Figure 6.6 compares the differences in total sugar contents between the original and replicate wood samples from Lysimeters 1, 3 and 4.

The percentage differences in total sugar contents among the wood samples and their replicates at all depths in Lysimeters 1, 3 and 4 were <6 %. This suggests that degradation was similar throughout the horizontal profile of the lysimeters owing to the homogenous nature of the peat. However, the percentage differences in sugar contents between the wood samples in Lysimeter 4 at 0.32 m depth were 23 %, suggesting that there was an elevated degree of decay in one particular wood sample, caused by chemical hydrolysis and/or holocellulose biosynthesis.

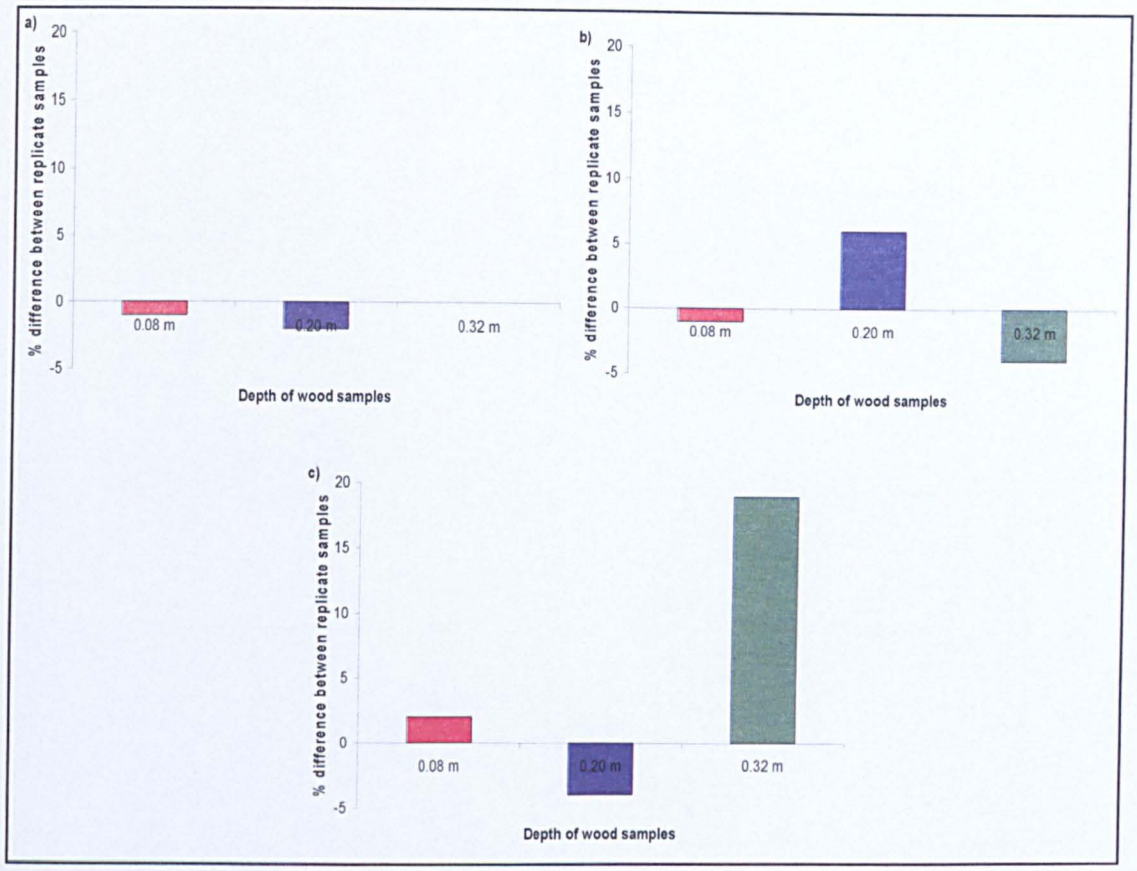

Figure 6.6: Differences in total sugar contents between the original and the replicate wood samples from Lysimeters 1(a), 3(b) and 4(c).

The replicate wood samples from Lysimeters 6 and 10 (as shown in Figure 6.7) display different trends to those from Lysimeters 1, 3 and 4. The percentage differences in total sugar contents at 0.08 m depths range from 1 % to 8 %, whilst at 0.32 m depths sugar losses range from 4 % to 16 %. This data suggests that the heterogeneous nature of the landfill waste within Lysimeters 6 and 10 exerts an influence on the biological, chemical and physical processes responsible for wood decay.

Figure 6.8 compares the differences in total sugar contents in Lysimeters 1 and 12 which were permanently saturated by water and leachate respectively, and highlights the differences in sugar contents in Lysimeters 3 and 8 which had their water/leachate

levels manipulated. This figure is representative of the patterns obtained from the majority of the lysimeters.

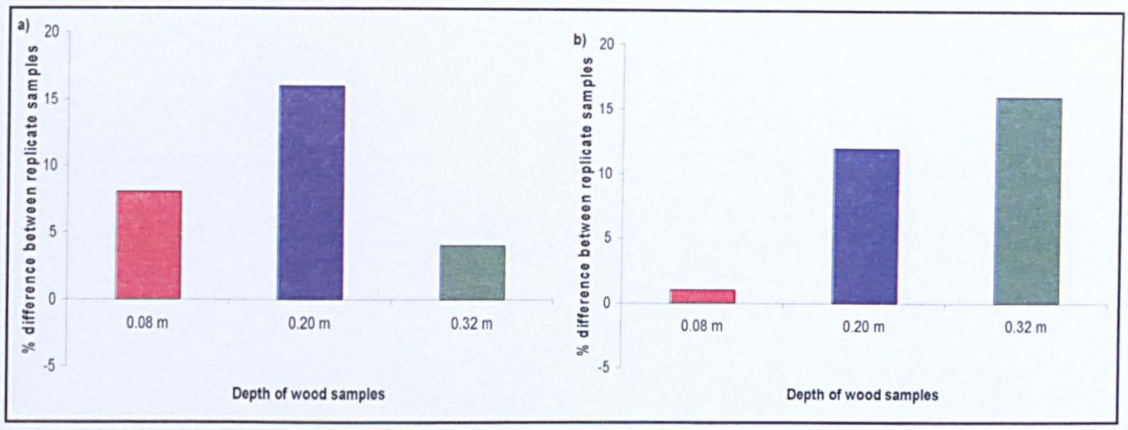

Figure 6.7: Differences in total sugar contents between the original and the replicate wood samples from Lysimeters 6(a) and 10(b).

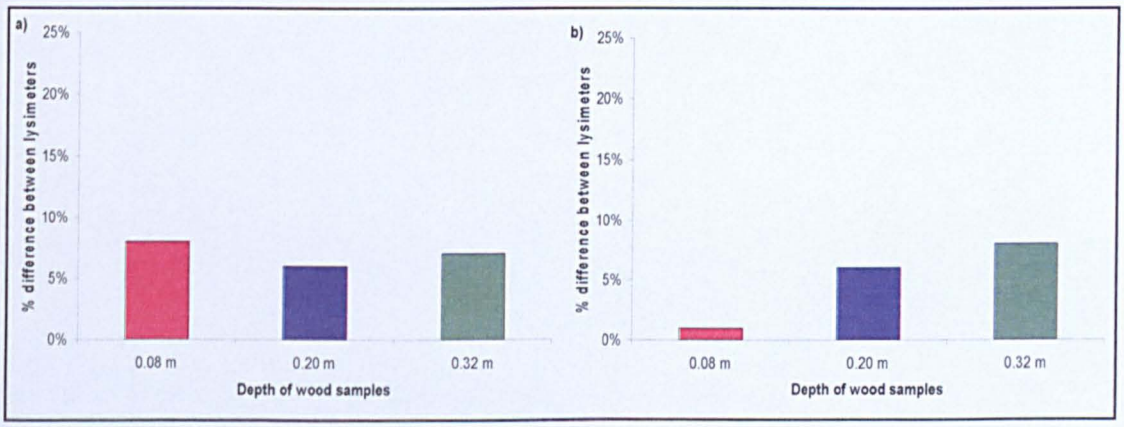

Figure 6.8: Differences in total sugar contents between Lysimeters 1 and 12(a) and Lysimeters 3 and 8(b).

The variation in total sugar contents among the different sediment types within each set of lysimeters has indicated comparable values varying by <8 %. These results suggest that the sediment composition in the lysimeters does not influence the extent of wood degradation.

Two lysimeters display differences in total sugar contents that do not conform to the previous trend. Figure 6.9 shows a significant difference in the sugar contents from

Lysimeters 2 and 6 at 0.08 m and 0.32 m depths. This suggests that preferential wood degradation, promoted by chemical hydrolysis and/or holocellulose biosynthesis, does not take place within the first year of the experiment.

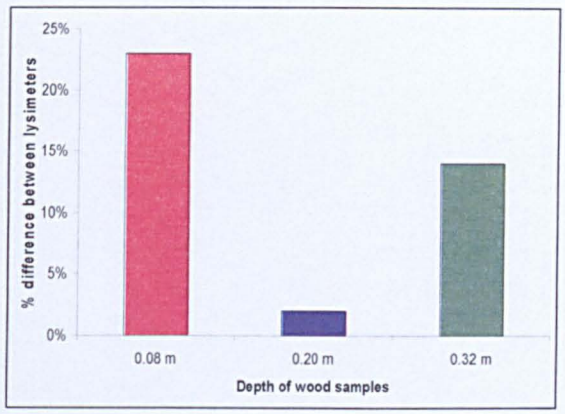

Figure 6.9: Differences in total sugar contents between Lysimeters 2 and 6.

In order to understand the extent of cellulose and hemicellulose degradation within different sediments, the proportional weights of the primary cellulose (glucose) and hemicellulose (xylose) in Lysimeters 1 and 12, and 3 and 8 are shown for comparison in Figures 6.10 and 6.11, respectively. The results show that throughout the profile of all the lysimeters the weights of glucose and xylose remain similar, irrespective of sediment type.

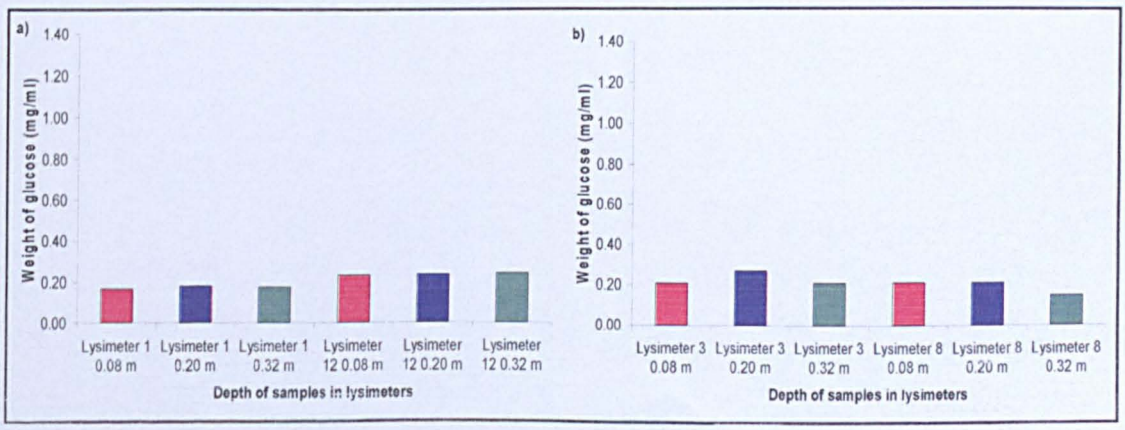

Figure 6.10: Proportional weights of glucose for Lysimeters 1 and 12(a) and Lysimeters 3 and 8(b).

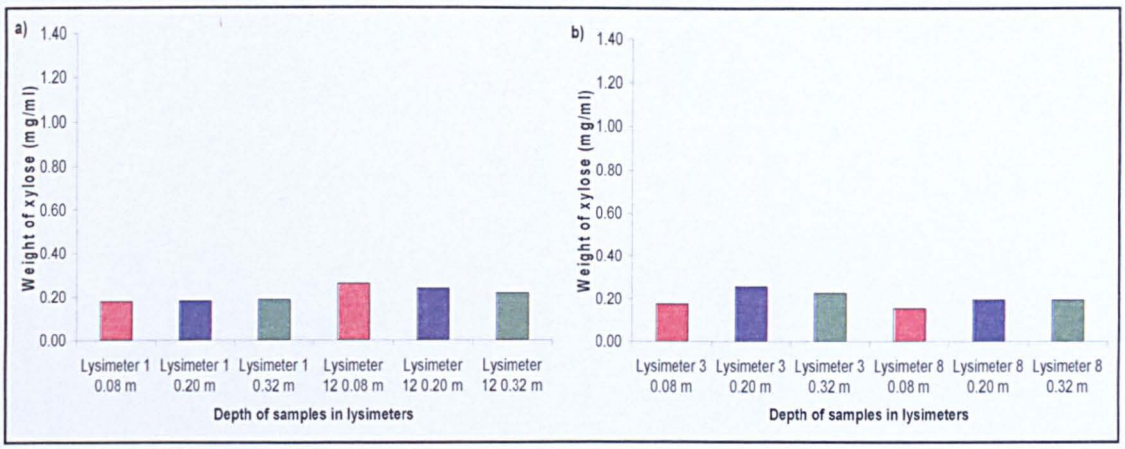

Figure 6.11: Proportional weights of xylose for Lysimeters 1 and 12(a) and Lysimeters 3 and 8(b).

Figure 6.12 shows the proportional weights of glucose and xylose for Lysimeters 2 and 6. Although the weights of xylose are comparable between Lysimeters 1, 3, 8 and 12 at all three depths, the weights of glucose display variability to the previous trend. The results show that after the first year of monitoring the weights of glucose indicate variable losses, both at different depths in the same lysimeter and between lysimeters. There may be two reasons for this disparity; firstly, the early stages of glucose degradation do not occur at a constant rate in Lysimeters 2 and 6; and secondly, chemical hydrolysis and cellulose biosynthesis associated with water/leachate level manipulation is more pronounced in the first year of monitoring.

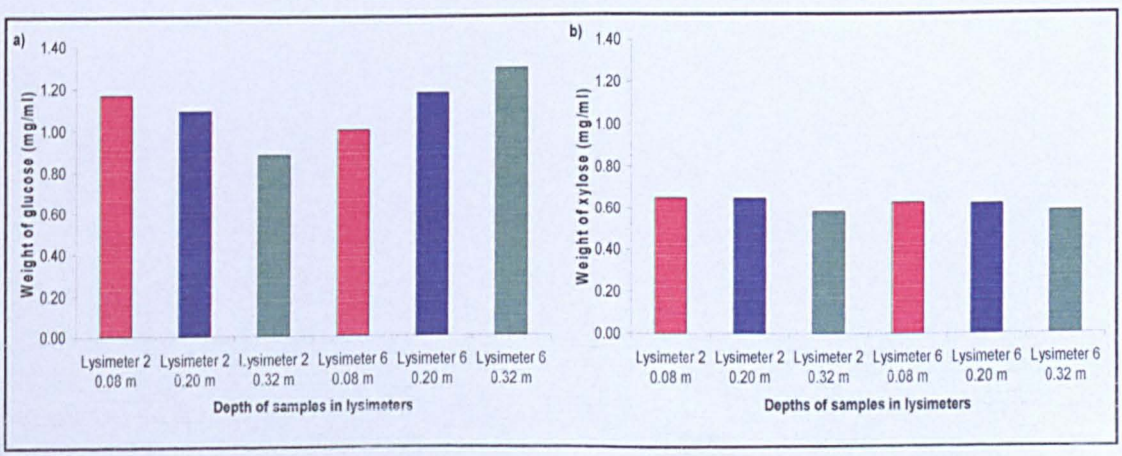

Figure 6.12: Proportional weights of glucose(a) and xylose(b) for Lysimeters 2 and 6.

Figure 6.13 shows the proportional weights of glucose and xylose from the wood samples obtained from the '*in situ*' wetland archaeological sites. The weights of glucose and xylose highlight similar trends to those previously identified in the lysimeter study. However, the wood samples obtained from Rolls Farm 1 and 3 show that as degradation proceeds, the weights of glucose and xylose decrease within the same sample, with the outer samples indicating a greater amount of decay than the inner samples. This suggests that if degradation were to continue, the weights of glucose and xylose would become comparable with their equivalent sugars from Flag Fen and Nigtevecht. In this context, the differences in sediment types would not significantly affect the pattern and rate of wood decay. This finding confirms the results obtained from the different sediments in the lysimeter study which have been described previously.

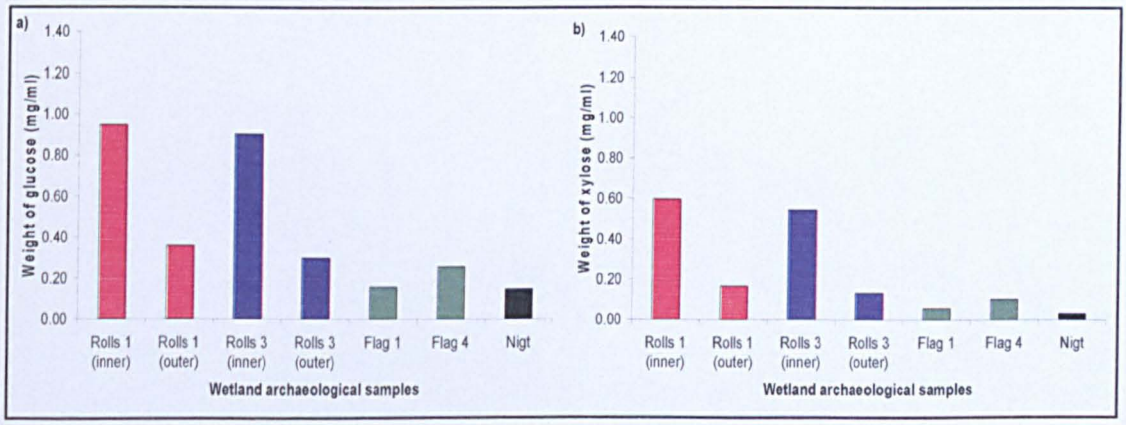

Figure 6.13: Proportional weights of glucose(a) and xylose(b) for the '*in situ*' wetland archaeological samples. Flag 1 and 4 = Flag Fen; **Rolls 1 and 3 =** Rolls Farm; **Nigt =** Nigtevecht.

6.5 Length of time in the burial environment

Figure 6.14 shows the percentage total sugar losses against time for Lysimeters 9-12. It is representative of the patterns obtained from the majority of the lysimeters. In the first year of monitoring total sugar losses from the wood samples taken from

Lysimeters 9 and 10 all averaged <20 %. However, the wood samples taken from Lysimeter 11 indicate a higher rate of loss (75-80 %) during the next six months. The samples obtained from Lysimeter 12 at the end of the experiment displayed similar values to those taken from Lysimeter 11.

The results show that rates of sugar losses increase over the duration of the experiment, with the majority of sugar losses occurring in the second year. It is suggested that changes in the extent of wood decay between the first and second year of the lysimeter study are associated with the onset of the accelerated methane production phase (3) in the cycle of decomposition. During this phase the microbial population increases significantly with methanogens, acetogens and cellulolytics degrading the wood samples (Barlaz 1997, Barlaz *et al.* 1989a). However, by the end of the lysimeter study the microbial population stabilized as the final phase of methanogenesis was entered (Barlaz *et al.* 1989a).

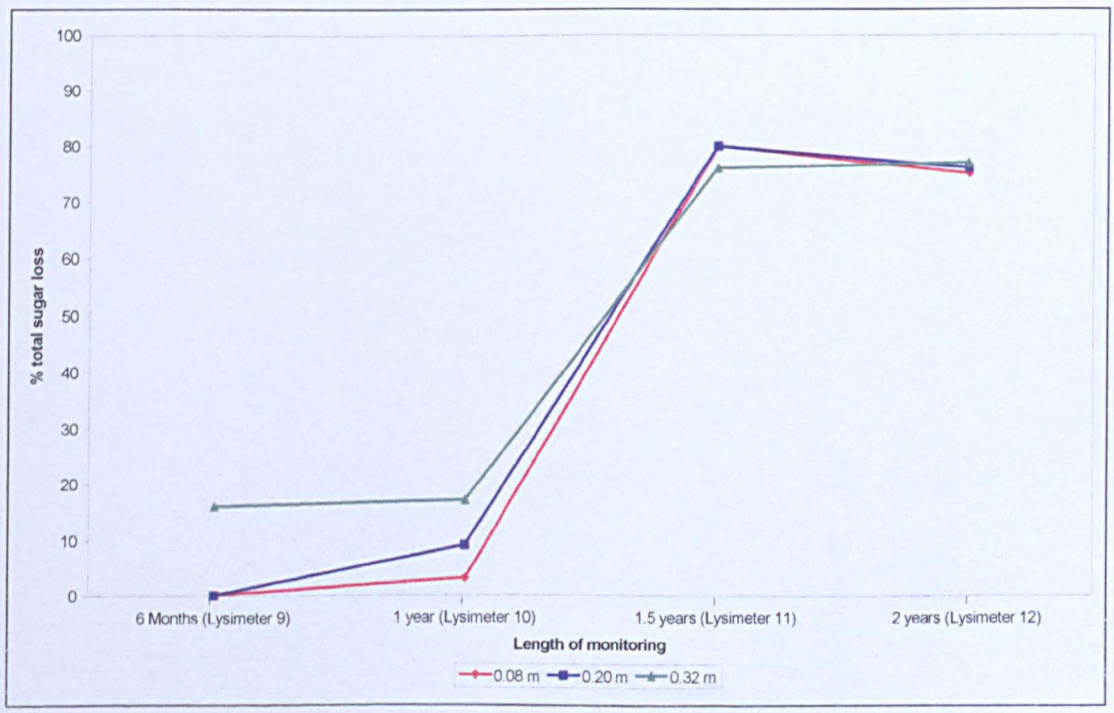

Figure 6.14: Linear plots of total sugar losses for Lysimeters 9-12.

Figure 6.15 shows the proportional weights of the primary sugars for Lysimeters 9-12 at 0.08 m, 0.20 m and 0.32 m depths. The weights of xylose, galactose and mannose (which correspond to the primary hemicellulose fractions within the wood samples) decrease at all three depths over the duration of the experiment. However, the weights of glucose do not follow this trend. After the first six months of the experiment at 0.32 m depth the weight of glucose is 0.9 mg/ml, which is less than the weights of glucose at 0.08 m and 0.20 m depths. At the end of the second six months of the experiment the weight of glucose at 0.32 m depth increased to 14 mg/ml, whereas the weights of glucose at 0.08 m and 0.20 m depths decreased. It is suggested that the nature of the burial environment surrounding the wood sample at 0.32 m depth may be different to that surrounding the wood samples at 0.08 and 0.20 m depths, owing to variability in the decomposition of the landfill waste (Barlaz 1997, Barlaz *et al.* 1989a).

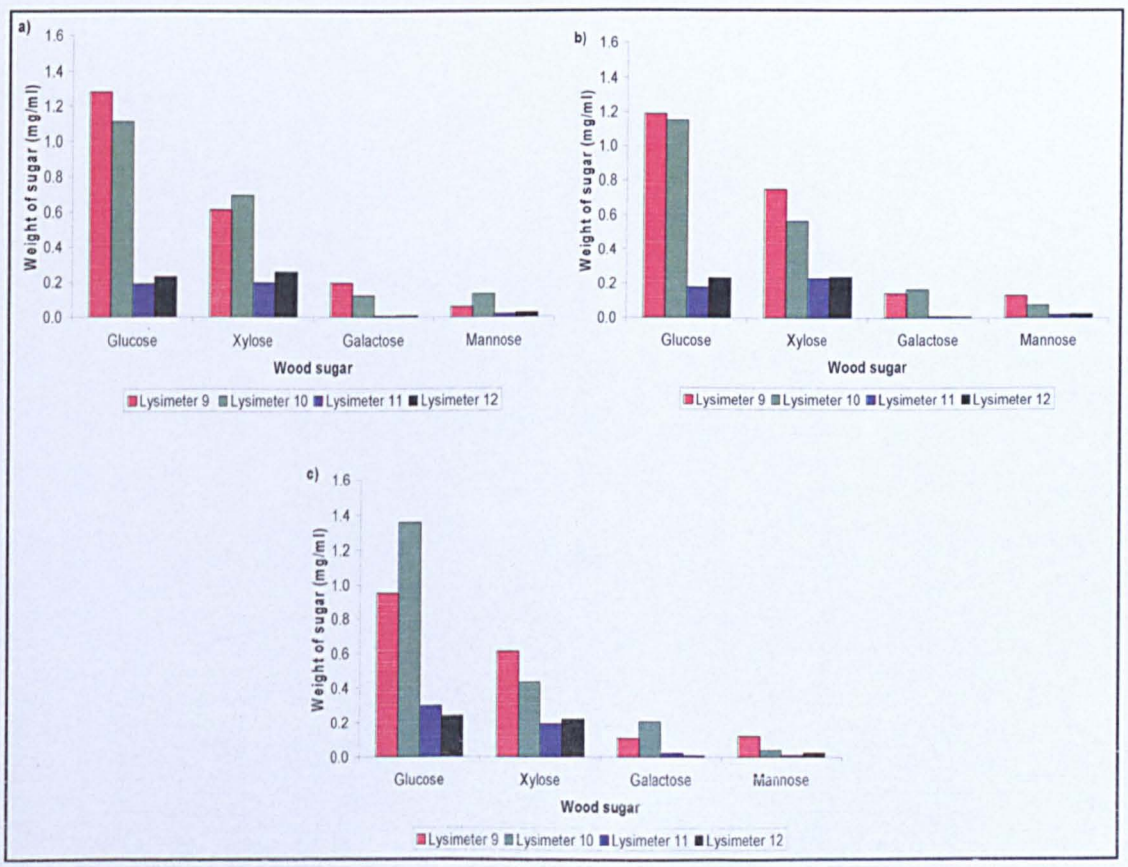

Figure 6.15: Proportional weights of primary sugars for Lysimeters 9-12 at 0.08 m(a), 0.20 m(b) and 0.32 m(c) depths.

Table 6.5 shows the Pearson correlation coefficients between the proportional weights of the secondary wood sugars and the total weights of wood sugars for Lysimeters 9-12. The R-squared values show that there is a significant correlation between the weights of ribose and the total weights of wood sugars. Despite the fact that a correlation exists between the weights of ribose and rhamnose, and between the weights of rhamnose and the total weights of wood sugars, the R-squared values do not indicate a strong level of significance. These results suggest that the majority of the secondary hemicelluloses degrade consistently at all depths over the duration of the experiment and that the rate of decay between ribose and rhamnose is similar.

Table 6.5: Relationship between the proportional weights of secondary wood sugars and the total weights of wood sugars for Lysimeters 9-12.

		total sugars	arabinose	ribose	rhamnose
Pearson Correlation	total sugars	1.000[a]	0.311	0.968	0.659
	arabinose		1.000	0.181	-0.075
	ribose			1.000	0.729
	rhamnose				1.000[a]

[a] Similarity 1.000 by definition

Figure 6.16 shows the proportional weights of the primary sugars for Lysimeters 5-8. As can be seen, a similar trend to that in Figure 6.15 is in evidence. Although the weights of xylose and galactose decreased over the duration of the experiment, the weights of glucose at 0.32 m depth increased in the second six months of the experiment. This mirrors the trends seen in Lysimeters 9-12 (as shown in Figure 6.15).

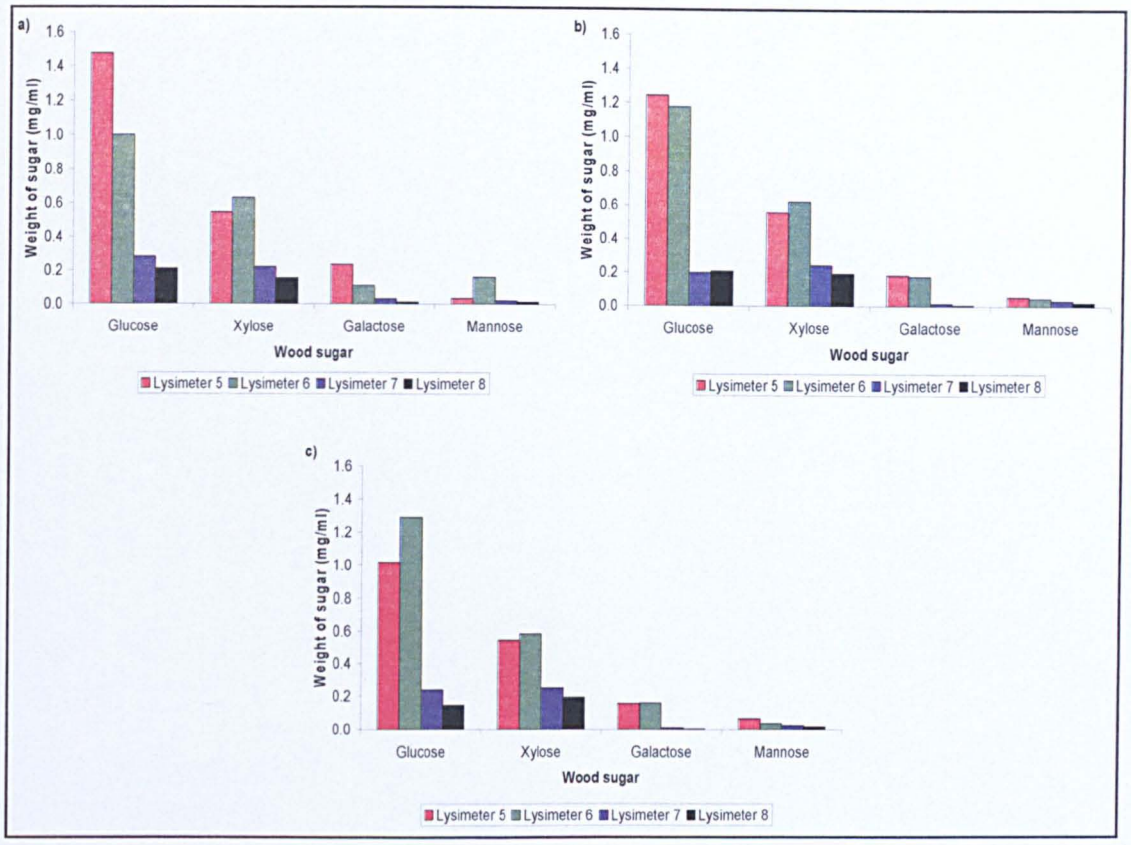

Figure 6.16: Proportional weights of primary sugars for Lysimeters 5-8 at 0.08 m(a), 0.20 m(b) and 0.32 m(c) depths.

6.6 Discussion of wood degradation results

The results of the gas chromatographic (GC) analysis for all the wood samples from the lysimeter study and the '*in situ*' wetland archaeological sites have highlighted a number of trends in the data. These can be divided into two sections. The first set of trends discussed in Section 6.6.1 highlights the inter-relationship between the various wood sugars and their association with the total wood sugars. The second set of trends determines the factors responsible for the degradation of the wood samples (as described in Section 6.6.2).

6.6.1 The pattern of wood decay

The analysis of the wood samples has highlighted two main trends among the seven different wood sugars as decay proceeded. The first trend shows that the main

cellulose (glucose) and two of the main hemicelluloses (xylose and galactose) degraded at a constant rate when compared with the total amount of sugars in a wood sample. However, the remaining primary hemicellulose (mannose) and the three secondary hemicelluloses (arabinose, ribose and rhamnose) did not indicate a similar relationship. This variation may be due to several factors, such as chemical hydrolysis, hemicellulose biosynthesis, and/or the molecular structure and nature of bonding between the sugars. The second trend showed that the proportional weights of glucose and xylose decreased consistently with each other as decay progressed, irrespective of the biological, chemical and physical factors influencing wood decay.

In addition, the inner and outer wood samples taken from Rolls Farm 1 and 3 indicate differences in the proportional weights of glucose and xylose. These results have shown that the outer samples have experienced a greater amount of decay than the inner samples, indicating that degradation proceeded inwards in a radial manner to the core of the wood (Hoffmann 1981, Paajanen & Viitanen 1988).

6.6.2 Factors responsible for wood degradation

There are two main factors responsible for the degradation of the wood samples in both the lysimeter study and the wetland archaeological sites; these are sediment composition and the length of time in the burial environment.

6.6.2.1 Sediment composition

The analysis of the total sugar contents for the wood samples from the lysimeters which contained different sediments and which had their water/leachate levels manipulated, and the proportional weights of glucose (the primary cellulose) and xylose (the primary hemicellulose) from the lysimeter study and the wetland

archaeological sites, have both shown that sediment composition does not influence the degree of wood degradation.

In contrast to the above finding, the results from the replicate wood samples, taken in order to assess whether the rate of wood decay remained constant within similar sediments and at similar depths in the lysimeter study, produced contrasting conclusions. The percentage differences in total sugar contents between the wood samples and their replicates at all depths in the lysimeters which contained peat and water reflected the homogenous nature of the peat in relation to wood degradation (as shown in Figure 6.6). In contrast, the percentage differences in sugar contents between the wood samples and their replicates at all depths in the lysimeters which contained waste and leachate suggested that the sediment was heterogeneous in nature (as shown in Figure 6.7). These results suggest that the heterogeneity of the waste influences the biological, chemical and physical processes responsible for wood decay at different rates within a particular lysimeter.

6.6.2.2 Length of time in the burial environment

The analysis of the wood samples obtained from all the lysimeters showed that there were significant losses in both the proportional and total weights of the seven wood sugars during the second year of monitoring when compared with the first year (as shown in Figures 6.14 and 6.15, and Table 6.5). These results suggest that the sediments contained in the lysimeters were entering the different phases in the cycle of decomposition, where a change in the microbial population of the sediment produced considerable changes in the degradation of the wood samples. Leaching of the soluble compounds in the first year of monitoring was replaced by the loss of

structural carbon (cellulose and hemicellulose) from the cell walls, owing to microbial activity, during the second year of monitoring (Barlaz *et al.* 1989a).

6.7 Summary

This chapter has presented the results of the gas chromatographic (GC) analysis of the oak wood samples recovered from the lysimeter study and the '*in situ*' wetland archaeological sites. The interpretation of this analysis highlights several distinct patterns:

1) The degradation of the wood samples produces changes in the total sugar content of the wood.
2) The cellulose and main hemicellulose fractions of the wood samples which correspond to glucose, xylose and galactose respectively, degrade at a linear rate as degradation proceeds.
3) The cellulose and the primary hemicellulose (xylose) fractions decrease in a similar manner.
4) Water/leachate level management did not influence the rate of wood degradation.
5) The homogeneous nature of peat and the heterogeneous nature of landfill waste produced differing rates of wood decay.
6) Degradation proceeds inwards in a radial manner to the core of the wood.
7) The cellulose and hemicellulose fractions in wood decrease significantly after one year within the different sediments.

The following chapter will present a discussion of the results from the physico-chemical, microbiological and wood degradation studies.

Chapter 7 Discussion

7.1 Introduction

This chapter outlines trends in the data as generated through the integration of, and by drawing comparisons between, the different techniques presented in Chapters 4 to 6. In brief, Chapter 4 outlined the results of the techniques used to characterize the sediments in the lysimeters, and Chapter 5 presented the microbiological results from denaturing gradient gel electrophoresis (DGGE) of the sediment and wood samples obtained from the lysimeter study and the '*in situ*' wetland archaeological and landfill sites. Subsequently, Chapter 6 highlighted the results of the gas chromatographic (GC) analysis of the wood samples recovered from the lysimeter study and the '*in situ*' wetland archaeological sites.

It is anticipated that the identification of any trends in the data generated will enable the detection of the main factors responsible for wood degradation in the short-, medium- and long-term; and as a consequence, determine the most appropriate environmental conditions for the preservation of oak wood in wetland archaeological sites and the optimization of organic waste degradation in landfill sites.

A multi-disciplinary approach was used to assess the biological, chemical and physical nature of the sediments obtained from the lysimeters. This was undertaken alongside the assessment of the biological component of the sediments excavated from the '*in situ*' wetland archaeological and landfill sites, in order to facilitate the characterization of the differing burial environments and their component parts, thereby enabling comparisons to be drawn between the variables analyzed.

The results produced from the GC analysis of the wood samples recovered from the lysimeter study and the '*in situ*' wetland archaeological sites, provided important baseline information of the inter-relationship between the seven major oak wood sugars (which correspond to the cellulose and hemicellulose fractions) and their associations with the total wood sugars (as described in Section 6.2), during the process of decay.

As a corollary, this chapter will integrate the biological, chemical and physical data obtained from the characterization of the differing sediments in the lysimeters and the '*in situ*' wetland archaeological and landfill sites, with the results from the wood degradation studies, in order to identify the factors responsible for decay. These findings will subsequently identify the most appropriate environmental conditions which preserve and degrade oak wood over a variety of different time scales.

7.2 Factors responsible for wood degradation

Two factors have been identified from the biological and physico-chemical results which are considered to be the primary variables responsible for oak wood degradation; these are the length of time in the burial environment and the bio-degradation of the wood by bacterial activity. It appears that differences in sediment composition, levels of saturation and redox potential do not alter the rate and extent of oak wood decay under the experimental conditions deployed in this study.

These results contrast with the findings from previous research which has indicated that changes in the physico-chemical factors characterizing differing burial environments can affect the rate and extent of wood degradation (Caple *et al.* 1997, Caple & Dungworth 1995, Chapman and Cheetham 2002, Chectham 2004, Corfield

forthcoming, Hogan *et al.* 2001). As a consequence of this new information, the potential differences that exist between the current research and the results of these earlier studies will be assessed in Section 7.2.2.

7.2.1 Length of time in the burial environment

The results presented in Section 6.5 have highlighted a strong relationship between oak wood degradation and the length of time in the burial environment. Total sugar losses from the wood samples in all the lysimeters decrease over time. The majority of the total sugar contents of the wood samples is lost within the first two years of burial, with the rate of degradation increasing significantly between the first and second year of monitoring (as shown in Figure 6.14). These findings reinforce the results of the research undertaken by Barlaz and co-workers (1989b), who demonstrated that the maximum rate of organic decomposition in landfill sites occurs between the first and second year of monitoring, and Kenward & Hall (2000) and Björdal and co-workers (1999), who have suggested that the main phases of wood degradation in waterlogged contexts occur before, during, and for a 'short period' after burial.

During the course of the lysimeter study, the decomposition of the organic portion of the landfill waste also produced significant results which confirm the importance of the relationship between organic decay and the length of time in the burial environment (as described in Section 4.6). Organic decomposition was assessed by measuring the settlement drop in the height of the waste over the duration of the experiment (as shown in Figures 4.13-4.14). The decrease in the height of the waste corresponded to the three stages of waste settlement (*cf.* Morris & Woods 1990). The findings show a fall in all the waste heights within the lysimeters. However, the decrease in the heights of the waste in the aerobic lysimeters which had their leachate

levels manipulated was far more significant than that experienced in the lysimeters which were permanently saturated with leachate.

The findings presented above suggest that a drop in leachate levels allows air into the zones of aeration (1) and fluctuation (2) within the waste in the aerobic lysimeters, which consequently accelerates the biological degradation of the organic materials (Das *et al.* 2002, Hale Boothe *et al.* 2001, Wall & Zeiss 1995). This process will influence both the changes in the biological, chemical and physical composition of the waste (Read *et al.* 2001) and the rate at which the cycle of decomposition proceeds (Barlaz *et al.* 1989a, Stessel & Murphy 1992). The weakening of the waste matrix subsequently increases the rate of settlement drop in the height of the waste (as shown in Figures 4.13 and 4.14 [which correspond to Lysimeters 5-8 and Lysimeters 9-12, respectively]) (Stessel & Murphy 1992).

Previous investigations have highlighted the environmental implications of the aerobic bio-degradation of landfill waste (Read *et al.* 2001, Stessel & Murphy 1992, Yuen *et al.* 1999). Bacteria convert the organic portion of the waste to predominantly carbon dioxide (CO_2) and water (H_2O), with stabilized humus remaining (Yuen *et al.* 1999). This is in contrast to the increased bacterial production of methane (CH_4) which is generated by the reduction of CO_2 by hydrogen (H_2) under anaerobic conditions. The recirculation of leachate through the waste mass (which was controlled by the manipulation of water/leachate levels in the present study) increases biomass production and cellulolytic activity (Stessel & Murphy 1992). These processes can decrease the long-term impacts on the surrounding environment from full-scale landfill sites, improve post-closure maintenance costs and reduce methane production within the waste mass (Read *et al.* 2001).

The variation in the organic decomposition of the waste in the lysimeters was not reflected in the results from the investigation of the degradation of the wood samples in the same environment. A comparison between the weights of wood sugars and the manipulation in water/leachate levels indicated that the weights of total sugar losses (as shown in Figure 6.3) and the proportional weights of individual sugar losses (as shown in Figure 6.4) decreased at a constant rate, irrespective of the level of saturation present in the lysimeters. These findings suggest that the readily digestible holocellulose, slowly digestible crystalline holocellulose and recalcitrant cellulose in landfill waste may produce significantly different rates of degradation due to microbial digestion (AFRC Institute of Food Research 1988). This is in contrast to the holocellulose which is less easily digestible in the wood samples.

On the basis of the above findings, it is suggested that although the nature of oak wood enables it to be used as a general representation of the biodegradable portion of waste, future studies of this nature should investigate further the degradation of the different forms of holocellulose in wood and waste, in order better to understand the bioavailability and digestion kinetics of differing organic materials. These results would increase our knowledge of microbial holocellulose biosynthesis and any associated changes in substrate production; and as a consequence, highlight inter-relationships among variations in the biological, chemical and physical composition of the differing burial contexts and the organic material therein.

Changes in the biological and physico-chemical composition of the waste were confirmed by shifts in the pH values obtained from Lysimeters 8 and 12 which contained waste and leachate (as shown in Figure 4.12). These results suggest that a manipulation of leachate levels produces an increase in pH during the first year of the

experiment, which is followed by a decrease in pH in the second year of the experiment. As a consequence, the changes in pH alter the physico-chemical composition of the waste and trigger the development of new microbial populations. This subsequently influences the cycle of decomposition (as shown by the pH values for Lysimeters 8 and 12 [Figure 4.12], the denaturing gradient gel electrophoresis (DGGE) banding patterns [Figures 5.2 and 5.5] and the hierarchical cluster analysis [Figures 5.4 and 5.7] of the sediment and wood samples for Lysimeters 5-8 and Lysimeters 9-12, respectively). These findings corroborate and reinforce the results of the lysimeter studies undertaken by Barlaz (1997) and Barlaz and co-workers (1989a, 1989b), who demonstrated that changes in the microbial populations within waste are caused by variations in the value of pH.

Figure 4.12 shows that the first year of the lysimeter study was characterized by the anaerobic acid phase (2) in the cycle of decomposition, where carboxylic acids accumulated and pH decreased. The second year of monitoring was characterized by the accelerated methane production phase (3) in the cycle of decomposition, in which pH values and microbial populations significantly increased (Barlaz 1997, Barlaz *et al.* 1989a, 1989b). Leaching of soluble compounds from the cell walls of the organic portion of the waste by chemical hydrolysis in the first year was replaced by the loss of structural carbon (which primarily consisted of cellulose and hemicellulose) by microbial activity in the second year (as shown in Figures 6.14 and 6.15, and Table 4.1). These findings reinforce the results of previous work of Barlaz and co-workers (*op. cit.*), who demonstrate that the different phases in the cycle of decomposition produce changes in the microbial populations. As a consequence, this creates greater organic degradation of the waste by microbial digestion in the second year of

monitoring, as opposed to leaching of soluble compounds by chemical hydrolysis in the first year of monitoring.

The findings from the lysimeters which contained waste and leachate also highlighted different rates of organic decay within the cycle of decomposition (as shown by the pH values in Figure 4.12 and the four main phases in the cycle of landfill waste decomposition in Table 4.1). In the permanently saturated conditions of the anaerobic lysimeter (12) and in the saturated zones of the lysimeters which had their leachate levels manipulated (7 and 8), the pH values show that the cycle of decomposition occurred at a slower rate. However, in the zone of aeration in the lysimeters which were manipulated by leachate levels (7 and 8), decomposition occurred over a shorter time scale (Barlaz *et al.* 1989b, Stessel & Murphy 1992).

At the end of the experiment, in the zone of aeration within the lysimeters which had their leachate levels manipulated (7 and 8), the pH values suggest that the final phase of methanogenesis (4) had been entered (as shown in Figure 4.12 and Table 4.1). However, in the permanently saturated conditions present in the anaerobic lysimeter (12) and in the saturated zones of the lysimeters which were manipulated by leachate levels (7 and 8), the pH values indicate that the accelerated methane production phase (3) had been entered (as shown in Figure 4.12 and Table 4.1).

These results provide further confirmation that the rate of organic decay within the cycle of decomposition changed during the experiment as a consequence of the manipulation of leachate levels. Drops in leachate level introduce air into the system (as shown by the linear plots of sediment redox potentials and water levels in Figure 4.3) and promotes the biological degradation of the organic portion of the waste (as

shown by the settlement drop in the height of the waste over the duration of the experiment for Lysimeters 5-8 [Figure 4.13] and Lysimeters 9-12 [Figure 4.14]) (Das *et al.* 2002, Hale Boothe *et al.* 2001, Wall & Zeiss 1995).

The findings presented above demonstrate that the length of time in the burial environment is one of the primary factors responsible for the degradation of the wood samples obtained from the lysimeter study and the wetland archaeological sites. The majority of wood decay occurs before, during and shortly after burial (Björdal *et al.* 1999). This is highlighted by the results of the total sugar losses from the wood samples in all the lysimeters. The samples have shown that approximately 60 % of the total cellulose and hemicellulose present in wood degrades between the first and second year of monitoring. However, although the organic portion of the landfill waste confirmed the relationship between organic degradation and the duration of burial (Barlaz *et al.* 1989b), the findings have also indicated that changes in both the value of pH (Barlaz 1997, Barlaz *et al.* 1989a, 1989b) and the manipulation of leachate levels (Das *et al.* 2002, Hale Boothe *et al.* 2001, Wall & Zeiss 1995) increase the rate of organic decay within the cycle of decomposition.

The results from the current study also suggest that the factors responsible for the degradation of the wood samples and the decomposition of the organic portion of landfill waste are affected by the various forms of holocellulose present within differing types of original organic material. As a consequence, this variation can produce significantly different rates of decay even where similar processes of microbial digestion are in evidence.

7.2.2 Microbiological activity

The interpretation of the results from the previous three chapters has indicated a strong relationship between oak wood degradation and measured activity of bacteria. Comparisons among the total weights of wood sugars from all the lysimeters highlighted a number of distinct similarities. Although several environmental conditions were manipulated within the lysimeter study, the results from the analyses of the wood samples show that the degradation of cellulose and hemicellulose is similar, irrespective of the burial environment (as described in Section 6.4) or the level of saturation (as described in Section 6.3).

The above findings seem to contradict previous research on organic degradation in burial environments, where a change in the redox potential (Caple *et al.* 1997, Caple & Dungworth 1995, Corfield forthcoming, Hogan *et al.* 2001) and the saturation level (Chapman & Cheetham 2002, Cheetham 2004, Hogan *et al.* 2001) of sediment has been shown to have a direct influence on the '*in situ*' preservation of archaeological remains. However, unlike previous investigations where the primary objective of the research was to identify the optimum environmental conditions to preserve archaeological wood '*in situ*', one of the main aims of this study was to determine the major causes of oak wood decay. Therefore, on the basis of these contrasting aims, the findings from the current study significantly enhance our understanding of the factors responsible for wood degradation in differing burial contexts and over a variety of time scales.

With the exception of the study by Hogan and co-workers (2001), no other research has monitored the degradation of fresh wood from the initial stages of burial over a two-year period under controlled conditions. Previous investigations have only

recovered wood samples in more advanced stages of degradation and used these as the baseline for inferring the physico-chemical and biological data produced during a monitoring programme. Although the latter methodology can provide an insight into the patterns and processes responsible for wood decay, it is restricted in its approach, as it only uses degraded archaeological wood samples obtained during excavation with no knowledge of the burial history. As a consequence, it cannot determine the factors which may have influenced the wood from the time of burial prior to the '*in situ*' monitoring of the particular environment in question (Boddy & Ainsworth 1984). On the basis of this information, it is therefore suggested that although the collection of data solely from archaeological woods may help determine the optimum conditions necessary for preservation '*in situ*', it does not accurately assess the patterns and processes responsible for wood decay, particularly during the early stages of burial.

The similarities that exist between the total sugar losses in the majority of the lysimeters at all depths (as shown in Figure 6.3) indicated that the manipulation of the water/leachate levels in the lysimeters did not influence the degree of wood degradation. Comparison between this finding and the strong relationship that has been shown to exist between oak wood degradation and the activities of bacteria during the course of this study, confirms the research undertaken by Björdal & Nilsson (2002). Microscopy verifies the results of their work, namely that the chemical and morphological changes in the decay profile of waterlogged archaeological wood after burial results from microbial decay and not from chemical hydrolysis; as was previously assumed (Björdal *et al.* 1999).

These findings have also enhanced our understanding of the similarities between the factors responsible for the degradation of oak wood in differing burial environments,

i.e. wetland archaeological and landfill sites. The results obtained from the analysis of the denaturing gradient gel electrophoresis (DGGE) banding patterns (as shown in Figure 5.8), the hierarchical cluster analysis (Figures 5.7 and 5.10) and the principal-component analysis (Table 5.6) of the sediment and wood samples obtained from the majority of the lysimeters indicates the presence of similar bacterial populations at all depths, in different sediments and under differing physico-chemical conditions. These bacterial species must be responsible for the majority of the wood degradation identified during the current study. This finding is consistent with previous research undertaken by Björdal and co-workers (1999) who demonstrated that there are certain ubiquitous groups of bacteria which are responsible for wood degradation, irrespective of the different environmental conditions imposed upon the wood.

Unfortunately, it was not possible to determine the extent to which sediment composition affects wood in wetland archaeological and landfill sites, as precise environmental conditions prior to and after burial could not be established. However, the results that have been presented from the lysimeter study indicate that sediment type is unlikely to influence wood degradation during early burial. Therefore, on the basis of these findings, it is hypothesized that differing '*in situ*' burial environments are unlikely to affect wood decay; instead other patterns and processes such as cellulose and hemicellulose biosynthesis may be primarily responsible for the progress of the process.

The results from the DGGE banding patterns (as shown in Figure 5.2), the hierarchical cluster analysis (Figure 5.4) and the principal-component analysis (Table 5.2) of the sediment and wood samples from Lysimeters 5-8 show an increase in the similarity of the bacterial populations between the first and second year of monitoring, which may

be due to the different physico-chemical processes associated with waste decomposition. This observation corroborates the research undertaken by Barlaz and co-workers (1989a) who demonstrated that the relationships between bacterial communities converge over time as the environmental conditions within the waste change.

The proportional weights of glucose and xylose obtained from the surface and central wood samples recovered from the timbers of Rolls Farm wetland archaeological site (as shown in Figure 6.13) indicate that surface samples experience a greater degree of decay than those from the centre of the wood (Björdal & Nilsson 2002). This suggests that degradation proceeds in a radial manner to the core of the wood (Hoffmann 1981, Paajanen & Viitanen 1988) and it is primarily due to the activities of bacteria. The gradual penetration of fluids and bacteria into the wood from the sediment is highlighted using Sorensen's pairwise similarity coefficient and is shown in Tables 5.1 and 5.5. The tables show the percentage similarity among lanes within the DGGE gel for the sediment and wood samples obtained from Lysimeters 5-8 and Lysimeters 10-12, respectively. The interpretation of these data suggests that the sediment and wood samples display greater percentage similarities the longer they are contained within the lysimeters. Unfortunately, the thin sections which were cut from the surface of the wood samples in the lysimeter study were not of sufficient quality to confirm this radial pattern of decay over a different time scale.

The radial pattern of wood degradation is corroborated by the analysis of the DGGE banding patterns from the surface and central wood samples taken from Rolls Farm wetland archaeological site (as shown in Figure 5.8). The highest bacterial species diversity, as indicated by the number of bands (Oliveira 2003), is present in the

surface wood sample from Rolls Farm 3. This band profile contained three times the number of bands (15) when compared with the central wood sample (which only contained 5 bands).

The findings presented above have demonstrated that changes in the physico-chemical characteristics of the burial environment do not directly affect wood degradation. One of the most important factors affecting wood decay is the synthesis of cellulose and hemicellulose by the activities of bacteria. There are several ubiquitous bacterial species which are present in different sediments and under different environmental conditions. These are responsible for the majority of wood degradation in the burial environments used within the current study.

The research undertaken during this study has used oak wood as the complimentary organic biomarker to determine the factors responsible for organic degradation in both types of burial environments. However, it is recognised that the density, compression and water content of wood varies both between different species and among the same species (Schweingruber 1978). These factors can ultimately affect the resistance of a particular wood to decay (Barefoot & Hankins 1982), i.e. the softer the wood, the lower the density and compression resistance and the higher the water content. As a consequence, the biological, chemical and physical structure of the wood will affect the bacterial degradation of the holocellulose, which will result in different rates of decay. This is particularly important in waterlogged/water saturated archaeological excavations where oak wood is the primary material recovered from the burial environment and in landfill sites where soft woods dominate.

The surface to volume ratio of the wood and the surface area which has been exposed will also influence the rate of bacterial digestion (AFRC Institute of Food Research 1988, Cummings & Stewart 1994). An increase in the ratio between the surface area of the wood sample and the volume would improve the availability of the products necessary for organic decomposition (Driessen *et al.* 1995). As a consequence, this process would create superior environmental conditions for the biosynthesis of the wood by cellulolytic activity (AFRC Institute of Food Research 1988, Westlake 1989). The rate and extent of wood degradation would subsequently increase, resulting in a decrease in the length of time required for the complete removal of the cellulose and hemicellulose from the wood structure. However, the majority of the information available on surface to volume ratios and surface areas of organic materials is contradictory (AFRC Institute of Food Research 1988). As a consequence, the results of previous research should be treated with caution. It is therefore suggested that further detailed and controlled investigations are required in order to enhance the accuracy and validity of these findings.

On the basis of this information, it is difficult to use the results of the oak wood samples obtained throughout the course of this study, in order accurately to represent the potential degradation which may be experienced by other wood species over a similar time scale. Despite this, the generic patterns of degradation, which have been identified above as being primarily responsible for oak wood decay, will still be commensurate with other species.

The integration of the results of the sediment and the wood samples obtained during the current study and the data generated by the multi-disciplinary approach adopted to characterize the differing burial environments, has enabled a greater understanding of

two pairs of related processes: the relationship between the decay rate of oak wood to the pathway it follows *en route* to the burial environment; and the link between burial conditions and organic degradation over a variety of time scales (Kenwood & Hall 2000).

The results from the current study provide further evidence to help identify the most favourable environmental conditions necessary for the '*in situ*' stabilization of organic materials within wetland archaeological sites and the manipulation of the primary factors which are able to promote the optimization of organic waste decomposition in landfill sites.

The preservation of the waterlogged wood archive/record not only increases our understanding of our cultural heritage (Caple 1994) for use by future generations, but also enables the management and protection of Britain's buried archaeological remains by the promotion of '*in situ*' preservation, rather than excavation (Caple 1994, Chapman & Cheetham 2002).

Contrary to the '*in situ*' preservation of oak wood in wetland archaeological sites, optimizing the decomposition of the organic portion of landfill waste can decrease methane production (Yuen *et al.* 1999), reduce the levels of certain toxic organic materials in landfill leachate (Read *et al.* 2001), and as a consequence, decrease potential environmental problems (Das *et al.* 2002). Increasing organic waste decomposition will also promote long-term cost savings in post-closure maintenance costs (Yuen *et al.* 1999) and liability reduction (Read & Hudgins 2000) for many waste management companies.

7.3 Summary

This chapter has presented an overview of the results obtained from the three main strands of research undertaken during this study; the physico-chemical analyses, microbiological assessments and wood degradation studies. It has integrated each set of results in order to consider the effectiveness of the techniques used holistically to characterize the sediments in the lysimeter study from a biological, chemical and physical perspective, and to help identify the main factors responsible for the decay of the wood samples recovered from the lysimeter study and the '*in situ*' wetland archaeological sites. The two primary factors responsible for wood degradation are the length of time in the burial environment and the biosynthesis of the cellulose and hemicelluloses by bacteria.

The findings outlined in this chapter enhance our understanding of the complex biological, chemical and physical interactions that take place within a burial environment and between differing burial environments. These interactions affect the rate and extent of wood decay. By using oak wood samples of different ages, i.e. the short- (lysimeter study) and long-term (wetland archaeological sites), this study has also shown the inter-relationship between the individual wood sugars (which correspond to the cellulose and hemicelluloses) as decay proceeds. As a consequence, the integration of both sets of data further develops our understanding of the '*in situ*' preservation of organic remains in wetland archaeological sites.

By using oak wood as a representation of the organic portion of landfill waste, most of the primary factors responsible for organic waste decomposition have been established. However, owing to the different forms of cellulose and hemicellulose present among both types of organic materials, the use of wood has not identified all

the factors responsible for organic waste decay. Organic decomposition was also influenced by the addition of air into the waste matrix. This process increases the activities of bacteria which degrade the organic component of the waste more rapidly than under anaerobic conditions.

Nevertheless, on the basis of these findings, the current study has not only demonstrated that it is possible to enhance the rate and extent of organic degradation in landfill sites by the manipulation of a number of environmental variables, but it has further developed our understanding of the optimum conditions required for the organic decomposition of landfill waste.

The concluding chapter will briefly summarise the original aims of the research and discuss the extent to which these aims have been achieved. The results of the study will then be used to identify areas that would benefit from further research.

Chapter 8 Conclusions

8.1 Introduction

This chapter summarises the main findings resulting from the characterization of the sediments in the lysimeter study in order to determine the factors responsible for oak wood degradation in this study and in the '*in situ*' wetland archaeological sites investigated. These results are discussed in relation to the main research aims of the thesis which were:

1) To artificially manipulate a number of the key biological, chemical and physical parameters which characterize the differing sediments in the lysimeter study
2) To determine the key environmental parameters responsible for oak wood degradation over a variety of time scales by using samples obtained from the lysimeter study and a number of wetland archaeological and landfill sites
3) To identify the most appropriate environmental conditions under which to preserve wood in wetland archaeological sites and optimize organic waste degradation in landfill sites

The second part of this chapter will outline the recommendations for future research based on the main findings of the study and assess the appropriateness of using lysimeters to replicate the conditions occurring within '*in situ*' wetland archaeological and landfill sites.

8.2 Review of the main findings

Although the financial and time constraints associated with the monitoring of '*in situ*' burial environments prevented the physico-chemical assessment of the wetland

archaeological and landfill sites chosen for this study, it has still been possible to characterize the sediments in the lysimeter study and identify the patterns within these sediments which are implicated in the decay of the wood samples in both the lysimeters and the '*in situ*' wetland archaeological sites. As a result of these findings, this study has considered the optimum environmental conditions which preserve and degrade oak wood.

8.2.1 Characterizing the lysimeters using biological, chemical and physical measurements

The biological, chemical and physical data generated from a multi-disciplinary approach aimed at characterizing the sediments in the lysimeter study has proven effective in this respect. The trends in the data highlighted both general patterns of similarity and difference between the sediments in the lysimeters and have identified a number of the more complex interactions that take place between the different variables investigated.

The manipulation of water/leachate levels in the aerobic lysimeters resulted in changes in the level of saturation of the sediments, along with the sediment redox potential and the dissolved oxygen of the water itself. This has led to a greater understanding of the complex interactions that occur between these parameters and the cumulative effect that they have upon the bacterial degradation of oak wood; a supposition which previous researchers have strongly advocated (Brunning *et al.* 2000, Chapman & Cheetham 2002, Cheetham 2004, Hogan *et al.* 2001, Powell *et al.* 2001).

The data generated by measuring the dissolved oxygen of the water in the lysimeters which contained peat has provided further information on the characterization of burial environments that was not anticipated prior to the commencement and during the monitoring of this study. The results from the analysis of the dissolved oxygen values and water levels from all the lysimeters containing peat have indicated that there was a high level of dissolved oxygen in the water prior to a drop in water level. This finding suggests that an increase in water level may still undermine the preservation of the wood samples; an observation which has not been made by previous researchers.

Although the results from this study have indicated a correlation between the levels of saturation, the oxidizing-reducing potential of the waste and the bio-deterioration of the organic material therein, it does not identify a similar relationship between these parameters and the wood samples. However, the results do suggest that the different forms of holocellulose in waste may produce different rates of biosynthesis which contrast with the holocellulose present in the oak wood samples (AFRC Institute of Food Research 1988). In order to confirm this assertion, laboratory simulations which manipulate similar environmental conditions, and that contain oak wood, are required. The simulations need to be undertaken over longer time scales than were feasible in the present research. These can be subsequently compared with the results from the waste lysimeters in the current study.

The results obtained from the majority of the lysimeters which contained waste have also indicated the importance of monitoring pH. They have demonstrated that the value of pH can significantly influence the dynamics of the burial environment; and as a consequence, determine organic decomposition. The pH values obtained from the

waste can significantly affect its redox potential (Bohn 1971, British Standards Institute 1990, Cheetham 2004). Variation in the redox potential and pH can cause changes within the bacterial community (AFRC Institute of Food Research 1988, Barlaz 1997, Barlaz *et al.* 1989a). The current study has shown that bacterial population shifts subsequently alter the decomposition of the organic fraction of the waste.

The use of denaturing gradient gel electrophoresis (DGGE) has provided important baseline data for understanding changes in bacterial activity and population dynamics. The data that have been generated not only show a significant relationship between the bacterial communities present in the different sediments and the zones of saturation but also identify a number of ubiquitous bacterial types present in the lysimeters and the '*in situ*' wetland archaeological and landfill sites. These communities are responsible for the majority of wood degradation observed during this study.

The similarities that exist between the main findings from the biological, chemical and physical results obtained during the monitoring of the lysimeters and the biological results from the wetland archaeological and landfill sites have clearly demonstrated the effectiveness of using a laboratory-scale simulation to replicate the environmental conditions within differing '*in situ*' burial contexts. However, in order to further develop our understanding of the dynamics of these environments, it is necessary to undertake a multi-disciplinary '*in situ*' monitoring programme (which includes biological, chemical and physical assessments) to characterize each site studied. As a consequence, the results obtained from such a study would wholly represent the patterns and process responsible for the degradation of the wood samples directly

recovered from '*in situ*' burial contexts (-a situation that has not been possible during the current study).

8.2.2 The main factors responsible for oak wood degradation

The biological, chemical and physical data generated during the lysimeter study have been used in conjunction with the gas chromatographic (GC) analysis of the oak wood samples obtained from this study and the '*in situ*' wetland archaeological sites, in order to determine the main factors responsible for wood degradation.

The findings from the current study have identified two factors which are primarily responsible for wood decay; these are the duration of burial and the bio-degradation of the wood by a number of ubiquitous bacterial species which are present in differing sediments. The pattern of bacterial degradation proceeds in a radial manner into the core of the wood, degrading the cellulose and main hemicelluloses at a constant rate throughout the duration of burial.

The results also show that the rate and extent of oak wood degradation is not influenced by the physico-chemical parameters which define the sediments in the burial environments studied, i.e. level of saturation, pH, redox potential and dissolved oxygen.

8.2.3 The preservation and degradation of oak wood in differing burial environments

On the basis of the findings discussed above, it is imperative to control bacterial activity in order to either preserve or degrade oak wood. From the time of burial in the sediment, irrespective of the physico-chemical variables present, the activities of

bacteria will degrade the wood over time. As a consequence, it is vital to inhibit the activities of the bacterial species responsible for wood decay in order to promote the preservation of archaeo-organic remains in wetland archaeological sites, whilst also encouraging bacterial growth and activity in order to optimize organic material decomposition in landfill sites.

Contrary to the information obtained from the oak wood samples, the findings produced from the analysis of the settlement drop in the height of the waste in the lysimeters which contained leachate, indicated that the decomposition of the organic fraction was significantly influenced by the manipulation of leachate levels. Leachate level manipulation introduced air into the lysimeter system which consequently decreased the height of the waste by holocellulose biosynthesis. The results of the study show that the rate and extent of organic degradation is not only influenced by the duration of burial and the bio-degradation of the waste by a number of ubiquitous bacterial species but also by aeration. Therefore, on the basis of this information, it is not only necessary to encourage both bacterial growth and activity, but also to promote the continual addition of air into the waste mass, in order to optimize organic material degradation in landfill sites.

8.3 Recommendations for future research

It is important to expand upon the themes produced from this study in order to understand the burial environment in greater detail and with greater accuracy. This will enable a more considered appreciation of the complex patterns and processes responsible for the degradation of wood in differing sediments.

This section will consequently discuss the main factors responsible for wood degradation during the lysimeter study and indicate the appropriateness of using an experimental study to replicate the conditions occurring within '*in situ*' wetland archaeological and landfill sites. The main findings are subsequently discussed in order to give directions for future research into the understanding of wood decay.

The primary objective of the lysimeter study was to establish the extent to which the biological, chemical and physical interactions within differing sediments influenced oak wood degradation.

The findings from the experiment have provided an insight into the complex nature of two contrasting burial environments by replicating some of the environmental conditions that exist within wetland archaeological and landfill sites. By doing so, it has developed our understanding of the patterns responsible for wood decay in different sediments and under a variety of environmental conditions.

Two primary factors have been identified which are responsible for the degradation of the wood samples: these are the length of time in the burial environment and bacterial activity. However, recent research by Björdal & Nilsson (2002) has indicated that the pattern of degradation produced by the bacterial attack of fresh wood is different to the pattern of decay produced by the bacterial attack of archaeological wood. Therefore, in light of this information, it is suggested that further research be undertaken on the bacterial degradation of wood under laboratory simulations and within '*in situ*' sites. This can be achieved by genetically sequencing bands cut from a DGGE gel in order to produce a comprehensive profile of the bacterial species responsible for wood decay. (Pioneering work of this nature has recently been

undertaken by Helms & Kilstrup [2001]). The data generated can then be used in conjunction with conventional microscopic techniques which assess the ultra-structure of the wood, in order to produce a greater understanding of the complex interactions that occur between wood and the surrounding sediment, whilst also providing more detailed information on the specific microbial species involved in the degradation process over a variety of time scales.

On the basis of the results of the wood degradation studies undertaken during this research it is suggested that future investigations of a similar nature should explore differences in the density, compression and water content of wood, both between different species and within the same species. These experiments should be undertaken in differing burial environments and under a variety of environmental conditions. The results would increase our understanding of the bacterial degradation of the cellulose and hemicellulose fractions in differing woods and the subsequent affects that these factors have upon its biological, chemical and physical structure.

The determination of decay within the same species of wood can be developed further by assessing the impact of changes in the surface to volume ratio and the surface area, against the rate and extent of bacterial digestion. These results would not only increase our understanding of the conditions necessary for the degradation and the preservation of oak wood, but also the relationship between the decay rate and the burial conditions which influence microbial biosynthesis.

The results from the lysimeter study have demonstrated that the degradation of wood by bacterial activity occurs under a variety of environmental conditions. Therefore, in order to restrict this process, it is necessary to inhibit bacteria wherever possible.

Previous research from landfill sites has shown that high levels of toxic contaminants from certain heavy metals retards the activities of the microorganisms responsible for organic degradation (AFRC Institute of Food Research 1988).

On the basis of these findings, a logical suggestion would be that future investigations could assess the localised effect of adding differing concentrations of trace metals to organic material in a variety of burial environments, in order to monitor subsequent changes in the microbial community. However, whilst the utilization of such a strategy may improve the preservation of sensitive archaeo-organic remains in wetland archaeological sites, it is recognised that the environmental implications associated with the 'spiking' of natural burial environments with heavy metals may invalidate such an approach.

The results obtained from the lysimeter study have also demonstrated that oak wood can be used as a general representation of the organic portion of landfill waste. However, due to the different rates of holocellulose degradation between the organic materials in landfill waste, it is suggested that further investigations should be undertaken in order to enhance our understanding of the digestion kinetics of organics and the environmental factors responsible for the process of decay.

Another major finding from the lysimeter study is the observation that the three stages of landfill waste settlement (Wall & Zeiss 1995) were principally influenced by the manipulation of leachate levels. Leachate level manipulation introduced air into the system which consequently promoted the bacterial degradation of the organic portion of landfill waste (Boyd 1981, Hale Boothe *et al.* 2001). On the basis of these results, it is suggested that larger-scale and more representative lysimeters be constructed in

order to optimize the decomposition of organic material. The results would help to generate a greater understanding of the patterns and processes responsible for organic biosynthesis and therefore enable further research to be undertaken into the optimum physico-chemical conditions necessary for organic waste decomposition. As a consequence, these findings would reduce the time taken for landfill sites to be returned to post-productive use.

8.4 Summary

This thesis has presented the biological, chemical and physical data collected from the study and characterization of the sediments within a two-year lysimeter study in order to determine the factors responsible for oak wood degradation in '*in situ*' wetland archaeological and landfill sites. The techniques that have been used during the course of this study have been developed in order to gain a holistic understanding of the dynamics of contrasting burial environments and to consider the similarities that exist between the patterns responsible for both the preservation and the degradation of wood. It is hoped that the findings produced from this work will encourage further research into the prevention of the unwanted biological decay of archaeo-organic remains in wetland archaeological sites and help towards the optimization of organic waste decomposition in landfill sites.

References

AFRC Institute of Food Research (1988). *A Basic Study of Landfill Microbiology and Biochemistry.* DTI Report: ETSUB1159. Department of Trade and Industry, Northumberland.

Ainsworth, A. M. & Goulder, R. (2001). Denaturing Gradient Gel Electrophoresis used to Profile Bacterial Diversity in Landfill Leachate. Unpublished notes.

Albersheim, P., Nevins, D. J., English, P. D. & Kaar, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydrate Research* **5**, 340-345.

Allsopp, D. & Seal, K. J. (1986). *Introduction to Biodeterioration.* Edward Arnold, London.

Amann, R. I., Ludwig, W. & Scheiffer, K-H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews* **59**, 143-169.

American Society for Testing and Materials (1996). *Standard Test Method for Acid-Insoluble Lignin in Wood.* ASTM Designation: D1106-96. ASTM International, Pennsylvania, USA.

American Society for Testing and Materials (1996). *Standard Test Method for Dichloromethane Solubles in Wood.* ASTM Designation: D1108-96. ASTM International, Pennsylvania, USA.

American Society for Testing and Materials (1996). *Standard Test Method for Preparation of Extractive-Free Wood.* ASTM Designation: D1105-96. ASTM International, Pennsylvania, USA.

American Society for Testing and Materials (1995). *Standard Test Method for Ash in Wood.* ASTM Designation: D1102-84. ASTM International, Pennsylvania, USA.

American Society for Testing and Materials (1995). *Standard Test Method for 1 % Sodium Hydroxide Solubility of Wood.* ASTM Designation: D1109-84. ASTM International, Pennsylvania, USA.

Ashton, N. (2004). Hunting for the First Humans in Britain. *British Archaeology* **70**, 8-13.

Backa, S. & Brolin, A. (1991). Determination of pulp characteristics by diffuse reflectance FTIR. *Tappi Journal* **May 1991**, 218-226.

Baird, A. J., Surridge, B. W. J. & Money, R. P. (2004). An assessment of the piezometer method for measuring the hydraulic conductivity of a *Cladium mariscus* - *Phragmites australis* root mat in a Norfolk (UK) fen. *Hydrological Processes* **18**, 1-17.

Baird, A. J. & Waldron, S. (2003). Shallow horizontal groundwater flow in peatlands is reduced by bacteriogenic gas production. *Geophysical Research Letters* **30 (No. 20)**, 1-4.

Barefoot, A. C. & Hankins, F. W. (1982). *Identification of Modern and Tertiary Woods*. Oxford University Press, Oxford.

Barlaz, M. A. (1997). Microbial Studies of Landfills and Anaerobic Refuse Decomposition. In *Manual of Environmental Microbiology* (ed. C. J. Hurst), pp 541-557. American Society for Microbiology, Washington DC, USA.

Barlaz, M. A., Schaefer, D. M. & Ham, R. K. (1989a). Inhibition of Methane Formation from Municipal Refuse in Laboratory Scale Lysimeters. *Applied Biochemistry and Biotechnology* **20/21**, 181-211.

Barlaz, M. A., Schaefer, D. M. & Ham, R. K. (1989b). Bacterial Population Development and Chemical Characteristics of Refuse Decomposition in a Simulated Sanitary Landfill. *Applied and Environmental Microbiology* **55**, 55-65.

Behar, S. (1996). *Testing the Waters: Chemical and Physical Vital Signs of a River*. River Water Network, Oregon, USA.

Bergman, I., Lundberg, P., Preston, C. M. & Nilsson, M. (2000). Degradation of 13C-U-Glucose in *Sphagnum majus* Litter: Responses to Redox, pH and Temperature. *Soil Science Society of America Journal* **64**, 1368-1381.

Biostrategy Associates Ltd. (1992). *Review of the technique for monitoring the microbiology of landfill.* DTI Report ETSUB1316. Department of Trade and Industry, Northumberland.

Björdal, C. G. & Nilsson, T. (2002). Waterlogged archaeological wood - a substrate for white rot fungi during drainage of wetlands. *Biodeterioration & Biodegradation* **50**, 17-23.

Björdal, C. G., Nilsson, T. & Daniel, G. (1999). Microbial decay of waterlogged archaeological wood found in Sweden - Applicable to archaeology and conservation. *International Biodeterioration and Biodegradation* **43**, 63-73.

Blakeney, A. B., Harris, P. J., Henry, R. J. & Stone, B. A. (1983). A Simple and Rapid Preparation of Alditol Acetates for Monosaccharide Analysis. *Carbohydrate Research* **113**, 291-299.

Blanchette, R. A. (1995). Biodeterioration of archaeological wood. *CAB Abstracts* **9**, 113-127.

Blanchette, R. A. & Hoffmann, P. (1994). Degradation processes in waterlogged archaeological wood. In *Proceedings of the 5th ICOM Group on Wet Organic Archaeological Materials Conference* (ed. P. Hoffmann), pp 111-137. ICOM, Bremerhaven, Germany.

Blanchette, R. A., Nilsson, T., Daniel, G. & Abad, A. (1990). Biological Degradation of Wood. In *Archaeological Wood: Properties, Chemistry and Preservation* (eds. R.

M. Rowell & R. J. Barbour), pp 141-174. American Chemical Society, Washington DC, USA.

Boddy, L. & Ainsworth, A. M. (1984). Decomposition of Neolithic Wood from the Sweet Track. In *Somerset Levels Papers: Number 10* (eds. J. M. Coles, B. J. Orme & S. E. Rouillard), pp 93-96. Stephen Austin and Sons Ltd., Hertford.

Bogner, J. E., Miller, R. M. & Spokas, K. (1995). Measurement of microbial biomass and activity in landfill soils. *Waste Management and Research* **13**, 137-147.

Bohn, H. L. (1971). Redox Potentials. *Soil Science* **112**, 39-45.

Boyd, W. D. (1981). The controlled landfill project. *Procedures of the US Department of Energy/Agronne National Laboratory: 'Contractors' Research and Development Workshop' Converting Waste to Energy.* Argonne Natural Laboratory, Argonne, Illinois, USA.

Brinkhoff, T., Santegoeds, C. M., Sahm, K., Kuever, J. & Muyzer, G. (1998). A poly-phasic approach to study the diversity and vertical distribution of sulphur-oxidizing *Thiomicrospora* species in coastal sediments of the German Wadden Sea. *Applied and Environmental Microbiology* **64**, 4650-4657.

British Standard Institute (1990). *Methods of test for soils for civil engineering purposes.* Chemical and Electrochemical Tests: BS377-3. British Standards Institute, London.

Bronsius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence from a ribosomal gene from *Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America* **75**, 4801-4805.

Bruce, K. D., Strike, P. & Ritchie, D. A. (1999). DNA Extraction from Natural Environments. In *Methods in Biotechnology, Volume 12: Environmental Monitoring of Bacteria* (ed. C. Edwards), pp 97-107. Humana Press Inc., NJ, USA.

Brunning, R. (1999). The *in situ* preservation of the Sweet Track. In *Bog Bodies, Sacred Sites and Wetland Archaeology* (eds. B. Coles, J. Coles & M. S. Jørgensen), pp 33-38. Wetland Archaeology Research Project, Exeter.

Brunning, R., Hogan, D., Jones, J., Maltby, E., Robinson, M. & Straker, V. (2000). *Saving the Sweet Track. The in situ preservation of a Neolithic wooden trackway, Somerset, UK.* Conservation and Management of Archaeological Sites **4**, 3-20.

Budgell, D. R., Hayes, E. R. & Helleur, R. J. (1987). Direct identification of pentoses and hexoses by pyrolysis/capillary gas chromatography. *Analytica Chimica Acta* **192**, 243-253.

Caple, C. (2001). Overview - Degradation, Investigation and Preservation of Archaeological Evidence. In *Handbook of Archaeological Sciences* (eds. D. R. Brothwell & A. M. Pollard), pp 587-593. John Wiley & Sons Ltd., Chichester.

Caple, C. (2000). *Conservation Skills: Judgement, Method and Decision Making.* Routledge, London.

Caple, C. (1996). Parameters for monitoring anoxic environments. In *Preserving Archaeological Remains 'in situ'* (ed. M. Corfield), pp 113-122. Museum of London Archaeological Service, London.

Caple, C. (1994). Reburial of Waterlogged Wood, the Problems and Potential of this Conservation Technique. *International Biodeterioration and Biodegradation* **34**, 61-72.

Caple, C. (1993). Defining a Reburial Environment; Research Problems Characterising Waterlogged Anoxic Environments. In *Proceedings of the 5th ICOM Group on Wet Organic Materials Conference* (ed. P. Hoffmann), pp 407-421. Portland Press, Maine, USA.

Caple, C., Dungworth, D. & Clogg, P. (1997). Results of the characterisation of the anoxic waterlogged environments which preserve archaeological organic materials. In *Proceedings of the 6th Waterlogged Organic Archaeological Materials Conference* (eds. P. Hoffmann, T. Grant, J. A. Spriggs & T. Daley), pp 57-72. Druckerei Ditzen GmbH und Co. KG, Bremerhaven, Germany.

Caple, C. & Dungworth, D. (1995) Investigations into Waterlogged Burial Environments. In *Archaeological Sciences 1995: Proceedings of a conference on the application of scientific techniques to the study of archaeology* (eds. A. Sinclair, E. Slater & J. Goaulett), pp 233-240. Oxbow Monograph, Oxford.

Chapman, H. P. & Cheetham, J. L. (2002). Monitoring and Modelling Saturation as a Proxy Indicator for *in situ* Preservation of Wetlands - a GIS-based Approach. *Journal of Archaeological Science* **29**, 277-289.

Cheetham, J. L. (2004). *An assessment of the potential for* in situ *preservation of buried organic archaeological remains at Sutton Common, South Yorkshire.* Unpublished PhD Thesis, University of Hull.

Coles, B. & Coles, J. (1986). *Sweet Track to Glastonbury.* Thames and Hudson, London.

Coles, J. M. (1984). *The archaeology of wetlands.* Edinburgh University Press, Edinburgh.

Collins, H-J. & Spillmann, P. (1982). Lysimeters for Simulating Sanitary Landfills. *ASCE Journal of the Environmental Engineering Division* **108**, 852-863.

Corfield, M. C. (Forthcoming). Wetland Science. In *Wetland Archaeology and Environments: regional issues, global perspectives* (eds. M. C. Lillie & S. Ellis). Oxbow, Oxford.

Corfield, M. C. (1996). Preventative conservation for archaeological sites. In *Archaeological Conservation and its Consequences* (eds. A. Roy & P. Smith), pp 32-37. International Institute for Conservation of Historic and Artistic Works, London.

Cronyn, J. M. (2001). The Deterioration of Organic Materials. In *Handbook of Archaeological Sciences* (eds. D. R. Brothwell & A. M. Pollard), pp 627-637. John Wiley & Sons Ltd., Chichester.

Cronyn, J. M. (1990). *The Elements of Archaeological Conservation.* Routledge, London.

Cummings, S. P. & Stewart, C. S. (1994). *A study of cellulose degradation in landfills.* DTI Report ETSUB/B2/00198/REP. Department of Trade and Industry, Northumberland.

Das, K. C., Smith, M. C., Gattie, D. K. & Hale Boothe, D. D. (2002). Stability and quality of municipal solid waste compost from a landfill aerobic bioreduction process. *Advances in Environmental Research* **6**, 401-409.

Davis, M. F., Schroeder, H. A. & Maciel, G. E. (1994). Solid-State 13C Nuclear Magnetic Resonance Studies of Wood Decay. *Holzforschung* **48**, 301-308.

Dawson, J. E., Ravindra, R. & Lafontaine, R. H. (1981). A review of storage methods for waterlogged wood. In: *Proceedings of the ICOM Waterlogged Wood Working Group Conference* (ed. D. W. Grattan & J. C. McCawley), pp 227-235. ICOM-CC, Ottawa, Canada.

Dean, M. & Milligan, B. G. (1998). DNA conformational and denaturing gradient methods. In *Molecular Genetic Analysis of Populations: A Practical Approach* (ed. A. R. Hoelzel), pp 270-281. Oxford University Press, Oxford.

Dedysh, S. N., Panikov, N. I. & Tiedje, J. M. (1998). Acidophilic Methanotrophic Communities from Sphagnum Peat Bogs. *Applied and Environmental Microbiology* **64**, 922-929.

Delbrès, C., Godon, J-J. & Moletta, R. (1998). 16S rDNA Sequence Diversity of a Culture-Accessible Part of an Anaerobic Digester Bacterial Community. *Anaerobe* **4**, 267-275.

Ding, A., Zhang, Z., Fu, J. & Cheng, L. (2001). Biological control of leachate from municipal landfills. *Chemosphere* **44**, 1-8.

Driessen, J. H. A., Moura, M. L., Korzilius, E. P. E. & van der Sloot, H. A. (1995). The Sustainable Landfill. In *Proceedings of Sardinia '95, - 5th International Landfill Symposium* (eds. T. H. Christensen, R. Cossu & R. Stegmann), pp 15-24. Environmental Sanitary Engineering Centre (CISA), Cagliari, Italy.

Embley, M. & Widdick, D. (1991). An overview of the potential of nucleic acid technologies for analyzing landfill microbiology. *Landfill Microbiology: Research and Development Workshop II*, pp 137-142. Harwell Publishers, Oxford.

English Heritage (1991). *Exploring Our Past. Strategies for the archaeology of England.* English Heritage, London.

Environment Agency (2000). *A review of in situ composting to accelerate waste stabilisation.* Technical Report P419. Environment Agency, Bristol.

Eriksson, K-E. L., Blanchette, R. A. & Ander, P. (1990). Microbial and Enzymatic Degradation of Wood and Wood Components. In *Springer Series in Wood Science* (ed. T. E. Timell), pp 72-87. Springer-Verlag, Berlin, Germany.

Falk, R. H. (1980). Preparation of Plant Tissues for SEM. In *Preparation of Biological Specimens for Scanning Electron Microscopy* (eds. J. A. Murphy & G. A. Roomans), pp 79-87. Scanning Electron Microscopy Inc., Illinois, USA.

Faulkner, S. P., Patrick, W. H. & Gambrell, R. P. (1989). Field Techniques for Measuring Wetland Soil Parameters. *Soil Science Society of America Journal* **53**, 883-890.

Fengel, D. & Wegener, G. (1979). Hydrolysis of Polysaccharides with Trifluoroacetic Acid and its Application to Rapid Wood and Pulp Analysis. In *Hydrolysis of Cellulose: Mechanisms of Enzymatic and Acid Catalysis, Advances in Chemistry Series 181* (eds. R. D. Brown and L. Jurasek), pp 145-158. American Chemical Society, New York, USA.

Ferraz, A., Baeza, J., Rodriguez, J. & Freer, J. (2000). Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis. *Bioresource Technology* **74**, 201-212.

Ferris, M. J. & Ward, D. M. (1997). Seasonal Distributions of Dominant 16S rRNA-Defined Populations in a Hot Spring Microbial Mat Examined by Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology* **63**, 1375-1381.

Field, A. (2000). *Discovering Statistics Using SPSS for Windows*. SAGE Publications, London.

Fletcher, P. (1991). The role of research in the development of landfilling techniques - A researcher's perspective. *Landfill Microbiology: Research and Development Workshop II*, pp 105-112. Harwell Publishers, Oxford.

Gelsomino, A., Keijzer-Wolters, A. C., Cacco, G. & van Elsas, J. D. (1999). Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *Journal of Microbiological Methods* **38**, 1-15.

Gilardi, G., Abis, L. & Cass, A. E. G. (1995). Carbon-13 CP/MAS solid-state NMR and FT/IR spectroscopy of wood cell wall biodegradation. *Enzyme and Microbial Technology* **17**, 268-275.

Goodwin, T. W. & Mercer, E. I. (1983). *Introduction to Plant Biochemistry*. Pergamon Press, Oxford.

Gregory, D. (1998). Re-burial of Ship Timbers in the Marine Environment as a Method of *in situ* Preservation. In *Proceedings of the ICOM Group on Wet Organic Archaeological Materials Conference* (eds. C. Bonnot-Diconne, X. Hiiron, Q. Khôi Tran & P. Hoffmann), pp 78-83. ARC-Nucléart CEA, Grenoble, France.

Gregory, D., Mattjiesen, H. & Björdal, C. (2001). *In situ* preservation of artefacts in Nydam Mosc: Studies into environmental monitoring and the deterioration of wooden artefacts. In *Proceedings of the 8th ICOM Group on Wet Organic Archaeological*

Materials Conference (eds. P. Hoffmann, J. M. Spriggs, T. Grant, C. Cook & A. Recht), pp 213-230. Druckerei Ditzen GmbH und Co. KG, Bremerhaven, Germany.

Hale Boothe, D. D., Smith, M. C., Gattie, D. K. & Das, K. C. (2001). Characterization of microbial populations in landfill leachate and bulk samples during aerobic bioreduction. *Advances in Environmental Research* **5**, 285-294.

Halvadakis, C., Robertson, A. & Leckie, I. (1983). *Landfill Methanogenesis.* Technical Report Number 271. Department of Civil Engineering, Stanford University, California, USA.

Ham, R. & Bookter, T. J. (1982). Decomposition of Solid Waste in Test Lysimeters. *ASCE Journal of the Environmental Engineering Division* **108**, 1147-1170.

Hastings, R. (1999). Application of Denaturing Gradient Gel Electrophoresis to Microbial Ecology. In *Methods in Biotechnology, Volume 12: Environmental Monitoring of Bacteria* (ed. C. Edwards), pp 175-186. Humana Press Inc., NJ, USA.

Hatfield, G. R., Maciel, G. E., Erbatur, O. & Erbatur, G. (1987). Qualitative and Quantitative Analysis of Solid Lignin Samples by Carbon-13 Nuclear Magnetic Resonance Spectrometry. *Analytical Chemistry* **59**, 172-179.

Hedges, J. I. (1990). The Chemistry of Archaeological Wood. In *Archaeological Wood: Properties, Chemistry and Preservation* (eds. R. M. Rowell and R. J. Barbour), pp 111-140. American Chemical Society, Washington DC, USA.

Hedges, J. I. & Ertel, J. R. (1982). Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. *Analytical Chemistry* **54**, 174-178.

Helleur, R. J., Budgell, D. R. & Hayes, E. R. (1987). Identification of the composition of oligosaccharides and polysaccharides by pyrolysis/capillary gas chromatography. *Analytica Chimica Acta* **192**, 367-372.

Helms, C. A. & Kilstrup, M. (2001). DNA based Identification of Bacteria Inhabiting Waterlogged Wooden Artefacts from the Nydam Bog. In *Proceedings of the 8th ICOM Group on Wet Organic Archaeological Materials Conference* (eds. J. A. Spriggs, T. Grant, C. Cook & A. Recht), pp 249-258. Druckerei Ditzen GmbH und Co. KG, Bremerhaven, Germany.

Heyer, K., Hupe, K., Heerenklage, J., Ritzkowski, M., Dalheimar, F. & Stegman, R. (1999). Aeration of landfills as an innovative method process enhancement and remediation. In *Proceedings of Sardinia '99 - 7th International Waste Management and Landfill Symposium*, pp 35-47. Environmental Sanitary Engineering Centre (CISA), Cagliari, Italy.

Hill, A. R. (1976). The environmental impacts of agricultural land drainage. *Journal of Environmental Management* **4**, 251-274.

Hobson, P. (1988). *A Basic Study of Landfill Microbiology and Biochemistry.* DTI Report: ETSUB115. Department of Trade and Industry, Northumberland.

Hoffmann, P. (1981). Chemical Wood Analysis as a means of Characterising Archaeological Wood. In *Proceedings of the ICOM Waterlogged Wood Working Group Conference* (eds. D. W. Grattan & J. C. McCawley), pp 73-83. ICOM-CC, Ottawa, Canada.

Hoffmann, P. & Jones, M. (1990). The Structure and Degradation Process for Waterlogged Archaeological Wood. In *Archaeological Wood: Properties, Chemistry and Preservation* (eds. R. M. Rowell & R. J. Barbour), pp 35-65. American Chemical Society, Washington DC, USA.

Hogan, D. V., Simpson, P., Jones, A. M. & Maltby, E. (2001). Development of a protocol for the reburial of organic archaeological remains. In *Proceedings of the 8th ICOM Group on Wet Organic Archaeological Materials Conference* (eds. P. Hoffmann, J. M. Spriggs, T. Grant, C. Cook & A. Recht), pp 187-213. Druckerei Ditzen GmbH und Co. KG, Bremerhaven, Germany.

Howard, A. G. (1998). *Aquatic Environmental Chemistry*. Oxford University Press, New York, USA.

Howitt, D. & Cramer, D. (1997). *A guide to computing statistics with SPSS for Windows*. Prentice Hall/Harvester Wheatsheaf, London.

Iiyama, K., Kasuya, N., Tuyet, L. T. B., Nakano, J. & Sakaguchi, H. (1988). Chemical Characterisation of Ancient and Buried Wood. *Holzforschung* **42**, 5-10.

Jagels, R. (1981). A deterioration evaluation procedure for waterlogged wood. In *Proceedings of the ICOM Waterlogged Wood Working Group Conference* (ed. D. W. Grattan & J. C. McCawley), pp 69-72. ICOM-CC, Ottawa, Canada.

Jeperson, K. (1985). Extended storage of waterlogged wood in nature. In *Waterlogged Wood, Study and Conservation, Proceedings of the ICOM Waterlogged Wood Working Group Conference* (eds. R. Ramiere & M. Colardelle), pp 39-54. Centre D'etude et de Traitement des Bois Gorges D'eau, Grenoble, France.

Johnson, N. F. & Jackson, E. (1999). *Woldgate Former Landfill, Bridlington.* Report Number: 9694. Strata Surveys Limited, Middlewich, Cheshire.

Kaar, W. E., Cool, L. G., Merriman, M. M., & Brink, D. L. (1991). The Complete Analysis of Wood Polysaccharides using HPLC. *Journal of Wood Chemistry and Technology* **11**, 447-463.

Kegley, S. E. & Andrews, J. (1998). *The Chemistry of Water.* University Science Books, California, USA.

Kelly, J., Mackey, M. & Helleur, R. J. (1991). Quantitative analysis of saccharides in wood pulps by quartz-tube pyrolysis-polar phase gas chromatography. *Journal of Analytical and Applied Pyrolysis* **19**, 105-117.

Kenward, H. & Hall, A. (2000). Decay of delicate organic remains in shallow urban deposits: are we at a watershed? *Antiquity* **74**, 519-525.

Kim, Y. S. (1990). Chemical Characteristics of Waterlogged Archaeological Wood. *Holzforschung* **44**, 169-172.

Kuske, C. R., Banton, K. L., Adorada, D. L., Stark, P. C., Hill, K. K. & Jackson, P. J. (1998). Small-Scale DNA Sample Preparation Method for Field PCR Detection of Microbial Cells and Spores in Soil. *Applied and Environmental Microbiology* **64**, 2463-2472.

Lawson, T., Hopkins, D. W., Chudek, J. A., Janaway, R. C. & Bell, M. G. (2000). The Experimental Earthwork at Wareham, Dorset after 33 Years: 3. Interaction of Soil Organisms with Buried Materials. *Journal of Archaeological Science* **27**, 273-285.

Lehmann, J. (1998). *Carbohydrates: Structure and Biology.* Gutmann, Talheim, Germany.

Leikam, K. & Heyer, K. U. (1997). 'In-situ' stabilisation of completed landfills and old sites. In *Proceeding of Sardinia '97 - 6th International Landfill Symposium.* Environmental Sanitary Engineering Centre (CISA), Cagliari, Italy.

Leskovsek, H., Perko, S., Zigon, D. & Faganelli, J. (1994). Analysis of Carbohydrates in Marine Particulates by Gas Chromatography and Tandem Mass Spectrometry. *Analyst* **119**, 1125-1128.

Liesack, W., Weyland, H. & Stacjekbrandt, E. (1991). Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. *Microbial Ecology* **21**, 191-198.

Lindström, E. S. (1998). Bacterioplankton community composition in a boreal forest lake. *FEMS Microbiology Ecology* **27**, 163-174.

Loizidou, M. & Kapetanios, E. G. (1993). Effect of leachate from landfills on underground water quality. *Science of the Total Environment* **128**, 69-81.

Lopes, M. H., Neto, C. P., Barros, A. S., Rutledge, D., Delgadillo, I. & Gil, A. M. (2000). Quantitation of Aliphatic Suberin in *Quercus suber* L. Cork by FTIR Spectroscopy and Solid-State 13C-NMR Spectroscopy. *Biopolymers* **57**, 344-351.

Lorenz, K., Preston, C. M., Raspe, S., Morrison, I. K. & Feger, K. H. (2000). Litter decomposition and humus characteristics in Canadian and German spruce ecosystems: information from tannin analysis and 13C CPMAS NMR. *Soil Biology and Biochemistry* **32**, 779-792.

Lucas, D. L. (1982). On site packing and protection for wet and waterlogged wood. In *Proceedings of the ICOM Waterlogged Wood Working Group Conference* (eds. D. W. Grattan & J. C. McCawley), pp 51-56. ICOM-CC, Ottawa, Canada.

Maciel, G. E., O'Donnell, D. J., Ackerman, J. J. H., Hawkins, B. H. & Bartuska, B. (1980). A 13C NMR Study of Four Lignins in the Solid and Solution States. *Macromolecular Chemistry and Physics* **182**, 2297-2304.

McCarthy, A. J. (1987). Lignocellulose-degrading actinomycetes. *FEMS Microbiology Reviews* **46**, 145-163.

McKinley, V. L. & Vestal, J. R. (1985). Physical and Chemical Correlates of Microbial Activity and Biomass in Composting Municipal Sewage Sludge. *Applied and Environmental Microbiology* **50**, 1395-1403.

Meshitsuka, G. & Nakano, J. (1985). Structural characterisation of compound middle lamella lignin. *Journal of Wood, Chemistry and Technology* **5**, 391-404.

Meshitsuka, G., Lee, Z. Z., Nakano, J. & Eda, S. (1982). Studies on the nature of lignin-carbohydrate bonding. *Journal of Wood, Chemistry and Technology* **2**, 251-267.

Morris, D. V. & Woods, C. E. (1990). *Settlement and engineering considerations in landfill final cover designs*. Geotechniques of waste landfills - theory and practice. ASTM Designation: STP1070. ASTM International, Pennsylvania, USA.

Muzyer, G. (1999). DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology* **2**, 317-322.

Muyzer, G. & Ramsing, N. B. (1995). Molecular methods to study the organization of microbial communities. *Water Science and Technology* **32 (8)**, 1-9.

Muzyer, G., Dewaal, E. C. & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S ribosomal RNA. *Applied and Environmental Microbiology* **59**, 695-700.

Nayling, N. T. (1989). *The archaeological wood survey: a review of the potential and problems of waterlogged structural wood.* Ancient Monuments Laboratory Report: 62/89. English Heritage, London.

Newman, R. H., Hemmingson, J. A. & Suckling, I. D. (1993). Carbon-13 Nuclear Magnetic Resonance Studies of Kraft Pulping. *Holzforschung* **47**, 234-238.

Nilsson, T. (1999). Microbial degradation of wood: an overview with special emphasis on waterlogged wood. In *Proceedings of the 7th ICOM Group on Wet Organic Archaeological Materials Conference* (eds. C. Bonnot-Diconne, X. Hiron, Q. Khoi Tran & P. Hoffmann), pp 65-70. ARC Nucléart, Grenoble, France.

Oliveira, M. A. (2003). *Environmental impacts on epilithic microbial communities in streams of the Peak District and North Yorkshire.* Unpublished PhD Thesis, University of Hull.

Onay, T. & Pohland, P. (1998). *In-situ nitrogen management in controlled bioreactor landfills.* Elsevier Science, London.

Paajanen, L. & Viitanen, H. (1988). Microbiological degradation of wooden piles in building foundations. In *The 19th International Research Group on Wood Preservation.* International Research Group on Wood Preservation, Stockholm, Sweden.

Pan, D-R., Tai, D-S. & Chen, C-L. (1990). Comparative Studies on Chemical Composition of Wood Components in Recent and Ancient Woods of *Bischofia polycarpa*. *Holzforschung* **44 (1)**, 7-16.

Pandey, K. K. & Pitman, A. J. (2003). FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *International Biodeterioration and Biodegradation* **52**, 151-160.

Parfitt, K. (1994). The Dover Bronze Age Boat. *Current Archaeology* **133**, 4-12.

Parker-Pearson, M. & Sydes, R. (1997). The Iron Age enclosures and prehistoric landscape of Sutton Common, South Yorkshire. *Proceedings of the Prehistoric Society* **63**, 221-259.

Patrick, W. H. & Mahapatra, I. C. (1968). Transformation and availability to rice of nitrogen and phosphorous in waterlogged soils. *Advances in Agronomy* **20**, 323-359.

Pavlikova, H., Sykorova, I., Cerny, J., Sebestova, E. & Machovic, V. (1993). Spectroscopic Study of Degraded Woods from the Elbe Valley. *Energy and Fuels* **7**, 351-356.

Pennanen, T., Paavolainen, L. & Hantula, J. (2001). Rapid PCR-based method for the direct analysis of fungal communities in complex environmental samples. *Soil Biology and Biochemistry* **33**, 697-699.

Pepper, I. A. (1997). PCR: Applications for Plant and Soil Microbes. In *Manual of Environmental Microbiology* (ed. C. J. Hurst), pp 437-444. American Society for Microbiology, Washington DC, USA.

Peterson, L. E. (2002). CLUSFAVOR 5.0: hierarchical cluster and principal-component analysis of microarray-based transcriptional profiles. *Genome Biology* **3**, 21-28.

Pettersen, R. C. (1984). The Chemical Composition of Wood. *Advances in Chemistry Series* **207**, 56-126.

Pickup, R. W. (1991). Development of molecular methods for the detection of specific bacteria in the environment. *Journal of General Microbiology* **137**, 1009-1019.

Ponnamperuma, F. N. (1972). The chemistry of submerged soils. *Advances in Agronomy* **24**, 29-96.

Powell, K. L., Pedley, S., Daniel, G. & Corfield, M. (2001). Ultrastructural observations of microbial succession and decay of wood buried at a Bronze Age archaeological site. *International Biodeterioration and Biodegradation* **47**, 165-173.

Pryor, F. (2001). *Archaeology and Environment of the Flag Fen Basin.* English Heritage, London.

Rafferty, B. (1990). *Trackways through time: Archaeological Investigations on Irish Bog Roads, 1985-89.* Headline Publishing Ltd., London.

Raiswell, R. (2001). Defining the Burial Environment. In *Handbook of Archaeological Sciences* (eds. D. R. Brothwell & A. M. Pollard), pp 595-603. John Wiley & Sons Ltd., Chichester.

Rathje, W. & Murphy, C. (1992). *Rubbish: The Archaeology of Garbage*. Harper Collins Publishers, New York, USA.

Read, A. D., Hudgins, M. & Phillips, P. (2001). Aerobic landfill test cells and their implications for sustainable waste disposal. *The Geographical Journal* **167**, 235-247.

Read, A. D. & Hudgins, M. (2000). The aerox landfill concept: test cell results suggest greater sustainability in landfill practices. *Eco-Management and Auditing* **7**, 196-206.

Rondon, M. R., Goodman, R. M. & Handelsman, J. (1999). The Earth's bounty: assessing and accessing soil microbial diversity. *Tibtech* **17**, 403-409.

Saiki, R. K., Gelfand, D. H., Steffel, S., Scharf, S., Higuchi, R., Horn, G. T., Mullis, K. & Erlich, H. (1988). Primer directed amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487-491.

Sait, L., Galic, M., Strugnell, R. A. & Janssen, P. H. (2003). Secretory Antibodies Do Not Affect the Composition of the Bacterial Microbiota in the Terminal Ileum of 10-Week-Old Mice. *Applied and Environmental Microbiology* **69**, 2100-2109.

Sambrook, J., Fritish, E. F. & Maniatas, T. (1989). *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, New York, USA.

San, I. & Onay, T. T. (2001). Impact of various leachate recirculation regimes on municipal solid waste degradation. *Journal of Hazardous Materials* **87**, 259-271.

Santegoeds, C. M., Damgaard, L. R., Hesselink, G., Zopfi, J., Lens, P., Muyzer, G. & Beer, D. (1999). Distribution of sulphate-reducing and methanogenic bacteria in aerobic aggregates determined by microsensor and molecular analyses. *Applied and Environmental Microbiology* **65**, 4618-4629.

Santegoeds, C. M., Muyzer, G. & Beer, D. (1998). Biofilm dynamics studied with microsensors and molecular techniques. *Water Science and Technology* **37**, 125-129.

Santegoeds, C. M., Nold, S. C. & Ward, D. M. (1996). Denaturing Gradient Gel Electrophoresis Used To Monitor the Enrichment Culture of Aerobic Chemoganotrophic Bacteria from a Hot Spring Cyanobacterial Mat. *Applied and Environmental Microbiology* **62**, 3922-3928.

Schultz, T. P. & Burns, D. A. (1990). Rapid secondary analysis of lignocellulose: comparison of near infrared (NIR) and fourier transform infrared (FTIR). *Tappi Journal* **May 1990**, 209-212.

Schultz, T. P., Templeton, M. C. & McGinnis, G. D. (1985). Rapid Determination of Lignocellulose by Diffuse Reflectance Fourier Transform Infrared Spectrometry. *Analytical Chemistry* **57**, 2867-2869.

Schweingruber, F. H. (1978). *Microscopic Wood Anatomy.* Zürcher AG, Germany.

Sheffield, V. C., Cox, D. R., Lerman, L. S. & Myers, R. M. (1989). Attachment of a 40 base pair G + C rich sequence (GC clamp) to genomic DNA fragments by the polymerase chain reaction results in improving detection of single base pair changes. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 232-236.

Siaz-Jimenez, C., Boon, J. J., Hedges, J. I., Hessels, J. K. C. & De Leeuw, J. W. (1987). Chemical Characterisation of Recent and Buried Woods by Analytical Pyrolysis: Comparison of Pyrolysis Data with 13C NMR and Wet Chemical Data. *Journal of Analytical and Applied Pyrolysis* **11**, 437-450.

Silvey, P. & Blackall, L. L. (1995). A study of microbial ecology of MSW. In *Proceedings of Sardinia '95, - 5th International Landfill Symposium* (eds. T. H. Christensen, R. Cossu & R. Stagmann), pp 117-125. Environmental Sanitary Engineering Centre (CISA), Cagliari, Italy.

Singh, A. & Kim, Y. (2000). Micromorphological characteristics of wood biodegradation in wet environments: a review. *International Association of Wood Anatomists* **21 (2)**, 135-155.

Smit, A. (2004). The burial environment. In *A Pilot Study on the Monitoring of the Physical Quality of Three Archaeological Sites at the UNESCO World Heritage Site at Schokland, Province of Flevoland, the Netherlands* (eds. R. M. van Heeringen, G. V. Mauro & A. Smit), pp 47-58. National Service of Archaeological Heritage, Amersfoort, the Netherlands.

Smith, R. (1999). *A Multi-Disciplinary Approach to Oak Wood Degradation in Different Environmental Contexts.* Unpublished MSc Dissertation, University of Hull.

Steeling, C. & Petsom, A. (1987). Structural features of lignins and humic substances as revealed by spin-echo and broadband decoupled C-13 NMR spectroscopy. *Science of the Total Environment* **62**, 165-174.

Steffan, R. J. & Atlas, R. M. (1991). Polymerase Chain Reaction: Applications in Environmental Microbiology. *Annual Reviews in Microbiology* **45**, 137-161.

Stessel, R. I. & Murphy, R. J. (1992). A lysimeter study of the aerobic landfill concept. *Waste Management & Research* **10**, 485-503.

Stumm, W. H. & Morgan, J. (1981). *Aquatic Chemistry.* John Wiley & Sons, New York, USA.

Takatert, N., Sanchez-Perez, J. M. & Tremolières, M. (1999). Spatial and temporal variations of nutrient concentration in the groundwater of a floodplain: effect of hydrology, vegetation and substrate. *Hydrological Processes* **13**, 1511-1526.

Tchobanoglous, G., Theisen, H. & Vigil, S. A. (1993). *Integrated Solid Waste Management Engineering Principles and Management Issues.* McGraw Hill Inc., New York, USA.

Teske, A., Wawer, C., Muyzer, G. & Ramsing, N. B. (1996). Distribution of sulphate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by

most-probable number counts and DGGE of PCR-amplified ribosomal DNA fragments. *Applied and Environmental Microbiology* **62**, 1405-1414.

Tiedje, J. M., Asuming-Brempong, S., Nusslein, K., Marsh, T. L. & Flynn, S. J. (1999). Opening the black box of soil microbial diversity. *Applied Soil Ecology* **13**, 109-122.

Trofymow, J. A. (1998). *The Canadian Intersite Decomposition Experiment (CIDET): Project and Site Establishment Report.* Canadian Forest Service, British Colombia, Canada.

Trofymow, J. A., Preston, C. M. & Prescott, C. E. (1995). Litter Quality and its Potential Effect on Decay Rates of Materials from Canadian Forests. *Water, Air and Soil Pollution* **82**, 215-226.

Tsui, C. K-M., Hyde, K. & Hodgkiss, I. J. (2001). Colonization patterns of wood-inhabiting fungi on baits in Hong Kong rivers, with reference to the effects of organic pollution. *Antonie van Leeuwenhoek* **79**, 33-38.

Tuma, J. J. & Abdel-Hady, M. (1973). *Engineering soil mechanics.* Prentice-Hall, Inc., New Jersey, USA.

Vainio, E. J. & Hantula, J. (2000). Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research* **104**, 927-936.

Veneman, P. L. M. & Pickering, E. W. (1983). Salt bridge for field redox potential measurements. *Communications in Soil Science and Plant Analysis* **14**, 669-677.

Wall, D. & Zeiss, C. (1995). Municipal landfill biodegradation and settlement. *Journal of Environmental Engineering* **121**, 214-223.

Wentz, F. E., Marcy, A. D. & Gray, M. J. (1982). Analysis of Wood Sugars in Pulp and Paper Industry Samples by HPLC. *Journal of Chromatographic Science* **20**, 349-352.

Westlake, K. (1989). Cellulolytic Bacteria in Sanitary Landfill. In *Proceedings of Landfill Microbiology: R & D Workshop* (eds. P. Lawson & Y. R. Alston), pp 20-33. Harwell, Oxfordshire.

Westlake, K., Brooks, B. & Watkiss, P. R. (1992). *Microbial Metabolism in the Brogborough Landfill Gas Enhancement Test Cells*. AEA Environment and Energy, Harwell, Oxfordshire.

Wetzel, R. G. & Likens, G. E. (1991). *Limnological Analysis*. Springer-Verlag, London.

Wilson, M. A., Godfrey, I. M., Hanna, J. V., Robinson, A. Q. & Finnie, K. S. (1993). The degradation of old Indian Ocean shipwrecks. *Organic Geochemistry* **20**, 599-610.

Wise, M. G., McArthur, J. V. & Shimkets, L. J. (1999). Methanotroph Diversity in Landfill Soil: Isolation of Novel Type I and Type II Methanotrophs Whose Presence

Was Suggested by Culture-Independent 16S Ribosomal DNA Analysis. *Applied and Environmental Microbiology* **65**, 4887-4897.

Wright, E. (1990). The Ferriby Boats - Seacraft of the Bronze Age. *Nautical Archaeological Society* **20**, 173-174.

Yen, B. C. & Scanlon, B. (1975). Sanitary landfill settlement rates. *Journal of Geotechnical Engineering* **101 (5)**, 475-487.

Yuen, S., Styles, J., Wang, Q. & McMahon, T. (1999). Findings from a full-scale bioreactor landfill in Australia. In *Proceedings of Sardinia '99 - 7th International Waste Management and Landfill Symposium.* Environmental Sanitary Engineering Centre (CISA), Cagliari, Italy.

Zech, W., Johansson, M-B., Haummaier, L. & Malcolm, R. L. (1986). CPMAS 13C NMR and IR spectra of spruce and pine litter and of the Klason lignin fraction at different stages of decomposition. *Z. Pflanzenernahr Bodenk* **150**, 262-265.

Zeiss, C. A. & Atwater, J. (1989). Waste facility impacts on residential property values. *Journal of Urban Planning and Development* **115 (2)**, 64-80.

Zumstein, E., Moletta, R. & Godon, J-J. (2000). Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. *Environmental Microbiology* **2**, 69-78.

Appendix 1

Sediment redox potentials using a salt bridge

Tables A1-1 to A1-2, A1-3 to A1-4 and A1-5 to A1-6 present the raw sediment redox potentials (measured in mV) collected at 0.08 m, 0.20 m and 0.32 m depths respectively, during the course of the redox monitoring programme in Lysimeter 3. Two sets of redox values are shown for each monitoring date. One set of values was obtained without the use of the salt bridge and the other set of values was taken with the aid of the salt bridge.

The missing data at the start of Tables A1-1, A1-3 and A1-5 correspond to the first year of monitoring, prior to the installation of the salt bridge. The missing data within Table A1-1 corresponds to the shrinkage in the height of the peat. The further insertion of the probes into the peat enabled future readings to be taken.

The data in Tables A1-1 to A1-6 is only used to confirm the accuracy between redox potential readings obtained with/without the aid of the salt bridge and therefore the tables do not display the most extreme values denoted in red (as displayed in Appendix 3). See Section 2.4.3.5 for the methodology used to process the raw redox values.

Table A1-1: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.08 m depth during the first year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
22/10/2001	-386, -410, -355	
24/10/2001	7, 292, 23	
26/10/2001	216, 315, -329	
05/11/2001	508, 120, -255	
21/11/2001	313, 286, 348	
05/12/2001	180, 363, 101	
19/12/2001	521, 335, 123	
02/01/2002	286, 297, 384	
16/01/2002	288, 93, -343	
30/01/2002	323, 475, -363	
13/02/2002	309, 395, -380	
18/02/2002	245, 312, -387	
20/02/2002		
22/02/2002		
13/03/2002	19, 300, 498	39, 258, 410
25/03/2002	-413, 329, -399	-404, 318, -388
27/03/2002	-429, -77, -400	-416, -68, -390
29/03/2002	-429, -13, -399	-418, -14, -396
10/04/2002	-416, 81, -419	-405, 96, -403
24/04/2002	-392, 36, -450	-392, 36, -450
08/05/2002	-394, -184, -438	-385, -170, -443
22/05/2002	-359, 94, -357	-374, 100, -371
19/06/2002	-295, 137, -298	-290, 141, -295
03/07/2002	-378, 49, -464	-359, 101, -448
12/08/2002	-386, -125, -482	-384, -111, -458
15/08/2002	-117, -239, 165	-117, -239, 165
19/08/2002	107, -222, 196	102, -234, 198
02/09/2002	-212, -258, 295	-208, -250, 290
16/09/2002	-88, -337, 395	-90, -333, 392

Table A1-2: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.08 m depth during the second year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
23/09/2002	-429, -400, -411	-426, -397, -408
25/09/2002	-370, 19, -379	-370, 23, -380
30/09/2002	-419, 25, -370	-418, 30, -367
25/10/2002	-320, -276, 353, -401, 191	-319, -277, 354, -399, 190
20/11/2002	-322, -376, 246, -336, -301	-326, -374, 278, -338, -271
04/12/2002	-310, -276, 295, -117, -168	-311, -279, 295, -117, -169
20/12/2002	-216, -268, 323, -87, -200	-214, -268, 315, -87, -198
03/01/2003	-253, -287, -227, -1, -94	-225, -279, -226, -29, -93
20/01/2003	-244, -287, -262, -65, -180	-262, -287, -262, -63, -188
23/01/2003	-364, -33, 318, -25, 161	-352, -25, 325, -26, 164
30/01/2003	-280, -70, 435, 81, 367	-286, -62, 428, 77, 372
14/02/2002	-182, -42, 479, -129, 408	-181, -51, 480, -129, 400
03/03/2003	-412, -401, 116, -17, -50	-412, -401, 115, -16, -50
05/03/2003	-300, -294, 173, -10, -54	-300, -298, 180, -12, -51
10/03/2003	-188, -280, 298, 6, -53	-189, -278, 299, 7, -33
26/03/2003	-190, -280, 318, 12, 21	-192, -282, 315, 11, 21
11/04/2003	-150, -284, 323, 36, 44	-153, -281, 329, 40, 45
23/04/2003	-254, -280, 356, 102, -52	-252, -279, 355, 112, -52
19/05/2003	-314, -274, 320, 111, -146	-281, -275, 292, 115, -120
02/06/2003	-358, -271, 149, 86, -155	-361, -273, 142, 83, -157
18/06/2003	-357, -277, 389, 100, -162	-359, -278, 390, 100, -163
30/06/2003	-332, -324, 374, 191, -60	-335, -326, 377, 195, -57
07/07/2003	-312, -222, 320, 368, 187	-311, -217, 330, 358, 188
09/07/2003	65, -242, 480, 61, 348	68, -239, 478, 64, 352
11/07/2003	-47, -241, 499, -319, 340	-50, -244, 492, -318, 355
30/07/2003	-37, -69, 518, -164, 384	-43, -60, 518, -142, 383
15/08/2003	-52, 15, 543, 178, 397	-50, 18, 543, 172, 400
25/08/2003	-60, 22, 581, 273, 441	-65, 24, 584, 270, 438

Table A1-3: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.20 m depth during the first year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
22/10/2001	-363, -400, -406	
24/10/2001	-371, -356, -392	
26/10/2001	-359, -328, -394	
05/11/2001	-388, -371, -397	
21/11/2001	-399, -384, -448	
05/12/2001	-400, -381, -395	
19/12/2001	-408, -385, -401	
02/01/2002	-404, -388, -405	
16/01/2002	-395, -342, -397	
30/01/2002	-389, -391, -395	
13/02/2002	-397, -394, -404	
18/02/2002	-374, -352, -362	
20/02/2002	65, 158, -97	
22/02/2002	94, 247, -212	
13/03/2002	421, 346, -258	402, 378, -272
25/03/2002	-151, -284, -430	-139, -275, -417
27/03/2002	-142, -313, -433	-128, -303, -421
29/03/2002	-132, -331, -432	-119, -331, -413
10/04/2002	-381, -370, -438	-366, -353, -423
24/04/2002	-388, -356, -436	-381, -349, -429
08/05/2002	-384, -323, -431	-377, -317, -425
22/05/2002	-389, -332, -428	-384, -334, -424
19/06/2002	-338, -319, -370	-335, -317, -367
03/07/2002	-396, -396, -398	-394, -394, -423
12/08/2002	-403, -398, -437	-403, -396, -407
15/08/2002	260, -117, -8	261, -124, -7
19/08/2002	254, -176, -198	259, -173, -197
02/09/2002	254, 115, 131	243, 113, 136
16/09/2002	-178, 128, 143	-177, 130, 145

Table A1-4: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.20 m depth during the second year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
23/09/2002	-389, -338, -66	-386, -335, -65
25/09/2002	-389, -394, -70	-391, -395, -72
30/09/2002	-329, -414, -403	-329, -412, -401
25/10/2002	-402, -423, 209, 144, -396	-401, -423, 205, 143, -392
20/11/2002	-351, -389, 261, -179, -399	-351, -389, 265, -179, -399
04/12/2002	-397, -350, 286, 178, -378	-398, -352, 287, 177, -379
20/12/2002	-297, -357, 120, 162, -205	-297, -358, 121, 163, -209
03/01/2003	-404, -404, 155, 97, -63	-404, -406, 155, 95, -64
20/01/2003	-400, -421, -244, -104, -74	-400, -422, -216, -104, -64
23/01/2003	-400, -404, -241, 82, -89	-400, -404, -249, 82, -92
30/01/2003	-255, -368, -201, 76, -5	-250, -373, -202, 78, -3
14/02/2002	6, -428, 281, 103, -10	2, -398, 289, 73, -11
03/03/2003	-396, -378, 195, 20, -130	-396, -379, 196, 49, -124
05/03/2003	-383, -401, 179, 74, -96	-391, -403, 187, 80, -94
10/03/2003	-419, -407, 206, 133, -109	-419, -407, 206, 133, -109
26/03/2003	-399, -407, 261, 92, -170	-406, -406, 238, 99, -141
11/04/2003	-403, -401, 273, 144, -189	-400, -418, 239, 140, -189
23/04/2003	-401, -408, 163, 87, -99	-401, -408, 157, 88, -102
19/05/2003	-405, -414, 45, 92, -106	-400, -410, 53, 98, -113
02/06/2003	-401, -409, -398, 86, -412	-404, -412, -401, 84, -415
18/06/2003	-408, -414, -403, 93, -423	-409, -415, -403, 93, -424
30/06/2003	-410, -411, -404, 98, -381	-410, -411, -404, 98, -381
07/07/2003	-408, -418, -403, 101, -290	-411, -410, -401, 100, -278
09/07/2003	67, -405, -398, 47, -410	71, -403, -399, 47, -412
11/07/2003	43, -178, -404, 96, -417	39, -181, -406, 93, -420
30/07/2003	-130, -20, -430, 95, -410	-130, -9, -418, 95, -409
15/08/2003	176, 188, -419, 93, -68	171, 191, -423, 95, -65
25/08/2003	230, 225, -408, 118, -20	231, 228, -411, 115, -18

Table A1-5: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.32 m depth during the first year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
22/10/2001	-49, -301, 7	
24/10/2001	147, -318, -440	
26/10/2001	-225, -461, 172	
05/11/2001	197, -460, -385	
21/11/2001	-41, -381, -448	
05/12/2001	-33, -379, -444	
19/12/2001	-27, -251, -446	
02/01/2002	-264, -245, -352	
16/01/2002	-421, -359, -350	
30/01/2002	-419, -349, -351	
13/02/2002	-430, -214, -364	
18/02/2002	-387, -199, -325	
20/02/2002	-435, -290, -364	
22/02/2002	-430, -297, -373	
13/03/2002	-411, -300, -368	-425, -313, -382
25/03/2002	-423, -385, -266	-410, -373, -285
27/03/2002	-423, -276, -363	-423, -276, -363
29/03/2002	-429, -274, -365	-411, -265, -356
10/04/2002	-434, -275, -372	-418, -262, -357
24/04/2002	-415, -371, -400	-411, -364, -392
08/05/2002	-388, -320, -389	-383, -314, -384
22/05/2002	-433, -383, -421	-427, -377, -426
19/06/2002	-377, -333, -368	-373, -330, -365
03/07/2002	-434, -283, -430	-433, -281, -427
12/08/2002	-430, -260, -438	-438, -258, -428
15/08/2002	-437, -224, -427	-430, -219, -421
19/08/2002	-436, -247, -461	-433, -217, -439
02/09/2002	-435, -248, -421	-431, -235, -424
16/09/2002	-435, 199, -435	-434, 197, -432

Table A1-6: Comparison between sediment redox potentials using a salt bridge in Lysimeter 3 at 0.32 m depth during the second year of monitoring.

Date	Sediment redox potential	Redox potential using salt bridge
23/09/2002	-439, -391, -441	-436, -388, -437
25/09/2002	-438, -370, -440	-431, -372, -443
30/09/2002	-437, -282, -437	-435, -283, -435
25/10/2002	-443, -416, -439, 174, -240	-442, -415, -439, 174, -241
20/11/2002	-443, -386, -389, -161, 224	-441, -366, -410, -158, 226
04/12/2002	-449, -426, -447, -117, 206	-449, -427, -446, -117, 205
20/12/2002	-448, -402, -377, -97, 200	-448, -401, -380, -97, 200
03/01/2003	-447, -434, -420, -55, 189	-447, -434, -420, -55, 189
20/01/2003	-438, -432, -396, -93, 174	-448, -434, -421, -97 200
23/01/2003	-449, 431, -435, -71, 174	-449, 431, -435, -71, 173
30/01/2003	-429, -466, -442, -85, 162	-431, -470, -443, -81, 170
14/02/2002	-418, -423, -433, -337, 166	-418, -433, -434, -360, 180
03/03/2003	-444, -434, -445, -110, 163	-444, -434, -445, -110, 160
05/03/2003	-428, -431, -423, -113, 164	-428, -433, -425, -118, 165
10/03/2003	-447, -443, -449, -101, 164	-446, -443, -448, -100, 164
26/03/2003	-445, -430, -445, -105, 158	-445, -430, -445, -105, 158
11/04/2003	-451, -431, -447, -107, 159	-436, -442, -445, -106, 157
23/04/2003	-442, -420, -437, -108, 171	-442, -420, -437, -108, 171
19/05/2003	-441, -446, -442, -100, 171	-457, -430, -450, -111, 163
02/06/2003	-435, -415, -435, -127, 150	-438, -4318 -439, -130, 148
18/06/2003	-439, -414, -440, -104, 108	-440, -415, -444, -104, 107
30/06/2003	-439, -413, -442, -92, 114	-415, -438, -430, -92, 114
07/07/2003	-442, -415, -443, -70, 119	-443, -414, -448, -96, 117
09/07/2003	-440, -414, -442, -75, 121	-441, -414, -442, -69, 120
11/07/2003	-437, -403, -435, -99, 128	-437, -417, -445, -78, 125
30/07/2003	-441, -406, -441, -94, 125	-442, -410, -442, -96, 125
15/08/2003	-442, -410, -351, -88, 131	-446, -412, -353, -83, 132
25/08/2003	-441, -412, -307, -74, 142	-440, -409, -311, -79, 143

Appendix 2

Water/leachate level management data

This appendix contains the heights of the water/leachate levels (measured in metres) in each lysimeter collected during the course of the experiment. The water/leachate levels were confirmed using a manometer.

Tables A2-1 to A2-2, A2-3 to A2-4 and A2-5 to A2-6 present the values obtained from Lysimeters 1-4, 5-8 and 9-12 during the first and second year of monitoring, respectively.

The missing data in the tables corresponds to the duration of the lysimeter study.

Table A2-1: Heights of the water/leachate levels in Lysimeters 1 to 4 during the first year of monitoring.

Date	Lysimeter 1	Lysimeter 2	Lysimeter 3	Lysimeter 4
22/10/2001	0.382	0.378	0.38	0.365
24/10/2001	0.356	0.280	0.347	0.210
26/10/2001	0.355	0.280	0.282	0.187
05/11/2001	0.355	0.280	0.282	0.187
21/11/2001	0.355	0.280	0.282	0.187
05/12/2001	0.355	0.280	0.282	0.187
19/12/2001	0.355	0.280	0.282	0.187
02/01/2002	0.355	0.280	0.282	0.187
16/01/2002	0.355	0.280	0.282	0.187
30/01/2002	0.366	0.280	0.283	0.186
13/02/2002	0.366	0.280	0.283	0.186
18/02/2002	0.356	0.280	0.283	0.186
20/02/2002	0.356	0.146	0.149	0.144
22/02/2002	0.356	0.146	0.147	0.138
13/03/2002	0.356	0.146	0.147	0.138
25/03/2002	0.356	0.334	0.327	0.355
27/03/2002	0.356	0.280	0.282	0.268
29/03/2002	0.356	0.280	0.282	0.236
10/04/2002	0.356	0.280	0.282	0.230
24/04/2002	0.356	0.280	0.281	0.228
08/05/2002	0.356	0.280	0.281	0.225
22/05/2002	0.356	0.280	0.281	0.224
19/06/2002	0.356	0.280	0.280	0.221
03/07/2002	0.356	0.280	0.280	0.198
12/08/2002	0.356	0.254	0.279	0.192
15/08/2002	0.356	0.185	0.174	0.158
19/08/2002	0.356	0.152	0.165	0.118
02/09/2002	0.355	0.152	0.163	0.112
16/09/2002	0.355	0.153	0.163	0.107

Table A2-2: Heights of the water/leachate levels in Lysimeters 1 to 4 during the second year of monitoring.

Date	Lysimeter 1	Lysimeter 2	Lysimeter 3	Lysimeter 4
23/09/2002	0.355		0.315	0.294
25/09/2002	0.355		0.279	0.250
30/09/2002	0.351		0.258	0.223
25/10/2002	0.35		0.258	0.223
20/11/2002	0.35		0.258	0.221
04/12/2002	0.348		0.258	0.221
20/12/2002	0.347		0.143	0.220
03/01/2003	0.344		0.142	0.221
20/01/2003	0.343		0.142	0.219
23/01/2003	0.343		0.142	0.142
30/01/2003	0.343		0.142	0.128
14/02/2002	0.34		0.142	0.128
03/03/2003	0.339		0.348	0.343
05/03/2003	0.339		0.260	0.275
10/03/2003	0.339		0.257	0.264
26/03/2003	0.339		0.252	0.262
11/04/2003	0.339		0.246	0.257
23/04/2003	0.339		0.244	0.253
19/05/2003	0.338		0.238	0.252
02/06/2003	0.336		0.232	0.247
18/06/2003	0.336		0.230	0.241
30/06/2003	0.336		0.227	0.237
07/07/2003	0.334		0.223	0.232
09/07/2003	0.333		0.127	0.229
11/07/2003	0.332		0.123	0.224
30/07/2003	0.327		0.123	0.163
15/08/2003	0.321		0.324	0.103
25/08/2003	0.315		0.324	0.103

Table A2-3: Heights of the water/leachate levels in Lysimeters 5 to 8 during the first year of monitoring.

Date	Lysimeter 5	Lysimeter 6	Lysimeter 7	Lysimeter 8
22/10/2001	0.367	0.356	0.398	0.415
24/10/2001	0.262	0.278	0.275	0.273
26/10/2001	0.265	0.272	0.272	0.270
05/11/2001	0.265	0.272	0.275	0.271
21/11/2001	0.265	0.272	0.277	0.271
05/12/2001	0.265	0.273	0.276	0.270
19/12/2001	0.265	0.272	0.277	0.270
02/01/2002	0.265	0.272	0.278	0.271
16/01/2002	0.265	0.272	0.278	0.272
30/01/2002	0.265	0.272	0.279	0.272
13/02/2002	0.265	0.272	0.280	0.272
18/02/2002	0.265	0.273	0.281	0.272
20/02/2002	0.143	0.153	0.282	0.150
22/02/2002	0.144	0.146	0.148	0.146
13/03/2002	0.143	0.145	0.144	0.146
25/03/2002	0.369	0.317	0.300	0.330
27/03/2002	0.264	0.263	0.300	0.269
29/03/2002	0.254	0.261	0.263	0.264
10/04/2002		0.255	0.265	0.263
24/04/2002		0.248	0.263	0.261
08/05/2002		0.241	0.261	0.261
22/05/2002		0.234	0.257	0.260
19/06/2002		0.229	0.255	0.258
03/07/2002		0.222	0.253	0.258
12/08/2002		0.215	0.252	0.258
15/08/2002		0.208	0.153	0.162
19/08/2002		0.155	0.145	0.157
02/09/2002		0.126	0.145	0.155
16/09/2002		0.124	0.143	0.153

Table A2-4: Heights of the water/leachate levels in Lysimeters 5 to 8 during the second year of monitoring.

Date	Lysimeter 5	Lysimeter 6	Lysimeter 7	Lysimeter 8
23/09/2002			0.262	0.272
25/09/2002			0.262	0.271
30/09/2002			0.263	0.271
25/10/2002			0.260	0.271
20/11/2002			0.257	0.271
04/12/2002			0.253	0.270
20/12/2002			0.251	0.270
03/01/2003			0.248	0.270
20/01/2003			0.245	0.270
23/01/2003			0.150	0.168
30/01/2003			0.138	0.147
14/02/2002			0.138	0.147
03/03/2003				0.273
05/03/2003				0.270
10/03/2003				0.270
26/03/2003				0.263
11/04/2003				0.256
23/04/2003				0.250
19/05/2003				0.242
02/06/2003				0.233
18/06/2003				0.226
30/06/2003				0.220
07/07/2003				0.213
09/07/2003				0.211
11/07/2003				0.210
30/07/2003				0.163
15/08/2003				0.134
25/08/2003				0.111

Table A2-5: Heights of the water/leachate levels in Lysimeters 9 to 12 during the first year of monitoring.

Date	Lysimeter 9	Lysimeter 10	Lysimeter 11	Lysimeter 12
22/10/2001	0.385	0.378	0.372	0.390
24/10/2001	0.385	0.376	0.372	0.387
26/10/2001	0.384	0.375	0.366	0.386
05/11/2001	0.385	0.375	0.367	0.386
21/11/2001	0.384	0.375	0.367	0.386
05/12/2001	0.384	0.375	0.367	0.386
19/12/2001	0.384	0.375	0.368	0.386
02/01/2002	0.384	0.375	0.368	0.386
16/01/2002	0.384	0.374	0.367	0.385
30/01/2002	0.384	0.374	0.368	0.385
13/02/2002	0.384	0.374	0.368	0.385
18/02/2002	0.384	0.374	0.368	0.384
20/02/2002	0.384	0.374	0.368	0.384
22/02/2002	0.384	0.375	0.370	0.383
13/03/2002	0.383	0.375	0.370	0.383
25/03/2002	0.383	0.374	0.370	0.383
27/03/2002	0.383	0.374	0.370	0.406
29/03/2002	0.382	0.373	0.370	0.405
10/04/2002		0.374	0.370	0.401
24/04/2002		0.373	0.370	0.400
08/05/2002		0.373	0.370	0.397
22/05/2002		0.373	0.370	0.394
19/06/2002		0.373	0.370	0.392
03/07/2002		0.373	0.370	0.391
12/08/2002		0.373	0.370	0.387
15/08/2002		0.373	0.370	0.387
19/08/2002		0.373	0.370	0.387
02/09/2002		0.373	0.370	0.386
16/09/2002		0.373	0.370	0.385

Table A2-6: Heights of the water/leachate levels in Lysimeters 9 to 12 during the second year of monitoring.

Date	Lysimeter 9	Lysimeter 10	Lysimeter 11	Lysimeter 12
23/09/2002			0.370	0.380
25/09/2002			0.370	0.380
30/09/2002			0.370	0.380
25/10/2002			0.370	0.380
20/11/2002			0.367	0.380
04/12/2002			0.363	0.380
20/12/2002			0.361	0.380
03/01/2003			0.360	0.380
20/01/2003			0.359	0.380
23/01/2003			0.359	0.380
30/01/2003			0.359	0.380
14/02/2002			0.359	0.379
03/03/2003				0.378
05/03/2003				0.375
10/03/2003				0.374
26/03/2003				0.373
11/04/2003				0.371
23/04/2003				0.371
19/05/2003				0.369
02/06/2003				0.369
18/06/2003				0.368
30/06/2003				0.368
07/07/2003				0.367
09/07/2003				0.364
11/07/2003				0.359
30/07/2003				0.347
15/08/2003				0.339
25/08/2003				0.332

Appendix 3

Sediment redox potentials

Tables A3-1 to A3-12 present the raw sediment redox potentials (measured in mV) collected at 0.08 m, 0.20 m and 0.32 m depths during the course of the redox monitoring programme in the lysimeter study.

Redox equipment was only installed in Lysimeters 1-3 over the duration of the study owing to problems regarding the limited availability of redox probes and the time taken for the development and subsequent use of the salt bridges. However, more redox probes were inserted at each depth within the remaining lysimeters when they became available.

The missing data in the tables either corresponds to the periods where redox probes were not available for data generation or in the case of Lysimeter 3 at 0.08 m depth, where the shrinkage in the height of the peat exposed the tips of the redox probes. The probes were subsequently inserted further into the peat to enable future measurements to be obtained.

The decline in the height of the waste mass in Lysimeters 7 and 8 during the second year of monitoring prevented any further collection of redox readings at 0.08 m depths.

The red readings denote the values that were rejected from subsequent analyses. These values were the most extreme ones (outliers) collected at each depth.

Table A3-1: Sediment redox potentials in Lysimeter 1 during the first year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
22/10/01	-343, -274, -286	-175, -296, -295	-403, -407, -510
24/10/01	-342, -269, -87	-163, -307, -216	-394, -564, 562
26/10/01	-349, -88, -276	-338, -268, -258	-262, -449, 557
05/11/01	-296, -276, -295	-333, -218, 350	-238, -442, 535
21/11/01	-334, 293, -343	-462, 224, -329	-398, -419, 543
05/12/01	-281, -234, -359	-467, -264, -334	-410, -433, 555
19/12/01	-381, -145, -277	-471, -252, -337	-61, -441, 540
02/01/02	-275, -145, -200	-449, -239, -377	-157, -275, -402
16/01/02	-398, -153, -229	-441, -255, -329	-428, -365, -383
30/01/02	-396, -159, -259	-440, -265, -330	-435, -366, -410
13/02/02	-402, -158, -209	-443, -213, -335	206, -377, -295
18/02/02	-370, -115, -225	-365, -171, -284	240, -300, 372
20/02/02	-402, -177, -217	-443, -193, -213	234, -379, -368
22/02/02	-405, -169, -271	-200, -263, -443	-382, -337, 289
13/03/02	-410, -190, -319	-443, -274, -337	280, -387, -300
25/03/02	-413, -201, -307	-441, -245, -319	284, -368, -247
27/03/02	-413, -187, -319	-441, -246, -318	282, -372, -274
29/03/02	-412, -174, -315	-440, -257, -321	238, -366, -272
10/04/02	-417,-215, -342	-444, -231, -336	299, -392, -181
24/04/02	-314, -464, -316	-442, -244, -374	355, -414, -290
08/05/02	-414, -380, -233	-437, -236, -340	-342, -402, 45
22/05/02	-412, -389, -219	-434, -184, -356	-402, -396, -40
19/06/02	-359, -340, -263	-378, 318, -344	-371, -344, 154
03/07/02	-311, -300, -209	-326, -69, -255	-323, -274, 215
12/08/02	-420, -412, -485	-440, 280, -375	-447, 402, -295
15/08/02	-442, -412, -320	-440, -303, -355	-449, -405, -282
19/08/02	-423, -417, -301	-442, -287, -396	-449, -390, 284
02/09/02	-425, -424, -378	-448, 266, 13	-457, -399, 340
16/09/02	-423, -422, -312	-448, -282, -257	-457, -359, 331

Table A3-2: Sediment redox potentials in Lysimeter 1 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02	-424, -423, -308	-447, -377, -408	-457, -394, 358
25/09/02	-424, -424, -310	-448, -328, -377	-460, -394, 348
30/09/02	-423, -419, -308	-445, -299, -351	-458, -369, 336
25/10/02	-423, -205, -175	-395, -205, -400	-443, -191, 315
20/11/02	-426, -205, -242, 34, -347	-209, -192, -329, -414, -400	-393, -229, 299, -448, -40
04/12/02	-423, -458, -187, 177, -282	-430, -254, -320, -461, -403	-449, -282, 256, -449, -418
20/12/02	-438, 218, -197, 182, -280	-377, -271, -310, -454, -398	-456, -285, 317, -455, -383
03/01/03	-420, 196, -175, 246, -386	-444, -373, -323, -26, -350	-435, -386, 306, -471, -428
20/01/03	-439, 216, -179, 272, -414	-439, -308, -332, -444, -364,	-437, -212, 358, -443, -440
23/01/03	-414, 252, -80, 298, -398	-440, -381, -376, -23, -348	-462, -292, 357, -470, -422
30/01/03	-421, 203, -153, 186, -483	-314, -410, -461, -135, -442	-460, -282, -262, -506, -501
14/02/02	-424, 349, -197, 294, -400	-444, -418, 80, 295, -163	-431, -289, 320, -284, -428
03/03/03	-417, 286, -359, 325, -394	-437, -288, -176, 177, -343	-419, -240, 343, -443, -408
05/03/03	-425, 291, -367, 298, -403	-388, -452, -182, 184, -338	-436, -274, 328, -476, -444
10/03/03	-319, 367, -355, 357, -401	-190, -496, -189, 200, -358	-449, -372, 369, -457, -428
26/03/03	-408, 384, -372, 343, -396	-444, -289, -157, 214, -355	-452, 406, 354, -471, -424
11/04/03	-401, 389, -362, 352, -393	-439, -414, -201, 164, -362	-435, -411, 366, -486, -402
23/04/03	-416, 306, -347, 299, -509	-443, -354, -261, -135, -348	-453, -315, 337, -435, -424
19/05/03	-420, 254, -321, 253, -424	-435, -369, -289, -228, -341	-448, -402, 345, -413, -406
02/06/03	-412, 94, -202, -309, -386	-440, -379, -412, -289, -336	-449, -413, 329, -427, -414
18/06/03	-412, 76, -413, -366, -398	-445, -370, -405, -357, -376	-455, -424, 309, -459, -424
30/06/03	-411, -295, 195, -258, -397	-442, -392, -430, -318, -391	-453, -404, 263, -436, -417
07/07/03	-399, -336, 320, -313, -394	-438, -294, -417, -337, -375	-448, -392, 388, -433, -418
09/07/03	-406, -390, 303, -376, -472	-436, -392, -420, -372, -377	-445, -416, 343, -446, -414
11/07/03	-409, -396, 410, -386, -393	-439, -405, -422, -370, -381	-448, -417, 324, -449, -419
30/07/03	-405, -394, 440, -394, -395	-426, -397, -406, -356, -374	-431, -404, 391, -432, -406
15/08/03	-408, -382, 405, -401, -412	-436, -398, -415, -348, -377	-451, -402, 399, -435, -412
25/08/03	-463, -411, 540, -406, -392	-420, -404, -414, -387, -399	-449, -430, -432, -445, -437

Table A3-3: Sediment redox potentials in Lysimeter 2 during the first year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
22/10/01	-230, -392, -374	-340, -348, -138	510, 457, -410
24/10/01	164, 324, 62	-217, -157, -225	330, -267, 345
26/10/01	486, 325, 267	-258, -333, -125	509, -408, 434
05/11/01	389, 477, 577	-328, -270, -402	465, -405, 406
21/11/01	425, 531, 554	-312, -310, -405	40, -85, -410
05/12/01	423, 499, 528	-315, -309, -407	433, 397, -410
19/12/01	482, 532, 530	-328, -269, -413	484, 351, -411
02/01/02	496, 536, 532	-326, -392, -414	107, 347, -404
16/01/02	507, 515, 531	-316, -283, -392	-238, -98, -406
30/01/02	346, 381, 539	-376, -285, -387	-248, -123, -403
13/02/02	525, 519, 529	-381, 301, -407	233, 353, -416
18/02/02	245, 236, 480	-294, -240, -370	210, 335, -340
20/02/02	314, 288, 493	216, 294, -236	188, 369, -374
22/02/02	442, 451, 523	291, 310, -383	214, 347, -412
13/03/02	506, 504, 555	410, 265, -387	349, 347, -400
25/03/02	-211, -177, 85	70, -279, -385	342, 246, -360
27/03/02	-188, -211, -17	-71, -238, -372	354, 446, -346
29/03/02	294, -218, 44	-75, -233, -374	370, 423, -396
10/04/02	300, 166, 510	-108, -254, -388	413, 422, -391
24/04/02	-25, 382, 530	-346, -287, -376	494, 448, -421
08/05/02	-93, 3, 536	-376, -276, -174	456, 423, -422
22/05/02	-211, 76, 567	-393, -284, -246	-104, 50, -495
19/06/02	-244, 403, 570	-357, -208, -333	-148, 52, -362
03/07/02	-229, 473, 655	-305, -293, -379	-92, -59, -426
12/08/02	-385, 397, 611	-373, -235, -403	-356, -243, -441
15/08/02	-380, 390, 20	372, -127, -392	-348, -295, -482
19/08/02	309, 34, 206	425, -257, -401	-370, -325, -441
02/09/02	518, 523, 403	478, 338, -402	-366, -411, -440
16/09/02	496, 510, 219	484, 238, -335	-380, -400, -483

Table A3-4: Sediment redox potentials in Lysimeter 3 during the first year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
22/10/01	-386, -410, -355	-363, -400, -406	-49, -301, 7
24/10/01	7, 292, 23	-371, -356, -392	147, -318, -440
26/10/01	216, 315, -329	-359, -328, -394	-225, -461, 172
05/11/01	508, 120, -255	-388, -371, -397	197, -460, -385
21/11/01	313, 286, 348	-399, -384, -448	-41, -381, -448
05/12/01	180, 363, 101	-400, -381, -395	-33, -379, -444
19/12/01	521, 335, 123	-408, -385, -401	-27, -251, -446
02/01/02	286, 297, 384	-404, -388, -405	-264, -245, -352
16/01/02	288, 93, -343	-395, -342, -397	-421, -359, -350
30/01/02	323, 475, -363	-389, -391, -395	-419, -349, -351
13/02/02	309, 395, -380	-397, -394, -404	-430, -214, -364
18/02/02	245, 312, -387	-374, -352, -362	-387, -199, -325
20/02/02		65, 158, -97	-435, -290, -364
22/02/02		94, 247, -212	-430, -297, -373
13/03/02	19, 300, 498	421, 346, -258	-411, -300, -368
25/03/02	-413, 329, -399	-151, -284, -430	-423, -385, -266
27/03/02	-429, -77, -400	-142, -313, -433	-423, -276, -363
29/03/02	-429, -13, -399	-132, -331, -432	-429, -274, -365
10/04/02	-416, 81, -419	-381, -370, -438	-434, -275, -372
24/04/02	-392, 36, -450	-388, -356, -436	-415, -371, -400
08/05/02	-394, -184, -438	-384, -323, -431	-388, -320, -389
22/05/02	-359, 94, -357	-389, -332, -428	-433, -383, -421
19/06/02	-295, 137, -298	-338, -319, -370	-377, -333, -368
03/07/02	-378, 49, -464	-396, -396, -398	-434, -283, -430
12/08/02	-386, -125, -482	-403, -398, -437	-430, -260, -438
15/08/02	-117, -239, 165	260, -117, -8	-437, -224, -427
19/08/02	107, -222, 196	254, -176, -198	-436, -247, -461
02/09/02	-212, -258, 295	254, 115, 131	-435, -248, -421
16/09/02	-88, -337, 395	-178, 128, 143	-435, 199, -435

Table A3-5: Sediment redox potentials in Lysimeter 3 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02	-429, -400, -411	-389, -338, -66	-439, -391, -441
25/09/02	-370, 19, -379	-389, -394, -70	-438, -370, -440
30/09/02	-419, 25, -370	-329, -414, -403	-437, -282, -437
25/10/02	-320, -276, 353, -401, 191	-402, -423, 209, 144, -396	-443, -416, -439, 174, -240
20/11/02	-322, -376, 246, -336, -301	-351, -389, 261, -179, -399	-443, -386, -389, -161, 224
04/12/02	-310, -276, 295, -117, -168	-397, -350, 286, 178, -378	-449, -426, -447, -117, 206
20/12/02	-216, -268, 323, -87, -200	-297, -357, 120, 162, -205	-448, -402, -377, -97, 200
03/01/03	-253, -287, -227, -1, -94	-404, -404, 155, 97, -63	-447, -434, -420, -55, 189
20/01/03	-244, -287, -262, -65, -180	-400, -421, -244, -104, -74	-438, -432, -396, -93, 174
23/01/03	-364, -33, 318, -25, 161	-400, -404, -241, 82, -89	-449, 431, -435, -71, 174
30/01/03	-280, -70, 435, 81, 367	-255, -368, -201, 76, -5	-429, -466, -442, -85, 162
14/02/02	-182, -42, 479, -129, 408	6, -428, 281, 103, -10	-418, -423, -433, -337, 166
03/03/03	-412, -401, 116, -17, -50	-396, -378, 195, 20, -130	-444, -434, -445, -110, 163
05/03/03	-300, -294, 173, -10, -54	-383, -401, 179, 74, -96	-428, -431, -423, -113, 164
10/03/03	-188, -280, 298, 6, -53	-419, -407, 206, 133, -109	-447, -443, -449, -101, 164
26/03/03	-190, -280, 318, 12, 21	-399, -407, 261, 92, -170	-445, -430, -445, -105, 158
11/04/03	-150, -284, 323, 36, 44	-403, -401, 273, 144, -189	-451, -431, -447, -107, 159
23/04/03	-254, -280, 356, 102, -52	-401, -408, 163, 87, -99	-442, -420, -437, -108, 171
19/05/03	-314, -274, 320, 111, -146	-405, -414, 45, 92, -106	-441, -446, -442, -100, 171
02/06/03	-358, -271, 149, 86, -155	-401, -409, -398, 86, -412	-435, -415, -435, -127, 150
18/06/03	-357, -277, 389, 100, -162	-408, -414, -403, 93, -423	-439, -414, -440, -104, 108
30/06/03	-332, -324, 374, 191, -60	-410, -411, -404, 98, -381	-439, -413, -442, -92, 114
07/07/03	-312, -222, 320, 368, 187	-408, -418, -403, 101, -290	-442, -415, -443, -70, 119
09/07/03	65, -242, 480, 61, 348	67, -405, -398, 47, -410	-440, -414, -442, -75, 121
11/07/03	-47, -241, 499, -319, 340	43, -178, -404, 96, -417	-437, -403, -435, -99, 128
30/07/03	-37, -69, 518, -164, 384	-130, -20, -430, 95, -410	-441, -406, -441, -94, 125
15/08/03	-52, 15, 543, 178, 397	176, 188, -419, 93, -68	-442, -410, -351, -88, 131
25/08/03	-60, 22, 581, 273, 441	230, 225, -408, 118, -20	-441, -412, -307, -74, 142

Table A3-6: Sediment redox potentials in Lysimeter 4 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02			
25/09/02			
30/09/02			
25/10/02	289, 165, 435, 301, 333	-277, -158, -105, -143, -313	-203, -41, -463, -432, -35
20/11/02	342, 211, 425, 259, 301	-228, -164, -99, -160, -299	-167, 17, -462, -418, 37
04/12/02	338, 148, 454, 218, 293	387, -215, -87, -166, -302	-165, 28, -457, -165, 38
20/12/02	317, 216, 429, 230, 283	-269, -237, -88, -166, -271	-133, 22, -457, -398, 34
03/01/03	328, 198, 455, 257, 308	-314, -239, -84, -179, -313	-70, 26, -454, -397, 43
20/01/03	288, 255, 485, 179, 317	-382, -270, -87, -210, -305	-105, 27, -442, -391, 20
23/01/03	275, 198, 444, 178, 308	-263, -214, -81, -161, 307	-66, 31, -443, -393, 43
30/01/203	283, 202, 485, 59, 389	245, 325, 185, -380, 65	-43, 16, -428, -430, 20
14/02/02	265, 189, 447, 40, 360	-339, -319, 291, -112, -267	-47, 38, 204, -131, 36
03/03/03	102, -68, 235, 38, 66	-405, -433, 53, -401, -413	-140, 11, -384, -342, 14
05/03/03	215, -14, 313, 164, 218	-407, -441, 44, -400, -407	-132, 15, -379, -343, 20
10/03/03	353, 55, 432, 294, 295	-408, -449, -6, -399, -414	-108, 17, -373, -340, 23
26/03/03	-11, 40, 409, 301, 270	-428, -450, -29, -406, -420	-73, 5, -357, -344, 18
11/04/03	-50, 63, 432, 437, 282	-419, -445, -35, -410, -417	-88, 12, -378, -354, 25
23/04/03	249, 84, 284, 280, 288	-420, -443, -53, -415, -422	-64, 11, -359, -327, 50
19/05/03	215, 77, 294, 331, 223	-418, -444, 23, -410, -406	-71, 13, -335, -339, 61
02/06/03	228, 66, 324, 386, 74	-430, -441, 88, -409, -419	-53, 11, -360, -331, 81
18/06/03	205, 92, 333, 416, 25	-430, -441, 91, -370, -415	-84, 12, -299, -316, 90
30/06/03	203, 51, 308, 359, -159	-426,-438, 138, -378, -389	-247, 19, -286, -302, 92
07/07/03	227, 100, 341, 316, -159	-400, -432, 127, -234, -395	11, 24, -278, -295, 102
09/07/03	211, 97, 312, 425, -165	-416, -421, 58, -293, -394	27, 4, -278, -381, 106
11/07/03	221, 82, 347, 367, -193	-405, -254, -162, -27, -233	7, 15, -265, -305, 109
30/07/03	202, 93, 315, 437, -205	-170, -172, 147, -12, -206	10, 60, -248, -271, 121
15/08/03	186, 88, 313, 412, -57	-161, -183, 258, 53, -106	83, 45, -251, -234, 112
25/08/03	153, 56, 339, 405, 186	-167, -215, 397, 89, -55	140, 33, -243, -212, 105

Table A3-7: Sediment redox potentials in Lysimeter 7 during the first year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
22/10/01			
24/10/01			
26/10/01			
05/11/01			
21/11/01			
05/12/01			
19/12/01			
02/01/02			
16/01/02			
30/01/02			
13/02/02			
18/02/02			
20/02/02			
22/02/02			
13/03/02	-157, 44, 51	-475, -84, -90	-540, -474, -490
25/03/02	-481, -474, -177	-555, -432, -419	-570, -457, -525
27/03/02	-496, -471, -351	-566, -435, -523	-567, -481, -526
29/03/02	-395, -444, -144	-568, -432, -523	-568, -465, -525
10/04/02	-514, -364, -504	-572, -401, -520	-568, -477, -540
24/04/02	-478, -250, -190	-565, -227, -568	-506, -481, -536
08/05/02	-472, -413, -418	-563, -368, -554	-357, -475, -529
22/05/02	-466, -134, -384	-562, -391, -560	-550, -490, -536
19/06/02	-92, 7, -451	-507, -387, -523	-494, -435, -482
03/07/02	-403, -152, -426	-504, -437, -582	-471, -382, -432
12/08/02	-504, -508, -504	-551, -476, -613	-559, -506, -499
15/08/02	55, 35, 4	-548, 6, -92	-557, -502, -514
19/08/02	41, 108, -8	-529, 13, -182	-549, -493, -546
02/09/02	95, 39, 0	-578, 60, -128	-549, -492, -520
16/09/02	114, 51, 14	-606, 67, -198	-538, -473, -526

Table A3-8: Sediment redox potentials in Lysimeter 7 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02	-519, -422, -491	-575, -481, -465	-544, -471, -524
25/09/02	-524, -519, -527	-583, -472, -587	-547, -490, -528
30/09/02	-521, -516, -509	-588, -472, -583	-509, -504, -524
25/10/02		-491, -480, -497, -597, -461	-439, -502, -518, -452, -173
20/11/02		-491, -479, -510, -441, -464	-474, -490, -517, -464, -494
04/12/02		-494, -482, -506, -494, -442	-473, -493, -513, -466, -533
20/12/02		-497, -481, -506, -467, -465	-469, -477, -510, -472, -555
03/01/03		-496, -503, -511, -443, -460	-443, -472, -508, -468, -602
20/01/03		-492, -492, -507, -483, -472	-503, -452, -510, -471, -605
23/01/03		88, 34, 23, -152, -100	-452, -481, -496, -464, -602
30/01/03		68, 33, 66, -161, -128	-393, -489, -506, -465, -604
14/02/02		88, 68, 57, -163, -140	-469, -507, -448, -441, -645
03/03/03			
05/03/03			
10/03/03			
26/03/03			
11/04/03			
23/04/03			
19/05/03			
02/06/03			
18/06/03			
30/06/03			
07/07/03			
09/07/03			
11/07/03			
30/07/03			
15/08/03			
25/08/03			

Table A3-9: Sediment redox potentials in Lysimeter 8 during the first year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
22/10/01			
24/10/01			
26/10/01			
05/11/01			
21/11/01			
05/12/01			
19/12/01			
02/01/02			
16/01/02			
30/01/02			
13/02/02			
18/02/02			
20/02/02			
22/02/02			
13/03/02	12, 263, 52	-519, -408, -503	-552, -445, -592
25/03/02	-335, -421, -487	-518, -407, -502	-488, -489, -568
27/03/02	-216, -420, -486	-527, -466, -499	-491, -488, -570
29/03/02	-36, -262, -123	-525, -466, -500	-490, -491, -571
10/04/02	-208, -116, -408	-508, -510, -507	-492, -516, -572
24/04/02	64, -95, -448	-539, -508, -532	-502, -546, -572
08/05/02	-80, -339, -445	-545, -507, -530	-488, -542, -572
22/05/02	-83, -166, -162	-545, -506, -525	-511, -543, -574
19/06/02	-169, -27, -168	-497, -444, -447	-454, -412, -524
03/07/02	-438, -162, -102	-483, -459, -399	-429, -457, -470
12/08/02	-507, -491, -502	-550, -510, -490	-530, -542, -577
15/08/02	115, 118, 50	-153, -407, -389	-531, -542, -584
19/08/02	15, 192, 64	-127, -341, -471	-528, -526, -582
02/09/02	23, 220, 52	-260, -471, -382	-523, -554, -581
16/09/02	23, 180, 49	-449, -471, -483	-505, -499, -579

Table A3-10: Sediment redox potentials in Lysimeter 8 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02	-470, -439, -457	-449, -478, -513	-514, -552, -576
25/09/02	-475, -473, -486	-451, -486, -516	-522, -540, -583
30/09/02	-478, -484, -476	-614, -492, -477	-504, -554, -588
25/10/02		-468, -465, -473, -459, -459	-471, -549, -518, -418, -331
20/11/02		-471, -332, -476, -462, -339	-375, -590, -581, -433, -232
04/12/02		-470, -462, -476, -462, -493	-443, -538, -579, -438, -249
20/12/02		-472, -463, -470, -463, -463	-432, -533, -574, -406, -271
03/01/03		-466, -477, -463, -449, -466	-426, -547, -570, -438, -347
20/01/03		-533, -477, -473, -462, -462	-414, -535, -579, -461, -379
23/01/03		-51, 134, 57, -127, -246	-424, -551, -577, -452, -372
30/01/03		-1, 55, 50, -108, -226	-419, -538, -535, -419, -320
14/02/02		-4, 37, -19, -97, -182	-437, -559, -636, -436, -318
03/03/03		-112, -138, -211, -457, -473	-431, -540, -459, -451, -383
05/03/03		-145, -172, -219, -451, -549	-440, -524, -469, -432, -381
10/03/03		-167, -226, -268, -447, -606	-455, -504, -543, -445, -380
26/03/03		-458, -458, -447, -453, -521	-470, -536, -530, -461, -409
11/04/03		-424, -439, -450, -452, -581	-458, -524, -521, -443, -388
23/04/03		-466, -463, -469, -464, -487	-414, -560, -546, -471, -424
19/05/03		-453, -466, -470, -471, -492	-454, -550, 534, -483, -414
02/06/03		-479, -479, -487, -483, -500	-440, -537, -537, -494, -480
18/06/03		-485, -476, -489, -485, -541	-471, -511, -599, -503, -493
30/06/03		-481, -467, -495, -473, -538	-461, -540, -606, -502, -546
07/07/03		-490, -487, -416, -495, -511	-486, -520, -608, -499, -487
09/07/03		36, 202, 67, 46, -535	-477, -469, -592, -501, -472
11/07/03		56, 159, 48, 72, -526	-467, -488, -595, -507, -474
30/07/03		56, 277, 23, 82, -562	-479, -476, -602, -504, -465
15/08/03		43, 304, 55, 96, -501	-481, -512, -578, -511, -476
25/08/03		9, 381, 62, 93, -484	-492, -566, -486, -521, -478

Table A3-11: Sediment redox potentials in Lysimeter 11 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02			
25/09/02			
30/09/02			
25/10/02	-481, -469, -452, -516, -43	-431, -515, -545, -41, -443	-92, -71, -396, -502, -537
20/11/02	-507, -501, -510, -487, -450	-467, -458, -463, -449, -548	-458, -453, -451, -551, -419
04/12/02	-510, -501, -516, -483, -452	-466, -457, -461, -549, -584	-462, -458, -462, -656, -497
20/12/02	-507, -512, -506, -474, -595	-465, -490, -480, -459, -601	-462, -466, -464, -665, -510
03/01/03	-512, -523, -513, -509, -465	-471, -477, -496, -464, -592	-463, -465, -469, -657, -465
20/01/03	-520, -576, -517, -513, -469	-471, -477, -474, -479, -523	-474, -469, -457, -664, -514
23/01/03	-518, -527, -527, -516, -466	-471, -488, -470, -491, -521	-464, -471, -457, -651, -534
30/01/03	-510, -541, -525, -517, -467	-471, -485, -478, -495, -521	-466, -479, -457, -665, -529
14/02/02	-531, -418, -535, -523, -612	-471, -470, -509, -465, -473	-463, -497, -411, -576, -565
03/03/03			
05/03/03			
10/03/03			
26/03/03			
11/04/03			
23/04/03			
19/05/03			
02/06/03			
18/06/03			
30/06/03			
07/07/03			
09/07/03			
11/07/03			
30/07/03			
15/08/03			
25/08/03			

Table A3-12: Sediment redox potentials in Lysimeter 12 during the second year of monitoring.

Date	0.08 m depth	0.20 m depth	0.32 m depth
23/09/02			
25/09/02			
30/09/02			
25/10/02	-489, -376, -481, -138, -43	-378, -102, -572, -295, -37	-489, 245, -135, -138, -477
20/11/02	-81, -284, -378, -360, -54	-460, -172, -550, -59, -86	-461, -89, -62, -59, -506
04/12/02	-79, -396, -357, -372, -74	-496, -289, -565, -62, -74	-458, -164, -58, -372, -483
20/12/02	-164, -387, -381, -283, -30	-407, -240, -570, -123, -127	-390, -346, -98, -58, -482
03/01/03	-333, -497, -448, -327, -402	-458, -232, -450, -82, -107	-415, -382, -407, -67, -461
20/01/03	-415, -464, -515, -293, -407	-283, -260, -299, -98, -86	-358, -388, -471, -64, -427
23/01/03	-430, -423, -424, -89, -415	-330, -243, -296, -88, -85	-355, -389, -375, -61, -458
30/01/03	-428, -415, -445, -200, -423	-291, -234, -278, -90, -89	-348, -388, -397, -72, -447
14/02/02	-462, -481, -466, -449, -433	-511, -376, -418, -74, -79	-475, -366, -469, -77, -484
03/03/03	-367, -477, -458, -376, -441	-319, -445, -352, -72, -78	-321, -391, -476, -99, -478
05/03/03	-421, -473, -468, -436, -449	-412, -437, -389, -77, -84	-351, -388, -476, -104, -480
10/03/03	-441, -475, -468, -457, -448	-451, -431, -417, -67, -96	-443, -398, -476, -102, -482
26/03/03	-466, -474, -458, -479, -489	-436, -518, -479, -68, -110	-464, -410, -480, -103, -486
11/04/03	-453, -476, -459, -483, -475	-439, -471, -483, -72, -109	-444, -403, -482, -102, -481
23/04/03	-460, -476, -460, -181, -488	-448, -545, -322, -69, -107	-426, -417, -480, -476, -450
19/05/03	-472, -471, -469, -476, -483	-508, -521, -484, -92, -111	-454, -422, -480, -237, -479
02/06/03	-486, -497, -491, -484, -493	-533, -501, -521, -103, -105	-470, -438, -503, -198, -481
18/06/03	-488, -509, -503, -500, -516	-575, -498, -526, -175, -195	-489, -449, -522, -379, -467
30/06/03	-496, -504, -507, -482, -506	-579, -482, -529, -418, -384	-503, -442, -537, -381, -442
07/07/03	-505, -164, -511, -491, -517	-570, -504, -525, -444, -432	-491, -452, -522, -395, -479
09/07/03	-511, -513, -515, -493, -512	-581, -503, -534, -467, -437	-469, -447, -525, -428, -487
11/07/03	-517, -517, -514, -502, -515	-576, -504, -497, -482, -452	-496, -479, -528, -464, -492
30/07/03	-510, -481, -510, -500, -509	-572, -488, -471, -457, -430	-467, -474, -529, -485, -474
15/08/03	-514, -494, -508, -505, -513	-581, -495, -482, -464, -446	-491, -479, -526, -477, -483
25/08/03	-483, -479, -450, -499, -342	-555, -482, -453, -454, -476	-479, -486, -551, -495, -473

Appendix 4

Dissolved oxygen data

Tables A4-1 to A4-12 present the dissolved oxygen values (measured in mg/ml) collected during the course of the experiment from Taps 1, 2 and 3 for Lysimeters 1-12, respectively.

The liquid samples from Taps 1 and 2 were obtained prior to a drop in the water/leachate levels. The samples from Tap 3 were taken prior to an increase in the water/leachate levels. For comparative purposes, dissolved oxygen measurements were obtained from the permanently saturated lysimeters at the same time as the measurements taken from the lysimeters which had their water/leachate levels manipulated.

The high cost of the Vacu-Vials prevented the generation of data for all the lysimeters prior to a drop in the water/leachate levels. The missing data in the tables corresponds to these high costs.

Table A4-1: Dissolved oxygen values of water from Taps 1, 2 and 3 in Lysimeter 1 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	11	20.1	20.1
18/02/02	11.8		
13/03/02			
25/03/02	14.3		0.9
12/08/02	7		7.3
16/09/02	9		9
23/09/02			
25/10/02			
04/12/02			
20/01/03	10.3		4.1
03/03/03			
10/03/03	10.7		13
07/07/03	9.7		7.8
25/08/03			9.3

Table A4-2: Dissolved oxygen values of water from Taps 1, 2 and 3 in Lysimeter 2 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	17.5	19.4	20.1
18/02/02	11.4		
13/03/02			20.1
25/03/02	14.8		
12/08/02	13.9	5.7	
16/09/02			2

Table A4-3: Dissolved oxygen values of water from Taps 1, 2 and 3 in Lysimeter 3 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	14.8	14.8	14.8
18/02/02		12.6	
13/03/02			15.8
25/03/02			
12/08/02		12.9	2.9
16/09/02	4.8		
23/09/02	14.3		
25/10/02			
04/12/02	15.8		
20/01/03			
03/03/03	13		12.6
10/03/03			
07/07/03		8.7	8.1
25/08/03			6

Table A4-4: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 4 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	8.4		
18/02/02		18.7	
13/03/02			
25/03/02			20.1
12/08/02			15.8
16/09/02			20.1
23/09/02	20.1		
25/10/02			
04/012/02			
20/01/03	20.1		
03/03/03	10.7		20.1
10/03/03			
07/07/03			20.1
25/08/03			20.1

Table A4-5: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 5 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	13.4		
18/02/02		15.8	20.1
13/03/02			
25/03/02	20.1		19.4

Table A4-6: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 6 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	20.1		
18/02/02		14.3	
13/03/02			
25/03/02			20.1
12/08/02			20.1
16/09/02			20.1

Table A4-7: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 7 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	3.3		
18/02/02		14.8	
13/03/02			
25/03/02	20.1		20.1
12/08/02			20.1
16/09/02			20.1
23/09/02	20.1		
25/10/02			
04/12/02			
20/01/03	20.1		

Table A4-8: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 8 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	10.3		
18/02/02		15.3	
13/03/02			
25/03/02			20.1
12/08/02		15.8	16.9
16/09/02			13.9
23/09/02	20.1		
25/10/02			
04/12/02			
20/01/03			
03/03/03	19.4		20.1
10/03/03			
07/07/03	20.1		
25/08/03			20.1

Table A4-9: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 9 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	20.1		20.1
18/02/02		9	
13/03/02			
25/03/02	20.1		9.3

Table A4-10: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 10 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	18.1		
18/02/02		16.9	
13/03/02			
25/03/02			20.1
12/08/02			20.1
16/09/02			20.1

Table A4-11: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 11 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	20.1		
18/02/02		14.3	
13/03/02			
25/03/02			18.7
12/08/02			20.1
16/09/02			20.1
23/09/02			
25/10/02			20.1
04/12/02			
20/01/03	20.1		

Table A4-12: Dissolved oxygen values of leachate from Taps 1, 2 and 3 in Lysimeter 12 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	20.1		
18/02/02		14.3	
13/03/02			
25/03/02			18.7
12/08/02	10.7		9.7
16/09/02	7		9
23/09/02			
25/10/02	7		9
04/12/02			
20/01/03	13.9		14.3
03/03/03	15.3		16.9
10/03/03			
07/07/03	20.1		
25/08/03			20.1

Appendix 5

Sediment pH values

Tables A5-1 to A5-2 present the pH values collected from the surface of the sediment in Lysimeters 1-3 over the duration of the experiment.

Lysimeters 1 and 3 were monitored over a period of two years, whilst Lysimeter 2 was monitored over a period of one year. The difference in the length of monitoring between the lysimeters corresponds to the missing pH values for Lysimeter 2 in Table A5-2.

Table A5-1: Sediment pH values for Lysimeters 1-3 during the first year of monitoring.

Date	Lysimeter 1	Lysimeter 2	Lysimeter 3
22/10/2001	4.85	4.73	4.86
24/10/2001	4.85	4.78	4.86
26/10/2001	4.83	4.85	4.78
05/11/2001	4.72	4.75	4.79
21/11/2001	4.77	4.69	4.76
05/12/2001	4.68	4.56	4.90
19/12/2001	4.88	4.36	4.75
02/01/2002	4.83	4.51	4.80
16/01/2002	4.80	4.48	4.60
30/01/2002	4.90	4.50	4.51
13/02/2002	4.83	4.13	4.85
18/02/2002	4.75	4.38	4.75
20/02/2002	4.62	3.79	4.01
22/02/2002	4.53	3.78	3.9
13/03/2002	4.69	3.85	3.91
25/03/2002	4.89	3.85	4.34
27/03/2002	4.76	4.75	4.13
29/03/2002	4.82	4.59	4.19
10/04/2002	4.76	4.96	4.65
24/04/2002	4.57	4.89	4.65
08/05/2002	4.65	4.73	4.37
22/05/2002	4.49	4.47	4.49
19/06/2002	4.51	4.53	4.46
03/07/2002	4.53	4.42	4.02
12/08/2002	4.54	4.45	3.98
15/08/2002	4.60	4.46	4.02
19/08/2002	4.54	4.25	3.98
02/09/2002	4.31	3.95	3.84
16/09/2002	4.49	4.21	3.66

Table A5-2: Sediment pH values for Lysimeters 1-3 during the second year of monitoring.

Date	Lysimeter 1	Lysimeter 2	Lysimeter 3
23/09/2002	4.45		3.93
25/09/2002	4.47		3.82
30/09/2002	4.65		3.83
25/10/2002	4.59		3.71
20/11/2002	4.54		3.60
04/12/2002	4.53		3.58
20/12/2002	4.62		3.54
03/01/2003	4.53		3.30
20/01/2003	4.46		3.55
23/01/2003	4.29		3.31
30/01/2003	4.42		3.24
14/02/2002	4.69		3.20
03/03/2003	4.36		4.10
05/03/2003	4.40		4.02
10/03/2003	4.32		3.99
26/03/2003	4.50		3.62
11/04/2003	4.46		3.57
23/04/2003	4.47		3.63
19/05/2003	4.41		3.60
02/06/2003	4.38		3.70
18/06/2003	4.15		3.72
30/06/2003	4.24		3.84
07/07/2003	4.24		3.80
09/07/2003	4.46		3.82
11/07/2003	4.37		3.79
30/07/2003	4.32		3.80
15/08/2003	4.29		3.82
25/08/2003	4.88		3.86

Appendix 6

Water/leachate pH values

Tables A6-1 to A6-12 present the pH values of the water/leachate collected during the course of the experiment from Taps 1, 2 and 3 for Lysimeters 1-12, respectively.

The pH values of the water/leachate were obtained at the same time as the dissolved oxygen values. Liquid samples from Taps 1 and 2 were taken prior to a drop in the water/leachate levels, whilst samples from Tap 3 were obtained prior to an increase in the water/leachate levels. For comparative purposes, liquid pH values were taken from the permanently saturated lysimeters at the same time as those obtained from the lysimeters which had their water/leachate levels manipulated.

The missing data in the tables corresponds to the missing dissolved oxygen values.

Table A6-1: Water pH values from Taps 1, 2 and 3 in Lysimeter 1 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	4.8	4.8	4.8
18/02/02	4.2		
13/03/02			
25/03/02	5.1		6.2
12/08/02	4.2		5.2
16/09/02	4		4.2
23/09/02			
25/10/02			
04/12/02			
20/01/03	4.2		4.1
03/03/03			
10/03/03	4.6		5.2
07/07/03	4.4		5.3
25/08/03			5.3

Table A6-2: Water pH values from Taps 1, 2 and 3 in Lysimeter 2 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	4.2	4.2	4.2
18/02/02	4		
13/03/02			5.6
25/03/02	5		
12/08/02	4.5	5.4	
16/09/02			5.2

Table A6-3: Water pH values from Taps 1, 2 and 3 in Lysimeter 3 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	4.4	4.4	4.4
18/02/02		4	
13/03/02			4.7
25/03/02			
12/08/02		3.6	3.7
16/09/02	3.7		
23/09/02	4.5		
25/10/02			
04/12/02	3.6		
20/01/03			
03/03/03	4.6		5.3
10/03/03			
07/07/03		3.8	3.7
25/08/03			4.1

Table A6-4: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 4 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	7.1		
18/02/02		5.8	
13/03/02			
25/03/02			7.2
12/08/02			7.1
16/09/02			7.1
23/09/02	7.7		
25/10/02			
04/012/02			
20/01/03	7.6		
03/03/03	5.1		6
10/03/03			
07/07/03			7.2
25/08/03			7.5

Table A6-5: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 5 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.6		
18/02/02		4.8	6.5
13/03/02			
25/03/02	7.6		6.2

Table A6-6: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 6 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.6		
18/02/02		7.6	
13/03/02			
25/03/02			6.4
12/08/02			6.3
16/09/02			6.3

Table A6-7: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 7 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.8		
18/02/02		6.1	
13/03/02			
25/03/02	7.2		6.2
12/08/02			6.3
16/09/02			6.3
23/09/02	7.7		
25/10/02			
04/12/02			
20/01/03	7.7		

Table A6-8: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 8 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.5		
18/02/02		6.2	
13/03/02			
25/03/02			6.1
12/08/02		6.3	6.0
16/09/02			5.8
23/09/02	7.7		
25/10/02			
04/12/02			
20/01/03			
03/03/03	7.6		6.6
10/03/03			
07/07/03	7.4		
25/08/03			7.2

Table A6-9: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 9 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.5		6.5
18/02/02		6.2	
13/03/02			
25/03/02	6.3		6.2

Table A6-10: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 10 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.5		
18/02/02		6.5	
13/03/02			
25/03/02			6.4
12/08/02			6.4
16/09/02			6.4

Table A6-11: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 11 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.4		
18/02/02		5.8	
13/03/02			
25/03/02			6.6
12/08/02			7.4
16/09/02			7.4
23/09/02			
25/10/02			7.4
04/12/02			
20/01/03	7.7		

Table A6-12: Leachate pH values from Taps 1, 2 and 3 in Lysimeter 12 during the course of the experiment.

Date	Tap 1	Tap 2	Tap 3
22/10/01	6.4		
18/02/02		5.8	
13/03/02			
25/03/02			6.8
12/08/02	5.7		5.7
16/09/02	5.6		5.6
23/09/02			
25/10/02	5.6		5.6
04/12/02			
20/01/03	6.1		6
03/03/03	6.3		6
10/03/03			
07/07/03	6.8		
25/08/03			7.4

Appendix 7

Settlement drop in waste height

Tables A7-1 and A7-2 present the settlement drop in the height of the waste mass (measured in metres) during the course of the experiment for Lysimeters 5-8 and 9-12, respectively.

The height of the waste was measured one week after the drop in the leachate levels. The missing data in the tables corresponds to the duration of the experiment.

Table A7-1: Settlement drop in waste height for Lysimeters 5-8 during the course of the experiment.

Date	Lysimeter 5	Lysimeter 6	Lysimeter 7	Lysimeter 8
22/10/01	0.420	0.410	0.435	0.435
05/11/01	0.340	0.345	0.340	0.347
22/02/02	0.340	0.302	0.285	0.331
29/03/02	0.295	0.295	0.285	0.294
19/08/02		0.290	0.265	0.268
16/09/02		0.290	0.265	0.265
30/09/02			0.265	0.282
30/01/03			0.244	0.255
10/03/03				0.270
11/07/03				0.265
25/08/03				0.245

Table A7-2: Settlement drop in waste height for Lysimeters 9-12 during the course of the experiment.

Date	Lysimeter 9	Lysimeter 10	Lysimeter 11	Lysimeter 12
22/10/01	0.445	0.405	0.420	0.435
05/11/01	0.395	0.400	0.394	0.418
22/02/02	0.384	0.384	0.368	0.410
29/03/02	0.360	0.379	0.367	0.405
19/08/02		0.375	0.366	0.405
16/09/02		0.375	0.366	0.405
30/09/02			0.366	0.405
30/01/03			0.355	0.387
10/03/03				0.388
11/07/03				0.390
25/08/03				0.392

Appendix 8

Gas chromatographic analysis of oak wood

Table A8-1 presents the proportional weights of individual sugars and the total weights of wood sugars (measured in mg/ml) obtained from the fresh wood samples and the samples taken from Lysimeters 1-6 over the duration of the experiment.

Table A8-2 presents the proportional weights of individual sugars and the total weights of wood sugars (measured in mg/ml) from the wood samples obtained from Lysimeters 6-12 over the duration of the experiment and the samples taken from the wetland archaeological sites used during this study.

Replicate wood samples were taken from Lysimeters 1, 3, 4, 6 and 10 in order to assess the potential variability of preservation within the sediment in each lysimeter.

Table A8-1: Weights of sugars in wood samples obtained from fresh wood and Lysimeters 1-6. L1–L6 = lysimeter; **.08, .20, .32** = depth; **Glu** = glucose; **Xyl** = xylose; **Gal** = galactose; **Man** = mannose; **Ara** = arabinose; **Rib** = ribose; **Rha** = rhamnose.

Sample	Glu	Xyl	Gal	Man	Ara	Rib	Rha	Total
Fresh	1.101	0.612	0.141	0.188	0.054	0.053	0.068	2.217
Fresh	1.111	0.613	0.083	0.161	0.029	0.016	0.026	2.039
Fresh	1.170	0.711	0.148	0.205	0.039	0.044	0.052	2.369
L1 .08	0.168	0.177	0.005	0.014	0.000	0.008	0.003	0.375
L1 .20	0.182	0.181	0.004	0.016	0.000	0.007	0.003	0.393
L1 .32	0.176	0.187	0.006	0.008	0.000	0.010	0.003	0.390
L1 .08	0.179	0.193	0.006	0.008	0.000	0.010	0.004	0.400
L1 .20	0.196	0.208	0.011	0.014	0.009	0.003	0.003	0.444
L1 .32	0.193	0.185	0.008	0.007	0.000	0.010	0.003	0.406
L2 .08	1.167	0.652	0.261	0.111	0.083	0.074	0.099	2.447
L2 .20	1.091	0.649	0.115	0.164	0.028	0.028	0.035	2.110
L2 .32	0.883	0.581	0.157	0.122	0.028	0.007	0.026	1.804
L3 .08	0.207	0.175	0.012	0.006	0.000	0.009	0.004	0.413
L3 .20	0.270	0.257	0.007	0.021	0.010	0.002	0.004	0.571
L3 .32	0.207	0.227	0.023	0.029	0.026	0.020	0.026	0.558
L3 .08	0.172	0.202	0.010	0.019	0.014	0.007	0.010	0.434
L3 .20	0.173	0.198	0.008	0.009	0.000	0.012	0.005	0.405
L3 .32	0.216	0.224	0.006	0.017	0.000	0.010	0.004	0.477
L4 .08	0.182	0.188	0.008	0.014	0.000	0.012	0.004	0.408
L4 .20	0.112	0.108	0.010	0.007	0.000	0.013	0.004	0.254
L4 .32	0.452	0.340	0.114	0.018	0.023	0.005	0.009	0.961
L4 .08	0.158	0.163	0.010	0.011	0.000	0.014	0.005	0.361
L4 .20	0.231	0.216	0.006	0.015	0.000	0.009	0.005	0.482
L4 .32	0.277	0.225	0.007	0.021	0.000	0.011	0.006	0.547
L5 .08	1.474	0.543	0.233	0.032	0.016	0.061	0.025	2.384
L5 .20	1.241	0.554	0.184	0.053	0.022	0.012	0.022	2.088
L5 .32	1.012	0.545	0.155	0.065	0.000	0.027	0.016	1.820
L6 .08	0.998	0.630	0.110	0.160	0.029	0.014	0.013	1.954
L6 .20	1.175	0.622	0.177	0.045	0.026	0.010	0.025	2.080
L6 .32	1.291	0.582	0.159	0.037	0.000	0.033	0.015	2.117

Table A8-2: Weights of sugars in wood samples obtained from Lysimeters 6-12 and the wetland archaeological sites. L6-L12 = lysimeter; **.08, .20, .32** = depth; **RO1 and RO2** = Rolls Farm; **I and O** = inner and outer; **FLAG 1 and 2** = Flag Fen; **NIGT** = Nigtevecht; **Glu** = glucose; **Xyl** = xylose; **Gal** = galactose; **Man** = mannose; **Ara** = arabinose; **Rib** = ribose; **Rha** = rhamnose.

Sample	Glu	Xyl	Gal	Man	Ara	Rib	Rha	Total
L6 .08	0.928	0.525	0.156	0.083	0.018	0.047	0.02	1.777
L6 .20	0.950	0.504	0.149	0.063	0.017	0.025	0.016	1.724
L6 .32	1.418	0.603	0.200	0.036	0.016	0.052	0.028	2.353
L7 .08	0.280	0.220	0.029	0.020	0.021	0.011	0.018	0.599
L7 .20	0.199	0.243	0.015	0.032	0.019	0.013	0.014	0.535
L7 .32	0.238	0.250	0.009	0.025	0.014	0.005	0.008	0.549
L8 .08	0.210	0.154	0.010	0.013	0.000	0.010	0.004	0.401
L8 .20	0.209	0.193	0.005	0.020	0.000	0.009	0.004	0.440
L8 .32	0.146	0.193	0.004	0.017	0.000	0.009	0.004	0.373
L9 .08	1.279	0.611	0.193	0.061	0.015	0.039	0.022	2.220
L9 .20	1.189	0.748	0.140	0.129	0.000	0.038	0.012	2.256
L9 .32	0.951	0.607	0.106	0.121	0.019	0.032	0.012	1.848
L10 .08	1.108	0.695	0.122	0.133	0.020	0.042	0.013	2.133
L10 .20	1.149	0.561	0.164	0.076	0.017	0.032	0.013	2.012
L10 .32	0.988	0.529	0.210	0.027	0.000	0.040	0.036	1.830
L10 .08	1.105	0.670	0.131	0.144	0.009	0.041	0.014	2.114
L10 .20	0.976	0.505	0.124	0.083	0.000	0.030	0.02	1.738
L10 .32	1.357	0.430	0.202	0.038	0.000	0.045	0.023	2.095
L11 .08	0.189	0.196	0.007	0.018	0.012	0.006	0.007	0.435
L11 .20	0.178	0.229	0.008	0.019	0.000	0.012	0.005	0.451
L11 .32	0.298	0.190	0.020	0.008	0.000	0.012	0.008	0.536
L12 .08	0.231	0.260	0.009	0.025	0.013	0.004	0.007	0.549
L12 .20	0.234	0.238	0.007	0.024	0.000	0.011	0.006	0.520
L12 .32	0.241	0.218	0.006	0.023	0.000	0.010	0.004	0.502
RO1 I	0.945	0.596	0.031	0.112	0.015	0.035	0.015	1.749
RO1 O	0.357	0.167	0.031	0.040	0.005	0.065	0.017	0.682
RO2 I	0.902	0.544	0.089	0.100	0.000	0.035	0.013	1.683
RO2 O	0.299	0.132	0.031	0.027	0.007	0.067	0.019	0.582
FLAG 1	0.158	0.058	0.118	0.019	0.008	0.051	0.022	0.434
FLAG 2	0.259	0.103	0.098	0.034	0.000	0.042	0.015	0.551
NIGT	0.147	0.032	0.024	0.000	0.000	0.021	0.014	0.238