The effect of irrigation with wastewaters on the abundance of bio-indicators in established short rotation coppice willow plantations

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Abstract

This study investigated the effect of irrigation with wastewaters on the abundance of earthworms, mites and springtails in established short rotation coppice willow plantations. The study examined two different sites in Northern Ireland over two consecutive irrigation periods in 2012 and 2013. Site one ($8{,}100m^2$) was located at Culmore, Co. Derry and was irrigated with primary treated effluent from a nearby wastewater treatment plant at a rate of $30m^3$/ha/d. Site two ($23{,}700m^2$) was located at Hillsborough, Co. Down and was irrigated at variable rates (18, 34 and 44 m^3/ha/d) with dairy parlour washings from an on-site farm. Earthworms were extracted by a combination of chemical extraction (mustard solution) and hand-sorting. Mites and springtails were extracted using Berlese-Tullgren funnels.

Earthworms proved to be useful bio-indicators to monitor the impact of irrigation with dairy wastewater at site two since their abundance significantly decreased at the highest irrigation rates used at this site (i.e. 34 and 44 m^3/ha/day). The abundance of earthworms was not significantly affected by irrigation with municipal wastewater at site one. A variety of earthworm species were recovered in sites one and two (n=8 and n=11, respectively) but the majority of these were present in low numbers. Acid-tolerant earthworm species occurred in greatest numbers at both sites. The abundance of mites and springtails was not affected by irrigation with wastewater in sites one or two, regardless of application rate.

Previous land-use significantly affected the abundance of earthworms and mites at site one. A greater abundance of earthworms was observed in plots that had been previously planted with grassland prior to SRC willow conversion in 2010, while a greater abundance of mites was observed in plots that had been previously planted with poplar. No interaction factor was evident between previous cropping history and irrigation.

Declaration

I declare that this thesis is entirely my own work, except where otherwise stated and has not been previously submitted to any other institute or university

James Feighan

answer
Agricultural Need for Sustainable Willow Effluent Recycling

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Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Methodology

Chapter 4: Results

Appendix

Appendix A

Appendix B

Appendix C

Appendix D

Appendix E

List of Plates

Chapter 2: Literature Review

Chapter 3: Methodology

Abbreviations and Organisations

Abbreviations

AITC: Allyl Isothiocyanate

D.P.W: Dairy Parlour Washings

MGP: Mixed Genotype Plantation

NREAP: National Renewable Energy Action Plan

SGP: Single Genotype Plantation

SMC: Soil Moisture Content

SRC: Short Rotation Coppice

Organisations

AFBI: Agri-Food and Biosciences Institute

BSI: British Standards Institute

EEA: European Environmental Agency

EC: European Commission

ETCSIA: European Topic Centre for Spatial information and Analysis

EU: European Union

ISO: International Standards Organization

NSEP: National Swedish Environmental Protection Board.

SEAI: Sustainable Energy Authority of Ireland

Chapter 1 Introduction

The European Union Directive on the promotion of the use of energy from renewable energy sources (2009/28/EC) sets a target of 20% of energy to come from renewable sources by 2020 (EEA, 2009). It is important that this target be achieved in a sustainable manner and the European Commission (EC) has provided some guidance to member states in this regard (European Commission, 2010). Energy crops, such as oilseed rape, perennial grasses, sunflowers and short rotation coppice trees, are part of the EU initiative to provide sustainable renewable energy sources (Haughton *et al.* 2009). Short Rotation Coppice (SRC) refers to high yield trees (e.g. poplar and willow) grown under a coppicing regime whereby harvesting occurs every few years rather than when the plant is fully grown (ETC/SIA, 2013). Fig. 1.1 shows that SRC trees (also known as short rotation trees) account for 1% of the total 5.5 m ha land area currently planted with energy crops in the EU with this value varying between EU member states (Fig. 1.2). Larger cropping areas exist in Denmark, Germany, France, Sweden, Poland and the United Kingdom (UK) with a total combined EU energy potential of 440 KtOE/year (Appendix A, Table A1)

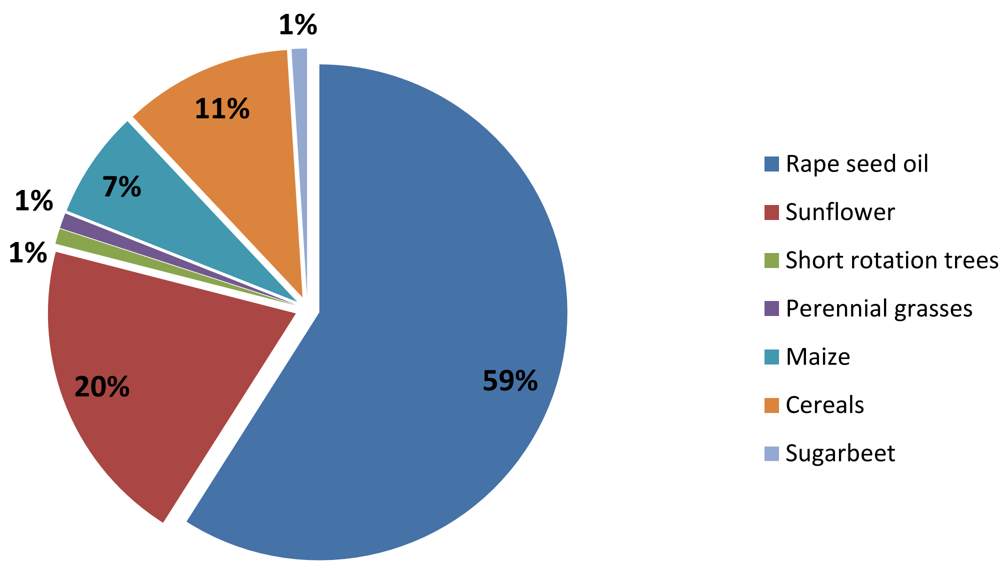

Figure 1.1: Mix of energy crops in EU 2006-2008 (ETC/SIA, 2013)

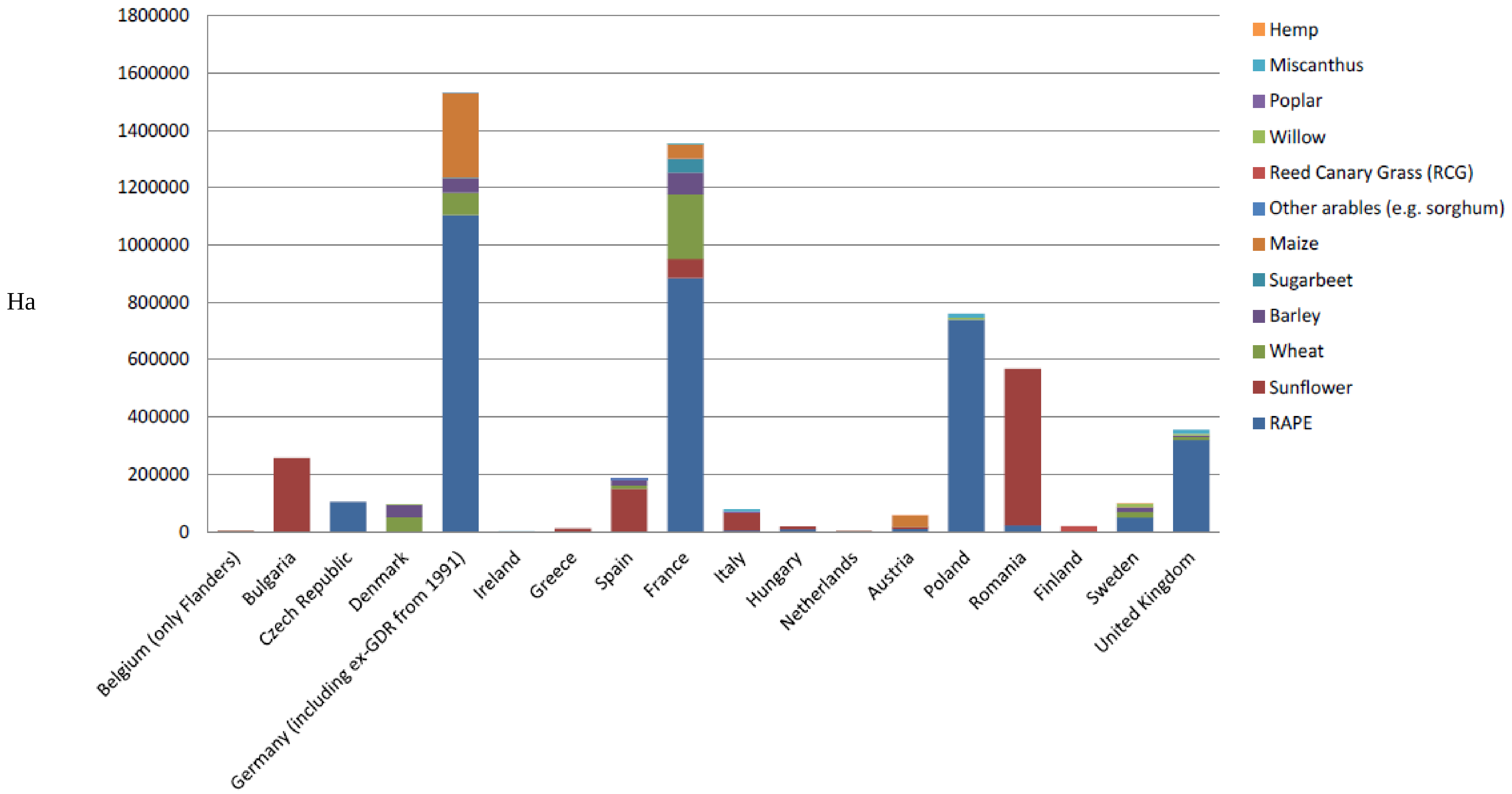

Figure 1.2: Energy crop distribution (ha) in EU countries in 2008 (EEA, 2013 and Panoutsou *et al.* 2011)

In 2007, the European Environment Agency (EEA) undertook a review of the environmental compatibility of EU energy cropping patterns. In this review it was reported that energy cropping patterns were not *'environmentally compatible'* according to the criteria of the study and a more environmentally compatible cropping scenario was developed for the period up to 2020 (EEA, 2007). This scenario includes a much larger share of SRC trees in the total energy crop mix (Fig. 1.3).

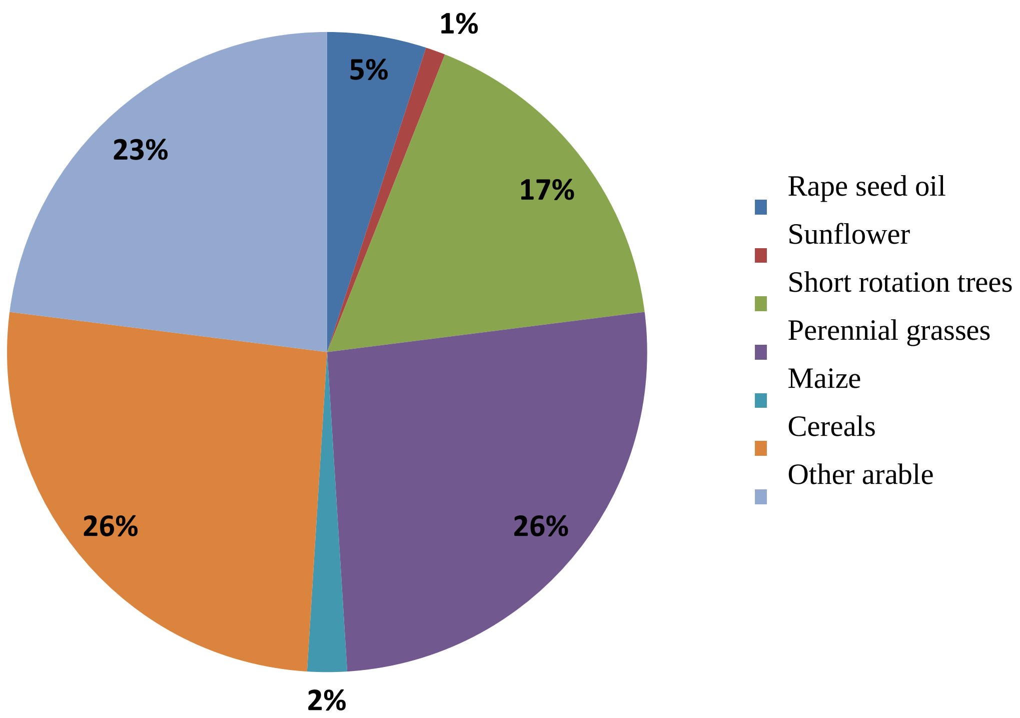

Figure 1.3: The 'environmentally compatible' energy cropping scenario developed by the EEA for 2020 (EEA 2007)

In 2010, individual member states adopted National Renewable Energy Action Plans (NREAPs) to outline how they aimed to reach the target set in the Renewable Energy Directive (2009/28/EC). A review of these plans revealed that a number of member states propose to use woody crops including willow to achieve their 2020 renewable energy targets. The U.K. NREAP states that *'our analysis of perennial energy crops such as short rotation coppice willow, and miscanthus indicates that there is a* *theoretical potential for around 700,000 hectares to be planted by* 2020' (European Commission, 2014). The Irish NREAP states that energy crops will include willow, miscanthus and eucalyptus and that grant support will be given for planting these crops (European Commission, 2014). In addition, the Sustainable Energy Authority of Ireland (SEAI) predicts that by 2020 up to 350,000ha of land could be planted with energy crops including willow, with a further 200,000 ha planted post-2020 (SEAI, 2012). Sweden and Germany also propose to increase the land area under SRC trees. The Swedish Board of Agriculture predict a short-term increase of SRC trees from 14,000 ha to 30,000 ha (Dimitriou *et al*, 2009). In Germany, SRC tree cultivation is expected to increase markedly during the next decade due to a changing subsidy policy and the identification of high cultivation potentials for certain areas (Dimitriou *et al*, 2009).

This investigation focuses specifically on SRC willow due to the projected increase, by the EEA, in its use as an energy crop over the next 10-15 years (Table 1.1). As previously mentioned, the SEAI also predict an increase in the contribution of SRC willow to energy requirements in Ireland in the coming decades (SEAI, 2012). Willow has a number of desirable features as an energy crop including its ease and pace of establishment, rapid growth rate, high biomass production, reliable coppicing ability, tolerance of high planting density, low disease and pest susceptibility and an ability to grow in harsh sites. (Volk *et al*, 2006, AFBI, 2010 and Galbally *et al*, 2013). A further advantage of SRC willow is that it has the ability to remove potentially polluting substances from wastewaters when applied, via an irrigation system, at a rate that satisfies the water and nutrient requirements of the plantation. This is known as bio-remediation. Willow may be particularly suitable for bioremediation owing to its high water and nutrient uptake; during the growing season, 75-95% of nitrogen and phosphorus in wastewater can be removed by willow where the wastewater application rate is between 500 and 1000mm/ha/yr. (Guide *et al.* 2007). The benefits of using willow for bioremediation have been demonstrated in a number of countries, i.e. Sweden, Poland, Denmark, U.K. and Estonia (NSEP, 1993; Perttu, 1993; Kutera and Soroko, 1994; Kowalik and Randerson, 1996; Rosenqvist and Dawson, 2005; EEA, 2007 and AFBI, 2010).

Table 1.1: Percentage contribution of SRC willow to total energy crops in selected EU member states in 2000 together with projected increases for 2020 and 2030 (EEA, 2007)

Year	2000	2020	2030
Czech Republic	5	10	15
Estonia	5	10	10
Finland	0	5	5
France	0	5	5
Germany	5	5	15
Hungary	5	10	15
Latvia	5	5	10
Lithuania	5	5	10
Poland	5	10	10
Slovakia	5	10	15
Slovenia	5	10	15
Sweden	5	10	15
United Kingdom	0	5	10

The EEA have stated that they would like to see a move towards the use of willow as an energy crop and its use for bioremediation purposes as this would help achieve both the targets set in the aforementioned Renewable Energy Directive and also in the Water Framework Directive (2000/60/EC) i.e. to achieve good water quality status in member states by 2015 (EEA, 2007). Under proper land management conditions SRC willow should contribute to the natural capital of the local area and provide important ecosystem services (Rowe *et. al*, 2009; AFBI, 2010 and Bullock and Hawe, 2014). The EEA, however, warns of potential associated environmental pressures and in a publication entitled '*EU Bioenergy Potential from a Resource Efficiency Perspective'* (EEA, 2013) it is stated that the creation of perennial biomass plantations requires careful planning with detailed knowledge of the production system and the local environmental situation including biodiversity.

A number of workers have reported on the compatibility of SRC willow (and other energy crop) plantations with biodiversity (EEA, 2007; Eggers *et al*, 2009; Rowe *et al*, 2009; Fargione, 2010 and Pedroli *et al,* 2013) and with specified soil invertebrates (Sage and Tucker, 1998; Sage, 1998; Minor *et al,* 2004; Baum *et al,* 2009 and Dimitriou *et al,* 2009) although some knowledge gaps still exist, particularly in relation to the full life-cycle of the plantation (Dimitriou *et al,* 2009 and Rowe *et al,* 2009). The compatibility of irrigated SRC willow plantations with soil biodiversity and with specified soil invertebrates has not been investigated despite the use of this practice in a number of countries over a number of decades.

Particular difficulties arise in this type of research insofar as there are a number of other factors, aside from irrigation, that may impact on soil invertebrates in an SRC willow plantation e.g. site preparation techniques and the use of pesticides/fertilisers (Minor *et al.* 2004). In order to eliminate any potential impacts associated with the establishment phase of an SRC willow plantation, two established plantations were used in the study. The sites chosen were dedicated research sites owned and maintained by the Agri-Food and Biosciences Institute in Northern Ireland. The experimental set-up at the sites was such that selected plots within the plantation were irrigated with wastewater (primary treated municipal wastewater at Site 1 and dairy parlour washings at Site 2) while other plots were not irrigated and served as controls.

The main objectives of the project are to;

- identify a number of soil invertebrates for use as bio-indicators which can be used to provide an early warning system of environmental changes arising from irrigation of SRC willow plantations;
- determine whether the abundance of selected bio-indicators differs between irrigated and non-irrigated plots in an established SRC willow plantation in receipt of (a) primary treated municipal wastewater and (b) dairy parlour washings;
- measure the moisture content and pH of the soil in irrigated and non-irrigated plots and determine whether these parameters are affected by irrigation;
- determine whether there is any correlation between soil moisture content/pH and the abundance of selected bio-indicators; and
- Compare the impact on selected bio-indicators due to additional factors within test sites i.e. planting history and genotype of willow.

Chapter 2 Literature Review

2.1 Short Rotation Coppice Willow Plantations

Willow belongs to the family Salicacea, genus *Salix* and comprises approx. 450 species worldwide, distributed mainly in the Northern hemisphere. Willow can be grown by the Short Rotation Coppice (SRC) method which involves planting trees that are cut back (coppiced) every two years. It is ideally suited to this cultivation method because of its toughness and good yields (AFBI, 2010 and Galbally *et al*, 2013). The growth of SRC willow is a process that has a lot more in common with traditional agricultural activities than forestry as it has a regular harvesting routine. Even though willow plantations typically do not require as high value farmland as other crops, there are a few factors that must be considered when planting a site including;

- Planting area and access: a minimum planting area of 5 ha is generally required and this should be in a minimum of 2 ha blocks to facilitate large harvesting machinery (Venendaal *et al.* 1997 and AFBI, 2010);

- Soil depth: a minimum cultivation depth of 20-25cm is required to allow for mechanical plantation (AFBI, 2010 and Ederfelt *et al*, 2013);

- pH: willow typically require a soil pH value between 5 and 7 (AFBI, 2010 and Galbally *et al*, 2013);

- precipitation levels: willow requires a typical annual rainfall of 900-1100mm if the site does not receive water from other sources e.g. irrigation (AFBI, 2010);

- water holding capacity; soils used for willow plantations must have a good moisture holding capacity (Venendaal *et al*, 1997 and AFBI, 2010).

2.1.1 Life Cycle

There are three distinct phases involved in growing an SRC willow plantation and these are site preparation, planting and maintenance and coppicing

Site preparation

Some pre-planting preparation is necessary to ensure the success of the willow crop. Heavy vegetation present must be removed prior to ploughing. This may be achieved by mechanical or chemical means. For economic reasons, chemical treatment is recommended (Baum *et al*, 2009). Glyphosate (a non-selective, systemic broad spectrum herbicide) is commonly used at an application rate of 4.0 to 5.0l/ha with additional use of Chlorpyrifus (3.0l/ha) to control leatherjacket pest population (AFBI, 2010). The site is ploughed 10 days after pesticide application. Depending on the history of the site, the level of ploughing can be reduced to avoid soil compaction which is detrimental to willow establishment but a minimum plough depth of 20-25cm is required to ensure an adequate root structure (AFBI, 2010 and Edelfeldt *et al,* 2013). SRC willow plantations typically have a life-span of 25 years and therefore site preparation will only occur upon initial establishment of the plantation and every 25 years thereafter.

Planting and maintenance

Typically a number of different *Salix* genotypes are planted to help increase growth and reduce risk from disease, with some typical varieties including ‘Tora’, ‘Olaf’ and ‘Beagle’. Planting occurs ideally in early spring as this allows early establishment of the crop. Approximately 18,000 cuttings per ha are planted, which results in a final established crop of around 15,000 per ha. Once planted, willow will quickly develop an extensive root structure that does not usually penetrate the soil too deeply.

Minimal or no fungicides or insecticides are generally applied to SRC willow plantations following establishment although herbicides are needed during the establishment phase due to the weak competitiveness of young willow (Baum *et al.*

2009 and Fry and Slater, 2009). A post-planting application of herbicide may be applied at 1.0-3.5l/ha (AFBI, 2010).

SRC willow plantations rarely require the use of fertilisers during their rotation. Where there is a good nutrient supply from former land use (e.g. arable) nutrient fertilization is not required even during the establishment year (Kahle *et al*, 2007; Fry and Slater 2009 and Baum *et al.* 2009). The bulk of nutrients is allocated to the leaves and therefore remains in the field after leaf fall. Plantations may be fertilized with sewage sludge, municipal wastewater or other wastewaters but this is mainly for the purposes of wastewater management rather than to meet nutrient requirements.

Coppicing

An initial cutback of the coppice is necessary within the first year of growth (to facilitate future growth). Thereafter coppicing occurs every 2-4 years for commercial harvesting, for a period of approximately 25 years (Plate 2.1). Coppicing can be manual or mechanical. Manual coppicing is much slower but less damaging to the site since the use of heavy machinery, which may cause compaction, is avoided (AFBI, 2010).

Plate 2.1: Harvesting of willow crop (AFBI, 2010)

2.1.2 Irrigation of SRC willow plantations

It has been reported that the economic return of willow as an energy crop can be increased if it is irrigated with wastewater effluent (Rosenqvist and Dawson 2005; Sharma and Ashwath, 2006 and Zema *et al*, 2012). This process also cleans or bioremediates the effluent since the willow extracts potentially polluting nutrients. The effluent is added to the soil beneath the willow by an irrigation system at a rate that satisfies the water and nutrient requirements of the plantation but does not cause flooding. It has been calculated that willow coppice can use up to one million litres of water per tonne of dry matter produced annually-an average of 35-45% more water than similar arable areas growing potatoes or cereals (AFBI, 2010). Willow is very suitable for bioremediation purposes due to this high water uptake and also due to a highly complicated, but shallow, root system which allows for an excellent absorption of surface liquids and prevents seepage of applied wastewaters to groundwater. Excessive application, at a rate that cannot be absorbed by the root system, can however result in contamination of waterways (AFBI, 2010). Studies indicate that the evapotranspiration rate of an SRC willow plantation during the growing season is 3.47-6.65 mm/ha/d (AFBI, 2010). In an EEA publication entitled *'Estimating the environmentally compatible bioenergy potential from agriculture-EEA Technical report No 12/2007'* it is stated that specified bioenergy crops such as willow have the ability to successfully treat nutrient-rich wastewaters e.g. municipal wastewaters, since the average nutrient content in municipal wastewaters corresponds closely to the nutrient requirements of growing willow (EEA, 2007).

The benefits of using willow for bioremediation has been demonstrated in a number of countries such as the UK, Sweden, Poland, Denmark and Estonia (Venendaal *et al.* 1997; Rosenqvist and Dawson, 2005; Faaij, 2006; I.E.A, 2007; I.E.A, 2011 and Holm and Heinsoo, 2013). While the EEA would like to see a move away from annual energy crops to perennials and the use of these plantations for bioremediation of wastewaters, the '*EU Bioenergy Potential from a Resource Efficiency Perspective'* report (EEA, 2013) warns of associated potential environmental pressures and states that the creation of perennial biomass plantations requires careful planning with detailed knowledge of production systems and the local environmental situation including biodiversity, water regime and nutrient cycles (EEA, 2013).

Biodiversity is the encompasses of all species, food chains and biological system within an environmental system (Paoletti, 1999b). From an agricultural standpoint, biodiversity comprises the planned biodiversity i.e. crops and livestock and the unplanned biodiversity which is composed of all other biota in the system (Brussaard *et al.* 2007). Although human activities do not always necessarily work against biodiversity, there is a risk that certain land management practices e.g. mono-cropping, tillage, fertilisation and irrigation, can reduce the biodiversity of an area (Paoletti *et al.* 1998 and Brussaard *et al.* 2007). This is especially true of soil biodiversity and preserving soil biota is perhaps the most important and challenging task for sustainability of land use (Hagvar 1998, Bengtsson *et al.* 2000 and de Goede and Brussaard 2002).

2.2 Soil monitoring using bio-indicators

The sustainable management of soils requires soil monitoring, including the use of biological indicators or bio-indicators that can relate land use and management to soil functioning and ecosystem services (Pulleman *et al.* 2012). A bio-indicator can be defined as a species or assemblage of species that is particularly well-matched to specific features of the landscape and/or reacts to impacts and changes within that landscape (Paoletti, 1999b). The use of bio-indicators can be very useful in providing an early warning system of environmental changes and may also be used to diagnose the cause of an environmental problem (Dale and Beyeler, 2001 and Cairns *et al*, 1993).

Edwards *et al.* (1996), Dale and Beyeler (2001) and Pulleman *et al.* (2012) identify several characteristics required of a useful soil bio-indicator in that they must;

1. relate to important ecological functions;
2. be easily measured in a cost and time-efficient manner;
3. have a good spatio-temporal coverage;
4. respond to stress in a predictable and measurable manner; and
5. predict changes that can be averted by management actions.

Faber *et al.* (2013) in an investigation of the use of soil bio-indicators at a European level, state that;

> '*over the past decade, developments in environmental monitoring and risk assessment [has] converged toward the use of indicators and endpoints that are related to soil functioning and ecosystem services.*

These workers recognize that a large number of bio- indicators have been proposed over the years and many have been applied in monitoring schemes across Europe. Common soil bio-indicators include nematodes, enchytraeids, ground beetles, earthworms, springtails and mites (Faber *et al.* 2013) and these are reported to be the most important soil invertebrate groups in temperate regions (Kibblewhite *et al.* 2008 and Gardi *et al.* 2009). The advantages and disadvantages associated with a number of these indicators have been reported upon by many workers as follows; nematodes (Bongers and Ferris, 1999; Ekschmitt *et al.* 2001 and Pulleman *et al.* 2012); enchytraeids (Didden and Römbke, 2001 and Römbke, 2003) and ground beetles (Axelsen and Kristensen, 2000; Allegro and Sciaky, 2003 and Cluzeau *et al*, 2012). The use of earthworms, springtails and mites as bio-indicators shall be further discussed in subsequent sections (2.3, 2.4 and 2.5). This group of organisms has been chosen for further discussion as they represent the broad functional assemblages that act at different spatio-temporal scales in soil and which relate to different ecosystem functions (Bispo *et al*, 2009). In addition, some previous studies have investigated the use of earthworms and mites in SRC willow plantations.

2.3 Earthworms

Earthworms (Annelida: Oligochaetae) are one of the most important organisms found in soil, not only in making up the dominant component of animal biomass, but also due to their important role in decomposition and changing the physical structure of the soil (Edwards and Bohlen, 1996). Earthworms are extremely important ecosystem engineers and are very effective at mixing and aerating the soil whilst burrowing and digesting detritus.

Earthworms are widely reported to meet the criteria required of a bio-indicator. Edwards and Bohlen (1996), Paoletti (1999a) and Cenci and Jones (2009) concur that earthworms possess a number of features that make them ideal bio-indicators including;

- they are globally distributed but have a fairly low species distribution on site;
- their biology and ecology is thoroughly understood;
- their size makes these organisms very easy to observe;
- identification keys are available and identification is comparatively simple;
- standardized guidelines have been developed for their extraction and collection;
- they are in contact with both the solid and aqueous phases of soil;
- most species are not extremely sensitive to low levels of contamination and their response to stress are measurable and reproducible;
- they are active throughout the growing seasons of plants;
- they are long-living in contrast to other possible soil bio-indicators;
- they frequently have low mobility which means that they are representative of the habitat being sampled.

There are several families of earthworms with many being location-specific**.** Within the British Isles, the two most important families are Lumbricidae and Megascolecidae (Edwards and Bohlen 1996). The earthworm populations in crop-growing areas in temperate regions are far more likely to be Lumbricidae than any other family (Edwards and Bohlen, 1996). It is reported that there are approximately 200-220 species of Lumbricidae in Europe (Bouche, 1972 and Sims and Gerard, 1999) with some common species including *Lumbricus terrestris*, *Eiseniella tetraedra* and *Aporrectodea rosea* (Sims and Gerard, 1999).

2.3.1 Identifying Features

Earthworms have the shape of a cylindrical tube (Fig. 2.1) and vary in length from 2-30 cm in the British Isles (Edwards and Bohlen 1996). They are bilaterally symmetrical, externally segmented with a corresponding internal segmentation (Sims and Gerard 1999). The number of segments, which may reach 100-150, vary between species but is fairly constant within a species (Barnes and Ruppert 1994; Edwards and Bohlen, 1996 and Sims and Gerard, 1999).

There a number of distinguishing marks on earthworms that are essential for identification purposes and while these marks are always located along the body of the earthworm, their placement within segments differ from species to species. The most recognizable feature of an earthworm is the glandular swelling commonly referred to as the clitellum, a modification to the epidermis (Fig. 2.2.) Within the clitellum, glandular ridges often develop which are known as *tibercula pubertatis*. These appear in a specimen as it is undergoing puberty. The earthworm can then be classified as an adult although it is not yet capable of producing eggs. The location of the *tibercula pubertatis* seldom varies within species and is a useful tool for identification purposes.

Figure 2.1: Earthworm (Rutgers *et al.* 2008)

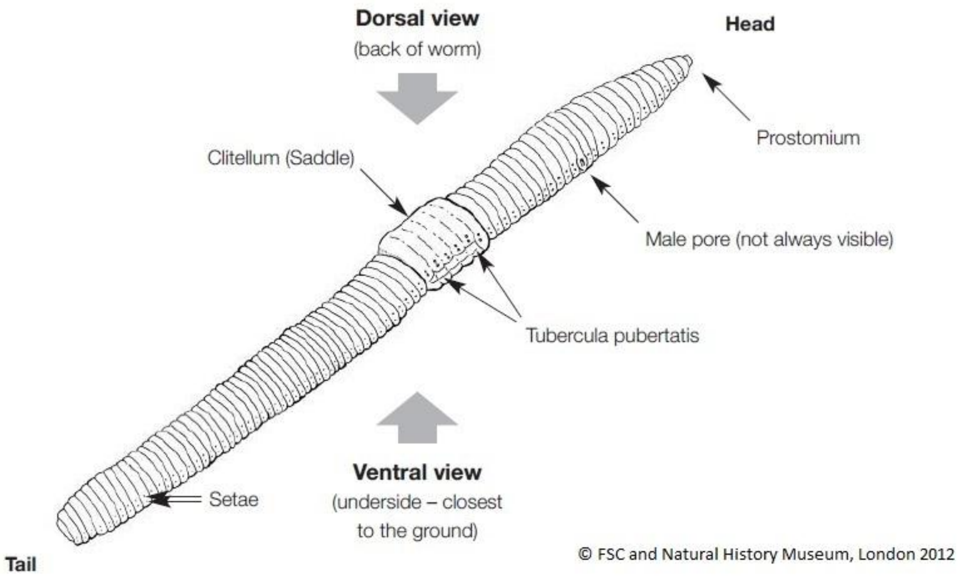

Figure 2.2: Anatomy of an Earthworm (FSC, 2015)

2.3.2 Seasonal Population Trends

Earthworms are continuous or semi-continuous breeders, producing eggs at most times of the year (Edwards and Bohlen, 1996). The preferred location for reproduction differs between species with some reproducing within the soil and others on the soil surface. Reproduction always results in an egg-capsule. The capsule may contain up to 20 eggs but usually only one egg will reach full development. Although eggs may be produced all the year round, egg capsules are usually laid when temperatures begin to fall in the autumn and they will not hatch until temperatures reach an appropriate level, usually early to mid-spring. The lowest abundance of earthworms are observed during the winter and early spring months as the adults hibernate deep within the soil (Sims and Gerard 1999). The process of hibernation is referred to as 'quiescence' and is a period of inactivity of earthworms owing to unfavourable conditions. Following active hatching periods, the proportion of immature earthworms greatly outnumbers adult earthworms. In general, earthworm populations are reported to be most active during late spring and early autumn, as this coincides with favourable temperature and soil moisture conditions, with a decline in activity reported during dry periods in summer

(Curry, 1994). Other studies suggest that the seasonal population structure of earthworm differs considerably depending on species and site conditions, particularly soil temperature and soil moisture. Edwards and Bohlens (1996) cite studies by Rhee (1967) who compared the population structure of five earthworm species and observed that the ratio of immature earthworms to adults differed within each species at any given time during the study. These workers also observed that for all but one of the earthworm species investigated, immature earthworms greatly outnumbered adult earthworms.

2.3.3 Feeding Habits

Earthworms feed on micro-organisms and decaying plant matter in soil, along with the faeces of larger animals. They are often categorised into ecological functional groups or ecotype depending on their feeding preferences and the types of burrows they produce (Bouche, 1972). Figure 2.3 shows the typical location and characteristics of epigeic, endogeic and anecic earthworm species. Epigeic species live and feed only at the surface of the soil and leaf litter while endogeic species live and feed in the mineral soil layers up to a depth of approximately 20 cm. Anecic earthworms produce deep burrows and come to the surface layers to feed, dragging leaves and other organic matter from the surface through the soil horizons to depths of approximately 1 m.

2.3.4 Factors Affecting the Abundance of Earthworm

A number of factors affect the abundance of earthworms in soils including predation, soil temperature, soil moisture content, soil pH, porosity and access to food. Anthropogenic activities such as agriculture and forestry have also been shown to affect earthworm abundance (Gerard, 1967 and Chan and Barchia, 2007). Table 2.1 presents the abundance of earthworms (per m^2 of soil) in selected habitats.

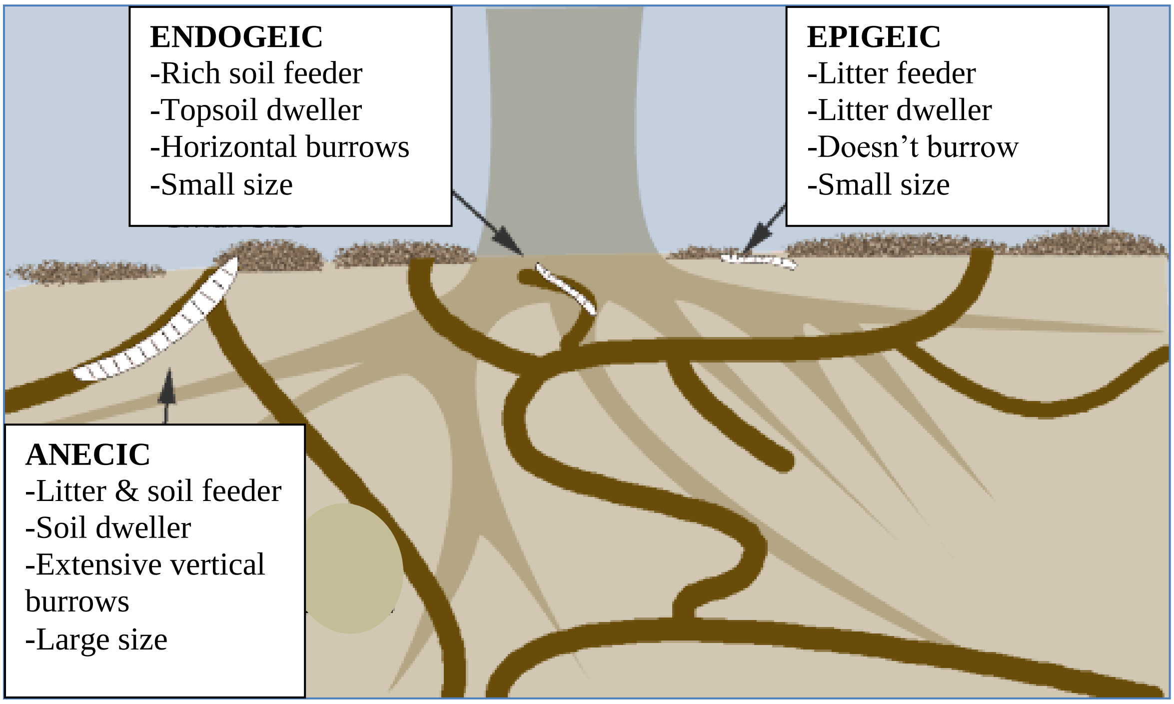

Figure 2.3: Burrowing habits of different ecological functional groups of earthworm (Minnesota, 2011)

Table 2.1: Abundance of earthworms per m^2 of soil in selected habitats

Habitat	**Earthworm abundance (m^2)**	**Reference**
Arable land- minimum cultivation	1160	Curry *et al.* 2002
Arable land -yearly cultivation	320	Curry *et al.* 2002
Arable land- intensive cultivation	25	Curry *et al.* 2002
Tilled organic cropping systems	344	Smith *et al*, 2008
Average household lawn	30	Sims and Gerard 1999
Beech Woodland	165	Phillipson *et al.* 1978
Coniferous forest	160	Smith *et al*, 2008
Old growth deciduous woodland	700	Smith *et al*, 2008
Poplar Woodland (test site)	12-30	Salehi *et al*, 2013
SRC willow receiving sewage sludge	30	Kocik *et al.* 2007

Predation

Earthworms have many predators. The larger of these predators tend to be vertebrates and earthworms are a staple diet of varying importance to moles, badgers, hedgehogs, shrews and foxes. Earthworms are also an important food source for a variety of birds including blackbirds, thrush, robins, gulls and starlings. Some invertebrates also feed on earthworms including ants and several ground beetles (Gerard, 1967 and Edwards and Bohlen, 1996).

In recent years, earthworms have become prey to two invasive species, the Australian flatworm (*Australoplana sanguinea alba*) and the New Zealand flatworm *(Arthurdendyus triangulatus*). These are carnivorous flatworms that have become widespread throughout the British Isles. The New Zealand Flatworm first appeared in Britain in the 1960s and is now widespread throughout Ireland (Murchie *et al.* 2003). The New Zealand flatworm has no natural predators and is well-adjusted to the mild and wet climate of the British Isles. In its introduced range, it is a predator of Lumbricid earthworms with increased numbers evident in areas of high earthworm biomass. New Zealand flatworm densities of as low as 0.8 per m^2 can result in a reduction of 20% of the total earthworm biomass,-the bulk of which is a reduction in anecic species (Murchie and Gordon, 2013). In particular, it is considered that *A. triangulatus* poses a serious risk to *L. terrestris* with attendant implications for soil functioning and indigenous earthworm-feeding wildlife (Haria *et al.* 1998; Christensen and Mather 2001 and Mather and Christensen, 2003).

The Australian Flatworm, *Australoplana sanguinea alba,* was first recorded in England in the late 1970s and has since spread to Ireland, although to a lesser degree than the New Zealand Flatworm. The Australian flatworm is also well suited to the climate of the British Isles and has no natural predators. Although smaller than the New Zealand flatworm, the Australian flatworm, if present, may also result in a significant loss of earthworm abundance (Santoro and Jones 2001, Mather and Christensen, 2003).

Soil pH

Earthworms exist at a number of pH ranges with some species being more acid- tolerant than others. The ideal pH range for some common earthworms is shown in Table 2.2. A recent study by Natural England (2014) showed that a number of earthworm species exist and thrive in acidic soil pHs including *L. rubellus, E. tetraedra and A. Limicola*. It is reported that *L. terrestris* prefers a more neutral pH but will also survive at somewhat lower pHs (Laverack, 1961 and Muys and Granval, 1997).

Table 2.2: Ideal pH range for various earthworm species

Species Name	Ecotype	Preferred pH range
Apporrectodea limicola	endogenic	3.7-7.0
Apporrectodea rosea	endogenic	4.9-9.8
Eisenia fetida	epigeic	4.3-7.5
Eiseniella tetraedra	epigeic	4.6-8.5
Lumbricus castaneus	epigeic	3.9-8.4
Lumbricus eisenia	epigeic	3.6-7.6
Lumbricus festivus	epigeic	4.5-8.2
Lumbricus rubellus	epigeic	3.5-8.4
Lumbricus terrestris	anecic	6.2-10
Octolasion tyrtaeum	endogenic	4.3-8.1
Satchellius mammalis	epigeic	4.3-8.2

Soil pH may affect earthworms indirectly owing to its influence on metal solubility and uptake which may have toxic effects for many earthworm species (Yong and Phadungchewit 1993; van Vliet *et al.* 2005 and Leveque *et al.* 2013).

Soil temperature

Temperature is important to earthworms as it dictates reproduction and respiration rates and also whether quiescence occurs. The optimum temperature and range of tolerances differs from species to species but a soil temperature of 10.6^{o}C is considered low for respiration to occur, with a temperature of 15^{o}C considered optimum for many temperate species such as *L. rubellus* and *L. terrestris* (Edwards and Bohlen, 1996; Uvarov and Scheu, 2004 and Uvarov *et al.* 2011). Earthworms will continue respiration, though at a much reduced rate down to 5^{o}C (Crockett *et al*, 2001 and Khan *et al*, 2012) but below 5^{o}C they will undergo quiescence and retreat to lower soil layers. Soil temperatures above 18^{o}C can be detrimental to earthworms e.g. *L. terrestris* slows down their heart rate at this temperature and may suffer lethal hyperosmotic stress once soil temperatures reach upwards of 23^{o}C. Temperatures impact on reproduction rates in a similar way to respiration rates with higher reproduction rates at temperatures of optimum respiration for earthworms and reproduction ceasing once the minimum threshold for respiration is reached (Sims and Gerard, 1999 and Svendsen *et al.* 2007).

Physical Disturbance

Physical disturbance of soil may have a significant effect on earthworms for a number of reasons but particularly due to the possible effects on soil porosity. The porosity of soil refers to the fraction of void space within a soil that is occupied by air or water. Earthworms require a loose, porous soil in which to burrow and maintain adequate moisture levels (Jégou *et al,* 2002; Chan and Barchia, 2007 and Ernst *et al,* 2009). The porosity of soil is reduced by several anthropogenic activities including any activity that involves the use of heavy machinery (e.g. for farming and forestry) resulting in soil compaction and increased bulk density. A loss of earthworm abundance and their burrowing action has a knock-on effect of further reducing soil porosity (Whalley *et al.* 1995; Chan and Barchia, 2007; Capowiez *et al*, 2009 and Bottinelli *et al.* 2014). Earthworms may also be crushed by heavy machinery (Jégou *et al.* 2002, Chan and Barchia 2007). Tillage practices have, in general, a negative effect on earthworm abundance as it interferes with all deep-burrowing and litter-dwelling worms including Lumbricidae with reports that larger species of worm may disappear completely following tillage (Paoletti, 1999a). These effects are reduced when tillage practices are limited to the top 15 cm of soil (Stinner and House 1990). Earthworms are shown to exhibit a very slow recovery from compaction with a minimum of four years required to show any form of recovery (Bottinelli *et al*, 2014).

Soil Moisture

Soil moisture is essential for gas exchange in earthworms and to regulate respiration. Soil moisture has also been shown to influence the deposition and hatching of egg capsules; if the soil is very moist, earthworms will deposit their capsules near the surface, placing them much deeper in the soil when it is dry (Edwards and Bohlen, 1996 and Ernst *et al*, 2009). Dry soil conditions will also delay hatching of eggs. Drought can be highly damaging to earthworms either forcing anhydrobiosis or causing death (Edwards and Bohlen, 1996). Earthworms will attempt to leave soils of lower soil moisture content than they can withstand, but this limit is highly variable depending on species, location and soil type (Doube and Styan, 1996). Many species found in the British Isles have a preference for extremes of dry or wet soil with very little preference found in the mid-range moisture contents (Doube and Styan, 1996; Edwards and Bohlen 1996; Berry and Jordan, 2001) e.g. *E. fetida* displays optimum growth at moisture levels between 9-16% (Loehr *et al.* 1985; Edwards and Bohlen, 1996 and Berry and Jordan, 2001); *A. rosea* is active is soils with a moisture content of 10%. *L. terrestris* has optimum growth at approximately 30% moisture content while *E. andrei and E. tetraedra* favour very high moisture contents of 85% or above (Natural England, 2014).

It is important to note that the presence of earthworms, specifically anecic earthworms such as *L. terrestris*, help regulate the moisture content in soil due to the larger and vertical macropores that they create. Epigeic worms increase water infiltration and reduce soil pooling through their action on soil litter (Kocik *et al.* 2007, Ernst *et al.* 2009).

<u>*Application of pesticides*</u>

Pesticides are not traditionally formulated to target earthworms but they can have quite an adverse effect on their populations due to the mixture of formulations and residues that work their way into the soil post-application (Edwards and Bohlen 1992, Zhou *et al.* 2013 and Schnug *et al.* 2014a).

The herbicide glyphosate which is commonly used to kill grasses, woody plants and perennials (e.g. in SRC willow plantations) has been reported by some workers to have varying toxicities to earthworms. In general, negative effects on feeding behaviour, DNA and reproduction have been demonstrated causing a range of symptoms from delayed development to increased death (Springett and Gray 1992; Paoletti 1999a,; Verrell and Van Buskirk, 2004; Casabé *et al.* 2007, Solomon *et al.* 2007, Yasmin and D'Souza 2007; Correia and Moreira 2010; Piola *et al*, 2013 and Zhou *et al.* 2013).

Earthworms are affected by specific types of insecticides with carbamate and organophosphates reported to exert highly toxic effects (Edwards and Bohlen 1992). Chlorpyrifos, an organophosphate insecticide used to control crop pests (e.g. leatherjacket pests in willow plantations) has been shown to have varying effects on earthworms impacting on growth, reproduction and cholinesterase activity. The toxicity of chlorpyrifos to earthworms increases with increased concentration of the chemical and many investigations have focussed on exposure to higher concentrations as compared to typical doses applied in the field which is generally in the order of 4 mg/kg (approx. 4 litres of 40% chlorpyrifos/ha). Booth and O'Halloran (2001) report that exposure to chlorpyrifos affects maturation rates and fecundity of earthworms at concentrations of 28mg/kg and observed a significant decrease in cocoon production and viability of *A. caliginosa* at this concentration. Furthermore, these workers found that earthworms exposed as juveniles appeared to be more sensitive than those exposed as adults (Booth and O'Halloran, 2001). Zhou (2007) reported adverse effects on growth and fecundity in earthworms exposed to 5 mg/kg chlorpyrifos after eight weeks and observed that earthworms avoided soil containing concentrations of 40 mg/kg chlorpyrifos. From this study, it appeared that earthworms were not able to escape from pesticide-contaminated soil and hence were exposed continuously to elevated concentrations of pesticides. There appears to be a species-related variation in chlorpyrifos toxicity to earthworms, with *L. rubellus* being the most sensitive of six earthworms species investigated by Ma and Bodt (1993) including *A. calignosa, A. longa, L. rubellus, L.terrestris, E.fetida and E.veneta*. The recovery of earthworms to chloropyrifos exposure is an important aspect to consider with some workers reporting that the insecticide is rapidly eliminated following a cessation of exposure however a recovery of cholinesterase activity is much slower (Aamodt *et al.* 2007; Collange *et al.* 2010; Zhou *et al.* 2013 and Schnug *et al.* 2014a)

Application of organic fertilisers

Earthworms generally respond favourably to organic-based fertilisers such as manures, slurries and wastewaters. This is however, dependent on the levels and type of fertilizer applied, for example there is evidence to suggest that high application rates of pig and cattle slurry can be toxic to earthworm populations (Cotton and Curry 1979 and Paoletti 1999a).

The recovery of soils from applied organic wastes is greatly enhanced by the presence of earthworms. When soil containing these wastes are ingested and passed through the body of an earthworm, the nutrients, nitrogen, potassium, phosphorous and calcium are more readily processed by plants and micro-organisms (Devliegher and Verstraete, 1997 and Kocik *et al.* 2007). This action also results in a favourable change in the carbon to nitrogen (C:N) ratio, pH and moisture content levels of the soil (Atiyeh *et al*, 2000).

Unfavourable earthworm-induced changes arising from the use of organic fertiliser include an increase in the bioavailability of heavy metals to plants with some species of earthworm such as *E.fetida* shown to significantly increase bioavailability (Rodríguez *et al*, 2006 and Udovic and Lestan, 2007). Earthworms can bio-accumulate high concentrations of heavy metals and this may be passed along the food chain resulting in earthworm predators ingesting toxic levels of heavy metals (Spurgeon and Hopkin, 1996 and Bradham *et al.* 2006).

2.3.5 Earthworm Sampling Methods

There are four main methods used to expel earthworms from soil; these are passive hand-sorting, chemical expulsion, heat extraction and electrical extraction. None of these methods are capable of recovering all the earthworms present in the soil and as such, a combination of methods is typically used (B.S.I., 2011).

<u>Passive Hand-Sorting</u>

Hand sorting is the simplest sampling method for earthworms and requires that a specified area of soil is dug up and sorted to extract the various earthworm specimens present. The method is very time-consuming and can be highly labour-intensive depending on the structure of the soil. Hand-sorting is also a physically destructive method, which means that the method in itself is not acceptable to be carried out in places that suffer from long-term integrity issues. Passive hand-sorting may even be technically impossible due to the presence of large numbers of stones or a dense root network. The method is quite effective in the recovery of juvenile worms from soil but has a lesser recovery rate for adults (B.S.I., 2011 and Bartlett *et al.* 2010). The method is not appropriate for the recovery of large anecic earthworms as this group typically escape into deep-reaching burrows once the soil is disturbed however it is reported to have good recovery rates for epigeic and endogeic earthworms, which predominantly produce horizontal burrows (Čoja *et al.* 2008). Hand-sorting is recommended to be carried out in conjunction with other methods to maximise earthworm recovery rates (Pelosi *et al.* 2009 and Rajapaksha *et al.* 2014).

Heat Extraction

Heat extraction is a method that requires a combination of hand-sorting and extraction through the use of a light source that also emits heat. This is usually accomplished through the use of a Tullgren funnel or the more specialised Kempson apparatus (discussed in Section 2.4.7). The use of a Kempson apparatus along with hand-sorting yields the highest earthworm biomass from soil, mainly due to a very high efficiency rate for the recovery of juvenile earthworms (Čoja *et al.* 2008). Unfortunately this method may not always be appropriate to use as it destroys large volumes of soil-even more so than passive hand-sorting. The method can also be more costly in terms of the equipment required and the timescales necessary to conduct this method of extraction. The method is not recommended to be used in wooded or agricultural areas (Bartlett *et al.* 2010).

Chemical Extraction

Chemical extraction is the most commonly used extraction method for earthworms and is the preferred method cited in conjunction with hand-sorting by the International Standards Organization (ISO 23611- Part 1). Chemical extraction works through the application of an irritant that drives earthworms to the soil surface (B.S.I 2011and Čoja *et al.* 2008). These methods tend to be highly effective for the collection of anecic earthworms and some workers report that the method is biased in terms of the recovery of same (Bartlett *et al.* 2010). When deployed on its own, chemical extraction does not physically disturb the soil although the chemicals used can impact on soil vegetation and other organisms living in the soil.

The method involves the application of a chemical to a specified area of soil of known dimensions, typically set at 50 x 50cm. This area can be adjusted depending on recovery rates and the properties of the soil. Once a set amount of time has passed, typically 30 minutes, the earthworms on the surface are collected and the area is sorted by hand with additional use of the irritant to encourage any worms remaining in the soil to come to the surface (Southwood and Henderson, 2000; Römbke *et al*, 2005 and B.S.I, 2011).

Formalin is the most common chemical used to extract earthworms, even though it has been shown to exert toxic effects on humans, local vegetation, bacteria and micro-arthropods (Eichinger *et al.* 2007). While formalin has a very negative effect on the surrounding environment it degrades quite rapidly and does not persist in the soil (Eichinger *et al,* 2007 and Čoja *et al,* 2008). The effects of formalin on vegetation tend to be quite varied ranging from reduced transpiration and germination rates to reduced shoot dry weight and necrosis (Eichinger *et al,* 2007). However this is documented as being species specific and no publications were found on the effects of formalin on *Salix spp.*

Other chemical extractants have been researched by other workers and these include potassium permanganate, detergents and mustard. The use of potassium permanganate results in similar problems as formalin, particularly in that it may damage local vegetation. The recovery rate of detergents seems to be significantly less than formalin (Bartlett *et al,* 2006) and also damage and disfigure the collected earthworms. The use of detergents is not recommended if a study requires extensive identification work (East and Knight, 1998). In recent years, allyl isothiocyanate (AITC) has gained considerable interest as an alternative to formaldehyde (Zaborski, 2003). As a suspension, it is easily degraded in soil, with a half-live of 80–120h. AITC has the same irritant effects of formalin for earthworms but preliminary research has shown that it does not appear to carry the range of side-effects associated with formalin use (Eisenhauer *et al,* 2008; Pelosi *et al,* 2009 and Bartlett *et al.* 2010). Since AITC is a plant allelochemical it is considered a very feasible and much more environmentally-friendly option to currently used chemical extractants (Pelosi *et al,* 2009).

Commercial mustard powder contains allyl isothiocyanate (AITC) and solutions of mustard powder (10g/l) have been used in a number of earthworm studies (Gunn, 1992; Hogger, 1993; Chan and Munro, 2001; Muramoto and Werner, 2002, Pelosi *et al,* 2009 and Rajapaksha *et al.* 2014). Some questions arise over the reproducibility of recovery rates when using commercial mustard solution and a possible bias has been reported for large, sexually mature, anecic earthworms (Bartlett *et al.* 2006), A number of studies have shown that mustard solution is more effective than formalin for the recovery of earthworms (Gunn, 1992 and Chan and Munro, 2001). Pelosi *et al.* (2009) found that the recovery of earthworms using mustard solution was increased significantly when this expellant was used in combination with hand-sorting. Čoja (2008) states that mustard is attractive for researchers worried about the toxicity of formalin. Eisenhauer *et al.* (2008) state that the advantages of using mustard as an extractant include its ease of application and high extraction rates for deep-burrowing anecic species but they point out that it may be less effective for some earthworm species, with recovery being heavily dependent on soil type and soil moisture content.

<u>Electrical Extraction</u>

Electrical extraction (known as the Octet method) works on the principle of applying an electrical current to soil for a short period of time (typically 30 minutes) to force earthworms from the soil. It is a specialised extraction method and has a low recovery rate for adult earthworms as compared to juveniles which is problematic for identification. It is difficult for the method to be quantitative as the exact volume of soil being sampled is unknown (Southwood and Henderson, 2000). Electrical extraction is the least destructive of all earthworm extraction methods (B.S.I., 2011).

2.4 Mites

Soil mites represent the largest group of Acariformes (Arachnida; Acari) and are a very diverse group in terms of morphology and behaviour. A nationwide study of soil biota in Great Britain suggested that mites are the most frequently recorded group in soils, occurring in 94% of all soil samples (Black *et al,* 2003 as cited by Gulvik, 2007). Oribatid mites (sub-order Oribatida) are the most numerous and species-rich mite group in soil; there are about 7,000 species worldwide and over 1,000 species in Europe. Van Straalen (1998) states that Oribatid mites (along with springtails) make up the bulk of soil biodiversity. Oribatid mites appear to be the most widely studied of all mite groups in soils.

A number of researchers report on the value of soil mites as bio-indicators (van Straalen, 1998; Altieri, 1999; Behan-Pelletier, 1999; Paoletti 1999b; Ruf and Beck, 2005; Gulvik, 2007; Eeva and Penttinen, 2009 and Skubała and Zaleski, 2012). Some useful characteristics of soil mites as soil bio-indicators include;

- they are present in soil in high numbers;

- they have well-developed sampling methodologies;
- they are easy to sample in all seasons;
- they are in contact with both the solid and aqueous phases of soil;
- mite abundance is reported to decline rapidly when their habitat is damaged, a characteristic which allows detection of environmental degradation;

2.4.1 Identifying Features

Soil mites are small, commonly 0.1-1mm in length (Gulvik, 2007). Mites roughly resemble spiders and share some common features in that they have eight legs instead six, common in arthropods and they also have two other pairs of appendages that are unique to arachnids, chelicerae (used in feeding and defence) and pedipals (used in feeding, locomotion and reproduction) (Krantz, 1978). Their body form is divided into two sections (known as tagmata), a front section known as the gnathosoma and a rear abdomen section known as the idiosoma (Fig. 2.4). Mites differ from other arachnids in that they do not possess a narrow waist region (pedicle) joining both tagmata nor do they possess spinnerets (finger-like appendages) or distinct segmentation on the rear tagmata. A number of features are particularly useful for mite classification including location of stigmata (openings in the exoskeleton of a mite forming part of the respiratory system) which differs between sub-orders and, the structure and location of setae (Krantz, 1978). Oribatid mites have much less pronounced pedipals than other mites and possess a rostrum (part of the exoskeleton that projects over the mouthparts like a hood). The characterisation of mites to species level is difficult and a high level of expertise is required. Specimens must be mounted and flattened on a slide and viewed under a compound microscope at 200-1000x. Some specimens require dissection for complete classification.

2.4.2 Feeding Habits

Soil mites play a very important role in the decomposition of organic matter and recycling of nutrients (Krantz, 1978). They feed primarily on decomposing higher plant material and on the soil microflora. Mites that feed on soil microflora can be categorised as mycophages, phycophages and bacteriophages. Mycophages feed on fungi, phycophages feed on algae and bacteriophages feed on bacteria. Their consumption of nutrient-rich food makes them highly nutritious and they are an important food for other predators in the environment (Behan-Pelletier, 1999).

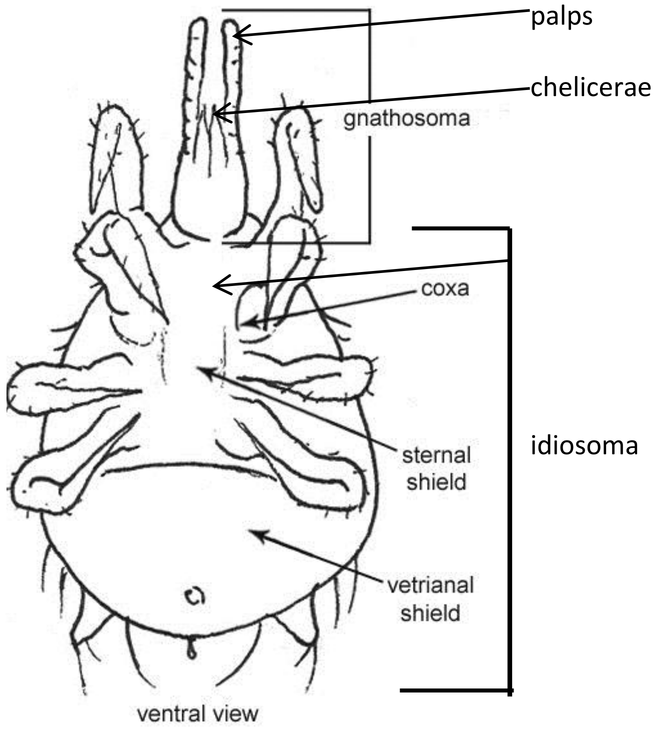

Figure 2.4: Mite showing gnathsoma, idiosmoma, palps and chelicerae (UBC, 2015)

2.4.3 Seasonal Population Trends

Reproduction in mites is usually sexual and the process differs depending on the species and the area inhabited. Thousands of eggs are typically fertilized during reproduction (Krantz, 1978) and once set in place, the eggs develop at a variable rate that can range from days to several years in temperate climates depending on species (Behan-Pellier, 1999). The abundance of mites is generally low in winter and peaks from March to July when soil conditions are most favourable (Larink, 1997). In agricultural systems, mite abundance usually decreases between July and August as a result of harvesting (Larink, 1997).

2.4.4 Abiotic Factors Affecting the Abundance of Mites

Typical mite abundance within soil varies depending on site conditions (particularly pH) and land-use and may range from a few hundred individuals per m^2 to hundreds of thousands of individuals per m^2 (Krantz, 1978; Curry, 1994; Behan-Pelletier, 1999; Behan-Pelletier and Kanashiro, 2010). The main abiotic factors that influence the abundance of mites are soil pH, temperature, soil moisture content, physical disturbance and the application of fertilisers and pesticides.

pH

The tolerance of soil mites to pH is variable depending on the species. Some acidophilic mites are reported to thrive in soils that have been somewhat acidified as the lower pH assists in their reproductive process and also lowers competition (Hagvar and Gunnar, 1980 and Hagvar, 1990). For example, while Oribatid mites are reported to be abundant in virtually any forest system, they reach much higher abundance values in acidic soils (200,000 individuals per m^2) than alkaline soils (20,000 individuals per m^2). A pH below 2.9 is considered detrimental to the majority of mites (Hagvar, 1990, Davey *et al.* 1995, van Straalen and Verhoef 1997).

Temperature

The effect of temperature on mites is highly variable with different species able to withstand different temperature gradients depending on their environment (Krantz, 1978). Larink (1997) reported that a temperature of 15^{o}C is ideal for temperate-climate mites. A temperature of 10^{o}C is considered to be the minimum threshold below which respiration is affected in temperate climates; temperatures below 6^{o}C are reported to result in a decrease in abundance (Cannon, 1987; Hart *et al,* 2002; Wekesa *et al,* 2010 and Beckett, 2011). Upper temperature tolerance is also species-dependent with losses in abundance generally observed at soil temperatures above 25^{o}C (Nguyen and Amano, 2009 and Wekesa *et al*, 2010).

Moisture

Moisture content does not regulate the abundance of mites as readily as temperature and pH. Larink (1997) reports that soil moistures of around 15% are ideal for mites when most of the pores are air-filled. Loss of abundance may occur only in extended drought conditions; this is credited to an impermeable exoskeleton which makes them more tolerant to desiccation than other soil invertebrates (Oliver *et al*, 2000, Lindberg *et al*, 2002 and Taylor and Wolters 2005). In water-saturated soils, mites can survive in enclosed air bubbles for some days and are able to change their metabolism under anaerobic conditions (Larink, 1997). Water pooling for extended periods of time results in a loss of abundance in some species (Lóšková *et al*, 2013) although Lindberg *et al* (2002) report that the abundance of Oribatids increase in areas receiving irrigation. Long-term irrigation is reported by some workers to result in a lack of diversity (Tsiafouli *et al*, 2005).

Physical Disturbance

Mites are reported to be adversely affected by a number of land management techniques (Davey *et al.* 1995; Behan-Pellier, 1996; Bedano *et al.* 2006 and Behan-Pelletier and Kanashiro, 2010) but particularly those that mechanically disturb the soil by compaction which results in reduced porosity (Bedano *et al*, 2006 and Cao *et al*, 2011). Adequate porosity is important to navigate the soil layers and access food sources. A decrease in soil porosity is reported to cause a reduction in species richness and density (Ducarme *et al*, 2004) although the effects are less marked than for larger soil invertebrates such as earthworms. Recovery is highly dependent on the group and the local environment but an estimation of 8-10 years is applied (Behan-Pelletier, 1999).

Application of fertilizers and pesticides

The use of fertilisers is reported to cause variable effects on the abundance of mites depending on species type, the type of fertilizer being applied, above-ground vegetation, effects on the microbial community and general soil properties including accumulation of specific nutrients (Cao *et al*, 2011 and Nielsen *et al*, 2012). A significant amount of research undertaken in this area focuses on Oribidata and Mesostigmata. Cao *et al.* (2011) observed a decrease in Oribatids over an 11 year fertiliser application period and attributed this to an increase in soil phosphorus levels which reduced fungal food sources; an associated increase in Mesostigmata was also observed due to increased food sources under nutrient-rich conditions. Oliver *et al.* (2005) observed an increase in Oribatids in grassland under increased nitrogen, phosphorus and potassium levels and attributed this increase to a decrease in predators as opposed to direct effects of fertiliser application.

The tolerance of mites to pesticide treatments is reported to be species-specific but, generally speaking, pesticides have a negative impact on abundance, particularly for Oribatid and Gamasid mites. This effect is short-lived and numbers will recover quickly in the absence of pesticide application (Minor *et al,* 2004 and Bedano *et al,* 2006). According to Benamú *et al* (2010) the direct and indirect effects of the herbicide, glyphosate on arthropods (including mites) has not been extensively researched. It seems that for mites the most extensive effect observed in the application of glyphosate is not a direct one but indirect due to changes in the local environment e.g. to vegetation, microclimate and food sources. Sub-lethal effects have been reported by a number of workers (Guiseppe *et al.* 2006; Schneider *et al,* 2009 and Benamú *et al,* 2010) as the application of glyphosates affects prey consumption, fertility and development of young. The latter effect is suggested as the herbicide mimics specific hormones and disrupts the endocrine system of mites (Cauble and Wahner, 2005). It appears that any loss of abundance due to the application of glyphosate is negated within a few months of application (Minor *et al,* 1994; Benamú *et al,* 2010 and Bosch-Serra *et al.* 2014).

Use of the insecticide chlorpyrifos has been reported to be toxic to mites but generally not at concentrations applied in the field (Prischmann *et al.* 2005 and Shi *et al.* 2008). The abundance of mites may be indirectly impacted by the application of chlorpyrifos owing to changes in the environment e.g. to vegetation and prey (Al-Assiuty *et al.* 2014). Oribatids appear to be most sensitive (Al-Assiuty *et al,* 2014).

2.4.5 Extraction of Mites

Extraction methods used for mites are divided into two categories: physical extraction and chemical extraction. Physical extraction methods are the most commonly used and recommended in ISO 23611 (B.S.I, 2006). Chemical extraction methods are influenced by soil composition and are recommended only as an alternative to physical extraction methods within ISO 23611 and will not be discussed further here.

Physical extraction methods derive from the initial step of taking a soil core sample to a minimum of 10cm below the soil horizon and extracting the organisms from the sample by exploiting soil behavioural habits. These methods force the insects to leave the soil through the addition of stimuli such as heat, moisture or the use of a chemical. The method is particularly useful when soil samples contain a lot of plant and detrital matter. The most commonly used method for extraction of mites (and springtails) is the use of a temperature gradient which uses the combined effect of heat and light to dry out the samples and create a differential moisture content in the soil from which the invertebrates will attempt to escape but by doing so, will fall into a collection container containing a preservative. This type of method can be divided into two sub-categories: dry extractors and wet extractors. Wet extraction is not suited for the extraction of mites or springtails (Southwood and Henderson, 2000). Dry extractors include the Berlese-Tullgren Funnel, Macfadyen extractor and Kempson Bowl Extractor.

The Berlese-Tullgren funnel, and its various modifications, is the most commonly used method for separating small arthropods such as mites from soil (Behan-Pellier, 1999). The Berlese-Tullgren funnel is based on the principle that a heat source (usually a tungsten light bulb) is placed above the soil sample which is located on a mesh. The soil sample is heated by the bulb and the specimens within the sample move through the soil sample and mesh and fall down a funnel into preservation liquid. The Berlese-Tullgren funnel can be modified depending on the required conditions, for example, the apparatus can be adapted for extraction of specimens from loose samples or from cores; the mesh size can be increased for larger insects; and the gradient of the funnel can be increased for faster moving invertebrates. In the case of soil cores, it is important that the core remains intact and inverted to ensure that animals can leave the sample by natural passageways (Haarlov, 1947 and Hubert *et al*, 2009). The Macfadyen extractor operates on the same principle as the Berlese-Tullgren funnel with the exception that a water bath is used to maintain the soil samples at a higher humidity level and a more pronounced temperature stratification (Macfadyen, 1961 and Block, 1966). The Kempson Bowl extractor also uses a water bath and the major difference between it and the Macfadyen apparatus is in the use of a pulsed light source rather than a continuous one (Southwood and Henderson, 2000).

2.5 Springtails

Springtails (Arthropoda: Collembola) are one of the oldest and most widespread terrestrial arthropods on Earth with between 6,500 and 8,000 species recorded world-wide (Hopkin,1997; Schneider *et al.* 2011 and Bellinger *et al,* 2013). Within the British Isles there over 350 species of springtails, of varying degrees of rareness (Hopkin, 1997 and Hopkin, 2005). Some common species of note are *Ceratophysella denticulate* (a widespread species found in damp habitats), *Hypogastrura purpurescens* (found in areas of sewage) and *Onychiurus ambulans* (common in soil, caves and on leaf litter) (Hopkin, 2005)

Springtails are useful bio-indicators as they are the most valuable member of the soil fauna involved in decomposition processes. They are reported to be susceptible to the effects of contamination by a variety of sources (Fontanetti *et al,* 2011). Their high abundance in soil and their ease of collection mean that they can be recovered in high numbers. They also have short life-cycles making them respond quickly to environmental change (Hopkin, 2005 and Greenslade, 2007). In the context of useful bio-indicators, Bispo *et al,* 2009 classifies springtails as one of three priority levels to detect environmental change, the other two priority levels being earthworms (or enchytraeids) and microbial respiration.

2.5.1 Identifying Features

Springtails are referred to as micro-arthropods owing to their small size, which is typically between 2-4mm in length (Hopkin, 1997). Springtails, in general, have the biology of a typical arthropod in that their body is divided into three tagmata, a head, thorax and abdomen and they possess six legs (Fig. 2.5). Several body types (and colours) exist ranging from stout, short-legged animals to elongated, long-legged ones. The segments of the abdomen possess a series of ventral tubes, which have a function in fluid exchange and a tail-like springing abdominal appendage (the furca) used by some species for locomotion (Hopkin, 1997). The common name of springtail is derived from this springing furca. The size of the furca can be quite variable, being quite large in the case of surface-dwelling springtails with soil-dwelling species having a very short furca or none at all (Hopkin, 1997). Springtails also possess a characteristic ventral tube, known as a collophore that plays an important role in moisture balance.

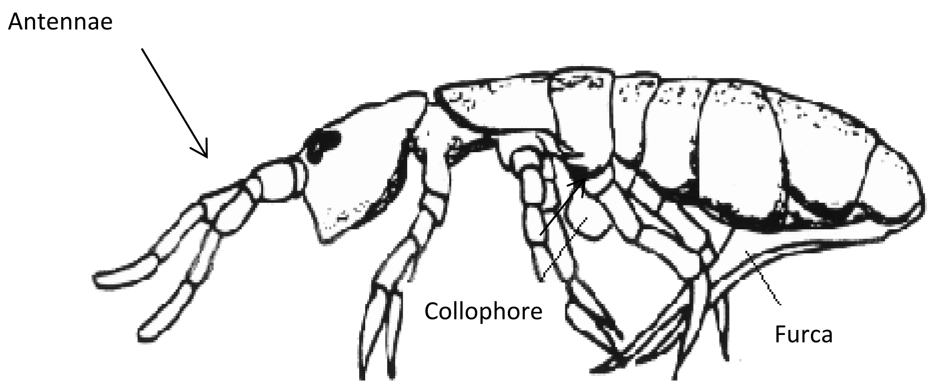

Figure 2.5: Springtail, showing antennae, collophore and furca (N.C. University, 2009)

2.5.2 Feeding Habits

Springtails are extremely important within soil as decomposers, predator and prey. In general, they are polyphagous, ingesting whatever decomposing vegetation, fungi, lichens, protozoa and nematodes that is available within the soil, together with animal exudates such as faecal droppings (Hopkin 1997 and Jørgensen *et al*, 2008). Other studies have indicated that springtails have specific feeding preferences that differ between species (Block, 1966; Bodvarrso, 1970; Gilmore and Raffensperger, 1970; Takeda and Ichimura, 1983; Leinaas and Ambrose, 1985; Al-Safadi, 1988 and Hishi *et al,* 2007). Springtails are also an important source of food for other organisms with some vertebrates, e.g. birds and frogs, preying on them in their local environment. The majority of springtail predators tend to be other invertebrates including hunting spiders, pseudo scorpions and ants that prey on springtails along with other invertebrates as part of a generalist diet. Some species of beetles are specialised to prey on springtails (Hopkin, 1997). In soil, where the furca in springtails tends to be reduced or absent, springtails are known to be vulnerable to predation by mites (Heckmann *et al,* 2007).

2.5.3 Seasonal Population Trends

The lifecycle of a springtail can be quite variable between species, and this includes when they lay eggs and hatch as certain species may lay eggs and hatch in late spring while others may overwinter to hatch in early spring. Multivoltine (multiple hatchings) may be possible within a one year cycle, though this is species-dependent and influenced by soil ambient temperatures and the availability and uptake of food (Hopkin, 1997). In some species, eggs are capable of a long diapause which is a dormant period of delayed development brought on by regularly and recurring periods of adverse environmental conditions. The life span of springtails is generally short, usually less than a year. Different species reach maximum and minimum abundance at different times of the year and population fluctuations have been reported to reflect species-specific strategies as adaptations to various soil conditions in various soil layers (Van Straalen, 1998).

2.5.4 Abiotic Factors that Affect the Abundance of Springtails

The abundance of springtails in soil varies depending on site conditions and land management practices and can range from tens of thousands of individuals per m^2 to hundreds of thousands of individuals per m^2. In undisturbed grassland areas, springtail abundance is reported to range from 30,000 and 100,000 per m^2 of soil with lower abundances (1000-1600 per m^2 of soil) observed in agricultural areas of high intensity (Curry, 1994 and Hopkin, 1997). A number of abiotic factors affect the abundance of springtails in soils including soil pH, temperature, soil moisture content, physical disturbance and the application of pesticides and fertilisers.

pH

In general, pH range preferences of springtails are species-specific and fairly narrow range, although some species of springtails are reported to exhibit a uniform distribution across the pH scale (van Straalen 1998 and Garnier and Ponge, 2004). In general, acidified environments does not adversely affect springtail abundance with some species reported as tolerating a pH as low as 2 (van Straalen 1998).

Temperature

Springtails are directly affected by soil temperature insofar as each species has a preferred range with the lethal minimum temperature being referred to as the super cooling point (SCP) and the lethal maximum temperature referred to as the thermostupor point (TSP) where the heat causes the animal to stop motion and eventually expire. The preferred temperature range of different species differs radically depending on location. In temperate climates, a soil temperature of 15^{o}C is considered optimum for most species to carry out feeding and development (Hopkin, 1997 and Larink, 1997).

Moisture

Springtails prefer wet or damp conditions and lack of moisture is reported to have a highly negative effect on abundance and species diversity (Hopkin, 1997). In field situations this can be quite difficult to test due to the influence of other abiotic factors but adverse effects have been demonstrated in experimentally simulated droughts (Pflug and Wolters, 2001 and Jucevica and Melecis, 2006). It is reported that some springtails species can negate the effects of drought through a combination of allowing for passive absorption of water vapour together with the accumulation of sugars from localised plant matter, allowing them to remain active and even lay eggs during drought for quick population recovery when optimum conditions prevail (Waagner *et al,* 2011). Natural and artificial precipitation has been shown to increase springtail abundance as it causes them to move to the surface of the soil and avail of increased food sources (Rodríguez *et al,* 2006). Frampton *et al* (2000) states that artificial precipitation/irrigation can be useful during drought conditions as it helps to preserve some food sources and stimulate hatching.

Physical disturbance

Physical disturbance directly affects springtails as they are highly dependent on the natural porosity of the soil to navigate their environment (Larink, 1997). Springtails are highly dependent on the burrowing activities of other soil organisms such as earthworms. The distribution of springtails is affected by soil compaction and they tend to avoid the decreased pore size of compacted soils (Wickenbrock and Heisler, 1997; Larsen *et al,* 2004 and Son *et al,* 2011). It is reported that physical disturbance due to compaction and tillage is more likely to have an adverse effect on the abundance of springtails than the application of chemicals in intensively managed farming systems (Hopkin, 1997; Bedano *et al*, 2006 and Ponge *et al*, 2013).

Application of fertilisers and pesticides

The application of organic fertilisers is reported to have a positive effect on the abundance of springtails resulting in increased abundance, particularly in areas that receive an input of nitrogen (Reekedar *et al.* 2006, Leroy *et al.* 2007 and Schutz *et al.* 2008).

Several pesticides are documented to affect the abundance of springtails however this effect can be quite variable depending on the chemical being used and its concentration; toxic effects are generally observed at concentrations higher than those typically applied in the field (Alves *et al,* 2014; Schnug *et al.* 2014a and Schnug *et al,* 2014b). The abundance of springtails is negatively affected by the application of herbicides with a loss of abundance reported directly upon application to an area however this loss of abundance is more readily attributed to loss of food sources as opposed to direct toxicity effects (Fox, 1964, Hopkin, 1997 and Alves *et al,* 2014). Guiseppe *et al.* (2006) and Hammad and Gurkan (2012) report that the herbicide glyphosate does not cause lethal effects for micro-invertebrates such as springtails but may cause sub-lethal effects as applications impact on food sources. This can have knock-on effects for fertility and the development of young (Guiseppe *et al,* 2006; Schneider *et al*, 2009, and Benamú *et al,* 2010).

The effects of the insecticide, chlorpyrifos on springtails is twofold since application of this chemical affects food sources and egg development when it is chronically applied to a site (Fountain *et al.* 2007; Jager *et al.* 2007; Schnug *et al.* 2014a and Schnug *et al,* 2014b).

2.5.5 Extraction of Springtails

The three main ways to study springtails are (a) by direct observation of live individuals in their natural environment (b) by trapping the animals in pitfall traps for subsequent exanimation and (c) by collection of soil cores/leaf litter and extraction of the springtails. Soil core extraction, as recommended by ISO 23611, covers both the extraction of springtails and mites and has been discussed previously in Section 2.4.5.

2.6 The Impact of SRC Willow Plantations on Soil Invertebrates

The abundance and diversity of soil invertebrates is influenced by activities above and below the soil surface. The most important factors to consider when assessing impacts to soil invertebrates as a result of the establishment and maintenance of SRC willow plantations are (a) previous land-use; and (b) site preparation and maintenance (including coppicing regime and use of pesticides/fertilisers).

Site preparation and maintenance have been discussed previously in Section 2.1.1 and these activities together with land-use shifts may influence soil invertebrates because of potential changes to soil characteristics including bulk density and porosity (which affects water-holding capacity and aeration) soil pH and organic matter. These factors have been discussed previously with respect to their effects on earthworms, mites and springtails.

Many studies report on soil quality impacts of SRC willow plantations particularly from land-use shifts from intensive agricultural practices (Makeschin 1994; Reicosky *et al.* 1995; Borjesson, 1999; Jug *et al.* 1999 and Rowe *et al.* 2009). In general, a shift from intensive agricultural practices to SRC willow plantations has beneficial effects for soil quality including increased organic matter content, decreased leaching of nutrients and reduced erodibility. All of these factors should benefit soil invertebrates in an area. If a change in soil characteristics is desirable to soil invertebrates then beneficial effects should be observed in terms of species diversity and abundance. Borjesson (1999) states that;

> *'it is predicted that when SRC willow plantations sites replace an annual cropping system, the overall effect for soil invertebrates is beneficial mainly since there is a reduction in the use of heavy machinery and agrochemicals'.*

This is however, dependent on the nature of site preparation for SRC willow as well as the use of herbicides and other pesticides, fertilisation and frequency and method of coppicing. Campbell *et al* (2012) report that it is expected that SRC willow plantations are more beneficial to the preservation of biodiversity than traditional annual crops with a greater diversity and occurrence of soil fauna, especially decomposers.

A lack of research in relation to soil-dwelling invertebrates in SRC willow plantations has been noted by a number of workers (Dimitriou *et al*, 2009 and Rowe *et al*, 2009) in comparison to the number of studies carried out on aerial canopy invertebrates (Sage and Tucker, 1998; Borjesson, 1999; Haughton *et al*, 2009 and Rowe *et al*, 2009). Dimitriou *et al* (2009), in a review of animal diversity in SRC plantations states that *'most studies of animal species diversity in SRCs have dealt mainly with vertebrates and left invertebrates largely neglected'* and comment that due to the large numbers of invertebrates that reside in soil, investigations have been limited to individual indicator groups e.g. earthworms and ground beetles, and that more research into other soil dwellers is required. Previous scientific investigations in this area can be classified into three main types;

(a) comparison of the abundance of selected soil invertebrates in SRC willow plantations as compared to adjacent fields;
(b) comparison of changes in selected soil invertebrates in the aftermath of land-use shifts to SRC willow; and
(c) observation of changes in abundance of selected soil invertebrates during, but not for the entire duration of, the SRC rotation period.

Mackeschin (1994), in a study of SRC sites in Germany, reported that the abundance of earthworms, woodlice and harvestmen were increased under SRC willow when compared to adjacent arable fields. Dimitriou *et al* (2009) observed a lower diversity of ground beetles in SRC willow plantations as compared to adjacent arable fields. Baum *et al* (2009) cite studies who observed an increase in the abundance of harvestmen (Opilionida), woodlice (Isopoda) and earthworms (Lumbricidae) following conversion of arable soils to SRC willow and poplar in experimental sites but a decrease in the abundance of carabids (Carabidae) and spiders (Araneida). No changes in centipedes (Chilopoda) and millipedes (Diplopoda) were detected. Minor *et al.* (2004) found that the abundance and diversity of soil mites (Oribatida and Gamasida) was initially negatively affected during the first year after conversion of arable land to SRC but increased in the long term. Coates and Say (1999), in a study of five sites of SRC willow in southern England found that earthworm numbers decreased over the 6 years of the study. Work by AFBI in 2010 observed an initial decrease in earthworm numbers under SRC willow, though it was stated that this may change in longer established plantations (AFBI, 2010). Given the variability of findings from different studies, it is clear that more work is required in this area with specific focus on previous land-use and the nature of SRC site preparation and management, including the use of heavy machinery and pesticides.

2.7 The Impact of Irrigated SRC Willow Plantations on Soil Invertebrates

The impact of SRC willow on soil invertebrates is further complicated when the site is irrigated with wastewaters. Irrigation can improve the biomass yield from energy crops, including willow (Sharma and Ashwath, 2006 and Zema *et al*, 2012), however it can also impact on soil characteristics and water regime in the local environment which in turn can impact on the local biodiversity (Singh *et al*, 2012). The combined impact of SRC willow plantation and irrigation with wastewater on soil invertebrates has not been adequately researched and following an extensive review of the literature no published papers were available in this research area. The lack of research in this area was noted by Britt *et al* (2002) and there has been no progress since that time. In contrast, the effect on microbial communities has been more extensively researched (Filip *et al.* 2000, Carlander *et al.* 2009, Truu *et al.* 2009 and Zhang *et al.* 2010). Some value may exist in research undertaken of wastewater irrigation of soils under different forms of land management (e.g. agriculture and forestry) and Britt *et al.* (2002) notes that;

> *'provisional assumptions must be based on an interpretation of indirectly relevant experience (e.g. biodiversity impacts of waste applications to agricultural or forest crops), within the context of conditions prevailing within 'typical' energy crops'.*

A review of available literature suggests that biodiversity impacts of wastewater applications to agricultural/forest crops are dependent on a number of factors including;

(a) the volume of wastewater applied;

(b) organic matter and/or nutrient content of the wastewater;

(c) the presence of toxic contaminants in the effluent; and

(d) wastewater pH and the pH of the receiving soil.

The rate of wastewater application must satisfy the water requirements of the target crop and must not be applied at a rate that will cause changes to the soil water regime or pose a threat to surface and groundwater systems. In general, irrigation of soils and changes to soil moisture content causes loss of diversity in organisms that do not tolerate this activity while increasing the abundance of organisms that find irrigation beneficial (Doube and Styan, 1996; Frampton *et al.* 2000; Pflug and Wolters, 2001; Lindberg *et al.* 2002; Jucevica and Melecis, 2006 and Lóšková *et al.* 2013). The impact of soil moisture and irrigation on earthworms, mites and springtails has been discussed previously in Sections 2.3.4, 2.4.4 and 2.5.4.

The rate of wastewater application must also be matched to the nutritional requirements of the growing crop. If nutritional requirements are exceeded, then a build-up of organic matter and nutrients may occur in the soil. This will be influenced by the organic and nutrient loading applied and also the physical, chemical and biological characteristics of the soil. An important factor will be the rate of decomposition and mineralisation by resident soil organisms. In general, the soil invertebrate community responds favourably to increased organic matter and nutrients however, the rate of addition is critical (Cotton and Curry 1979; Curry, 1994 and Paoletti 1999a). As previously mentioned, the recovery of soils from applied organic wastes (including wastewaters) is greatly enhanced by the presence of earthworms. (Devliegher and Verstraete, 1997 and Kocik *et al.* 2007). The impact of additions of organic matter and nutrients on earthworms, mites and springtails has been discussed previously in Sections 2.3.4, 2.4.4 and 2.5.4

A variety of substances (including heavy metals and organic chemicals) may be present in wastewaters at concentrations that can exert toxic effects on soil invertebrates. When soils are irrigated with wastewaters containing these substances, resident invertebrates may experience chronic or acute toxic effects by direct or indirect exposure. A number of workers have studied the effects of these contaminants on soil invertebrates e.g. earthworms (Van Gestel and Ma, 1990; Spurgeon and Hopkin 1996; Rodríguez *et al,* 2006; Spurgeon et al, 2006; Brandham et al, 2006 and Udovic and Lestan, 2007) and micro-arthropods (Streit 1984; Ludwig et al, 1991 and Broerse, 2010). It is apparent from these studies that a significant number of factors influence the response of soil invertebrates to potential wastewater contaminants including physical, chemical and biological parameters in soil together with contaminant loading and the nature/duration of exposure.

Irrigation of a soil with wastewater may cause changes to soil pH by a number of mechanisms i.e. the direct addition of hydrogen ions from the applied wastewater, the generation of hydrogen ions during the decomposition of organic matter and/or the leaching of base cations, in particular calcium and magnesium. The buffering capacity of the receiving soil is an important factor to consider when assessing short or long-term changes to soil pH. Soil pH may control the distribution of soil invertebrates and most soil organisms survive within a fairly narrow pH range. Soil pH not only affects the living conditions of soil invertebrates but also influences the decomposition of organic matter and availability of nutrients and the solubility of potentially toxic pollutants (Ma and Bodt, 1983; Yong, 1993). The impact of soil pH on earthworms, mites and springtails has been previously discussed in Section 2.3.4, 2.4.4 and 2.5.4.

Chapter 3 Methodology

3.1 Culmore Site Description

The Culmore SRC willow plantation is owned and operated as an investigation site by the Agri-Food and Biosciences Institute (AFBI) and is located outside Culmore, County Londonderry. The plantation is located on a 2.5ha site and is irrigated with primary treated wastewater from an adjacent waste water treatment plant which treats effluent from Culmore town and the surrounding area (Plate 3.1). The willow plantation is visible at the centre background of the photo. The area is irrigated at a rate of 30m^3/ha/day during the irrigation periods. In 2012 the irrigation period extended from May to mid-October. In 2013, the irrigation period extended from May to October.

Plate 3.1: Wastewater treatment facility, Culmore, Co. Londonderry. Picture supplied by AFBI (Private use)

The Culmore SRC willow plantation was planted in 2010 on a former AFBI research site. The site is divided into two blocks of six plots each; within each block three plots are irrigated with municipal waste water while the remaining three are not irrigated. This investigation focussed on sampling from Block 2 only (Fig. 3.1), due partly to time and sampling equipment constraints but also because Block 1 was temporarily flooded with rainwater at the start of the investigation. The six plots in Block 2 are numbered from 7-12 (Fig 3.1). Each plot in the plantation is 30 x 45m. There is a 4m corridor of grass between Blocks 1 and 2 but only a small gap of 1m exists between each of the plots (Plate 3.2). Plots 8, 10 and 12 are irrigated with primary treated municipal wastewater at a rate of 30m^3/ha/day. Irrigation is by means of perforated pipes (Plate 3.3). The plantation site was previously planted with poplar, grass and willow before the site was uprooted in 2009 and re-planted with willow in 2010. Figure 3.1 shows that Plots 7 and 10 were previously planted with poplar, Plots 8 and 11 were previously planted with grass and Plots 9 and 12 were previously planted with willow. The site has been irrigated with municipal effluent from May to September/October every year since 2007 (AFBI, *pers comm, 2013*).

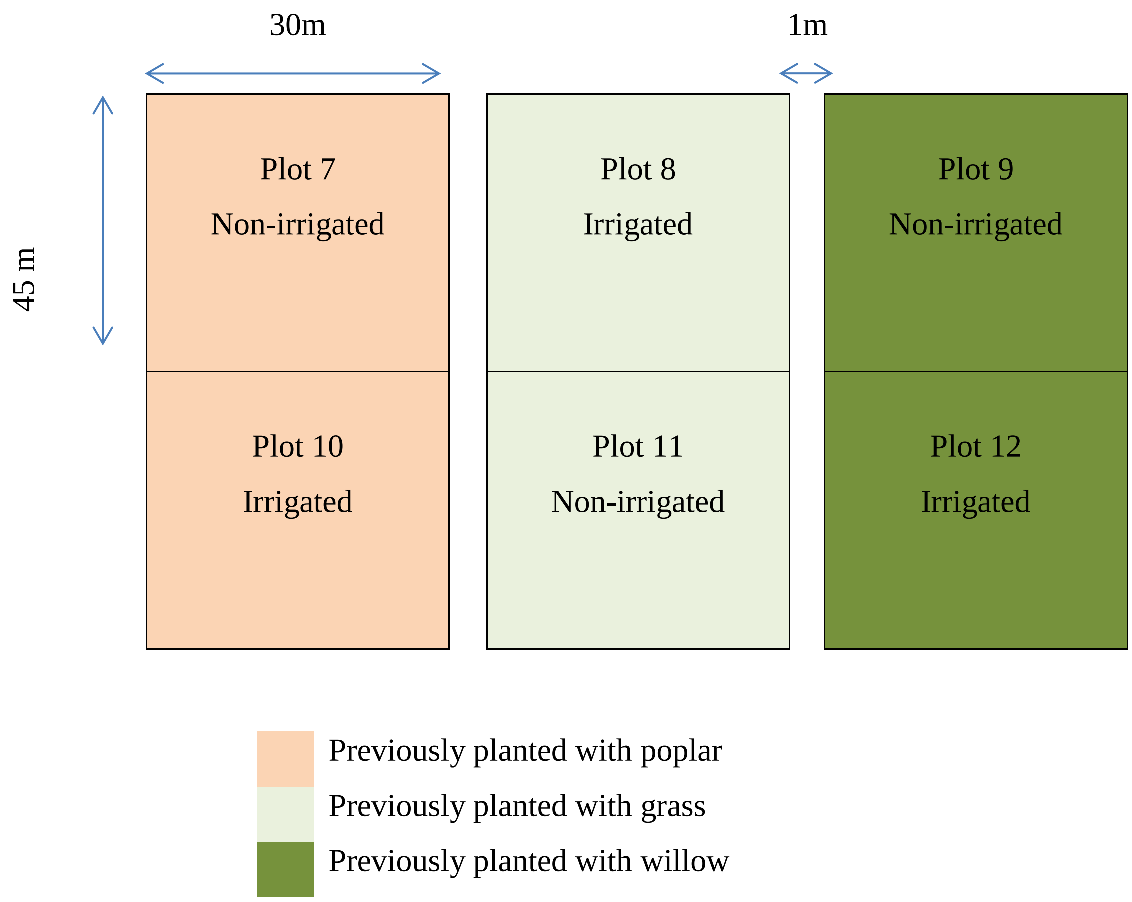

Figure 3.1: Block 2 at SRC Willow Plantation Culmore, Co. Londonderry

The topography of the Culmore SRC willow plantation is predominantly flat (Plate 3.2) with a gentle undulating slope towards Block 2. The soil in the Culmore plantation is heavy mineral clay. An analysis of the soil was undertaken by AFBI throughout 2012 and 2013 and available data is included in Table 3.1. Summary characteristics of primary treated effluent used for irrigation at Culmore were provided by Northern Ireland Water, who operates the wastewater treatment plant (Table 3.2).

Plate 3.2: SRC Willow Plantation at Culmore, Co. Londonderry showing Plot 8 and 9 and the 1m corridor between the plots

Plate 3.3: Irrigation pipes at Culmore SRC willow plantation

Table 3.1: Summary data for soil characteristics (mean and range of values) in irrigated and non-irrigated plots at Culmore SRC willow plantation for irrigated periods 2012 & 2013

		TC%	TN%	P (mg/l)	K (mg/l)	Mg (mg/l)	S (mg/l)
Irrigated Plots	Mean	9.8	0.44	15.5	47.5	113.5	18.3
	Range	9.6-10.1	0.41-0.46	12.1-20.3	29.7-60.8	75.7-210.8	9.7-35.1
Non-Irrigated Plots	Mean	10.6	0.48	13.7	48.9	88.0	12.3
	Range	9.9-11.1	0.46-0.50	10.6-17.6	35.8-63.3	74.8-117.7	8.6-18.2

(Source: AFBI, *pers comm*, 2014*)*

Table 3.2: Summary characteristics of primary treated effluent (mean and range of values) used for irrigation at Culmore wastewater treatment plant

	pH	BOD (mg/l)	Total N (mg/l)	Total P (mg/l)	Total K (mg/l)	Iron (mg/l)	Zinc (mg/l)
Mean	7.1	200	21.6	4.9	27.2	2.91	0.36
Range	6.7-7.9	150-280	8.4-42.7	1.9-10.2	8.1-49.8	0.52-19.34	0.02-2.24

(Source; N.I. Water, *pers comm, 2014*)

3.2 Hillsborough Site Description

The second SRC willow plantation site is located within a larger research facility run by AFBI at Hillsborough, Co. Down. The plantation is irrigated with dairy parlour washings (D.P.W.) originating from a working dairy farm located within the facility. The D.P.W. is stored in a large tank (Plate 3.4) to ensure a steady supply of D.P.W. to the SRC willow plantation during the irrigation season of May to September/October.

Plate 3.4: Storage tank used for dairy parlour washings at Hillsborough, Co. Down

The Hillsborough SRC willow plantation site is divided into two blocks with four plots in each block. This investigation focussed on Block 1 only due to time and sampling equipment constraints. Each plot within Block 1 is 57 x 100m and receives varying levels of D.P.W. via a perforated pipe. The irrigation levels are referred to as treatments and there are four levels of treatment as shown in Fig 3.2; Treatment 1 (0 m^3/ha/d- this is a control plot); Treatment 2 (18m^3/ha/d); Treatment 3 (34m^3/h/d); Treatment 4 (44m^3/h/d) (AFBI, *pers comm, 2014*).

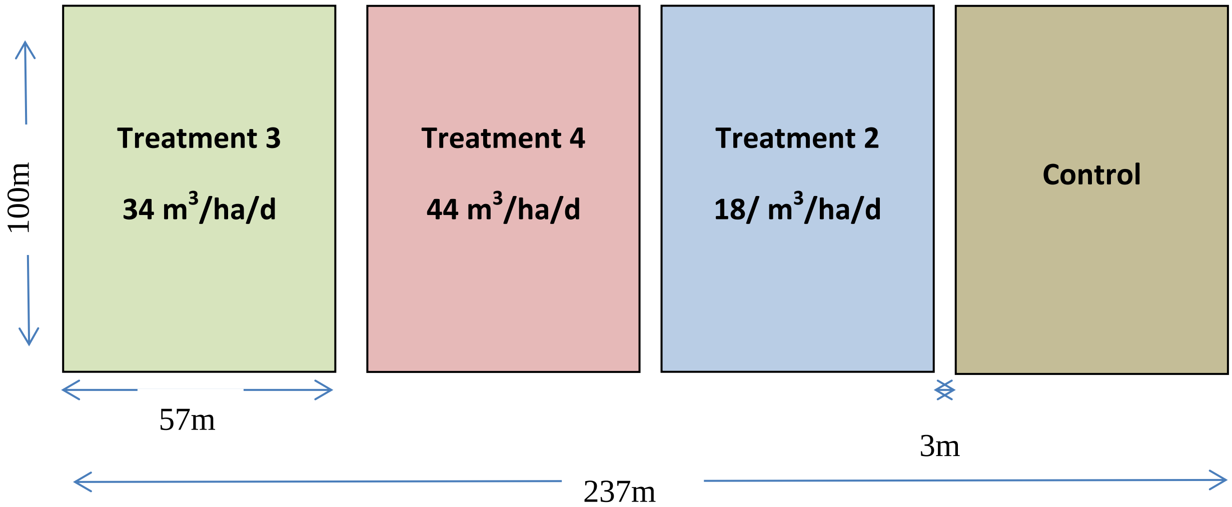

Figure 3.2: Block 1 at Hillsborough SRC willow plantation showing plots in receipt of varying amounts of D.P.W

The Hillsborough site was planted in 2007 and was previously a grassland area. The land has a sloped topography with the slope being more pronounced towards the Treatment 3 and Treatment 4 plots. The soil type is clay loam and contains a large number of small stones. There are six willow genotypes planted at Hillsborough SRC willow plantation as per the lay-out shown in Figure 3.3. The configuration is such that a single genotype (e.g. 'Beagle'*)* is planted in a particular segment (7m x100m) and a different genotype (e.g. 'Tora') is planted alongside this in another discrete segment- these segments are known as single genotype plots (SGP). A mixture of a number of genotypes planted in a double segment (14m x100m) and this is known as a mixed genotype plot (MGP).

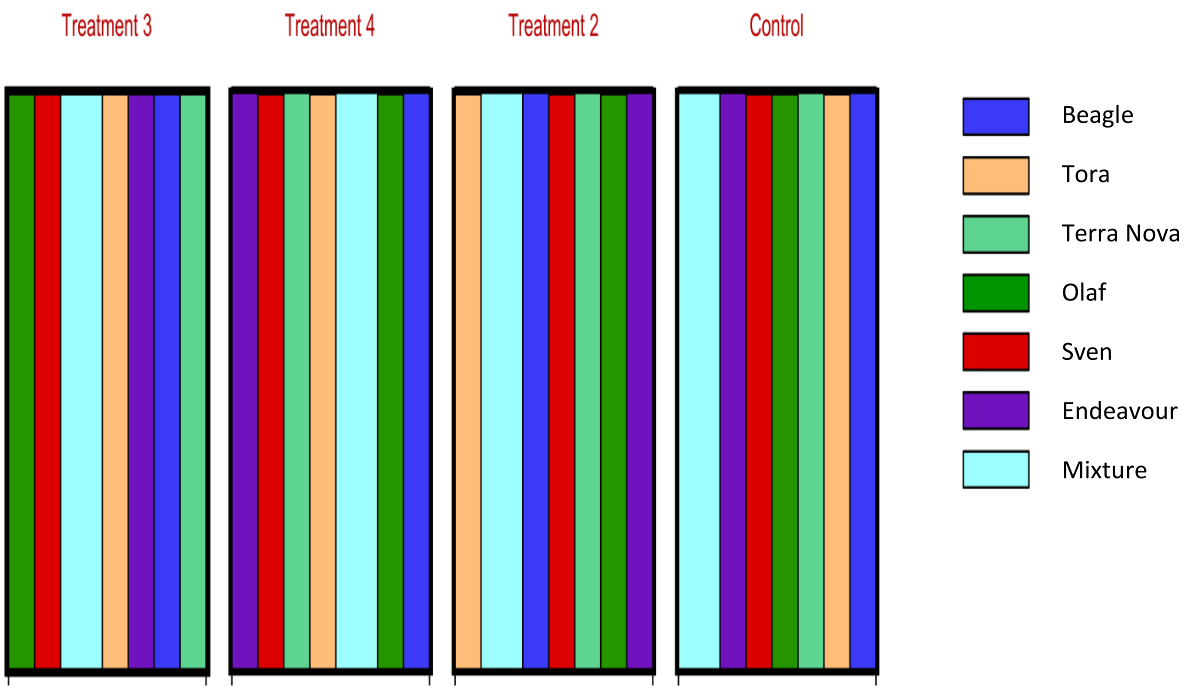

Figure 3.3: Planting scheme, Hillsborough SRC willow plantation

The SRC willow plantations have been irrigated with D.P.W. since 2010 (AFBI, *pers comm, 2013*). The D.P.W. is applied at different volumes as previously explained. Summary characteristics of the dairy washings are shown in Table 3.3.

Table 3.3: Summary characteristics of D.P.W. (mean and range of values) at Hillsborough

	pH	BOD (mg/l)	Total N (mg/l)	Total P (mg/l)	K (mg/l)
Mean	6.7	2815	178	56.3	576
Range	6.6-6.8	2150-3100	46.2-227	8.7-82.7	300-802

(Source: AFBI, *pers comm*, 2014)

3.3 Choice of Bio-Indicator

Following an extensive review of literature, three bio-indicators were chosen to detect potential environmental changes in irrigated SRC willow plantations, namely earthworms, mites and springtails. These bio-indicators were chosen as they relate to different ecosystem functions. The value of each of these soil invertebrates as bio-indicators has been discussed previously in Sections 2.2, 2.3 and 2.4.

3.4 Sampling Regime

3.4.1 Culmore

The 2012 irrigation season in Culmore commenced in May and ceased in mid-October. Earthworm sampling did not begin until July (due to difficulties in choosing a suitable extractant that would not interfere with other research on-going at the site by AFBI) and continued on a monthly basis until the end of the irrigation period. Earthworm sampling was extended beyond the irrigation period in 2012 to assess earthworm abundance outside of this time. Sampling ceased in December 2012 due to low soil temperatures. The 2013 irrigation period was between May and September. Earthworm sampling commenced in March 2013 to assess earthworm abundance prior to the 2013 irrigation period. Sampling ceased at the end of August 2013 which marked the end of the ANSWER project study period for this investigation. A total of 14 unique sampling events occurred in Culmore throughout 2012 and 2013, with 9 of these occurring during irrigated periods. On each sampling occasion, two samples were taken from each of the six plots in Block 2. All samples were taken at random through the use of a random number generator. Earthworm extraction and collection was carried out in the field from two discrete 0.5m x 0.5m areas in each plot. Extraction was undertaken in accordance with ISO 23611-1:2006, part 1 (Section 3.4). It was difficult to sample for earthworms in the thick undergrowth at the base of the willow coppice (Plate 3.5a) and therefore a small clearing was made to facilitate earthworm sampling (Plate 3.5b).

Sampling commenced for mites and springtails in the 2012 irrigation season in August 2012. This was a late start considering that irrigation had been on-going since May however; there was a number of difficulties in the supply of sufficient quantities of Berlese-Tullgren funnels that were required for extraction of micro-arthropods from the soil. Sampling extended beyond the irrigation period until December 2012 when sampling ceased for the winter. Sampling resumed in March 2013 on a monthly basis to determine the activity of mites and springtails prior to the 2013 irrigation period. Irrigation began in May 2013 and sampling continued on a monthly basis until August 2013, which marked the end of the ANSWER project study period for this investigation. The frequency of sampling increased to twice monthly in July and August 2013 to compensate for the lack of sampling in September. A total of 13 sampling events occurred in Culmore, with 8 of these occurring during irrigation periods. On each sampling occasion, three soil core samples were taken from each of the 6 plots for the extraction of micro-arthropods. These samples were transferred back to the lab in polythene bags on the day of sampling for heat extraction in Tullgren funnels as per ISO 23611-1:2006 -Part 2 (Section 3.6).

3.4.2 Hillsborough

The 2012 irrigation season in Hillsborough commenced in May and ended in mid-October. Earthworm sampling did not begin until July (due to difficulties in choosing a suitable extractant that would not interfere with other research on-going at the site) and continued until the end of the irrigation period. Earthworm sampling was extended beyond the irrigation period in 2012 to assess earthworm abundance outside of this time. Sampling ceased in December 2012 due to low soil temperatures. The 2013 irrigation period was between May and October. Earthworm sampling commenced in March 2013 to assess earthworm abundance prior to the 2013 irrigation season. Sampling ceased at the end of August 2013 which marked the end of the study period.

Plate 3.5: (a) Typical working conditions between rows (b) Earthworm extraction

A total of 14 sampling events occurred in Hillsborough, with 9 of these occurring during irrigation periods. On each sampling occasion, two samples were taken from each of the treatment plots in Block 1. All samples were taken at random through the use of a random number generator. Two samples were taken from a random single genotype plot and mixed genotype plot at each treatment level, resulting in a total of four samples from each treatment level. Earthworm extraction and collection was carried out in the field, with two discrete 0.5 x 0.5m areas being delineated in each plot. Extraction was undertaken in accordance with the guidelines given in ISO 23611-1:2006, part 1 (Section 3.4).

Sampling commenced for mites and springtails in the 2012 irrigation season in September 2012. This late start was due to a number of difficulties encountered in the supply of sufficient quantities of Berlese-Tullgren funnels required for the investigation. Sampling extended beyond the irrigation period until December 2012 when sampling ceased for the winter. Sampling resumed in March 2013 on a monthly basis to determine the activity of mites and springtails prior to the 2013 irrigation period. Irrigation began in May 2013 and monthly sampling continued until August 2013, which marked the end of the ANSWER study period. The frequency of sampling increased to twice monthly in July and August 2013. A total of 12 sampling events occurred in Hillsborough, with 7 of these occurring during irrigation periods. Three samples were taken from a single genotype plot and mixed genotype plot at each treatment level, resulting in a total of six samples from each treatment level. These samples were transferred back to the lab in polythene bags for heat extraction in Tullgren funnels as per ISO 23611-1:2006-Part 2 (Section 3.5).

3.5 Earthworm Extraction

A number of methods can be used to extract earthworms from soil. These methods are described in ISO 23611-1:2006 (Part 1) and are summarised in Section 2.3.5. Heat extraction methods were ruled out immediately due to the level of soil destruction that would ensue with this method. Electrical extraction was also ruled out due to the health and safety risks associated with this method and also because of the remoteness of the sites. This limited options to passive hand-sorting and/or chemical extraction. An initial trial test run at the Culmore site indicated that hand-sorting alone would not be a suitable method for the extraction of earthworms due to the dense structure of willow roots. These limitations meant that it would prove almost impossible to reach the soil depths recommended by the ISO method. This left chemical extraction as the only remaining option. ISO 23611-1:2006 (Part 1) recommend a combination of extraction of earthworms using formalin and hand-sorting. Due to the documented effects that formalin can exert on plant growth and soil micro-organisms, other alternative chemicals were considered and reviewed in light of complimentary on-going research by other students at the AFBI investigation sites. ISO 23611-1:2006 (Part 1) provides for an alternative method which involves extraction of earthworms using commercial mustard powder solution and this method was deemed to be compatible with other research activities on-going at the AFBI SRC willow plantations. Extraction was conducted using 100g of Coleman’s mustard powder dissolved in 10 litres of water. The solution was then applied to a 0.5 x 0.5m area at each sampling site using a quadrat to delineate the area. If an area had excess surface vegetation this was cleared away by hand prior to the application of mustard solution to ensure optimal visibility of the sample area and provide for maximum recovery of earthworms. When 30 minutes had elapsed, the top layer of soil was dug up and passive hand-sorting undertaken to ensure that all specimens were recovered. All specimens were stored in 70% ethanol and transported back to the lab for identification. Earthworms were identified under the microscope using 40-100x magnification and an LCD light system. Samples were identified to species level using an identification key (Sims and Gerard, 1999).

3.6 Extraction of Springtails and Mites

Springtails and mites were extracted from collected soil cores in accordance with ISO 23611-2:2006 (Part 2). This method involves the extraction of the top 15cm of soil from an area using soil cores of 5cm diameter and storing the soil samples in labelled plastic bags for subsequent heat extraction using a Berlese-Tullgren funnel (Plate 3.6). Heat extraction occurred over a period of nine days. The recovered specimens were fixed in a 50% ethylene glycol solution. Owing to the high level of expertise required for the classification of mites/springtails (even to Family level) and the time-consuming nature of the process, classification of mites/springtails was not undertaken in this study.

Instead, mites and springtails were extracted and enumerated separately and recorded as total mites or springtails per soil core sample. These values were subsequently converted to appropriate units for comparison purposes, as described below. The correct categorisation of each specimen as a mite/springtail required the use of a stereomicroscope (Leica M60) at 400x magnification and LCD light source and the use of identification keys i.e. springtails (Hopkins, 1997); mites (Krantz, 1978).

Plate 3.6: Bank of Berlese-Tullgren Funnel used for extraction of micro-invertebrates

ISO 23611-2:2006 (Part 2) states that the number of springtails/mites can be reported in various units (per kg of dry soil, per m^2 of soil or per cm^3 of soil). Due to the nature of this investigation and in particular the possibility of dealing with soil samples of widely varying soil moisture content, the unit of individuals/kg of dry soil was chosen. It was felt that by using this unit, more accurate comparisons could be made between springtail and mite abundance in irrigated vs non-irrigated areas which was the prime focus of the investigation. The dry weight of soil was determined by placing each soil core sample in a separate soil container following the extraction process and drying it in an oven at $105^{o}C$ for 24 hours as per ISO 23611-2:2006 (Part 2).

Abundance values were also calculated per m^2 of soil in order to compare the results of this investigation with those published reports that used the unit of individuals/m^2. This was achieved by multiplying the number of springtails or mites recovered from each soil core by a value of 509 as required in ISO 23611-2:2006 (Part 2).

3.7 Physical/Chemical Analysis

Soil temperature, soil pH and percentage soil moisture content (%SMC) was determined for each plot sampled at Culmore and Hillsborough on each sampling occasion. Soil temperature was determined in-situ using a soil thermometer. The soil thermometer was inserted to a depth of 10cm.

It was necessary to extract soil samples for the determination of pH and %SMC. Soil core samples were taken to a depth of 15cm and transferred to labelled and sealed plastic bags for transportation to the lab. Soil pH determination was determined in accordance with ISO 10390:2005 using 0.01M $CaCl_2$. %SMC was determined in accordance with ISO 11465:1993-the samples were dried in soil containers in an oven at 105^{o}C for 24 hours.

3.8 Statistical Analysis

All data generated from the investigation were entered onto Microsoft Excel and this programme was used to calculate average, maximum and minimum values.

Species-area curves were prepared for earthworms in PC-ORD to indicate that a sufficient sample size was achieved during the investigation to account for all earthworm species present.

When the datasets were shown to be non-parametric, data transformation was attempted using square root, log, log + 1, Box-cox and Johnson. The deficiency was not overcome by these transformations and statistical analysis relied on non-parametric methods. Two statistical packages were used for statistical analysis of the data. These were;

(a) Minitab (version 16): The significance of the various data sets was tested using Kruskal-Wallis. Correlation tests were performed using Ranked Spearman Correlation Tests.

(b) PC-ORD (version 6). The data was tested using PerMANOVA, which is a multivariate test for determining variance. PerMANOVA analysis requires the use of a distance matrix. Euclidean distance was chosen due to structure of data.

3.9 Limitation of Experimental Set-up

This investigation was part of larger investigation known as the ANSWER project (Agricultural Need for Sustainable Effluent Recycling). I.T. Sligo was one of the partners in the project and the lead partner was AFBI in Northern Ireland. AFBI have a number of demonstration/research sites throughout Northern Ireland and this investigation centred on two of these, as previously mentioned. Despite the benefits to the study of using already established SRC willow plantations (thereby avoiding a lengthy and costly lead-in period to the investigation) the experimental set-up brought a number of limitations to the study as follows;

- Alteration of the earthworm sampling method to use mustard solution instead of formalin. Formalin was the main chemical extractant used in other studies researched in literature but could not be used at AFBI research sites at Culmore and Hillsborough since these sites were being used by other AFBI scientists. While this author is satisfied that mustard solution achieved a sufficiently high recovery of earthworms (as supported by an extensive literature review), it must be noted that the results from this investigation was compared in certain instances to results from other studies that used formalin.
- The established SRC willow site at Culmore had previously been planted with a mix of other crops (i.e. poplar, willow and grass). This had the potential of introducing a variable other than irrigation into the investigation.
- The increments of irrigation were quite high at Hillsborough SRC willow plantations at 18, 34 and 44 m^3/ha/day as this site was established to test the upper limits of SRC willow for irrigation purposes.
- Sampling of micro-invertebrates didn't commence until August 2012 and September in Hillsborough due to issues with equipment and this meant that valuable data was lost for the early months of the 2012 irrigation period.
- The statistical tests used were for non- parametric data and could only test to a confidence level of 95%.

Chapter 4 Results

4.1 Abundance of Bio-Indicators at Culmore SRC Willow Plantation

Three plots (Plots 8, 10 and 12) at Culmore SRC willow plantation were irrigated with primary treated municipal wastewater at a rate of 30 m^3/ha/day from May to mid-October in 2012 and from May to October in 2013. Three further plots (Plots 7, 9 and 11) were used as controls and did not receive any effluent. For ease of reporting the 2012 irrigation period will be referred to hereafter as Phase 1, the 2013 irrigation period will be referred to as Phase 3 and the interim period between irrigation will be referred to as Phase 2. The abundance of earthworms, springtails and mites in irrigated and non-irrigated plots were investigated in Phases 1, 2 and 3. The results of the investigation are presented separately for each bio-indicator.

4.1.1 Earthworms

Effect of irrigation with muncipal effluent on earthworm abundance

The numbers of earthworms recovered (per m^2 of soil) in irrigated and non-irrigated plots at Culmore are shown in Table 4.1 for specified sampling dates in Phases 1, 2 and 3. Each value represents an average of two samples taken from each plot. Sampling commenced in Phase 1 in July 2012 and continued until the end of the 2012 irrigation period. Samples were not taken in May and June 2012 due to difficulties in choosing a chemical extractant that was compatible with other investigations on-going at the research site. Samples were taken in Phase 2 to determine earthworm abundance outside of the irrigation period. Phase 3 sampling commenced in May and continued until the end of August. The previous planting history of the various plots is also shown in Table 4.1; the relevance of this information is discussed later.

The main points evident from Table 4.1 are as follows;

(a) No particular trend is immediately obvious in earthworm abundance between irrigated and non-irrigated plots; higher abundances are observed in non-irrigated plots on some sampling occasions and in irrigated plots on other sampling occasions. This is also evident in Fig. 4.1.

	Plot 7: Non-irrigated	Plot 10: Irrigated	Plot 8: Irrigated	Plot 11: Non-irrigated	Plot 9: Non-irrigated	Plot 12: Irrigated	Average Irrigated	Average Non-Irrigated
	History: Poplar		History: Grassland		History: Willow			
Phase 1								
06/07/2012	0	3	99	186	0	7	*36.3*	*62*
31/07/2012	3	12	22	71	0	3	*12.3*	*26.6*
29/08/2012	2	0	83	53	11	4	*29*	*22*
26/09/2012	0	0	42	42	0	0	*14*	*14*
Average for Phase 1	**1.3**	**3.8**	**61.5**	**88**	**2.8**	**3.5**	***22.9***	***31.2***
Phase 2								
23/10/2012	0	0	34	52	2	0	*11.3*	*18*
20/11/2012	0	0	15	44	1	14	*9.6*	*15*
05/03/2013	6	0	13	21	0	0	*4.3*	*9*
03/04/2013	0	0	6	16	0	0	*2*	*5.3*
30/04/2013	0	0	23	26	0	4	*8.6*	*9*
Average for Phase 2	**1.2**	**0**	**18.2**	**31.8**	**0.6**	**3.6**	***7.2***	***11.3***
Phase 3								
27/05/2013	0	0	50	44	0	0	*14.6*	*16.7*
25/06/2013	0	0	21	50	0	0	*16.7*	*7*
30/07/2013	0	23	14	40	12	0	*12.3*	*17.3*
06/08/2013	0	0	24	26	11	0	*8*	*12.3*
17/08/2013	4	2	54	8	0	0	*18.7*	*4*
Average for Phase 3	**0.8**	**5**	**32.6**	**33.6**	**4.6**	**0**	***14.1***	***11.46***

Table 4.1: Numbers of earthworms (per m^2) in irrigated and non-irrigated plots at Culmore SRC willow plantation during the three main sampling phases together with the averages for total irrigated vs. total non-irrigated plots. Previous planting history is also shown.

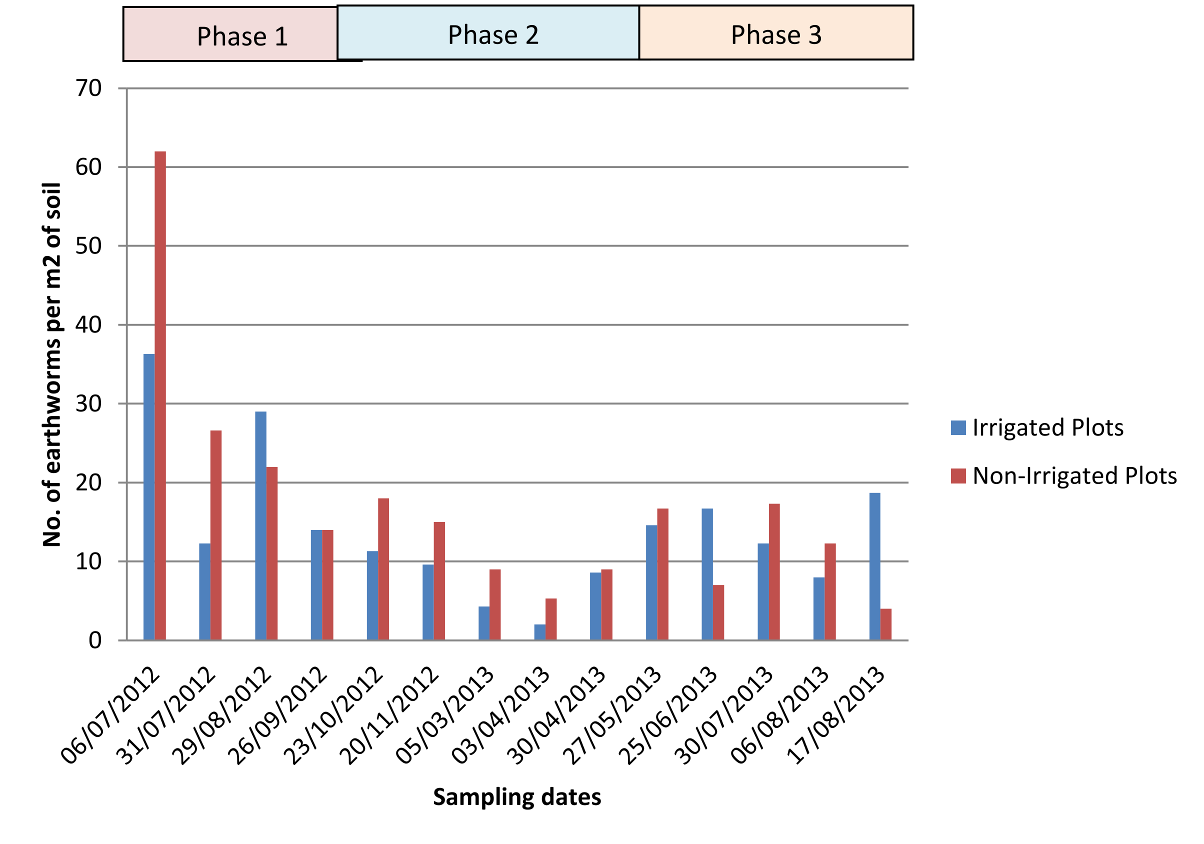

Figure 4.1: Average earthworm numbers (per m^2 of soil) in irrigated vs. non-irrigated plots

(b) The data obtained for the abundance of earthworms in irrigated and non-irrigated plots was statistically analysed to determine whether there was a significant difference between them. The data sets were non parametric and therefore Kruskal-Wallis was applied. The outcome of statistical analysis revealed no significant differences between irrigated and non-irrigated plots despite small differences being observed between averages in Table 4.1. This was the case when the data sets were tested for irrigated periods only (p=0.792: C.I. =0.05) and for all sampling events (p=0.585: C.I. =0.05). Detailed results of statistical outcomes are provided in Appendix D (Table D1).

(c) Several sampling results of zero recovery of earthworms are observed in Plots 7, 9, 10 and 12 during all Phases of the investigation. Earthworms were consistently recovered from Plots 8 and 11 in all sampling phases.

(d) Greater numbers of earthworms are present in Plots 8 and 11, regardless of irrigation status throughout Phases 1, 2 and 3. Although these plots are now planted with willow they have a different cropping history to other plots (as described in Section 3.3.1.).

(e) Lower numbers of earthworms were observed during the non-irrigated period (Phase 2) as compared to irrigated periods (Phase 1 and Phase 3).

(f) The only significant difference in earthworm abundance that emerged between Phase 1 and Phase 3 was in plots that were previously planted with grassland in both irrigated plots (*p*=0.004; C.I =0.05) and non-irrigated plots (*p*=0.034; C.I =0.05). Significant differences in earthworm abundance between Phase 1 and Phase 3 did not emerge in plots that were previously planted with poplar or willow. Detailed results of statistical outcomes are provided in Appendix D (Table D9).

Figure 4.2 is a species-area curve for earthworm sampling in Culmore. No predictions were made through jack-knife and Chao estimates when carrying out the species area curve, this indicates that no rare species are present at the site. The flattening of the curves in Fig 4.2 indicates that sufficient sampling was carried out (top line). The bottom line accounts for the abundance of the species present on the site and the flattening of that line indicates that the level of sampling carried out was sufficient to be representative of the species numbers.

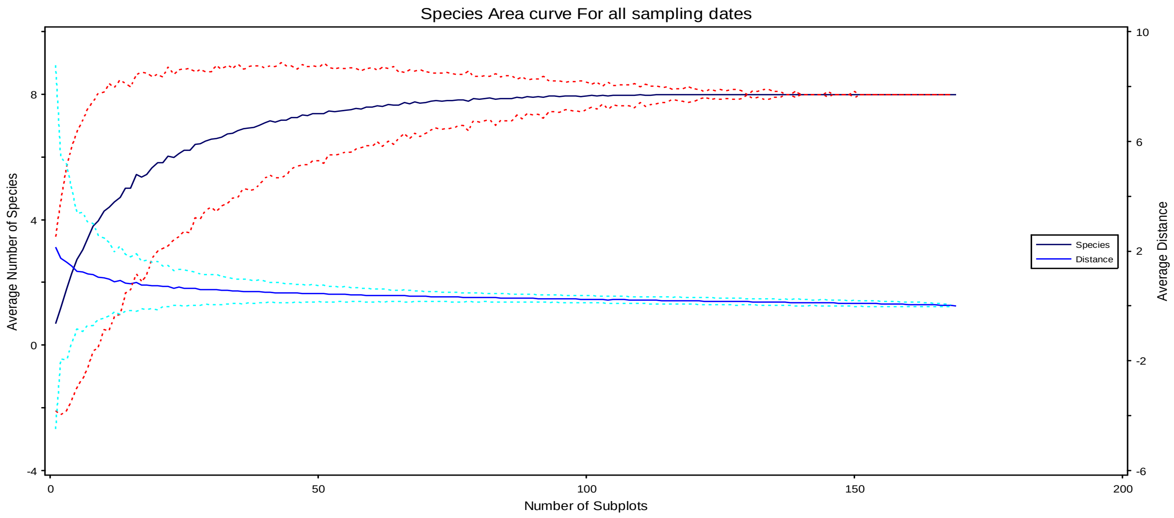

Figure 4.2: Species area curve for earthworms in Culmore SRC willow plantation

Eight earthworm species were observed across all sampling dates. The main species present were epigeic and included *E. tetraedra*, *L. eisenia*, *L. festivus*, *L. rubellus* and *S. mammalis.* Two endogeic species were present *(O. tyrtaeum* and *A. rosea)* but these were recovered in very low numbers throughout the investigation. One anecic earthworm species *(L. terrestris)* was also recovered but again in low numbers throughout the investigation. Relatively large numbers of non-clitellate earthworms were recovered in the Culmore plantation throughout the investigation which meant that these immature earthworms could not be identified to species level. The numbers of epigeic, endogeic, anecic and non-clitellate earthworms recovered on each sampling date is shown in Appendix B (Table B2).

Effect of Previous Cropping History on Earthworm Abundance

On inspection of Table 4.1, it is immediately apparent that earthworm abundance is substantially higher in Plots 8 and 11 as compared to other plots during Phases 1, 2 and 3 of the investigation; these plots were previously planted with grass but were uprooted and re-planted with willow in 2010. Other plots had previously been planted with poplar (Plots 7 and 10) or willow (Plots 9 and 10) but were uprooted and replanted with willow in 2010. This previous planting history is described in Section 3.1. Figure 4.3 shows the mean abundance of earthworms (per m^2 of soil) for combined irrigated and non-irrigated plots with a common planting history at Culmore. Average earthworm abundance values are consistently higher on each sampling occasion for Plots 8 and 11 than for all other plots (Table 4.1 and Figure 4.3).

The data sets were tested using Kruskal-Wallis and the outcome revealed that planting history significantly affected earthworm abundance. The data was further tested to ascertain if differences existed in different combinations of planting history. The outcomes are shown in Table 4.2 for irrigated and non-irrigated plots within irrigated periods only and also for all sampling events. The results show that regardless of irrigation, there is no difference in earthworm abundance between areas previously planted with poplar and willow. There is however a significant difference in earthworm abundance between areas previously planted with grass and those previously planted with poplar/willow and this difference is significant in both irrigated and non-irrigated plots.

Since a difference had emerged in earthworm abundance based on planting history, the data was further tested to ensure that planting history was not a factor in outcomes observed when statistically testing the significance of the abundance of earthworms in irrigated vs non-irrigated plots. PerMANOVA was used to determine if the variables of planting history and irrigation had a significant effect on each other. No interaction was observed for irrigated periods only (p=0.480; C.I. = 0.05) or for all sampling events (p=0.247: C.I. 0.05).

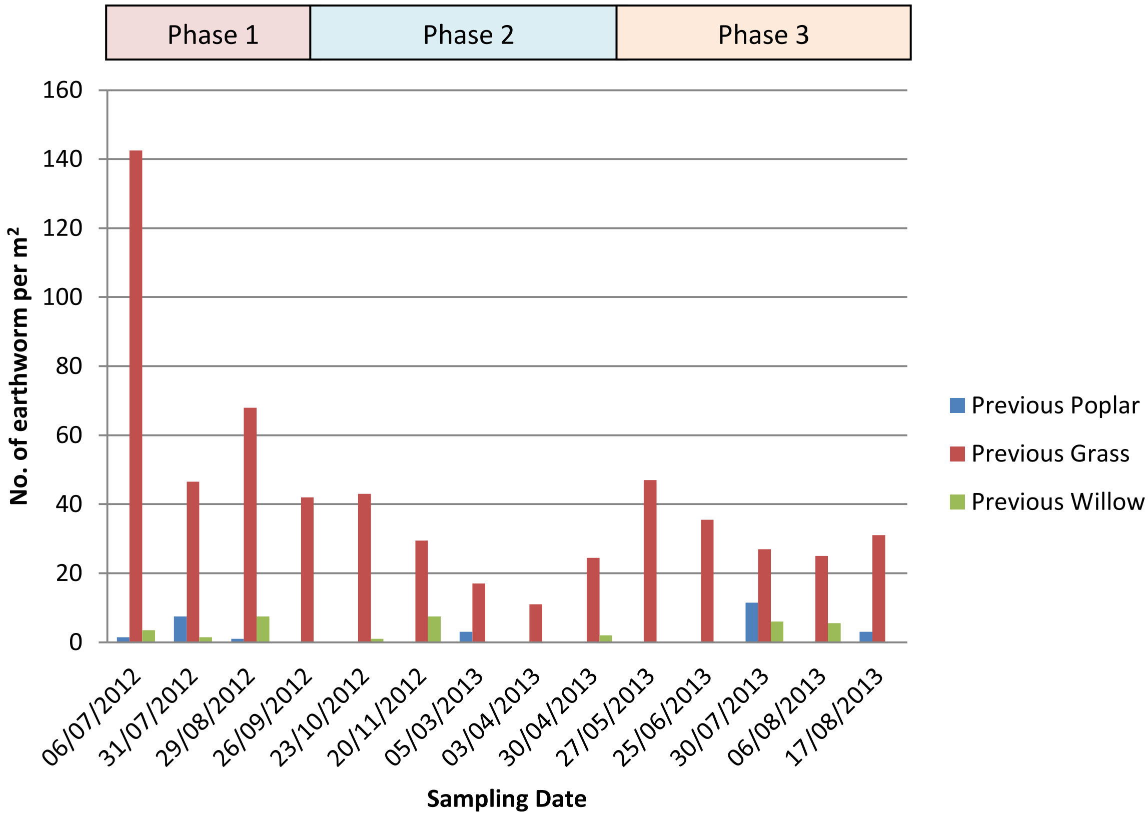

Figure 4.3: Average earthworm numbers (per m^2 of soil) for combined irrigated and non-irrigated plots with a common planting history at Culmore SRC willow plantation

Table 4.2: Outcome of statistical testing to determine significant differences in earthworm abundance in various combinations of planting history in irrigated and non-irrigated plots at Culmore SRC willow plantation

Irrigated plots only	**Phases 1, 2 and 3**	**Phases 1 and 3**
Poplar vs. Grass	$p = 0.001$	$p = 0.001$
Poplar vs. Willow	$p = 0.599$	$p = 0.873$
Willow vs. Grass	$p = 0.001$	$p = 0.001$
Non-irrigated plots only	**Phases 1, 2 and 3**	**Phases 1 and 3**
Poplar vs. Grass	$p = 0.001$	$p = 0.001$
Poplar vs. Willow	$p = 0.918$	$p = 0.875$
Willow vs. Grass	$p = 0.001$	$p = 0.001$

4.1.2 Mites

Effect of irrigation with muncipal effluent on the abundance of mites

The numbers of mites recovered per kg of dry soil on specified sampling dates in irrigated and non-irrigated plots at Culmore are shown in Table 4.3 for Phases 1, 2 and 3. Each value presented is the average of three samples taken in each plot. Eight sampling events are shown for irrigated periods (Phase 1 and 3) while five sampling events are shown for the non-irrigated period (Phase 2). Samples were taken in Phase 2 to determine abundance outside of the irrigation period. The main points evident from Table 4.3 are as follows;

a) In general, the highest average peak numbers of mites are observed in non-irrigated plots throughout the investigation. This is evident from Figure 4.4. However, when individual plots are viewed in isolation, no immediate trend is apparent in relation to peak numbers (Table 4.3) with high numbers recorded in various plots over the investigation period.

b) The data obtained for the abundance of mites in irrigated and non-irrigated plots in Culmore was statistically tested to determine whether there was a significant difference between them. The data sets were non-parametric so Kruskal-Wallis was applied. There was no significant difference in the abundance of mites in irrigated and non-irrigated plots when municipal effluent was applied at a rate of $30m^3$/ha/day both within irrigation periods only (p=0.091: C.I.=0.05) or on all sampling dates (p=0.198: C.I.=0.05). Detailed outcomes of statistical tests are provided in Appendix D (Table D2).

c) In Phase 1, the average number of mites in irrigated plots was 105/kg of dry soil while the value in non-irrigated plots was 191/kg of dry soil. The corresponding values in Phase 3 were 39/kg and 43/kg of dry soil, respectively. Although the abundance values appeared much lower in Phase 3 than Phase 1, it was not statistically valid to test for a significant difference since there were only two sampling events for mites in Phase 1.

	Plot 7: Non-irrig.	Plot 10: Irrigated	Plot 8: Irrigated	Plot 11: Non-irrig.	Plot 9: Non-irrig.	Plot 12: Irrigated	Average Irrigated	Average Non-irrig
	History: Poplar		**History: Grassland**		**History: Willow**			
Phase 1								
29/08/2012	268	110	179	49	342	16	*101.7*	*219.7*
26/09/2012	326	144	120	114	44	60	*108*	*161.3*
Average for Phase 1	**297**	**127**	**149.5**	**81.5**	**193**	**38**	***104.8***	***190.5***
Phase 2								
23/10/2012	91	45	54	10	45	107	*68.7*	*48.7*
20/11/2012	395	31	18	1	83	46	*31.7*	*159.7*
05/03/2013	128	139	29	75	0	131	*99.7*	*67.7*
03/04/2013	45	134	16	18	10	71	*73.7*	*24.3*
30/04/2013	181	24	12	35	58	5	*13.7*	*91.3*
Average for Phase 2	**168**	**74.6**	**25.8**	**27.8**	**39.2**	**72**	***57.5***	***78.4***
Phase 3								
27/05/2013	61	86	9	28	21	17	*37.3*	*36.7*
25/06/2013	92	26	26	14	64	17	*23*	*56.7*
16/07/2013	31	21	19	29	6	56	*32*	*22*
30/07/2013	36	21	18	48	29	55	*31.3*	*37.7*
06/08/2013	43	42	36	62	66	50	*42.7*	*57*
17/08/2013	56	55	51	71	33	107	*71*	*53.3*
Average for Phase 3	**53.2**	**41.8**	**26.5**	**42**	**36**	**50.3**	***39.6***	***43.9***

Table 4.3: Number of mites (kg of dry soil) in non-irrigated and irrigated plots at Culmore SRC willow plantation during the three main sampling phases and the averages for total irrigated vs. total non-irrigated plots. Previous planting history in each plot is also shown.

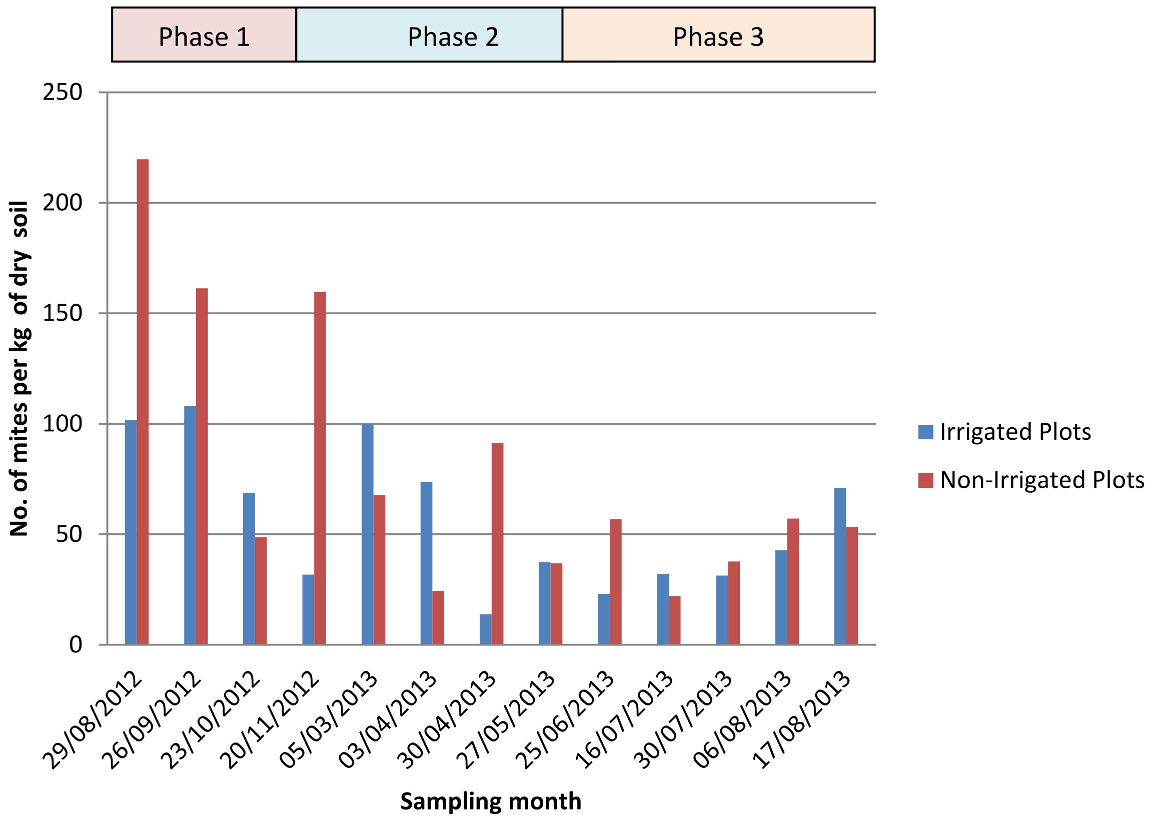

Figure 4.4: Average number of mites (per kg of dry soil) in irrigated and non-irrigated plots at Culmore

Effects of Previous Cropping History on Abundance of Mites

Fig 4.5 shows the average number of mites in combined irrigated and non-irrigated plots with a shared planting history at Culmore. There was a substantial variation in the abundance of mites in all plots with a common planting history but in general, higher peak numbers were observed in plots previously planted with poplar as compared to plots previously planted with grass or willow. Average values of mite abundance for each Phase of the investigation were also higher for plots previously planted with poplar than for those previously planted with grass and willow (Table 4.3). The significance of cropping history on the abundance of mites was tested using Kruskal-Wallis for combined irrigated and non-irrigated plots. The outcome of this statistical testing revealed a significant difference in mite abundance between different combinations of cropping history (p=0.001; C.I. =0.05) and therefore the data sets were tested individually for irrigated plots and also for non-irrigated plots. The results of statistical tests are shown in Table 4.4 and reveal that cropping history does not have a significant effect on the abundance of mites in irrigated plots regardless of the combination of planting history tested, however significant differences arise when non-irrigated plots are tested. These differences are significant when previously planted poplar plots are compared to other plots during all Phases of the investigation and for irrigated periods only.

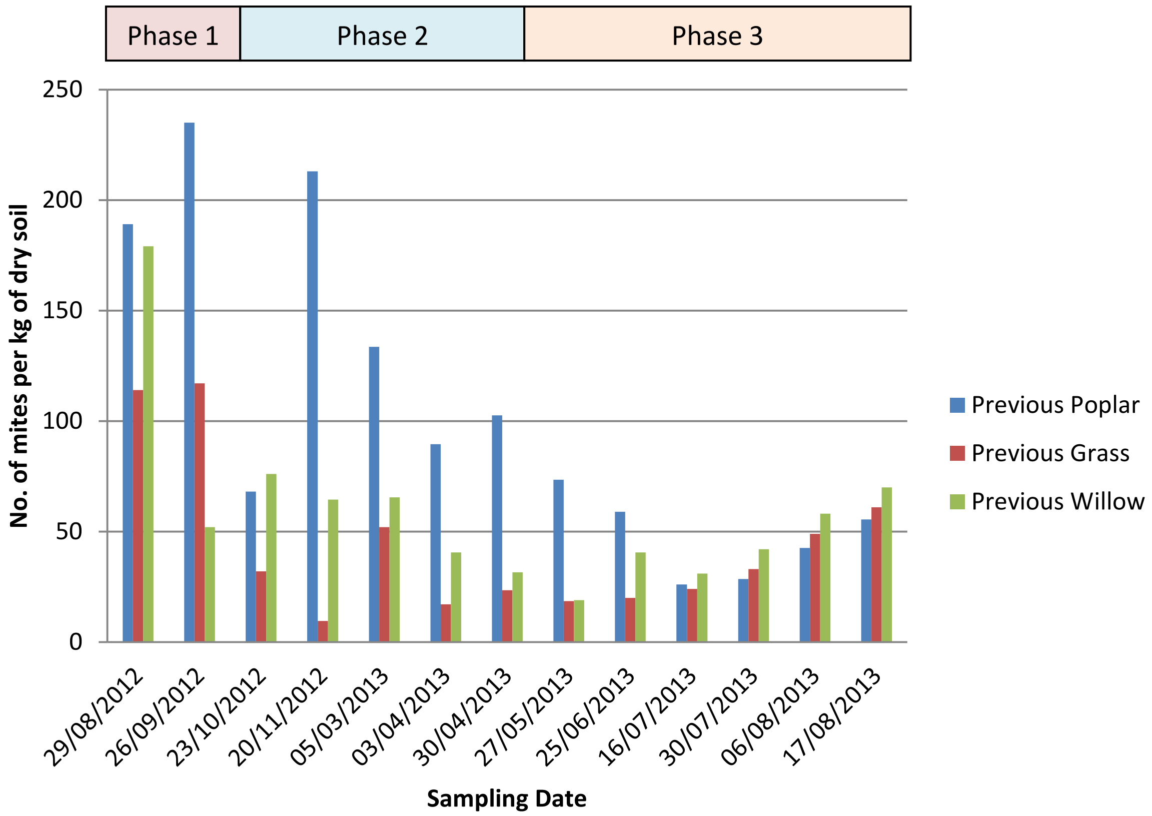

Figure 4.5: Average number of mites (per kg of dry soil) for combined irrigated and non-irrigated plots with a common planting history at Culmore SRC willow plantation

Table 4.4: Outcome of statistical testing to determine significant differences in mite abundance in various combinations of planting history in irrigated and non-irrigated plots in Culmore SRC willow plantation

Irrigated plots only	**Phase 1, 2 and 3**	**Phase 1 and 3**
Poplar vs. Grass	$p= 0.107$	$p= 0.103$
Poplar vs. Willow	$p= 0.323$	$p= 0.191$
Willow vs. Grass	$p= 0.580$	$p= 0.702$
Non-Irrigated plots only	**Phase 1, 2 and 3**	**Phase 1 and 3**
Poplar vs. Grass	$p= 0.001$	$p= 0.001$
Poplar vs. Willow	$p= 0.001$	$p= 0.005$
Willow vs. Grass	$p= 0.897$	$p= 0.884$

The data was further tested using PerMANOVA to ensure that planting history was not a factor in outcomes observed when statistically testing the significance of the abundance of mites in irrigated vs. non-irrigated plots. No combination effects emerged during irrigated periods only (p= 0.369; C.I. =0.05) or for all sampling events (p= 0.369; C.I. =0.05).

4.1.3 Springtails

Effect of Irrigation with Muncipal Effluent on Abundance of Springtails

The numbers of springtails recovered per kg of dry soil in irrigated and non-irrigated plots at Culmore are shown in Table 4.5 for specified sampling dates in Phases 1, 2 and 3. Each value presented is the average of three samples taken from each plot. Eight sampling events are shown for irrigated periods (Phase 1 and 3) while five sampling events are shown for the non-irrigated period (Phase 2). Samples were taken in Phase 2 to determine abundance outside of the irrigation period. The main points evident from Table 4.5 are as follows;

a) No particular trend is immediately obvious in the abundance of springtails between irrigated and non-irrigated plots with higher numbers observed in irrigated plots on some sampling occasions and higher numbers in irrigated plots on others, particularly during Phases 1 and 3. Similar values were obtained for the abundance of springtails in irrigated and non-irrigated plots during Phase 2 of the investigation. This is shown in Figure 4.6 which includes the average numbers of springtails/kg of dry soil in irrigated plots and non-irrigated plots throughout the entire sampling period. The highest average peak numbers recorded over the investigation period are in non-irrigated plots but there is no trend immediately apparent in relation to peak numbers in individual plots, with high numbers recorded in various plots over the investigation period. There are relatively few events of zero recovery or low recovery of springtails in the various plots with the exception of Plot 8.

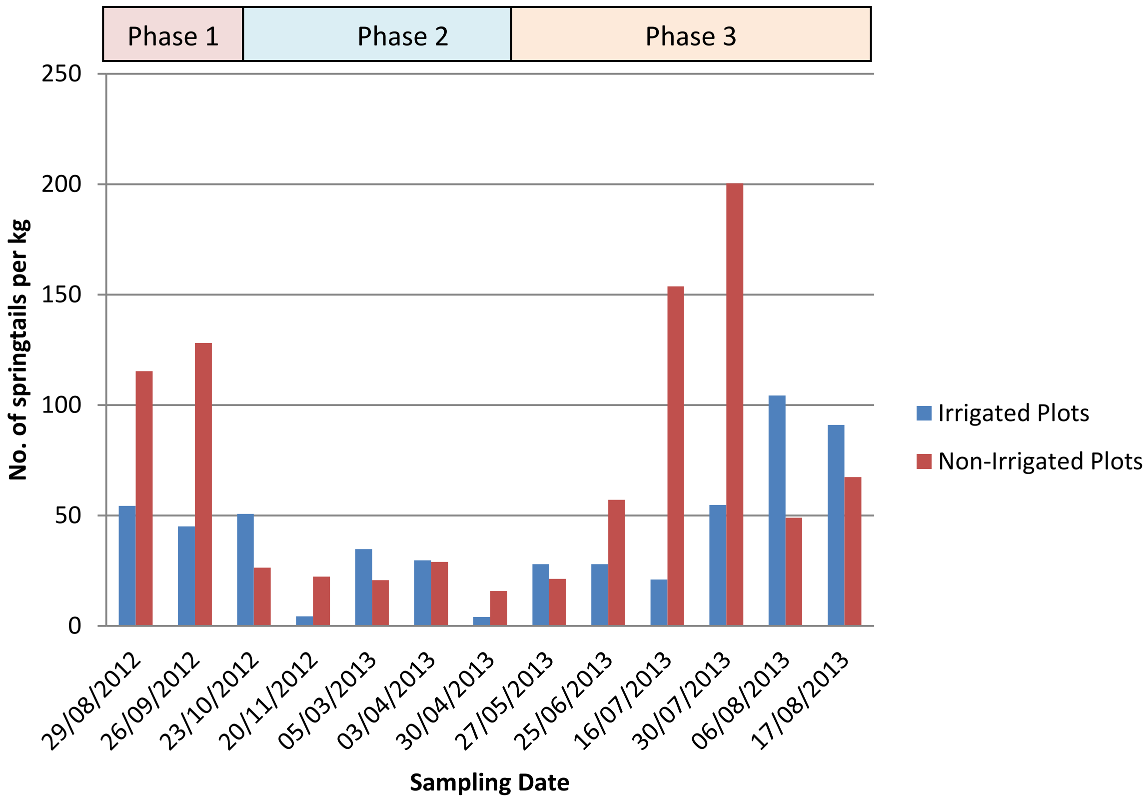

Figure 4.6: Average number of springtails (per kg of dry soil) in irrigated and non-irrigated plots at Culmore SRC willow plantation

b) The data obtained for the abundance of springtails in irrigated and non-irrigated plots in Culmore was statistically tested to determine whether there was a significant difference between them. The data sets were non-parametric so Kruskal-Wallis was applied. There was no significant difference in the abundance of springtails in irrigated and non-irrigated plots when municipal effluent was applied at a rate of $30m^3$/ha/day both during irrigated periods only (p=0.06: C.I.=0.05) or for all sampling events (p=0.065: C.I.=0.05) Detailed results of statistical tests are provided in Appendix D (Table D3).

c) In Phase 1, the average number of springtails in irrigated plots was 49.7/kg of dry soil and 121.7/kg of dry soil in non-irrigated plots. The corresponding values in Phase 3 were 54.5/kg and 91.4/kg of dry soil, respectively. Since there were only two sampling events for springtails in Phase 1, it was not statistically valid to determine significant differences between their abundance in Phase 1 as compared to Phase 3 although upon initial observation, the values appear quite similar.

	Plot 7: Non-Irr.	Plot 10: Irrigated	Plot 8: Irrigated	Plot 11: Non-Irr.	Plot 9: Non-irr.	Plot 12: Irrigated	Average Irrigated	Average Non-irrigated
	History: Poplar		History: Grassland		History: Willow			
Phase 1								
29/08/2012	270	46	104	20	56	13	*54.3*	*115.3*
26/09/2012	159	34	42	135	90	59	*45*	*128*
Average for Phase 1	**214.6**	**40.5**	**73**	**77.7**	**73**	**36.1**	***49.7***	***121.7***
Phase 2								
23/10/2012	25	13	44	18	36	95	*50.7*	*26.3*
20/11/2012	20	8	0	14	33	5	*4.3*	*22.3*
05/03/2013	45	49	11	13	4	44	*34.7*	*20.7*
03/04/2013	63	69	0	10	14	20	*29.7*	*29*
30/04/2013	5	10	0	42	0	2	*4*	*15.7*
Average for Phase 2	**31.7**	**29.7**	**10.9**	**19.3**	**17.4**	**33**	***24.7***	***22.8***
Phase 3								
27/05/2013	12	48	10	27	25	26	*28*	*21.3*
25/06/2013	75	8	63	47	49	13	*28*	*57*
16/07/2013	211	10	36	196	54	17	*21*	*153.7*
30/07/2013	143	60	93	211	247	11	*54.7*	*200.3*
06/08/2013	47	171	98	61	39	44	*104.3*	*49*
17/08/2013	84	105	124	102	16	44	*91*	*67.3*
Average for Phase 3	**95.3**	**67**	**70.6**	**107.5**	**71.6**	**25.4**	***54.5***	***91.4***

Table 4.5: Number of springtails (kg of dry soil) in non-irrigated and irrigated plots at Culmore SRC willow during the three main sampling phases and the averages for total irrigated vs. total non-irrigated plots. Previous cropping history is also shown

Effects of Previous Cropping History on Abundance of Springtails

Figure 4.7 shows the average abundance of springtails in combined irrigated and non-irrigated plots with a common planting history at Culmore. There was a substantial variation observed over the investigation period in the abundance of springtails in all plots with a common planting history. The number of springtails in plots previously planted with grass varied from a peak value of 211/kg of dry soil (30/07/2013) to zero recovery on three sampling occasions. The number of springtails in plots previously planted with poplar varied from a peak value of 270/kg of dry soil (29/08/2012) to the lowest value of 5/kg of dry soil (30/04/2013). The number of springtails in plots previously planted with willow varied from 247/kg of dry soil (30/01/2013) to zero (30/04/2013). Average values for springtail abundance was, in general, higher for plots previously planted with poplar than in other plots particularly in Phases 1 and 2. The significance of cropping history on the abundance of springtails was tested using Kruskal-Wallis for combined irrigated and non-irrigated plots. The outcome of this statistical testing revealed some significant differences in springtail abundance between different combinations of cropping history and therefore the data sets were tested individually for irrigated plots and also for non-irrigated plots. The outcomes, shown in Table 4.6 reveal that no significant differences were observed in springtail abundance in irrigated plots at Culmore, regardless of planting history during irrigated periods only or for all sampling events. A significant difference in springtail abundance emerged in non-irrigated plots when individual datasets were investigated but this significant difference emerged only when comparing abundances in plots previously planted with poplar to those previously planted with willow in Phases 1 and 3 only.

The data was further tested using PerMANOVA to ensure that planting history was not a factor in outcomes observed when statistically testing the significance of the abundance of springtails in irrigated vs. non-irrigated plots**.** No combination effects emerged during irrigated periods only (p=0.848; C.I. =0.05) or during the entire investigation period (p=0.754; C.I. =0.05).

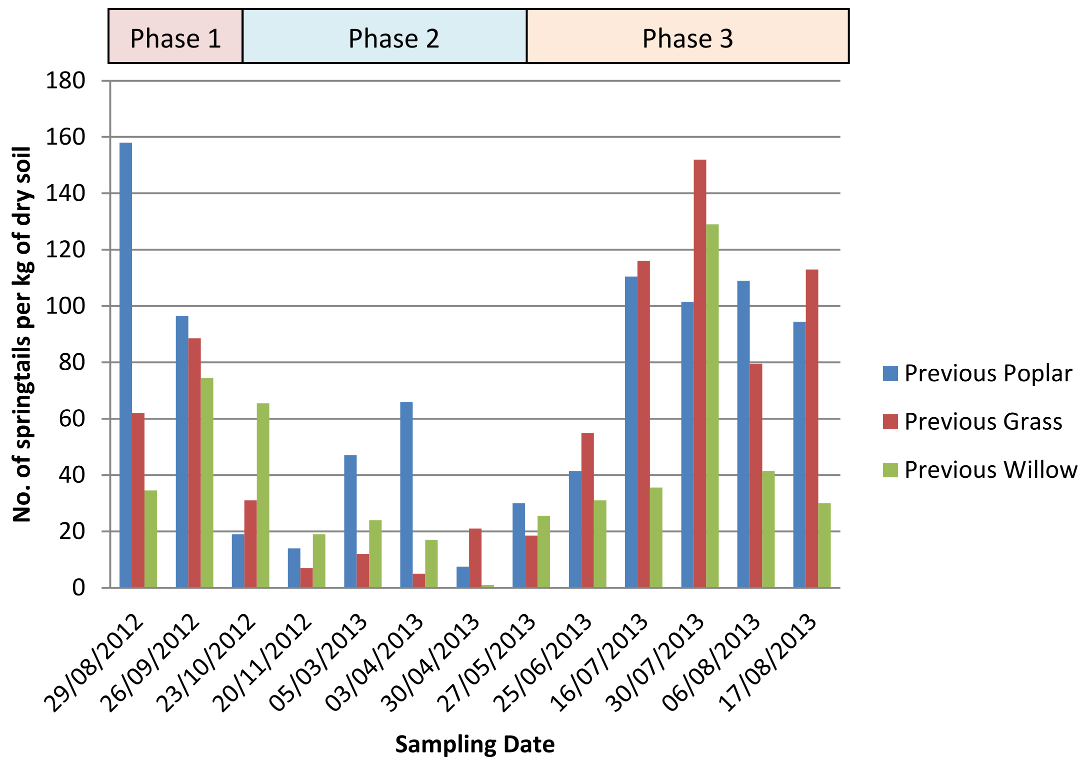

Figure 4.7: Average number of springtails (per kg of dry soil) in irrigated and non-irrigated plots with a common planting history at Culmore

Table 4.6: Outcomes of statistical testing for differences in the abundance of springtails in various combinations of planting history in irrigated and non-irrigated plots in Culmore SRC willow plantation

Irrigated	**Phase 1, 2 and 3**	**Phase 1 and 3**
Poplar and Grass	*p*= 0.263	*p*= 0.290
Poplar and Willow	*p*= 0.158	*p*= 0.114
Willow and Grass	*p*= 0.979	*p*= 0.943
Non-Irrigated	**Phase 1, 2 and 3**	**Phase 1 and 3**
Poplar and Grass	*p*= 0.107	*p*= 0.433
Poplar and Willow	*p*= 0.054	*p*= 0.043
Willow and Grass	*p*= 0.772	*p*= 0.724

4.1.4 Relationship between the Abundance of Earthworms, Springtails and Mites and Soil Characteristics

During the investigation period, measurements were taken on each sampling occasion of soil temperature. Samples were also taken and returned to the laboratory for pH and percentage soil moisture content (%SMC) determinations. Table 4.7 shows the summary results of these measurements. The full table of results can be seen in Appendix B (B13-15). The main points to note in relation to soil pH are as follows;

(a) A similar soil pH of 4.7 was observed during irrigated periods in irrigated and non-irrigated plots. This was also the case over the entire investigation period, where the average pH was 4.7 in irrigated plots and 4.6 in non-irrigated plots.

(b) No significant difference was observed in relation to soil pH between combined irrigated vs combined non-irrigated plots during irrigated months only (p=0.409; C.I=0.05) or across all sampling dates (p=0.209; C.I.=0.05).

(c) The pH range varied considerably over the investigation period in both irrigated and non-irrigated plots i.e. by 1.7 pH units in irrigated plots (4.1-5.8) and by 2 units in non-irrigated plots (3.7-5.7). The highest pH range was observed during irrigated periods in both irrigated (4.1-5.8) and non-irrigated plots (4.3-5.7).

(d) The soil pH decreased significantly from Phase 1 to Phase 3 across all plots (p= 0.001; C.I. =0.05) from an average pH of 5.0 to 4.4.

(e) A significant difference was observed in soil pH between individual irrigated plots, both during irrigated periods only (p=0.001; C.I=0.05) and also over the entire investigation period (p=0.001; C.I=0.05). This was due to the higher pH values observed in Plot 12 which had an average pH of 4.8 over the investigation period as compared to pH 4.6 for other irrigated plots. No significant difference in soil pH was observed between individual non-irrigated plots over the course of the investigation.

(f) A significant difference was observed in soil pH in plots with a different planting history over the entire investigation period (p=0.001; C.I. =0.05) with the lowest pHs observed in plots previously planted with poplar (i.e. Plots 7 and 10). The highest pH values were observed in plots previously planted with willow (i.e. Plots 9 and 12).

(g) When individual plots with a shared planting history were statistically tested to determine whether there was a significant difference in pH between irrigated and non-irrigated plots the only significant differences that arose were in plots previously planted with willow during irrigated periods (p=0.001; C.I = 0.05) and over the entire investigation period (p=0.003; C.I = 0.05). There was no significant difference in pH between irrigated and non-irrigated plots that had been previously planted with poplar or grass.

Detailed results of all aforementioned statistical analysis are presented in Appendix D (Table D7).

The main points to note in relation to soil temperature are as follows;

(a) During the investigation period, the temperature of the soil varied by 14^{o}C (5-19^{o}C). The lowest soil temperatures reached were 5^{o}C during April 2013 with the highest temperature observed in July 2012 (19^{o}C).

(b) The mean temperature in both irrigated and non-irrigated plots was higher during the irrigation period (15^{o}C) as compared to the non-irrigation period (8^{o}C). Similar average soil temperatures were observed in Phase 1 and Phase 3 of the investigation.

(c) No significant difference was observed in relation to soil temperature between irrigated and non-irrigated plots across all sampling dates (p=1.0; C.I = 0.05) or during irrigated months only (p=1.0; C.I = 0.05).

(d) No significant differences in soil temperature emerged between irrigated and non-irrigated plots with a shared planting history during irrigated periods only or during the entire investigation period.

Detailed results of all aforementioned statistical analysis are presented in Appendix D (Table D7).

The main points to note in relation to % SMC are as follows;

(a) The mean %SMC observed during irrigated periods in Culmore was 42.4% in irrigated plots and 42.7% in non-irrigated plots. A narrower range of % SMC values was observed in irrigated plots (i.e. 37.6-45.3%) as compared to non-irrigated plots (29.9-53.5%) during irrigated periods.
(b) Over the course of the investigation, similar mean %SMC values were observed in irrigated plots (i.e. 43.4%) as compared to non-irrigated plots (i.e. 43.9%) and a similar range of %SMC values were recorded (i.e. 33.8-66.4% in irrigated plots and 29.9-53.5% in non-irrigated plots).
(c) There was no significant difference observed in % SMC between irrigated and non-irrigated plots during irrigated periods only (p=0.950; C.I. =0.05) or across all sampling dates (p=0.718; C.I. =0.05).
(d) No significant difference was observed in the %SMC in soils with a different planting history during the irrigation period only (p=0.474; C.I. =0.05) or across all sampling dates (p= 0.944; C.I. =0.05).
(e) No significant differences in %SMC emerged between irrigated and non-irrigated plots with a shared planting history over irrigated periods only or during the entire investigation period.

Detailed results of all aforementioned statistical analysis are presented in Appendix D (Table D7).

Correlation tests were performed to determine whether any relationships existed between the various soil parameters monitored and the abundance of earthworms, springtails or mites over the entire investigation period. All correlation results are provided in Appendix E (Table E1) but the main outcomes were that the correlation between the abundance of earthworms, springtails and mites with % SMC (range 28.6% to 61.4%) and soil pH (range 3.7-5.8) were weak.

Table 4.7: Summary table of results for soil temperature, pH and % SMC in irrigated and non-irrigated plots at Culmore SRC willow plantation

Plot		**pH**		**Temperature(^{o}C)**		**% SMC**	
		Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
7 Non-irrigated	*All months*	4.0-5.3	4.6	5-19	11.8	36.0-52.4	44.7
	Irrigated months	4.4-5.3	4.7	11-19	15.4	36.0-52.4	44.3
	Non-irr. months	4.0-4.7	4.3	5-10	7.5	41.5-47.6	45.5
8 Irrigated	*All months*	4.1-5.8	4.6	5-19	11.8	33.8-53.3	46.0
	Irrigated months	4.1-5.8	4.8	11-19	15.4	38.7-53.3	46.1
	Non-irr. months	4.2-4.7	4.5	5-10	7.5	33.8-49.7	42.9
9 Non-irrigated	*All months*	4.2-5.7	4.7	5-19	11.8	32.2-50.6	42.6
	Irrigated months	4.3-5.7	4.8	11-19	15.4	32.2-50.6	41.4
	Non-irr. months	4.2-4.7	4.5	5-10	7.5	44.5-45.8	45.3
10 Irrigated	*All months*	4.1-5.6	4.6	5-19	11.8	29.9-52.6	42.4
	Irrigated months	4.2-5.6	4.7	11-19	15.4	29.9-52.6	43.0
	Non-irr. months	4.1-4.7	4.3	5-10	7.5	38.4-43.2	41.2
11 Non-irrigated	*All months*	3.7-5.7	4.6	5-19	11.8	30.1-53.5	44.4
	Irrigated months	4.5-5.7	4.8	11-19	15.4	30.1-50.7	42.3
	Non-irr. months	3.7-4.8	4.3	5-10	7.5	46.5-53.5	49.0
12 Irrigated	*All months*	4.4-5.6	4.8	5-19	11.8	28.6-61.4	42.7
	Irrigated months	4.5-5.6	4.9	11-19	15.4	28.6-49.6	40.0
	Non-irr. months	4.4-5.1	4.7	5-10	7.5	35.8-61.4	48.9
All Non-irrigated Plots	*All months*	3.7-5.7	4.6	5-19	11.8	29.9-53.5	43.9
	Irrigated months	4.3-5.7	4.7	11-19	15.4	29.9-53.3	42.7
	Non-irr. months	3.7-4.8	4.4	5-10	7.5	41.5-53.5	46.6
All Irrigated Plots	*All months*	4.1-5.8	4.7	5-19	11.8	33.8-61.4	43.4
	Irrigated months	4.1-5.8	4.7	11-19	15.4	37.6-45.3	42.4
	Non-irr. months	4.1-5.1	4.5	5-10	7.5	33.8-61.4	44.3

4.2 Abundance of Bio-Indicators in Hillsborough SRC Willow Plantation

Dairy parlour washings (D.P.W) were applied to selected plots in the Hillsborough SRC willow plantation at three different application rates i.e. $18m^3$/ha/day (T2), $34m^3$/ha/day (T3) and $44m^3$/ha/day (T4) from May to mid-October in 2012 and from May to October in 2013. A control plot was not irrigated. For ease of reporting, the 2012 irrigation period will be referred to as Phase 1, the 2013 irrigation period will be referred to as Phase 3 and the interim period between irrigation will be referred to as Phase 2. The abundance of selected bio-indicators (earthworms, springtails and mites) in irrigated and non-irrigated plots were investigated in Phases 1, 2 and 3. The results of the investigation are presented separately for each bio-indicator. The plantation set-up in Hillsborough provided for the planting of willow in single genotype plots (SGP) and mixed genotype plots (MGP) as previously described in Section 3.2 and the impact of this plantation set-up will also be reported upon separately.

4.2.1 Earthworms

Effect of irrigation with D.P.W. at different rates of application

The number of earthworms recovered (m^2 of soil) in control plots and plots in receipt of varying levels of D.P.W. are shown in Table 4.8 for both SGP and MGP for specified sampling dates in Phases 1, 2 and 3. Each value represents an average of two samples taken from each plot on a particular sampling date. Sampling commenced in Phase 1 in July 2012 and continued until the end of the 2012 irrigation period. Samples were also taken in the interim period between irrigations to determine earthworm abundance outside of the irrigation period. In Phase 3 sampling commenced in May and continued until the end of August. The main trends evident from Table 4.8 are as follows;

(a) The greatest numbers of earthworms (m^2 of soil) were present in the control and T2 plots as compared to T3 and T4 plots on all sampling dates. This trend is illustrated in Figure 4.8. The peak number of earthworms (m^2) observed in the control plots was 109/m^2 (13/08/2012 in MGP). The peak number of earthworms (m^2) observed in the T2 plot was 148/m^2 (20/07/2012 in MGP). In contrast, the peak number of earthworms (m^2) observed in the T3 and T4 plots were 19/m^2 (11/09/2012 in MGP) and 18/m^2 (11/09/2012 in MGP), respectively.

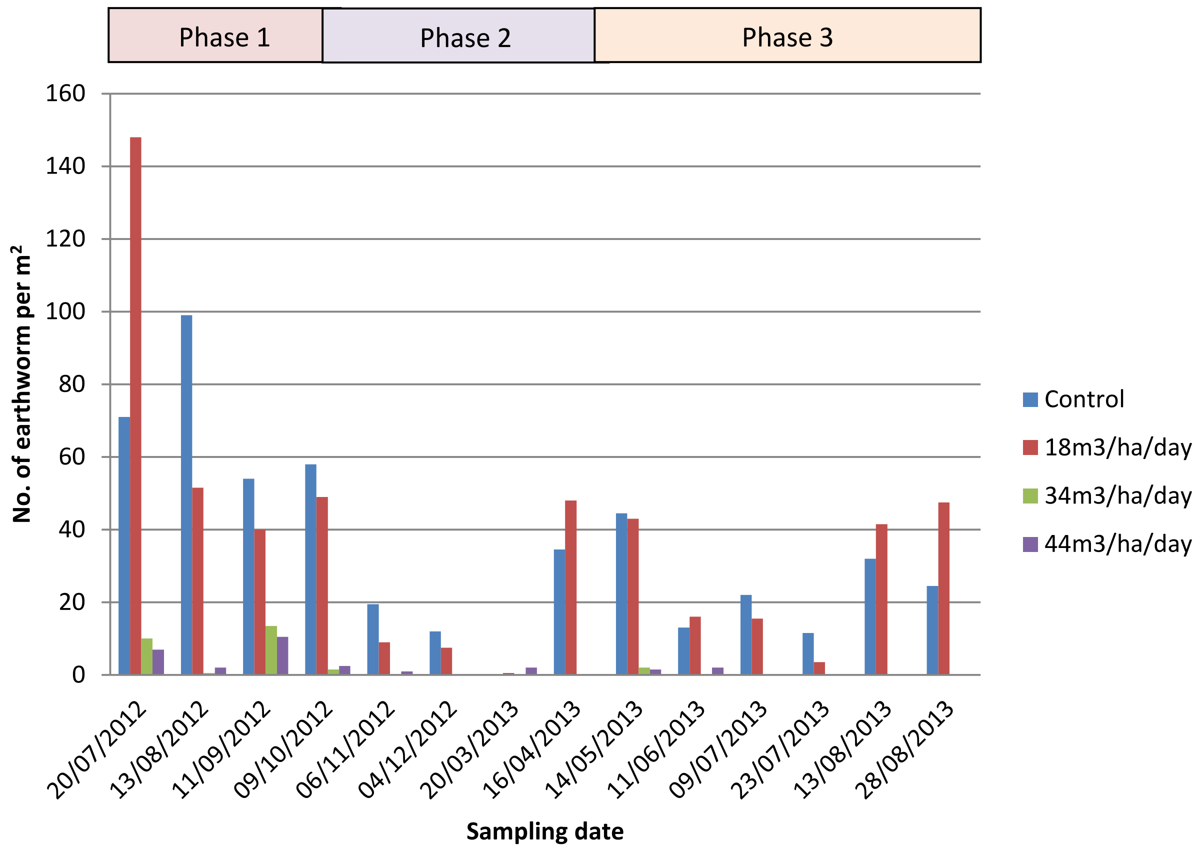

Figure 4.8: Average earthworm numbers (m^2) in control and various treatment plots (combined data for MGP and SGP) at Hillsborough willow plantation

(b) Data obtained for the abundance of earthworms in the various treatment plots in Hillsborough was statistically tested to determine whether there was a significant difference in the abundance of earthworms in each treatment plot as compared to the control plot. Since the data was non parametric. Kruskal-Wallis was applied. Results from Table 4.9 show that an irrigation rate of $18m^3$/ha/day (T2) had no significant effect on earthworm abundance as compared to the control plot during irrigated months only or for all sampling events. When the irrigation rate reached $34m^3$/ha/day (T3) and above, a significant effect on earthworm abundance was observed both during the irrigation periods and on all sampling dates. Detailed results of statistical tests are provided in Appendix D (Table D4).

	Control		18m^3/ha/day		34m^3/ha/day		44m^3/ha/day		Average MGP	Average SGP
	MGP	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**		
Phase 1										
20/07/2012	71	n.d	148	n.d	10	n.d	7	n.d	*59*	*ND*
13/08/2012	109	89	103	0	1	0	4	0	*54.3*	*22.3*
11/09/2012	51	57	31	49	19	8	18	3	*29.7*	*29.3*
9/10/2012	49	67	48	50	0	3	5	0	*25.5*	*30*
Average for Phase 1	**70**	**71**	**82.5**	**33**	**7.5**	**2.8**	**8.5**	**0.8**	***42.1***	***27.2***
Phase 2										
6/11/2012	22	17	7	11	0	0	0	2	*7.25*	*7.5*
4/12/2012	15	9	12	3	0	0	0	0	*18*	*6*
20/03/2013	0	0	1	0	0	0	4	0	*1.3*	*0*
16/04/2013	28	41	42	54	0	0	0	0	*38.5*	*23.8*
Average for Phase 2	**16.2**	**16.8**	**15.5**	**17**	**0**	**0**	**1**	**0.5**	***16.3***	***9.3***
Phase 3										
14/05/2013	32	57	37	49	4	0	3	0	*19*	*26.5*
11/06/2013	15	11	17	15	0	0	0	4	*8*	*7.5*
9/07/2013	21	23	11	20	0	0	0	0	*8*	*10.8*
23/07/2013	0	23	0	7	0	0	0	0	*0*	*7.5*
13/08/2013	31	33	18	65	0	0	0	0	*12.3*	*24.5*
28/8/2013	2	47	47	48	0	0	0	0	*12.3*	*23.8*
Average for Phase 3	**16.8**	**32.3**	**21.7**	**34**	**0.6**	**0**	**0.5**	**0.7**	***9.9***	***16.8***

Table 4.8: Number of earthworms (m^2) in control plot and various treatment plots at Hillsborough SRC willow plantation during Phase 1, 2 and 3 in SGP and MGP

Table 4.9: Outcome of statistical analysis to determine differences in earthworm abundance in control plots and plots receiving various volumes of D.P.W.

	Phase 1, 2 and 3	Phase 1 and 2
Control vs. $18m^3$/ha/day	*p*= 0.930	*p*= 0.832
Control vs. $34m^3$/ha/day	*p*= 0.001	*p*= 0.001
Control vs. $44m^3$/ha/day	*p*= 0.001	*p*= 0.001

(c) Higher average numbers of earthworms (m^2 of soil) were apparent during Phase 1 of the investigation across all treatment levels and in the control than were apparent during Phase 3. This was particularly noticeable for the control and T2 plot. There were frequent events of zero recovery of earthworms in T3 and T4 in Phase 3 as compared to the control or T2 plot. When the data was statistically analysed using Kruskal-Wallis for significant differences between Phase 1 and Phase 3, there was no significant difference evident across all treatment levels (p=0.378; C.I=0.05). The data was further tested for individual treatment levels as it was expected that a significant difference would arise in earthworm abundance between Phase 1 and 3 in the control plot and T2 plot. This however was not the case. Detailed results of statistical analysis are presented in Appendix D (Table D10).

Ten species of earthworm were recovered across all sampling dates. The majority of species present were epigeic and included *E. tetraedra, L. eisenia, L. festivus, L. rubellus* and *S. mammalis.* Four endogeic species were present on site *(O. tyrtaeum, A. rosea, A. limicola* and *A. icterica* but these were recovered in very low numbers. One anecic earthworm species, *L. terrestris* was also recovered but again in low numbers. Relatively large numbers of non-clitellate earthworms were recovered in Hillsborough. The numbers of epigeic, endogeic, anecic and non-clitellate earthworms recovered on each sampling date is shown in Appendix B (Table B7).

Figure 4.9 is a species-area curve for earthworm sampling in Hillsborough. No predictions were made through jack-knife and Chao estimates when carrying out the species-area curve, this indicates that no rare species are present at the site. The flattening of the curves in Fig. 4.9 indicates that sufficient sampling was carried out (top line). The bottom line account for the abundance of the species present on the site and the flattening of that line indicates that the level of sampling carried out was enough to be representative of the species numbers.

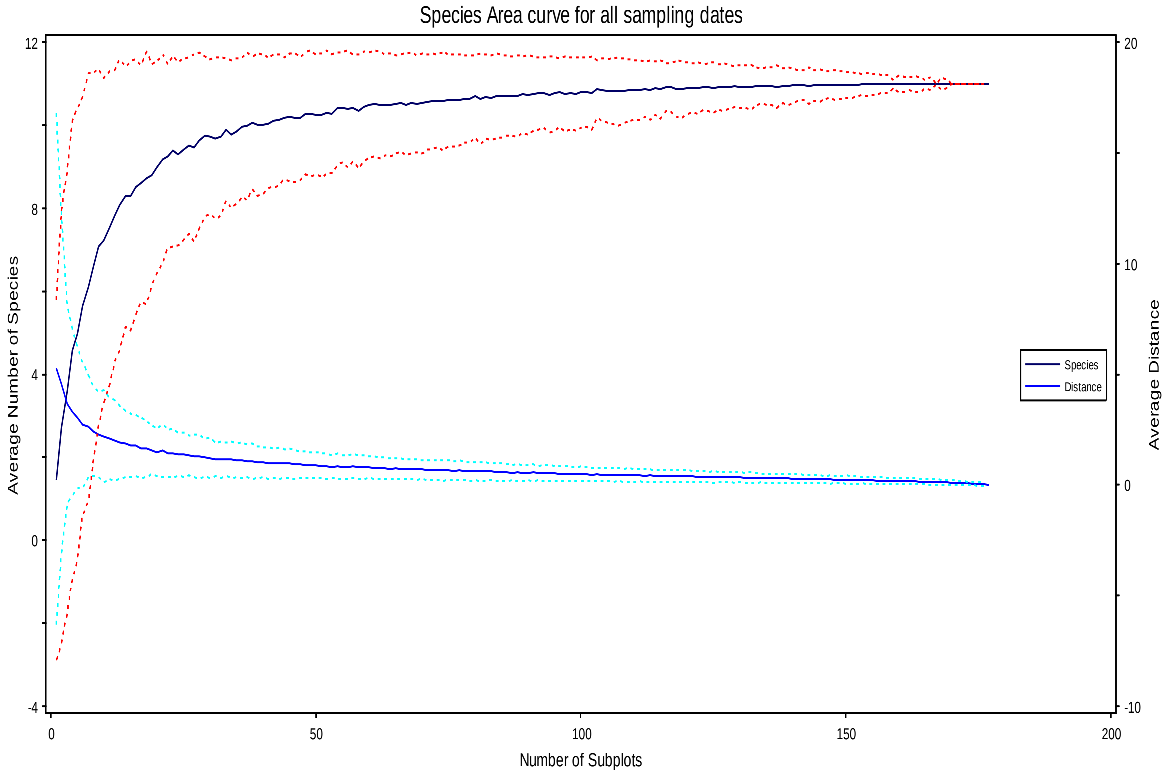

Figure 4.9: Species area curve for earthworms sampled at Hillsborough

__Effect of planting with mixed vs single willow genotypes on the abundance of earthworms__

From Table 4.8, no obvious trends are immediately apparent in relation to the abundance of earthworms in SGP vs. MGP with higher numbers present in MGP on some sampling occasions and in SGP on other sampling occasions. Figure 4.10 shows the average number of earthworms (per m^2 of soil) for each month sampled in SGP and MGP using combined abundance data for all treatment plots and the control plot. Results for July 2012 are not included in this figure as sampling could not take place in the SGP in July 2012.

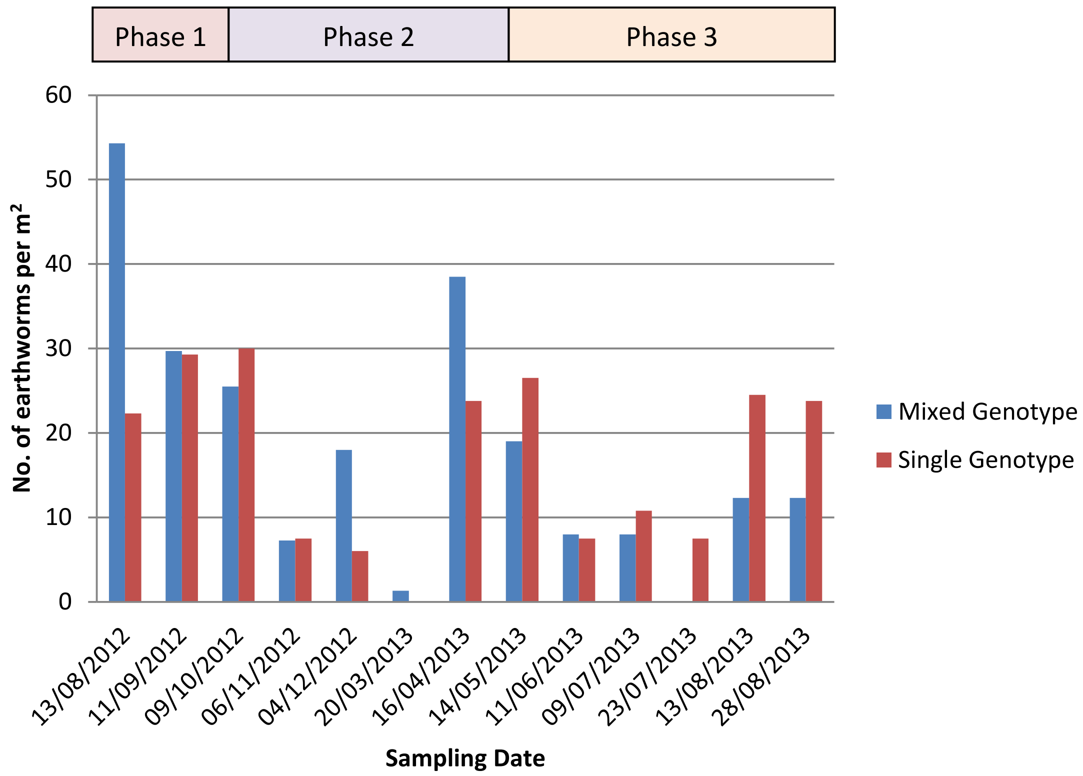

Figure 4.10: Average earthworm numbers (m^2 of soil) in MGP and SGP in Hillsborough SRC willow plantation

The data sets were tested for significant differences in earthworm abundance in SGP as compared to MGP using Kruskal-Wallis. There was no significant difference between earthworm abundance in SGP and MGP when all treatment plots were considered during irrigated periods only (p= 0.380; C.I. =0.05) or for all sampling events (p= 0.444; C.I. =0.05).

PerMANOVA was used to test the interaction between irrigation level and planting regime on the abundance of earthworms. The outcome of this test revealed that there was no interaction between planting regime and irrigation level when all plots were tested during irrigated periods (p=0.712; C.I.=0.05) or for all sampling events (p= 0.721; C.I.=0.05). Similarly, no interaction was evident within the control plot or various treatment plots when tested in isolation. Detailed results of statistical tests are provided in Appendix D (Table D4). As no interaction was evident it was possible to determine whether the earthworms may be better able to withstand irrigation at the higher rates in an MGP as compared to a SGP. The outcome of this statistical testing (using Kruskal-Wallis) reveals that earthworms in a MGP are no more resilient to higher irrigation levels at and above $34m^3$/ha/ day than those in a SGP. Detailed results of statistical tests are provided in Appendix D (Table D4).

4.2.2 Mites

<u>*Effect of irrigation with D.P.W. at different rates of application*</u>

The number of mites (per kg of dry soil) in the control plot and plots in receipt of varying levels of D.P.W. are shown in Table 4.10 for both SGP and MGP on specified sampling dates during Phases 1, 2 and 3. Each value represents an average of three samples taken from each plot. Eight sampling events are shown for irrigated periods (Phases 1 and 3) while four are shown for the non-irrigated period (Phase 2). The main trends evident from the data in Table 4.10 are as follows;

(a) There are large variations in the abundance of mites in all of the treatment plots and in the control over the duration of the investigation. This trend is evident in Figure 4.11 which shows the average abundance of mites in combined MGP and SGP plots at each treatment level over the duration of the investigation.

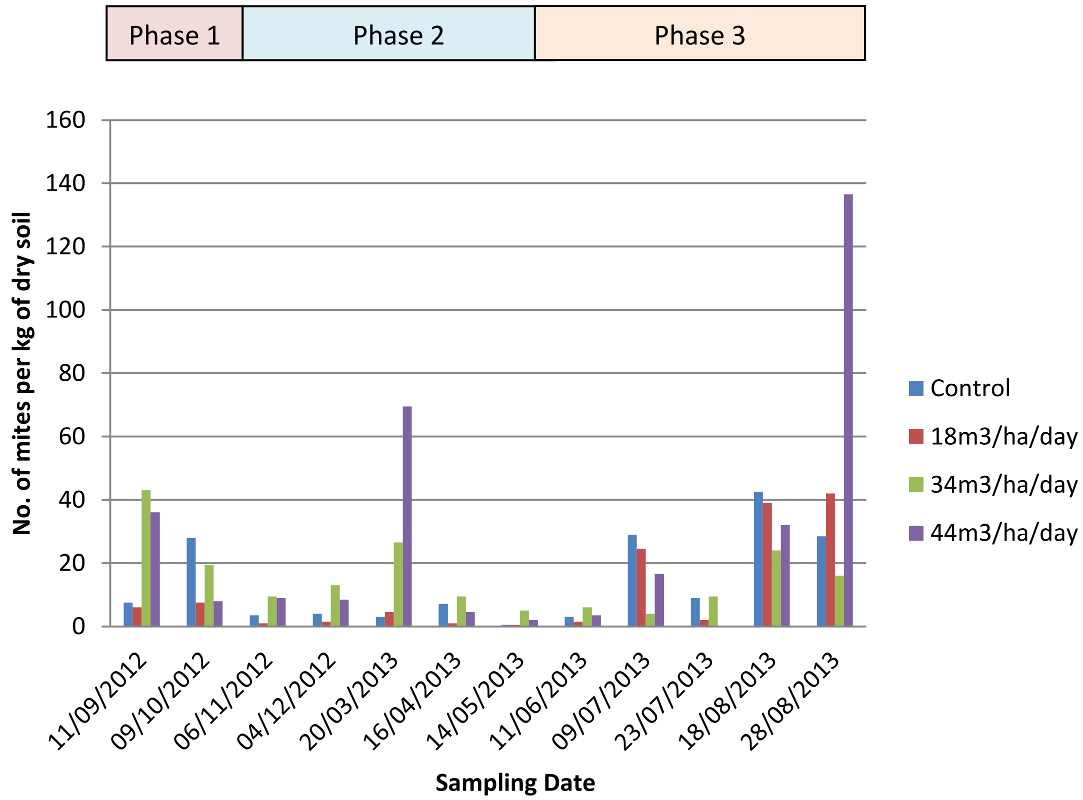

Figure 4.11: Average number of mites (per kg of dry soil) in control and various treatment plots (combined data for MGP and SGP) at Hillsborough

	Control		18m^3/ha/day		34m^3/ha/day		44m^3/ha/day		Average MGP	Average SGP
	MGP	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**		
Phase 1										
11/09/2012	9	6	6	6	46	40	42	30	*25.8*	*20.5*
9/10/2012	17	39	15	0	13	26	11	5	*14*	*17.5*
Average for phase 1	**13**	**22.5**	**10.5**	**3**	**29.5**	**33**	**26.5**	**17.5**	***19.9***	***19.0***
Phase 2										
6/11/2012	0	7	0	2	1	18	1	17	*0.5*	*11*
4/12/2012	0	8	3	0	7	19	17	0	*5.3*	*6.8*
20/03/2013	2	4	3	6	20	33	134	5	*39.3*	*12*
16/04/2013	7	7	0	2	14	5	9	0	*7.5*	*3.5*
Average for Phase 2	**2.3**	**6.5**	**1.5**	**2.5**	**10**	**18.9**	**40.3**	**5.5**	***13.2***	***8.3***
Phase 3										
14/05/2013	1	0	1	0	10	0	4	0	*4*	*0*
11/06/2013	2	4	2	1	11	1	2	5	*4.3*	*2.3*
9/07/2013	14	44	34	15	7	1	23	10	*19.3*	*17.5*
23/07/2013	3	15	3	1	8	11	0	0	*3.5*	*6.8*
18/08/2013	22	63	22	56	32	16	28	36	*26*	*42.8*
28/8/2013	47	10	45	39	28	4	261	12	*95.3*	*16.3*
Average for Phase 3	**14.8**	**22.7**	**17.8**	**22.4**	**16**	**5.5**	**53**	**10.5**	***25.4***	***14.3***

Table 4.10: Number of mites (kg of dry soil) in the control plot and various treatment plots at Hillsborough SRC willow plantation during Phase 1, 2 and 3 in SGP and MGP

(b) Relatively high abundance values are observed for mites in treatment plots receiving high irrigation volumes. The two highest abundance values observed during the investigation (261 mites/kg of dry soil -28/08/2013 in MGP and 134 mites/kg of dry soil-20/03/2013 in MGP) were in T4 in receipt of $44m^3$/ha/day.

(c) Data obtained for the abundance of mites in the various treatment zones in Hillsborough was statistically tested to determine whether there was a significant difference in the abundance of mites at each irrigation rate as compared to the control zone. Since the data was non parametric, Kruskal-Wallis was applied. There was no significant difference in the abundance of mites at any of the irrigation levels investigated during all sampling events or for irrigated periods only and using combined data for MGP and SGP or when MGP and SGP were tested in isolation. Detailed results of statistical tests are provided in Appendix D (Table D5).

(d) In general, the numbers of mites are greater during irrigated periods (Phase 1 and 3) as compared to Phase 2. Since there were only two sampling events for mites in Phase 1, it was not statistically valid to determine significant differences between the abundance of mites in Phase 1 as compared to Phase 3; however upon initial observation of the data it would appear that the abundance values are fairly similar.

(e) A number of sampling events yielded zero or low recovery of mites across all treatment plots.

Effect of planting with mixed vs single willow genotypes on the abundance of mites

From Table 4.10, no obvious trends are immediately apparent in relation to the abundance of mites (per kg of dry soil) in SGP vs. MGP with higher numbers present in MGP on some sampling occasions and in SGP on others. Figure 4.12 shows the mean number of mites per kg of dry soil for each month sampled in SGP and MGP using combined abundance data for all treatment plots and the control plot. When combined data is presented for all plots, greater peak numbers are evident for MGP than SGPs.

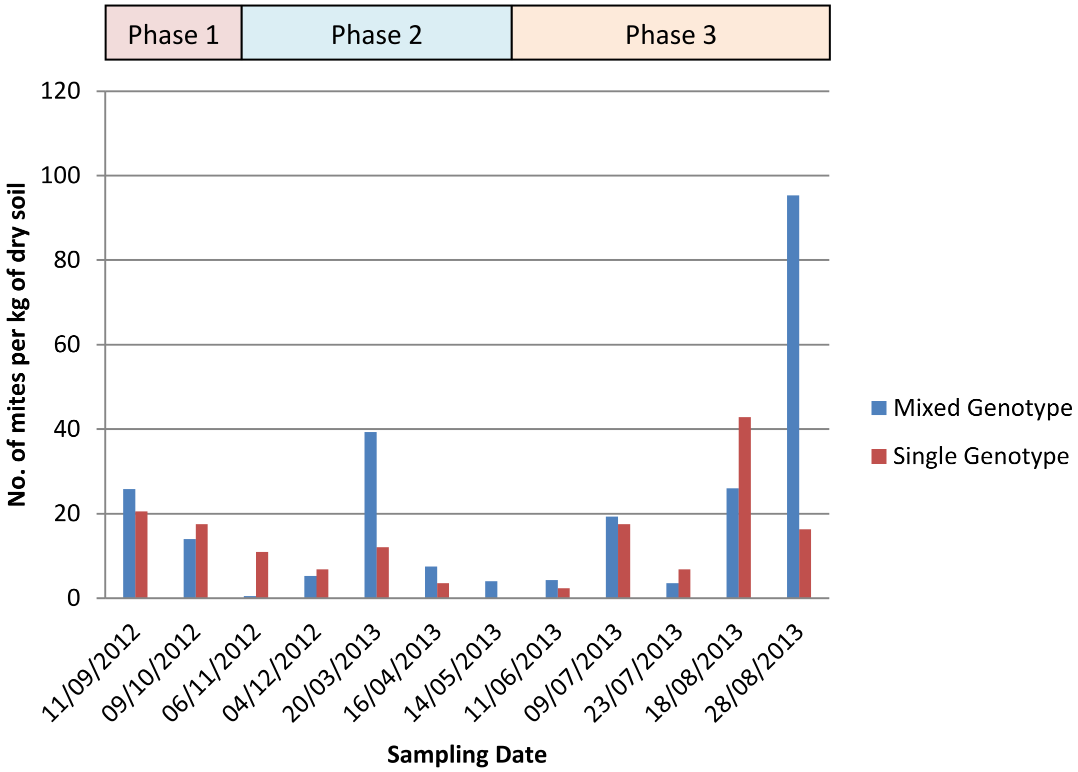

Figure 4.12: Average number of mites (per kg of dry soil) in MGP and SGP in Hillsborough SRC willow plantation

The data sets were tested for significant differences in the abundance of mites in SGP and MGP in each of the treatment zones at Hillsborough and in the control zone. There was no significant difference between the abundance of mites in SGP and MGP when the control and all treatment plots were considered during irrigated periods only (p=0.405; C.I.=0.05) or for all sampling events (p=0.560; C.I.=0.05).

PerMANOVA was used to test the interaction between planting regime and irrigation level on the abundance of mites. The outcome of this test revealed that there was no interaction between irrigation level and planting regime when all plots were considered over the entire investigation period (p=0.108; C.I. =0.05) or during irrigated periods only (p=0.094; C.I. =0.05). Similarly no interaction was evident when the control or various treatment plots were tested in isolation. Detailed results of statistical tests are provided in Appendix D (Table D5).

4.2.3 Springtails

Effect of irrigation with D.P.W. at different rates of application

The number of springtails (per kg of dry soil) in the control plot and plots in receipt of varying levels of D.P.W. are shown in Table 4.11 for both SGP and MGP on specified sampling dates during Phases 1, 2 and 3. Each value represents an average of three samples taken from each plot. Eight sampling events are shown for irrigated periods (Phases 1 and 3) while four are shown for the non-irrigated period (Phase 2). The main trends evident from the data in Table 4.11 are as follows;

(a) Variations occur in the abundance of springtails in all of the treatment plots and in the control over the duration of the investigation. This trend is evident in Figure 4.13 which shows the average abundance in combined MGP and SGP plots at each treatment level over the duration of the investigation. The highest overall peak numbers of springtails observed over the investigation period were in the control plot but there is no immediate trend apparent when individual sampling dates are considered i.e. all treatment plots experience peak springtail numbers on individual sampling dates over the investigation period.

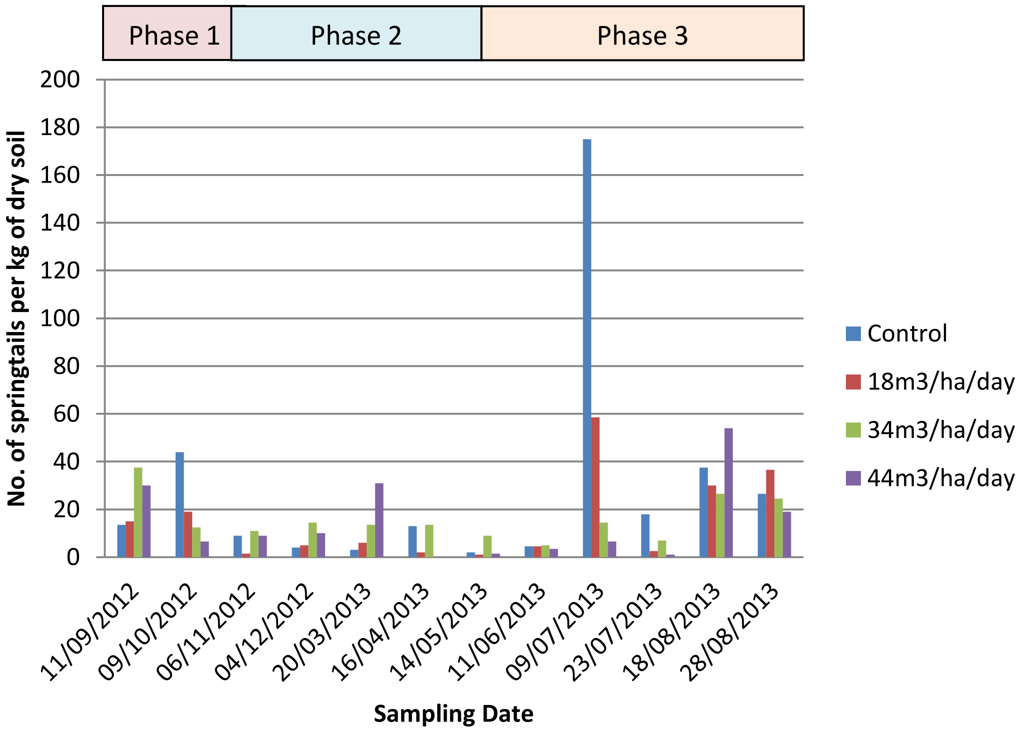

Figure 4.13: Average number of springtails (kg of dry soil) in control and various treatment plots (combined data for MGP and SGP) in Hillsborough

	Control		$18m^3$/ha/day		$34m^3$/ha/day		$44m^3$/ha/day		Average MGP	Average SGP
	MGP	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**	**MGP**	**SGP**		
Phase 1										
11/09/2012	13	14	7	23	40	35	42	18	*25.5*	*22.5*
9/10/2012	34	54	30	8	18	7	7	6	*22.3*	*18.8*
Average for Phase 1	**23.5**	**34**	**18.5**	**15.5**	**29**	**21**	**24.5**	**12**	***23.9***	***20.7***
Phase 2										
6/11/2012	3	15	0	3	2	20	1	17	*1.5*	*13.8*
4/12/2012	1	7	10	0	17	12	20	0	*10.8*	*4.8*
20/03/2013	2	4	2	10	7	20	58	4	*17.3*	*9.5*
16/04/2013	5	21	4	0	23	4	0	0	*8*	*6.3*
Average for Phase 2	**2.8**	**11.8**	**4**	**3.3**	**12.3**	**14**	**19.8**	**5.3**	***9.4***	***5.6***
Phase 3										
14/05/2013	4	0	2	0	18	0	3	0	*6.8*	*0*
11/06/2013	4	5	4	5	8	2	3	4	*4.8*	*4*
9/07/2013	150	200	106	11	26	3	10	3	*73*	*54.3*
23/07/2013	8	28	3	2	5	9	0	2	*4*	*10.3*
18/08/2013	15	60	48	12	38	15	25	83	*31.5*	*42.5*
28/8/2013	31	22	15	58	33	16	32	6	*27.8*	*25.5*
Average for Phase 3	**35.4**	**52.5**	**30**	**14.7**	**21.3**	**7.5**	**12.2**	**16.3**	***24.7***	***27.8***

Table 4.11: Number of springtails (kg of dry soil) in the control plot and various treatment plots at Hillsborough SRC willow plantation during the 2012 and 2013 irrigation periods and between irrigation periods in SGP and MGP

(b) Data obtained for the abundance of mites in the various treatment plots in Hillsborough was statistically tested to determine whether there was a significant difference in the abundance of springtails at each irrigation rate as compared to the control zone. Since the data was non parametric, Kruskal-Wallis analysis was applied. The outcome of this testing showed that irrigation with D.P.W. had no significant effect on the abundance of springtails at any of the irrigation levels investigated as compared to the control plot during all sampling events or for irrigated periods only using combined data for MGP and SGP or when MGP and SGP were tested in isolation. Detailed results of statistical tests are provided in Appendix D (Table D6).

(c) On initial inspection, it appeared that the numbers of springtails were greater during the irrigation periods (Phases 1 and 3) as compared to the non-irrigated period. Statistical analysis using Kruskal-Wallis did not, however, reveal a significant difference between these periods ($p=0.693$: C.I. =0.05). Since there were only two sampling events for springtails in Phase 1, it was not statistically valid to determine significant differences between the abundance of springtails in Phase 1 as compared to Phase 3 however upon inspection of the data in Table 4.11; the abundance values appear fairly similar.

(d) Relatively few sampling results of zero or low recovery of springtails are observed in any of the plots at Hillsborough and these occur only between November 2012 and May 2013, the non-irrigated period.

Effect of planting with mixed vs single willow genotypes on the abundance of springtails

Figure 4.14 shows the average number of springtails (per kg of dry soil) for each month sampled in SGP and MGP using combined abundance data for springtails in all treatment plots and the control plot. On initial inspection of Figure 4.14, it appears that the abundance of springtails was higher in MGP as compared to SGP however, when the data sets were tested (Kruskal-Wallis) there was no significant difference between them either during irrigated periods only ($p=0.642$: C.I.=0.05) or on all sampling dates ($p=0.493$: C.I.=0.05).

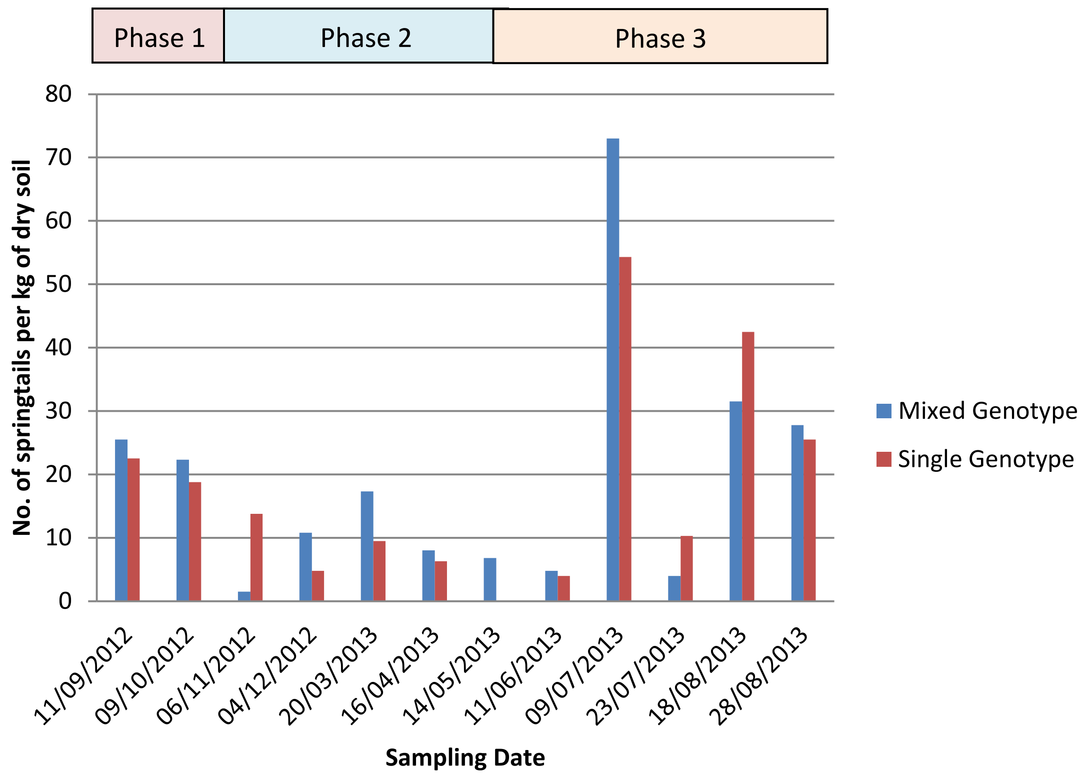

Figure 4.14: Average number of springtails (per kg of dry soil) in MGP and SGP in Hillsborough SRC willow plantation

PerMANOVA was used to test the interaction between irrigation level and planting regime on the abundance of springtails. The outcome of this test revealed that there was no interaction between irrigation level and planting regime using combined data for all treatment plots during irrigated periods only (p=0.784; C.I.=0.05) or for all sampling events (p=0.626; C.I.=0.05).

4.2.4 Relationship between Abundance of Bio-indicators and Soil Characteristics

During the investigation period, in-situ measurements were taken on each sampling occasion of soil temperature. Samples were also taken and returned to the laboratory for determination of soil pH and %SMC. Table 4.12 shows the summary results of these measurements and the full table of values can be found in Appendix B (Table B16-B18).

The main points to note in relation to soil pH are as follows;

(a) The mean soil pH in the control plot was 4.8 and varied from pH 4.4 to 5.3 over the investigation period with a general trend towards a decrease in soil pH from Phase 1 to Phase 3. The pH range was not significantly different between irrigated and non-irrigated periods (p=0.46: C.I. 0.05).

(b) The mean pH in the T2 plot was 4.8 and varied from pH 4.3 to 5.4 during both irrigated and non-irrigated periods, with a general trend towards a decrease in soil pH from Phase 1 to Phase 3. The pH range was not significantly different between irrigated and non-irrigated periods (p=0.62: C.I. 0.05).

(c) The mean pH in the T3 plot was 4.8 and varied from pH 4.3 to 5.7 during both irrigated and non-irrigated periods, with a general trend towards a decrease in soil pH from Phase 1 to Phase 3. The pH range was not significantly different between irrigated and non-irrigated periods (p=0.32: C.I. 0.05).

(d) The mean pH in the T4 plot was 4.8 and varied from pH 4.3 to 5.4 during both irrigated and non-irrigated periods, with a general trend towards a decrease in soil pH from Phase 1 to Phase 3. The pH range was not significantly different between irrigated and non-irrigated periods (p=0.85: C.I. 0.05).

(e) A significant difference in soil pH was observed between Phase 1 and Phase 3 (p=0.001; C.I = 0.05) across all treatment plots. The average pH in Phase 1 (all plots) was 5.0 while it was 4.5 in Phase 3 (all plots).

(f) A significant difference was observed in soil pH between the control and Treatment 4 plot (p=0.015; C.I= 0.05). This difference was only significant however when tested for all Phases of the investigation but not when tested for irrigated periods only (p=0.123; C.I. 0.05). There was no significant difference observed in soil pH between the control plot and T2 and T3 plots over the course of the investigation or during irrigated periods only.

The main points to note in relation to soil temperature are as follows;

(a) The soil temperature varied by 17^{o}C (4-21^{o}C) over the course of the investigation and by 10^{o}C (11-21^{o}C) during irrigation months only. The mean temperature in the control and various irrigated plots was higher during the irrigation period (15.9^{o}C) than the non-irrigated period (6.5^{o}C).

(b) No significant difference was observed in relation to soil temperature between the plots across all sampling dates (p=1.0; C.I = 0.05) or during irrigated months only (p=1.0; C.I = 0.05).

The main points to note in relation to %SMC are as follows;

(a) The average % SMC observed in the control plot was higher during the non-irrigated period (44.5%) than during irrigated periods (34.9%) however, the range of %SMCs observed was much greater during irrigated periods (22.5-45.1%) than during the non-irrigated period (39.5-48.4%).

(b) The average %SMC observed in the T2 plot was somewhat higher during the non-irrigated period (39.6%) than during the irrigated periods (36%) but the range of % SMCs observed was much greater during irrigated periods (24-49.9%) than during the non-irrigated period (34.4-43.1%).

(c) The average %SMC observed in the T3 plot was higher during the non-irrigated period (41.5%) than during irrigated periods (35.1%), however, but the range of %SMCs observed was much greater during irrigated periods (19.9-49.4%) than during the non-irrigated period (33.0-49.4%).

(d) The average %SMC observed in the T4 plot was much higher during the non-irrigated period (41.2%) than during irrigated periods (31.4%), however, but the range of %SMCs observed was much greater during irrigated periods (14.0-41.4%) than during the non-irrigated period (28.7-46.3%).

(e) There was no significant difference in the % SMC content observed during Phase 1 as compared to Phase 3 across any of the treatment plots (*p*=0.309; C.I = 0.05).

(f) There was a significant difference in %SMC observed between the control and all treatment plots over the entire investigation period (*p*=0.03; C.I. =0.05). This difference was not evident however during irrigated periods only when the control was compared to T2 (*p*=0.071; C.I. =0.05), T3 (*p*=0.259; C.I. =0.05), or T4 (*p*=0.114; C.I. =0.05).

(g) The planting regime of willow (MGP vs SGP) did not significantly affect %SMC during all sampling events (*p*=0.683; C.I. =0.05) or during the irrigation period only (*p*=0.778; C.I. =0.05).

Correlation tests were performed to determine whether any relationships existed between the various soil parameters monitored and the abundance of earthworms, springtails or mites over the entire investigation period. All correlation results are provided in Appendix E (Table E2) but the main outcomes were that the correlation between the abundance of earthworms, springtails and mites with % SMC (range 14-49.9%), temperature (range 4-21^{o}C) and soil pH (range 4.3-5.7) were weak.

Table 4.12: Summary table for pH, soil temperature and % SMC in control and various treatment plots at Hillsborough SRC willow plantation

Plot		**pH**		**Temperature (^{o}C)**		**% SMC**	
		Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Control	*All months*	4.4-5.3	4.8	4-21	13.2	22.5-48.4	37.7
	Irrigated months	4.4-5.3	4.8	11-21	15.9	22.5-45.1	34.9
	Non-Irr. months	4.4-5.3	4.8	4-9	6.5	39.5-48.4	44.5
T2: 18m^3/ha/d	*All months*	4.3-5.4	4.8	4-21	13.2	24.0-49.9	37.7
	Irrigated months	4.3-5.4	4.8	11-21	15.9	24.0-49.9	37.0
	Non-Irr. months	4.5-5.4	4.8	4-9	6.5	34.4-43.1	39.6
T3: 34m^3/ha/d	*All months*	4.3-5.7	4.8	4-21	13.2	19.9-49.4	35.4
	Irrigated months	4.4-5.7	4.7	11-21	15.9	19.9-49.4	35.1
	Non-Irr. months	4.3-5.7	4.9	4-9	6.5	33.0-49.4	41.5
T4: 44m^3/ha/d	*All months*	4.3-5.4	4.8	4-21	13.2	14.0-46.3	34.2
	Irrigated months	4.3-5.3	4.8	11-21	15.9	14.0-41.4	31.4
	Non-Irr. months	4.8-5.4	4.9	4-9	6.5	28.7-46.3	41.2

Chapter 5 Discussion

This investigation focused on the abundance of selected bio-indicators (earthworms, springtails and mites) in two established and irrigated SRC willow plantations in Northern Ireland, one in Culmore, Co. Londonderry and the other in Hillsborough, Co. Down. The use of established plantations was necessary to eliminate potential changes in earthworm, mite and springtail abundance that could be attributed to the establishment phase of the SRC willow plantation (Mackeschin, 1994; Borjesson, 1999; Minor *et al*, 2004; Dimitriou *et al*, 2009; Baum *et al.* 2009 and Campbell *et al*, 2010) as opposed to changes caused by irrigation with wastewater.

The SRC plantation in Culmore was established in 2010 and is divided into plots, some of which are irrigated with primary treated municipal effluent (at a rate of $30m^3$/ha/day) from a nearby wastewater treatment plant, generally from May to October. The SRC willow plantation in Hillsborough was established in 2007 and is also divided into plots; a number of these plots are irrigated with dairy parlour washings (D.P.W.) from an on-site farm at three different treatment levels generally from May to October each year. The SRC willow plots at Hillsborough were trial plots designed to test the upper limits of SRC willow for bioremediation purposes and as such were irrigated at much higher rates than what would be typical of commercial application. The BOD and nutrient concentrations of the D.P.W. used at Hillsborough were much higher (by an approximate magnitude of 10) than those in the primary treated municipal wastewater used at Culmore (Table 3.2 & 3.3).

5.1 Culmore

5.1.1 Effects of Irrigation on the Abundance of Earthworms, Mites and Springtails

In Culmore, the average number of earthworms observed across all irrigated plots over both irrigation seasons was $18.5/m^2$ while it was $21.3/m^2$ in non-irrigated plots. Statistical analysis of the data set did not yield a significant difference in earthworm abundance between irrigated and non-irrigated plots over the two irrigation seasons or for all sampling events. It should be noted that the average abundance values obtained in both irrigated and non-irrigated plots during the investigation were lowered by the relatively high frequency of low and zero recovery of earthworms in some plots, particularly Plots 7, 9, 10 and 11. This was not related to irrigation but to previous land management as will be discussed in Section 5.1.3.

Eight earthworm species were observed in Culmore SRC willow plantation but the majority of these were present in low numbers. All were common species throughout the British Isles (Sims and Gerard, 1999). It is important to note that the majority of earthworms recovered in Culmore did not possess a clitellum and therefore could not be identified to species level. A higher ratio of juvenile to adult earthworms has been reported in a number of studies and this varies depending on the time of year when sampling occurs (Edwards and Bohlen, 1996). The presence of large numbers of juvenile earthworms, mainly in the irrigation season, would appear to suggest that irrigation with primary treated municipal wastewater at a rate of 30m3/ha/day was not affecting the fecundity of earthworms. The most common earthworms present in Culmore were the surface-dwelling epigeic group which included *E. tetraedra*, *L. eisenia*, *L. festivus*, *L. rubellus* and *S. mammalis*. These were present throughout the investigation period and were present in highest numbers in Plots 8 and 11. Two endogeic species (*O. tyrtaeum* and *A. rosea)* were observed in very low numbers. The low recovered numbers of endogeic earthworms was not attributed to the earthworm extraction methods used in this study particularly since hand-sorting has been reported to have a high recovery rate for endogeic earthworms who predominantly produce horizontal burrows in the soil sub-surface (Čoja *et al.* 2008). The only anecic species present was *L. terrestris* in Plots 8 and 11 only. The low recovery rate for anecic earthworms in this study was not attributed to earthworm extraction methods. While it is noted that hand-sorting has a low recovery rate for anecic earthworms, the use of mustard solution has been reported to give a good recovery (Gunn, 1992 and Chan and Munro, 2001). In addition, Pelosi *et al.* (2009) found that the recovery of earthworms using mustard solution was increased significantly when this expellant was used in combination with hand-sorting. This combined extraction method was used in Culmore.

One possible reason for the low recovery of anecic earthworms at Culmore may have been due to the fact that the %SMC and pH range observed at Culmore would not be ideal for *L. terrestris* as will be discussed in Section 5.1.2. Another reason may be the presence of the New Zealand flatworm *(Arthurdendyus triangulatus*) observed across the site at Culmore throughout the investigation and is a known predator of Lumbricid earthworms. The New Zealand flatworm is documented as being found in the area according to the National Biodiversity Data Centre (Invasive Species, 2015). It is reported that flatworm densities of 0.8 per m^2 can result in a reduction of 20% of the total earthworm biomass, the bulk of which is a reduction in anecic species (Murchie and Gordon, 2013). In particular, it is reported that *A. triangulatus* poses a serious risk to *L. terrestris* (Haria *et al.* 1998; Christensen and Mather, 2001 and Mather and Christensen, 2003).

There was no significant difference between the abundance of mites in irrigated and non-irrigated plots at Culmore SRC willow plantation either during irrigated periods only or for all sampling events. The mean abundance of mites in irrigated plots in Phase 1 was 105/kg of dry soil while it was 190/kg of dry soil in non-irrigated plots. The corresponding mean abundance values in Phase 3 were lower i.e. 40/kg of dry soil and 44/kg of dry soil in the irrigated and non-irrigated plots, respectively. Unfortunately, there were only two sampling events in Phase 1 which restricted statistical analysis for significant differences in mite abundance between Phase 1 and 3. Since there was no significant difference between irrigated and non-irrigated plots in Phase 1 or 3, the observed reduction of mites in Phase 3 could not be attributed to irrigation but to other undetermined factors.

There was no significant difference between the abundance of springtails in irrigated and non-irrigated plots during irrigated periods (p=0.065; C.I. =0.05) or for all sampling events (p=0.06; C.I. =0.05) even though upon initial observation, numbers appeared to be higher in non-irrigated plots. This is reflected in the low significance values obtained which is close to 0.05. The mean abundance of springtails in irrigated plots in Phase 1 was 50/kg of dry soil while it was 122/kg of dry soil in non-irrigated plots. The corresponding abundance values in Phase 3 were 54/kg of dry soil in irrigated plots and 91/kg of dry soil in non-irrigated plots. The mite abundance values appear to be quite similar for Phases 1 and 3 but unfortunately there were only two sampling events in Phase 1 which restricted statistical analysis to prove this.

In summary, irrigation of selected plots within the Culmore plantation with primary treated municipal effluent ($30m^3$/ha/day) did not significantly affect the abundance of earthworms, mites or springtails at the site over the investigation period. A review of the literature revealed that irrigation with municipal effluent may affect the abundance of soil invertebrates depending on the volume of effluent applied and its constituents, particularly nutrients and persistent pollutant such as heavy metals (Krantz, 1978; Cotton and Curry; 1979, Edwards and Bohlen, 1992; Edwards and Bohlen, 1996; Sims and Gerard, 1999; Oliver *et al,* 2005; Bur *et al,* 2012, Meli *et al,* 2013 and Kim and An, 2014). Hydraulic overloading may also cause death of soil invertebrates by suffocation. The level of irrigation received by Culmore SRC willow plantation did not seem to be causing an issue in relation to any of these aspects when the abundance of bio-indicators in irrigated plots were compared to non-irrigated plots. An irrigation rate of 30m3/ha/d corresponds to an application of 3mm effluent ha/d. Willow has a reported evapotranspiration rate of between 3.47-6.65 mm of water per day during the summer months (Guide *et al.* 2007 and AFBI, 2010) and it is likely that many of the soluble constituents in municipal effluent would therefore have a rapid uptake in willow limiting their interaction with soil and the organisms dwelling therein.

5.1.2 Effect of Soil Moisture, pH and Temperature on Earthworms, Mites and Springtails

Soil temperature, moisture and pH were monitored in Culmore SRC willow plantation during the investigation period. Soil temperature was measured because this factor has been reported to affect the abundance of earthworm, springtails and mites and it was necessary to eliminate this as a factor that may influence bio-indicator abundance particularly during irrigated periods. Soil pH was measured for two reasons; firstly, to determine whether irrigation with municipal wastewater affected soil pH and secondly, to determine whether any changes in soil pH may be correlated with bio-indicator abundance. %SMC was measured to determine whether this parameter was significantly different in irrigated plots as compared to non-irrigated plots and whether any observed differences might impact on bio-indicator abundance.

There was no significant difference in the % SMC observed in irrigated plots as compared to non-irrigated plots in Culmore during irrigated periods. This is reflected in the similar %SMC observed of 42.4% in irrigated plots and 42.7% in non-irrigated plots during irrigated periods.

The relationship between %SMCs (range=29.9-61.4%) and the abundance of earthworms was weak at Culmore over the entire investigation period (r^2 = 0.087). Different earthworm species prefer different %SMC ranges (Loehr *et al*, 1985; Edwards and Bohlen, 1996; Domínguez and Edwards, 1997; Berry and Jordan, 2001 and Natural England, 2014) and the nature of the relationship that earthworms have with moisture content is also influenced by specific site factors (Edwards and Bohlen, 1996; Doube and Styan, 1996 and Berry and Jordan, 2001). A weak correlation between earthworm abundance and %SMC could therefore be expected unless correlation tests were performed separately for each species of earthworm observed on the site, which was attempted but proved unreliable due to the low population numbers observed for some species. The most common species recorded at Culmore was *E. tetraedra* which is recorded in a wide range of soils but especially in wet soils with a high organic matter content (Sims and Gerard, 1999 and Natural England, 2014). *L. andrei* is also reported to thrive in high moisture conditions but these were recovered in low numbers in irrigated and non-irrigated plots at Culmore. The %SMC observed at Culmore would not ideally suit a number of earthworms species e.g. *A. rosea* who are reported to prefer a %SMC of around 10%, which is below any %SMC value recorded at Culmore. Also *L. terrestris* are reported to exhibit optimum growth at approximately 30% SMC (Natural England, 2014) which is at the extreme lower end of %SMCs observed at Culmore (i.e. 29.9%-61.4%). Both *L. terrestris* and *A. rosea* were recovered in Culmore, but in very low numbers.

The relationship between %SMC (range=29.9-61.4%) and the abundance of mites and springtails at Culmore SRC willow plantation was weak with r^2 values of -0.032 and 0.091, respectively. This relationship was expected for the mid-range %SMC observed in Culmore since moisture content is reported not to affect the abundance of mites in soils as much as other soil parameters (e.g. pH and temperature) except under drought conditions or flooding for extended periods of time (Taylor and Wolters, 2005; Oliver *et al.* 2000 and Lóšková *et al.* 2013). A weak correlation was therefore expected since these extreme conditions were never experienced in Culmore. Springtails are shown to prefer moist soil conditions with a negative effect on their abundance being linked to areas of extended drought (Hopkin, 1997). The %SMC content at Culmore did not fall below 29.9% in irrigated plots or 30.1% in non-irrigated plots which would be favourable to springtails and should not negatively impact their abundance.

Statistical analysis revealed that there was no significant variation between the temperatures observed in irrigated and non-irrigated plots at Culmore during the irrigated period or during the entire investigation. The soil temperature range recorded at Culmore was 5-19^{o}C, with an average temperature of 15^{o}C observed in both irrigated and non-irrigated plots during irrigated periods. Soil temperature is linked to atmospheric conditions and ambient air temperatures are provided in Appendix C (Table C1) for the meteorological station near Culmore. The lowest ambient atmospheric temperatures were, as expected, during the non-irrigated period of October to May and this as reflected in soil temperatures.

Correlation tests yielded a weak correlation between soil temperature (range=5-19^{o}C) and the abundance of earthworms, springtails and mites at Culmore over the entire investigation period with r^2 values of values of 0.095, -0.181 and -0.004, respectively. Earthworm species vary in their optimum temperature requirements but most temperate species experience maximum respiration efficiency and reproduction at temperatures of 15^{o}C (Edwards and Bohlen 1996; Uvarov and Scheu 2004 and Uvarov *et al.* 2011). Temperatures above 18^{o}C negatively impact earthworms as their heart rate slows down and at temperatures beyond 23^{o}C they may suffer from lethal hyperosmotic stress (Khan *et al.* 2012). This extreme temperature was never recorded at Culmore. During irrigated periods, an average temperature of 15^{o}C was observed across all plots which would have been an ideal temperature for optimum growth of temperate-species earthworms (Edwards and Bohlen, 1996; Uvarov and Scheu, 2004 and Uvarov *et al.* 2011) and would not have adversely affected earthworm abundance. At low temperatures (<5^{o}C), earthworms undergo quiescence, a process whereby they retreat into soil layers and reduce their respiration rates to survive winter conditions (Edwards and Bohlen 1996 and Crockett *et al.* 2001). This period of quiescence was observed in November 2012 and sampling was suspended during this time until soil temperatures increased to 5^{o}C in March 2013.

The optimum temperature for mite respiration is highly species-dependent but at temperatures below 10^{o}C, respiration is reported to decrease and at temperatures above 25^{o}C, mites experience a lethal response (Krantz, 1978; Nguyen and Amano, 2009; Wekesa *et al*, 2010 and Beckett, 2011). The highest soil temperature recorded at Culmore was 19^{o}C and temperatures did not decrease below 10^{o}C during the irrigated period. It would therefore be reasonable to assume that soil temperatures should not have adversely affected mite abundance during irrigated periods. Mites do not undergo quiescence but at temperatures below 6^{o}C, abundance is affected in temperate-climate mites (Krantz 1978 and Beckett, 2011). A decrease in mite abundance was therefore expected and occurred for mites as soil temperatures decreased in Phase 2 presumably causing mites to burrow deeper into the soil and lower their respiration rates (Krantz 1978; Hopkin, 1997; Ulrich and Fiera, 2009 and Beckett, 2011). Recorded soil temperatures did not fall to very low levels during the investigation and the lowest temperature observed was 5^{o}C in April 2013. It was expected that a higher abundance of mites would be observed during the 2013 irrigation season as soil temperatures increased but this did not occur in either irrigated or non-irrigated plots, for reasons that were therefore unrelated to soil temperature.

The soil temperatures observed during irrigated periods in Culmore should not have adversely affected springtail abundance during this time. In temperate climates, a soil temperature of 15^{o}C is considered optimum for most species to carry out feeding and development (Hopkin, 1997 and Larink, 1997). This is the average soil temperature observed in Culmore during irrigated periods. The abundance of springtails is reported to decrease at temperatures below 10^{0}C since this is considered to be the threshold below which respiration is affected in temperate-climate springtails (Cannon, 1987; Hart *et al,* 2002; Wekesa *et al,* 2010 and Beckett, 2011). Soil temperature did not drop below 10^{o}C on any occasion during irrigated periods in Culmore. Temperatures below 10^{o}C were observed during Phase 2 of the investigation and coincided with lower springtail abundance. The abundance of springtails increased again during the 2013 irrigation season as soil temperatures increased.

Soil pH was also measured in the SRC willow plantation at Culmore throughout the investigation period. The soil pH was generally low throughout the site, with an average of 4.7 in non-irrigated and irrigated plots over the entire investigation period. This is lower than the pH of between 5 and 7 recommended for willow growth (AFBI, 2010 and Galbally *et al,* 2013). Statistical analysis revealed that there was no significant difference in soil pH between irrigated and non-irrigated plots at Culmore during irrigated periods or throughout the entire investigation. There was a significant difference in soil pH between Phase 1 and Phase 3. The mean pH observed in Phase 1 was 5.0 while this value dropped to a mean value of 4.4 during Phase 3. This drop in soil pH could be attributed to a number of factors but the most important factor from the perspective of this investigation could be the application of an acidic wastewater, however this was not believed to be a contributory factor since the average pH of the primary treated municipal wastewater used in this investigation was 7.1 with a range of 6.7-7.9, and the soil pH decreased equally in both non-irrigated and irrigated plots.

Since a significant drop in soil pH was observed in the Culmore SRC willow plantation between Phase 1 and 3, correlation tests were run for soil pH (range=pH 3.7-5.8) and the abundance of bio-indicators to rule this out as a factor in any observed changes. Weak correlations existed with r^2 values of -0.13, 0.171 and -0.136 for earthworms, mites and springtails, respectively. This would seem to indicate that the decreasing pH at Culmore SRC willow plantation is not yet adversely impacting bio-indicator abundance. It is important to note that correlation tests were run for total abundance values of all species of earthworms/mites/springtails on site as opposed to separate tests for individual species which, in the case of earthworms, proved unreliable due the low population numbers on-site for a number of species and was impossible for springtails and mites since these were not identified to species level. The observed pH values at Culmore SRC willow plantation would not be ideal for earthworm species that thrive in more neutral pHs, however it is within the range tolerated by many earthworm species (Table 2.2) e.g. *E. tetraedra, L. eiseni, O. tyrtaeum* and *A. rosea*. *E. tetraedra* was present in greatest numbers in Culmore with *L. eiseni*, *O. tyrtaeum* and *A. rosea* recovered in lower numbers. *L. terrestris* has a preferred pH of 6.2-10.0 and their numbers were expected to be limited by the acidity of the soil at Culmore (as well as the presence of the New Zealand flatworm, as previously mentioned). This was indeed the case with a peak number of only 6/m^2 observed over the investigation period.

Mites are reported to have a high tolerance to low soil pHs with loss of abundance only reported in highly acidic soils. Studies suggest soil pHs as low as 4.5 have no observable effect on the abundance of some species of soil mites (Hagvar and Gunnar, 1980; Davey *et al,* 1995 and Lóšková *et al,* 2013). The tolerance of springtails to low soil pHs is also well reported with some species thriving in pHs as low as 2. Many species of springtails show a uniform distribution throughout the pH gradient (van Straalen, 1998; Garnier and Ponge, 2004, and Alerding, 2013).

5.1.3 Effect of Cropping History on the Abundance of Earthworms, Mites and Springtails

SRC willow plantations are reported to both positively and negatively affect the abundance of soil invertebrates. Positive impacts from SRC willow plantations include the increased availability of leaf litter which increases the food supply for soil invertebrates (Poole, 1959; Bouche, 1972; Krantz, 1978; Takeda and Ichimura, 1983; Wise *et al.* 1988; Barnes and Ruppert, 1994; Colfer *et al.* 2004; Hopkin, 2005; Hishi *et al,* 2007; Stavrinides and Mills, 2009; Cakmak *et al.* 2009; Minnesota, 2011 and Fontanetti *et al,* 2011). The percentage soil carbon in Culmore SRC willow plantation is quite high with an average value of 9.8% (range 9.6-10.1%) in irrigated plots over the investigation period and an average value of 10.6% (range 9.9-11.1%) in non-irrigated plots (AFBI, 2013, *pers comm*). This would indicate that the soil, and presumably soil invertebrates, is benefiting from the leaf fall from willow in Culmore SRC willow plantation.

Negative impacts associated with SRC willow plantations arise from the use of heavy machinery during site preparation, planting and harvesting and this may lead to soil compaction causing reduced movement of soil invertebrates including earthworms and springtails (Wickenbrock and Heisler 1997; Jégou *et al.* 2002; Larsen *et al.* 2004; Chan and Barchia 2007 and Son *et al.* 2011). The SRC willow plantation in Culmore was planted in 2010. The site had a mixed cropping history, being used for the growth of poplar, willow and grass in different plots. The conversion of this mixed cropping pattern to SRC willow necessitated conventional mechanical and chemical treatments as outlined previously in Section 2.1.1. In particular, the area had to be uprooted, treated with pesticides and tilled to a depth of 25 cm. Following establishment of the newly planted SRC willow, the crop was coppiced in 2011 but was not coppiced throughout this investigation.

A significant difference was observed in the abundance of earthworms in areas that had a different previous cropping history. In particular, higher numbers of earthworms were observed in some plots (8 and 11) that had been historically planted with grassland prior to SRC willow conversion in 2010. A mean abundance of 45 earthworms/m^2 was calculated for previously planted grassland plots over the entire investigation period (irrigated and non-irrigated) with values of 75/m^2, 25/m^2 and 33/m^2 observed in Phase

1, 2 and 3, respectively. Plots that had been previously planted with poplar achieved a low mean abundance value (2/m^2) over the investigation period while plots that were previously planted with willow also yielded a low mean abundance value (3/m^2) over the investigation period. Statistical analysis of the datasets over the entire investigation period show that there was no significant difference observed in earthworm abundance between plots that had been previously planted with willow and poplar regardless of irrigation, however a significant difference emerged between previously planted grassland areas and other plots that had been planted with poplar/willow. The application of PerMANOVA did not yield a significant interaction factor between the effect of irrigation and planting history on the abundance of earthworms. This helps reinforce the finding that cultivation history affects earthworm abundance but irrigation does not at the rates applied at Culmore during this investigation.

The low abundance values in plots previously planted with poplar and willow decreased the average abundance of earthworms for the Culmore site in both irrigated and non-irrigated plots. The abundance of earthworms in previously planted grassland plots (irrigated and non-irrigated) at Culmore was compared to the abundance values in soils under a range of management practices (Table 2.1) and were close to values observed by other workers in intensively managed arable land (Curry *et al*, 2002), poplar stands (Salehi *et al*, 2013) and SRC willow plantations in receipt of sewage sludge (Kocik *et al*, 2007). The abundance values for earthworms are significantly below those observed in old growth deciduous woodland (Smith *et al*, 2008), beech woodlands (Phillipson et al, 1978) or even coniferous forest (Smith *et al*, 2008). The abundance of earthworms in plots previously planted with poplar and willow was, in general, below those values observed in intensively managed arable land, poplar stands and SRC willow plantations in receipt of sewage sludge.

The differences in earthworm abundance between plots with a different cropping history may be explained by the historic land management practices applied to various plots. Plots that were previously planted with poplar and willow underwent more intensive and deeper cultivation in the past than plots that had been previously planted with grass. Heavy machinery is reported to have a variety of impacts on soil invertebrates. In some cases soil invertebrates can be crushed and/or soils can be compacted thereby restricting their movement and feeding opportunities in the soil. The impacts of compaction are compounded by reduced air and water availability in diminished soil pores (Whalley *et* *al.* 1995 and Beylich *et al.* 2010). The effects of heavy machinery compaction can be alleviated, at least for earthworms, by confining agricultural practices to the top 15cm of the soil, this depth, however, is not sufficient for SRC willow cultivation which requires a depth of 20-25cm (Stinner and House, 1990 and AFBI, 2010). Soil compaction is reported to have a considerable effect on the abundance of earthworms and studies have shown that a long recovery period is often necessary, with larger species of earthworm frequently being totally lost from a site (Paoletti 1999b; Jégou *et al.* 2002; Chan and Barchia 2007 and Ernst *et al.* 2009). This appears to be the case in plots previously planted with poplar and willow at Culmore. Decreased earthworm abundance caused by soil compaction may persist while heavy machinery is being used on site e.g. during mechanical coppicing. In addition to the effects outlined above, the Culmore SRC willow plantation was also treated with the herbicide glyphosate and the insecticide chloropyrifos. Glyphosate has been reported by some workers to have varying toxicities to earthworms. In general, negative effects on feeding behaviour, DNA and reproduction have been demonstrated causing a range of symptoms from delayed development to increased death (Springett and Gray 1992; Paoletti 1999a; Verrell and Van Buskirk, 2004; Casabé *et al.* 2007, Solomon *et al.* 2007, Yasmin and D’Souza 2007; Correia and Moreira 2010; Piola *et al,* 2013 and Zhou *et al.* 2013). Chlorpyrifos, an organophosphate insecticide used to control leatherjacket pests in willow plantations, has been shown to have varying effects on earthworms, impacting on growth, reproduction and enzyme activity (Booth and O’Halloran, 2001 and Zhou, 2007). There appears to be a specific species-related variation in chlorpyrifos toxicity to earthworms, with *L. rubellus* being the most sensitive of six earthworms species investigated by Ma and Bodt (1993) including *A. calignosa, A. longa, L. rubellus, L. terrestris, E. fetida and E. veneta*. A number of these earthworm species were recorded in Culmore (i.e. *L. rubellus, L. terrestris* and *E. fetida)* with *E. fetida* occurring in greatest numbers. *L rubellus* was present in lower numbers but was more abundant than *L. terrestris.* The recovery of earthworms to chloropyrifos exposure is an important aspect to consider with some workers reporting that the insecticide is rapidly eliminated following a cessation of exposure however a recovery of enzyme activity is much slower (Aamodt *et al.* 2007; Collange *et al.* 2010; Zhou *et al.* 2013 and Schnug *et al.* 2014a). The Culmore SRC willow plantation was planted in 2010 and it is reasonable to assume that any adverse effects on earthworm abundance from pesticide applications no longer exist.

Mites are reported to be adversely affected by a number of land management techniques but particularly those that mechanically disturb the soil by compaction which results in reduced porosity (Bedano *et al,* 2006 and Cao *et al,* 2011). Adequate porosity is important to navigate the soil layers and access food sources (Ducarme *et al*, 2004) although the effects are less marked than for larger soil invertebrates such as earthworms. The tolerance of mites to pesticide treatments is reported to be species-specific but, generally speaking, pesticides have a negative impact on abundance. This effect is short-lived and numbers will recover quickly in the absence of pesticide application (Minor *et al,* 2004 and Bedano *et al,* 2006). According to Benamú *et al* (2010) the direct and indirect effects of the herbicide, glyphosate on arthropods (including mites) has not been extensively researched. It seems that for mites the most extensive effect observed in the application of glyphosate is not a direct one but indirect due to changes in the local environment e.g. to vegetation, microclimate and food sources. Sub-lethal effects have been reported by a number of workers (Guiseppe *et al.* 2006; Schneider *et al,* 2009 and Benamú *et al*, 2010) as the application of glyphosates affects prey consumption, fertility and development of young. It appears that any loss of abundance due to the application of glyphosate is negated within a few months of application (Minor *et al*, 1994; Benamú *et al,* 2010 and Bosch-Serra *et al.* 2014). Use of the insecticide, chlorpyrifos may be toxic to mites but generally not at concentrations applied in the field (Prischmann *et al.* 2005 and Shi *et al.* 2008). The abundance of mites may be indirectly impacted by the application of chlorpyrifos owing to changes in the environment e.g. to vegetation and prey (Al-Assiuty *et al.* 2014). As previously mentioned, the Culmore plantation was planted in 2010 and the application of pesticides is only required during plantation set-up and during early establishment (AFBI, 2010). Given the reported short-lived effects of the application of pesticides used in Culmore on the abundance of mites, the effects of pesticide application should not be an issue although compaction effects may not have improved since the plantation was established.

Minor *et al* (2004) found that the abundance of mites was initially negatively affected during the first year of SRC willow conversion from arable soil but found that abundance increased in the long term. Similar studies were not available for impacts on mite abundance following the conversion to SRC willow from other agricultural or horticultural land management practices. As previously, mentioned the mean abundance of mites in irrigated plots was 105/kg of dry soil in Phase 1 which is approx. 1,470/m^2. The latter value is calculated from the surface area of the soil core from which the mites were extracted as outlined in ISO 23611-1:2006-Part 2 (Section 3.6. The corresponding value for the abundance of mites in non-irrigated plots in Phase 1 was 190/kg of dry soil (2,660/m^2). The average abundance values observed for mites were somewhat lower in Phase 3 with values of 40/kg of dry soil (560/m^2) in irrigated plots and 44/kg of dry soil (616/m^2) in non-irrigated plots. When the abundance values observed for mites in Culmore SRC willow plantations are compared to other studies, they are low i.e. typical mite abundance are reported to range from a few hundred per m^2 to hundreds of thousands per m^2 depending on site conditions and land-management practices (Krantz, 1978; Curry, 1994; Behan-Pelletier, 1999; Behan-Pelletier and Kanashiro, 2010). As a typical example, oribatid mites are reported to reach densities of up to 200,000/m^2 in acidic soils in forested areas with lower densities of 20,000/m^2 in alkaline soils in forested areas. The abundance of mites appeared to decrease between Phase 1 and 3 but unfortunately, there were only two sampling events in Phase 1 which restricted statistical analysis to prove this point. The application of PerMANOVA did not yield a significant interaction factor between the effect of irrigation and planting history on the abundance of mites.

In Culmore, planting history was somewhat of a factor when examining the abundance of mites where higher numbers of mites were observed in areas previously planted with poplar as compared to plots previously planted with grassland or willow. An interesting outcome was that this effect was evident only in non-irrigated plots both during irrigated periods and also over the entire investigation. The reasons for this may be linked to soil pH since plots previously planted with poplar exhibited a significantly lower pH than other plots and it is indicated in literature that increasingly acidified environments may benefit mites due to competition and/or reproduction effects (Hagvar and Gunnar, 1980 and Hagvar, 1990).

Planting history was, in general, not an important factor when examining the abundance of springtails with no significant differences observed between plots with a different cropping history. Throughout this investigation, no published studies were found on springtails in SRC willow plantations however it is reported that physical disturbance (such as that experienced during conversion of land to SRC willow) directly affects springtails as they are highly dependent on the natural porosity of the soil to navigate their environment (Larink, 1997) and tend to avoid the decreased pore size of compacted soils (Wickenbrock and Heisler, 1997; Larsen *et al,* 2004 and Son *et al,* 2011). It is reported that physical disturbance due to compaction and tillage is more likely to have an adverse effect on the abundance of springtails than the application of chemicals in intensively managed farming systems (Hopkin, 1997; Bedano *et al,* 2006 and Ponge *et al,* 2013). Several pesticides are documented to affect the abundance of springtails however this effect can be quite variable depending on the chemical being used and its concentration. In addition, toxic effects are generally observed at concentrations higher than those typically applied in the field (Alves *et al,* 2014; Schnug *et al.* 2014a and Schnug *et al,* 2014b). The abundance of springtails is negatively affected by the application of herbicides with a loss of abundance reported directly upon application to an area however this loss of abundance is more readily attributed to loss of food sources as opposed to direct toxicity effects (Fox, 1964, Hopkin, 1997 and Alves *et al,* 2014). Guiseppe *et al.* (2006) and Hammad and Gurkan (2012) report that the herbicide glyphosate does not cause lethal effects for micro-invertebrates such as springtails but may cause sub-lethal effects, as applications impact on their food sources. This can have knock-on effects for fertility and the development of young (Guiseppe *et al,* 2006; Schneider *et al,* 2009, and Benamú *et al,* 2010). The effects of the insecticide, chlorpyrifos on springtails is twofold since application of this chemical affects food sources and egg development when it is chronically applied to a site though generally at concentrations higher than that applied in the field (Fountain *et al.* 2007; Jager *et al.* 2007; Schnug *et al.* 2014a and Schnug *et al,* 2014b). As previously mentioned, the Culmore plantation was planted in 2010 and the effects of pesticide application should not still be an issue for springtails.

The mean abundance of springtails in irrigated plots in Phase 1 was 50/kg of dry soil (700/m^2) while it was 122/kg of dry soil (1,708/m^2) in non-irrigated plots. The corresponding abundance values in Phase 3 were 54/kg of dry soil (756/m^2) in irrigated plots and 91/kg of dry soil (1,274/m^2) in non-irrigated plots. From a review of the literature, it appears that the abundance of springtails in soil varies depending on site conditions and land-management practices and can range from tens of thousands to hundreds of thousands per m^2. In undisturbed grassland areas, springtail abundance is reported to range from 30,000 to 100,000 per m^2 of soil with lower abundances (1000-1600 per m^2 of soil) observed in agricultural areas of high intensity (Curry, 1994 and

Hopkin, 1997). Springtail abundance in the Culmore SRC willow plantation was low and similar to that observed in intensively managed agricultural land.

5.2 Hillsborough

5.2.1 Effects of Irrigation on the Abundance of Earthworms, Mites and Springtails

The Hillsborough SRC willow plots were irrigated with D.P.W at a rate of 18, 34 and 44 m^3/ha/d from May to October. This corresponds to an application of 1.8, 3.4 and 4.4mm effluent ha/d. Throughout the discussion, the control plot will be referred to as the control, the plot receiving 18m^3/ha/d will be referred to as Treatment 2 (T2) while plots in receipt of 34 and 44m^3/ha/d, will be referred to as Treatment 3 (T3) and Treatment 4 (T4), respectively.

Earthworm abundance was significantly affected by the level of treatment received at Hillsborough SRC willow plantation but only at the higher irrigation levels received in T3 and T4 plots when earthworm abundance was drastically reduced to a mean value of less than 6/m^2 during all Phases in the T3 plot and to a mean value of less than 5/m^2 during all Phases in the T4 plot. There was no significant difference observed in earthworm abundance between the control and T2 plot during irrigation periods only or on all sampling dates. Earthworm abundance was quite high in the control and T2 plot with a mean value of 37/m^2 recovered in the control plot and 34/m^2 in the T2 plot over the entire investigation period. The abundance of earthworms was highest in Phase 1 with mean values of 71/m^2 and 58/m^2 in the control and T2 plots, respectively. These earthworm abundance values are higher than those observed in Culmore SRC willow plantation, particularly in Phase 1, and are somewhat above values observed by other workers (Table 2.1) in intensively managed arable land (Curry *et al*, 2002), poplar stands (Salehi *et al*, 2013) and SRC willow in receipt of sewage sludge (Kocik *et al*, 2007) however, they are still significantly below values observed in old growth deciduous woodland (Smith *et al*, 2008), beech woodlands (Phillipson et al, 1978) or even coniferous forest (Smith *et al*, 2008). The higher earthworm abundance values observed in Hillsborough as compared to Culmore may be due to the relatively longer recovery period that had elapsed since land-disturbance and planting of willow (i.e. five years in Hillsborough as compared to two years in Culmore). The necessity for long periods of time for earthworms to recover from land-disturbance and application of pesticides has been discussed previously in Section 5.1.3.

As previously mentioned, significant differences in earthworm abundance arose between the control plot and T3 plot and T4 plot. SRC willow has an evapotranspiration rate of between 3.47-6.65mm of water per day during the summer months (Guide *et al*, 2007 and AFBI, 2010) and the highest rate of application in the T4 plot was still within this range, therefore factors other than hydraulic overloading may account for the wipe-out of earthworms at the higher irrigation levels. This is confirmed by the on-going analysis of %SMC which will be discussed further in the next section but in summary confirmed that the willow plantation was able to absorb the applied effluent without increasing the %SMC of the soil in irrigated plots as compared to the control plot. The effluent constituents may still accumulate in soil by precipitation or other mechanisms and be ingested by earthworms before the effluent is fully absorbed by the willow and this aspect requires further investigation but was beyond the scope of this investigation.

A good diversity of earthworm species was present in the Hillsborough SRC willow plantation in the control and T2 plot but not in the T3 and T4 plots. In total, ten species of earthworm were observed at Hillsborough and all were common throughout the British Isles (Sims and Gerard, 1999). The majority of these species were present in low numbers. As in Culmore, a large proportion of earthworms present in Hillsborough were non-clitellate and therefore could not be identified to species level but their presence indicates that irrigation with D.P.W at levels of 18m^2/ha/d was not adversely affecting the fecundity of earthworms. A high ratio of juvenile to adult earthworms is commonly observed in habitats as previously discussed in Section 5.1.1.

The most common earthworms present in Hillsborough were the surface-dwelling epigeic group which included *E. tetraedra, L. eisenia, L. festivus, L. rubellus* and *S. mammalis*. These were present throughout the investigation period mainly in the control and T2 plots. The most common species observed in Hillsborough as in Culmore SRC willow plantation was *E. tetraedra*. Four endogeic species were also present *(O. tyrtaeum, A. icterica, A. limicola and A. rosea)* and these were present in very low numbers and mainly in the control and T2 plot. Only one anecic earthworm species was present (*L. terrestris)* and occurred mainly in the control and T2 plot during Phase 1 of the investigation. A low abundance of endogeic and anecic earthworm species had also been observed in Culmore. Low recovery rates for endogeic and anecic species in Hillsborough were not attributed to the earthworm extraction methods used, as previously discussed in Section 5.1.1., but may have been due to other factors e.g. unfavourable pHs or %SMC values observed on site, or the presence of the New Zealand flatworm which was present in Hillsborough throughout the investigation and is documented as being found in the area according to the National Biodiversity Data Centre (Invasive Species, 2015). While the constituents of D.P.W applied at Hillsborough may be attributed as a factor in the low recovery rates of endogeic and anecic earthworms in T3 and T4 plots, it was not responsible for the low recovery of these ecotypes in the control plot which did not receive irrigation.

No significant difference was observed in the abundance of mites when the control plot was compared to T2, T3 or T4 during the irrigated period only. This was also the case for all sampling events. The average number of mites calculated in the control plot over the entire investigation period was 17/kg of dry soil (238/m^2). In T2, T3 and T4, the corresponding values were 10/kg of dry soil (140/m^2), 23/kg of dry soil (322/m^2) and 29/kg of dry soil (406/m^2), respectively. When these values are contrasted to the average value of 57/kg of dry soil (798/m^2) for all plots in Culmore, a significant difference is evident with much higher abundance values observed in Culmore. The reported abundance of mites are typically of the order of a few hundred per m^2 to hundreds of thousands per m^2 depending on site conditions (Krantz, 1978; Curry, 1994; Behan-Pelletier, 1999; Behan-Pelletier and Kanashiro, 2010) and it is evident that the abundance values observed for mites in all plots at Hillsborough is at the lower end of this scale. High soil moisture conditions are not reported to affect the abundance of mites except during flooding for extended periods of time (Taylor and Wolters, 2005; Oliver *et al.* 2000 and Lóšková *et al.* 2013) and these conditions were not observed in Hillsborough. Other effects of irrigation, such as increased nutrient levels, may affect the abundance of mites directly due to the constituents of the wastewater (Bur *et al.* 2012, Meli *et al.* 2013, Kim and An, 2014) or indirectly due to effects on mite predators. These effects are reported to be species-specific. Mites were not identified to species level in this investigation therefore species-specific effects could not be determined, however, it is clear that the abundance of mites is quite low in Hillsborough

SRC willow plantation. The reasons for the low abundance of mites at Hillsborough was not determined during the investigation but were not attributed to irrigation with D.P.W since low abundance values were also observed in the control plot

Minor *et al* (2004) found that the abundance of mites was initially negatively affected during the first year of SRC willow conversion from arable soil but found that abundance increased in the long term. Mites are adversely affected by a number of land-management techniques but particularly those that mechanically disturb the soil by compaction which results in reduced porosity (Bedano *et al,* 2006 and Cao *et al,* 2011). Compaction effects at Hillsborough SRC willow plantation may still negatively affect mite abundance even though it has been five years since the plantation was established. The tolerance of mites to pesticide treatments is reported to be short-lived and numbers will recover quickly in the absence of pesticide application (Minor *et al,* 2004; Bedano *et al*, 2006 and Bosch-Serra *et al.* 2014). The Hillsborough SRC willow plantation was planted in 2007 and it is reasonable to assume that any adverse effects on mite abundance from pesticide application no longer exist.

No significant difference was observed in the abundance of springtails when the control plot was compared to T2, T3 or T4 during irrigated periods only. This was also the case for all sampling events. The average number of springtails calculated in the control over the entire investigation period was 24/kg of dry soil (336/m^2). In T2, T3 and T4, the corresponding values were 15/kg of dry soil (210/m^2), 22/kg of dry soil (308/m^2) and 19/kg of dry soil (266/m^2), respectively. In general the numbers of springtails observed in all plots at Hillsborough are much lower than Culmore, where an average value of 61/kg of dry soil (854m^2) was calculated for all plots over the investigation period. It was expected that springtails would thrive in the moist conditions observed at Hillsborough as they prefer wet or damp conditions (Hopkin, 1997). In addition, natural and artificial precipitation is reported to increase springtail abundance as it causes them to move to the surface of the soil and avail of increased food sources (Rodríguez *et al,* 2006). A review of the literature indicates that the abundance of springtails in grasslands can typically vary between 30,000 and 100,000 per m^2 of soil with lower abundances (1000-1600 per m^2 of soil) observed in agricultural areas of high intensity (Curry, 1994 and Hopkin, 1997). The average abundance values observed in Hillsborough SRC willow plantation are lower than those reported for intensively managed agricultural soils but this low abundance is not attributed to irrigation with D.P.W since low abundance values were also observed in the control plot and as previously mentioned, there were no significant differences in springtail abundances observed across all treatment plots.

Throughout this investigation, no published studies were found on springtails in SRC willow plantations however it is reported that physical disturbance (such as that experienced during conversion of land to SRC willow) directly affects springtails as they are highly dependent on the natural porosity of the soil to navigate their environment (Larink, 1997; Wickenbrock and Heisler, 1997; Larsen *et al*, 2004 and Son *et al,* 2011). It is reported that physical disturbance due to compaction and tillage is more likely to have an adverse effect on the abundance of springtails than the application of chemicals in intensively managed farming systems (Hopkin, 1997; Bedano *et al,* 2006 and Ponge *et al*, 2013) and effects of soil compaction may have affected springtail abundance in Hillsborough SRC willow plantation even though it has been five years since the plantation was established. A number of pesticides were used at the site preparation and establishment stage of the Hillsborough SRC willow plantation in 2007 but it is assumed based on researched literature (previously discussed in Section 5.1.3) that the effects of these would no longer affect springtail abundance.

In summary, irrigation of selected plots in Hillsborough SRC willow plantation with D.P.W. applied at variable rates does not affect the abundance of mites or springtails when compared to the abundance of these micro-arthropods in a control plot. The abundance of earthworms in Hillsborough was significantly affected by the higher levels of irrigation received in T3 and T4 plots and several events of zero or low recovery of earthworms were recorded in these plots. Earthworm abundance was not significantly affected at an irrigation rate of $18m^3$/ha/d. The uppermost level of irrigation with D.P.W tolerable by earthworms at Hillsborough was not determined in this investigation due to the fact that pre-determined irrigation levels were used at this AFBI research site. It can be stated however that the irrigation level of D.P.W tolerated by earthworms is greater than $18m^3$/ha/d and less than $34m^3$/ha/d.

5.2.2 Effect of Soil Moisture, pH and Temperature on the Abundance of Earthworms, Mites and Springtails

During the investigation period at Hillsborough, the %SMC was determined for each invertebrate sampling event in both SGPs and MGPs. Statistical analysis of the datasets did not reveal a significant difference in %SMC between SGPs and MGPs) in the control or in any of the treatment plots (as will be discussed later) and therefore results will be discussed in the context of %SMC in the various treatment plots (i.e. combined MGP and SGP) as compared to the control. Statistical analysis of the datasets did not reveal a significant difference in %SMC between the control and T2, T3 and T4 plot during the irrigated period. This indicates that the willow plantation is effectively absorbing the effluent applied at an equal rate throughout all plots. This may be expected since the irrigation rates applied in Hillsborough are within the evapotranspiration rates reported for willow i.e. 3.47-6.65mm of water per day during the summer months (Guide *et al*, 2007 and AFBI, 2010). The highest irrigation rate applied at Hillsborough is $44m^3$/ha/d which is equivalent to 4.4 m/ha/d.

The mean %SMC observed in the control plot during the irrigated period was 34.9% while the corresponding values in the T2, T3 and T4 plots were quite similar i.e. 37%, 35.1 % and 31.4%, respectively. The mean %SMC in all treatment plots was greater during the non-irrigated period than during the irrigation period (corresponding to higher rainfall levels observed during this time) but the range of %SMC values observed were greater during irrigated periods. The T3 and T4 plots experienced the highest range of %SMC values over the investigation period with values ranging from 19.9-49.1% in T3 and 14.0-41.4% in T4. It was expected that these plots, in receipt of higher irrigation volumes, would have the highest mean %SMC over the irrigated period but this was not the case. As a point of interest, the lowest moisture contents (15.6%) observed in Hillsborough over the entire investigation period were in June and July, 2013 in the T4 plot at the height of the irrigation period.

A weak correlation was obtained when %SMC (range=14.0-49.9%) was plotted against the abundance of earthworms (r^2=-0.129). There was a similar weak correlation observed in Culmore when correlation tests were performed for all species of earthworms over the range 29.9-61.4% SMC. The range of %SMC values and mean %SMCs were, in general, lower in Hillsborough than in Culmore. The relationship between %SMC and earthworms has been reported to be species-specific with different species surviving within a distinct %SMC range (Edwards and Bohlen 1996; Doube and Styan 1996, Berry and Jordan 2001). The most common earthworm species recorded at Hillsborough was *E. tetraedra*, an epigeic species also common in Culmore and which is best suited to soils of high moisture content (Sims and Gerard, 1999 and Natural England, 2014). The %SMC observed at Hillsborough should be more ideally suited to some earthworm species (e.g. *L. terrestris*) than those recorded at Culmore; *L. terrestris* are reported to exhibit optimum growth at approximately 30% SMC (Natural England, 2014) which is typical of the %SMC observed in all plots at Hillsborough during the irrigation period. While *L. terrestris* was recovered in higher numbers in Hillsborough than in Culmore (mainly in Phase 1), abundance values were still quite low in comparison to reported values in literature with lower pH values possibly influencing this or, as previously mentioned, the presence of the New Zealand flatworm.

The relationship between %SMC (range=14.0-49.9%) and the abundance of mites observed during the investigation period in Hillsborough SRC willow plantation revealed a weak correlation (r^2= -0.107) as had been the case in Culmore. It appears that the %SMC was not a strong factor affecting the abundance of mites across both sites within the range of %SMCs observed. Moisture content has been cited not to affect the abundance of mites in soils as much as other soil parameters (e.g. pH and temperature) except under drought conditions or flooding conditions for extended periods of time (Taylor and Wolters 2005, Oliver *et al.* 2000, Lóšková *et al.* 2013) and these conditions were never experienced at Hillsborough. The relationship between %SMC (range=14.0-49.9%) and the abundance of springtails observed during the investigation period also revealed a weak correlation (r^2= -0.144). A similar weak correlation was observed in Culmore. Springtails are shown to prefer wet conditions with a negative effect on their abundance being linked only to areas experiencing extended drought or flooding conditions (Hopkin 1997). These conditions were never observed at Hillsborough and in fact the %SMC range observed in Hillsborough (i.e. 14.0-49.9%) should be favourable to springtails. It could be assumed therefore that factors other than soil moisture were responsible for the low abundance values for springtails observed at Hillsborough.

The soil temperature range recorded at Hillsborough during irrigated periods was between 11-21^{o}C with an average temperature of 15.7^{o}C observed during irrigated periods. There was no significant difference between soil temperatures in the various treatment plots at Hillsborough. The highest soil temperature was recorded in July 2012 at 21^{o}C, but values of between 11-18^{o}C were recorded thereafter. The lowest soil temperatures were observed in winter 2012 and early spring 2013 (i.e. 4-5^{o}C) with an average temperature of 7^{o}C observed during the non-irrigated period. During the investigation, lower numbers of earthworms were observed during the non-irrigated period than irrigated periods. This was expected for reasons linked to low soil temperatures, which ranged from 4-11^{o}C, in the non-irrigated period. When quiescence temperatures were recorded in December 2012, sampling was suspended until soil temperatures increased again to 5^{o}C in March 2013. Temperatures above 18^{o}C are reported to negatively impact earthworm abundance as their heart rate slows down, and at temperatures above 23^{o}C, they may suffer from lethal hyperosmotic stress. A temperature of 21^{o}C was recorded only once during the investigation (in July 2012) but temperatures remained at or below 18^{o}C for the remainder of the investigation. An average soil temperature of 15.7^{o}C was observed in Hillsborough during irrigated periods, which is an ideal temperature for earthworm growth and activity in temperate regions (Edwards and Bohlen 1996; Uvarov and Scheu 2004 and Uvarov *et al.* 2011) and therefore should not have adversely affected earthworm abundance during irrigated periods.

Mites do not undergo quiescence but at temperatures below 6^{o}C, abundance is affected in temperate-climate mites (Krantz 1978 and Beckett 2011). Temperatures below 6^{o}C were recorded at Hillsborough in December, 2012 and March, 2013 and coincided with low mite abundance but temperatures increased steadily thereafter. The optimum temperature for mite activity is highly species-dependent but at temperatures below 10^{o}C, respiration begins to slow down in temperate-climate mites and at temperatures above 25^{o}C mites experience a lethal response (Krantz 1978, Wekesa *et al.* 2010, Nguyen and Amano 2009, Beckett 2011). Soil temperatures of 25^{o}C were not recorded during the investigation period at Hillsborough. Temperatures below 10^{o}C were not observed during irrigated periods and therefore should not have adversely affected mite abundance during this time. Similarly, soil temperatures should not have adversely affected springtail abundance during irrigated periods since in temperate climates, a soil temperature of 15^{o}C is considered optimum for most springtails to carry out feeding and development (Hopkin, 1997 and Larink, 1997). This is very close to the average soil temperature observed in Hillsborough during the irrigated period (15.7^{o}C). The abundance of springtails, as is the case for mites, is reported to decrease at temperatures below 10^{o}C (Cannon, 1987; Hart *et al,* 2002; Wekesa *et al,* 2010 and Beckett, 2011) which, as previously mentioned, was not observed during irrigated periods.

The soil pH in Hillsborough is acidic in nature with an average value of between 4.7-4.9 observed over the various treatment plots during the course of the investigation. This is similar to soil pHs observed in Culmore and is lower than the pH of between 5 and 7 recommended for willow growth (AFBI, 2010 and Galbally *et al,* 2013). Statistical analysis revealed that pH differences between the control and various treatment plots were insignificant with the exception of the control and T4 plot , with the latter having a slightly higher pH value than the control plot. There was a significant difference in soil pH between Phase 1 and 3 with an average value of 5.0 in Phase 1 which decreased to 4.5 in Phase 3 as was the case in Culmore. This drop in soil pH could be attributed to a number of factors but the most important factor from the perspective of this investigation could be the application of an acidic wastewater, however this was not the case, since the average pH of the D.P.W applied over the investigation period was in the range 6.6-6.8. In addition, a decrease in pH was also observed in the control plot which was not irrigated with D.P.W.

Since a significant drop in soil pH was observed in the Hillsborough SRC willow plantation between Phase 1 and 3, correlation tests were run for soil pH (range=4.3-5.7) and the abundance of earthworms to rule this out as a factor in any observed abundance changes. A weak correlations was obtained (r^2 =0.039). This would seem to indicate that the decreasing pH at Hillsborough SRC willow plantation is not adversely impacting bio-indicator abundance as had been the finding previously in Culmore SRC willow plantation. It is important to note that correlation tests were run for total abundance values of all species of earthworms as opposed to separate tests for individual species which proved unreliable due the low population numbers on-site for a number of species. As in Culmore, the observed pH values at Hillsborough would not be ideal for earthworm species that thrive in more neutral pHs, however it is within the range tolerated by many earthworm species e.g. *E. tetraedra, L. eisenia, O. tyrtaeum* and *A. rosea*. De Goede and Brussard (2002) state that a low pH will impact on the abundance of earthworms by limiting the abundance of less tolerant species and this seems to be the case in Hillsborough SRC willow plantation as the most numerous species recorded at Hillsborough were *E. tetraedra* (preferred range: 4.6-8.5) and *L. eiseni* (preferred range: 3.6-7.6). These species were also the most numerous in Culmore. Other earthworm species that prefer a more alkaline environment (e.g. *L. terrestris*) were present in much lower numbers as was the case in Culmore.

Mites and springtails are reported to have a higher tolerance to acidic soils than earthworms and should therefore not be adversely affected by the decreasing soil pHs observed in Hillsborough. This is confirmed by the weak relationships observed between soil pH (range=4.3-5.7) and the abundance of mites and springtails with r^2 values obtained of 0.156 and -0.146, respectively. Studies suggest that acidifying soil pHs down to a value of 4.5 has no effect on the abundance of soil mites (Hagvar and Gunnar 1980, Davey *et al.* 1995, Lóšková *et al.* 2013) while springtails also exhibit a high tolerance to low and changing soil pHs (van Straalen 1998, Garnier and Ponge 2004, Alerding 2013).

5.2.3 Effect of Planting Regime of SRC Willow on Abundance of Bio-indicators

The plantation design in Hillsborough allowed for sampling in both single and mixed genotype plots to determine whether this factor had an impact on the abundance of bio-indicators. Prior to testing for any significant differences in MGP as compared to SGP, PerMANOVA was applied to test whether an interaction factor existed between planting regime and irrigation level, No interaction was evident which meant that the datasets from Hillsborough could be tested separately for the effects of irrigation and planting regime.

The use of single or mixed *Salix* genotype plots did not significantly affect the abundance of earthworms in Hillsborough regardless of irrigation status. Higher earthworm abundance was evident in MGPs on some sampling occasions and in SGPs on others, but no trend was immediately apparent. The condition of the local environment and land-management activities are reported to affect earthworm abundance (Paoletti 1999b, Jégou *et al.* 2002, Chan and Barchia 2007, Ernst *et al.* 2009) however, the results of this study indicate that planting of single *Salix* genotypes as compared to mixed genotypes does not affect earthworm abundance.

The use of single or mixed genotype plots did not in general significantly affect the abundance of mites in Hillsborough regardless of irrigation status. Upon inspection of the data sets the effect of mixed vs. single genotype of *Salix* was only apparent in T3 during the irrigated period. However this was unique and no similar effect was recorded in the control, T2 plots or T4 plots. The use of single or mixed genotype plots did not in general significantly affect the abundance of springtails in Hillsborough regardless of irrigation status except again, in the T3 plot during the irrigated period. Similar to the case for mites, this response was unique and not observed in the control plot, T2 plot or T4 plot.

Chapter 6 Conclusions

- No significant difference was observed in the abundance of earthworms, springtails or mites between irrigated (30m^3 primary treated wastewater/ha/d) and non-irrigated plots at Culmore SRC willow plantation over two irrigation seasons (2012 & 2013).
- The %SMC was not significantly different between irrigated and non-irrigated plots at Culmore during irrigated periods indicating that the plantation is effectively absorbing all of the applied wastewater. The wastewater application rate (3.0mm/ha/d) is well below typical rates that can be evapo-transpired from willow during summer months (i.e. 3.47-6.65mm/ha/d).
- Previous land-use significantly affected the abundance of earthworms and mites in Culmore SRC willow plantation with a greater abundance of earthworms recovered from plots that had been previously planted with grassland prior to SRC willow conversion in 2010. The abundance of mites was greatest in plots that had been previously planted with poplar. No interaction factor was evident between previous cropping history and irrigation.
- Earthworms were useful bio-indicators to monitor the impact of irrigation with dairy parlour washings at Hillsborough SRC willow plantation as their abundance decreased significantly at higher irrigation rates (i.e. 34 and 44 m^3/ha/day). There was no significant difference in earthworm abundance between the control plot at Hillsborough and the plot in receipt of 18 m^3/ha/day. The abundance of mites and springtails was not significantly different in the control plot as compared to the various treatment plots at Hillsborough indicating that these soil invertebrates can withstand much higher irrigation levels that earthworms.
- The %SMC in the various treatment plots at Hillsborough did not differ significantly from the control plot with the exception of the T4 plot, which had a lower average %SMC that the control plot indicating that the plantation is effectively absorbing all of the applied wastewater even at application rates of 44m^3/ha/d which is within the evapo-transpiration range of SRC willow plantation during summer months.

- There was no significant difference between the recovery of earthworms, springtails or mites under different planting regimes i.e. MGP and SGPs. Earthworms in a MGP are no more resilient to higher irrigation levels (i.e. above $34m^3$/ha/d) than those in a SGP.
- The soil pH in Culmore and Hillsborough SRC willow plantations was lower than that recommended for willow growth (i.e. pH 5-7). In both sites, the soil pH decreased significantly over the investigation period i.e. in Culmore SRC willow plantation, the average soil pH decreased from 5.0 in 2012 to 4.4 in 2013 while in Hillsborough SRC willow plantation, the average soil pH decreased from 5.0 in 2012 to 4.5 in 2013. Soil pH was not significantly affected by irrigation at either site since the pH decreased equally across irrigated and non-irrigated plots. This may be connected to the buffering capacity of the soil but further research is necessary to determine if this is the case.
- The majority of earthworms recovered at both SRC willow plantations were non-clitellate but the most common adult earthworms species observed was *E. tetraedra*, an acid-tolerant earthworm. Earthworm species that require higher pH levels e.g. *L. terrestris* were present in much lower numbers at both sites.
- A higher abundance of earthworms was present in the Hillsborough SRC willow plantation (Control and T2) than in Culmore SRC willow plantation. The average abundance of earthworms at Hillsborough was typical of moderate-intensively managed agricultural land while earthworm abundance in Culmore was typical of intensively managed agricultural land. The Hillsborough plantation was established in 2007 while the Culmore plantation was established in 2010 providing a longer recovery period for earthworms since the plantation establishment phase. Lower numbers of both springtails and mites were observed in Hillsborough SRC willow plantation than in Culmore SRC willow plantation both in the control plot and in plots receiving varying levels of irrigation. The abundance of springtails and mites at both sites is typical of intensively managed agricultural land.

Chapter 7 Recommendations

- A resting period of at least 5 years is recommended for the Culmore and Hillsborough SRC willow plantations to allow earthworm, springtail and mite populations to increase before any other further sampling is undertaken. Thereafter the abundance of earthworms, mites and springtails should be further investigated to compare trends in abundance at a later stage in the SRC willow plantation.

- Further research is required on the impact of second and subsequent rotations of SRC willow on the abundance of earthworms, springtails and mites.

- Further research is required to determine the highest irrigation rate, using primary treated municipal effluent, which can be tolerated by earthworms, springtails and mites since these soil invertebrates were not affected at irrigation rates of $30m^3$/ha/d.

- Further research is required to determine the highest irrigation rate, using dairy parlour washings, which can be tolerated by earthworms since these were not affected at irrigation rates of $18m^3$/ha/d. Irrigation rates above this should not be applied at Hillsborough SRC willow plantation until this research is undertaken in order to protect earthworms at the site.

- The soil pH at Culmore and Hillsborough is below that recommended for SRC willow plantations and the benefits of increasing soil pH using lime should be investigated.

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Appendix

Appendix A: Bioenergy Cropping Area (ha) in E.U

Table A1: Bioenergy cropping area (ha) in E.U (2006-2008) (Panoutsou *et al.* 2011)

	Rape	**Sun flower**	**Wheat**	**Barley**	**Sugar beet**	**Maize**	**Reed Grass**	**Willow**	**Poplar**	**Miscanthus**	**Hemp**	**Other**
Belgium	959		1173	191		660						
Bulgaria		258094										
Czech Rep.	104000											
Denmark			51300	42750				2500				
Germany	1105000		78080	49920	3000	295000			500	300		
Ireland										2000		
Greece		11220										
Spain		11902	21159	150223					18			104
France	885687	66665	225000	75000	50000	50000		500		1500		
Italy	5200	59800							6000	7500		
Hungary	10175	8325										
Netherlands	2500					500						
Austria	10200	4800	855	645	0	40000					300	
Poland	740740							7000		13500		
Romania	22746	545912										
Finland	821		119	320			18700					
Sweden	50000		19600	15400			780	13000			390	
U.K	320542		10824	5093				5500		13500		
Total	3258571	1105038	398852	210479	53000	386160	19480	28500	6518	38300	690	104

Appendix B: Raw Data Recorded at the Culmore and Hillsborough Willow Plantations

Table B1: Recorded earthworm abundance per m^2 in Culmore SRC Willow Plantation

		Site			Site			Site	
Plot	**Date**	**A**	**B**	**Date**	**A**	**B**	**Date**	**A**	**B**
7	**06/07/12**	0	0	**20/11/12**	0	0	**25/06/13**	0	0
8	**06/07/12**	106	92	**20/11/12**	18	12	**25/06/13**	42	0
9	**06/07/12**	0	0	**20/11/12**	2	0	**25/06/13**	0	0
10	**06/07/12**	0	6	**20/11/12**	0	0	**25/06/13**	0	0
11	**06/07/12**	278	94	**20/11/12**	50	38	**25/06/13**	40	60
12	**06/07/12**	14	0	**20/11/12**	16	12	**25/06/13**	0	0
7	**31/07/12**	6	0	**05/03/13**	10	2	**30/07/13**	0	0
8	**31/07/12**	44	0	**05/03/13**	18	8	**30/07/13**	28	0
9	**31/07/12**	0	0	**05/03/13**	0	0	**30/07/13**	22	2
10	**31/07/12**	24	0	**05/03/13**	0	0	**30/07/13**	14	32
11	**31/07/12**	94	48	**05/03/13**	26	16	**30/07/13**	74	6
12	**31/07/12**	6	0	**05/03/13**	0	0	**30/07/13**	0	0
7	**29/08/12**	4	0	**03/04/13**	0	0	**06/08/13**	0	0
8	**29/08/12**	98	68	**03/04/13**	8	4	**06/08/13**	46	0
9	**29/08/12**	22	0	**03/04/13**	0	0	**06/08/13**	22	0
10	**29/08/12**	0	0	**03/04/13**	0	0	**06/08/13**	0	0
11	**29/08/12**	38	68	**03/04/13**	12	20	**06/08/13**	44	8
12	**29/08/12**	8	0	**03/04/13**	0	0	**06/08/13**	0	0
7	**26/09/12**	0	0	**30/04/13**	0	0	**17/08/13**	2	6
8	**26/09/12**	48	36	**30/04/13**	24	22	**17/08/13**	78	30
9	**26/09/12**	0	0	**30/04/13**	0	0	**17/08/13**	0	0
10	**26/09/12**	0	0	**30/04/13**	0	0	**17/08/13**	0	4
11	**26/09/12**	48	36	**30/04/13**	40	12	**17/08/13**	8	8
12	**26/09/12**	0	0	**30/04/13**	6	2	**17/08/13**	0	0
7	**23/10/12**	0	0	**27/05/13**	0	0			
8	**23/10/12**	40	28	**27/05/13**	60	40			
9	**23/10/12**	4	0	**27/05/13**	0	0			
10	**23/10/12**	0	0	**27/05/13**	0	0			
11	**23/10/12**	68	36	**27/05/13**	38	50			
12	**23/10/12**	0	0	**27/05/13**	0	0			

Table B2: Average epigeic (EP), endogeic (EN) and anecic (AN) and Non-Clitellate (NC) earthworms (per m^2) in various plots in Culmore plantation

Plot	Date	EP	EN	AN	NC	Date	EP	EN	AN	NC	Date	EP	EN	AN	NC
7	**06/07/12**	0	0	0	0	**20/11/12**	0	0	0	0	**25/06/13**	0	0	0	0
8	**06/07/12**	3	0	2	94	**20/11/12**	3	0	3	9	**25/06/13**	3	4	3	11
9	**06/07/12**	0	0	0	0	**20/11/12**	0	0	0	1	**25/06/13**	0	0	0	0
10	**06/07/12**	0	0	0	3	**20/11/12**	0	0	0	0	**25/06/13**	0	0	0	0
11	**06/07/12**	0	0	0	186	**20/11/12**	2	0	2	40	**25/06/13**	2	2	2	44
12	**06/07/12**	0	0	0	7	**20/11/12**	0	0	0	14	**25/06/13**	0	0	0	0
7	**31/07/12**	0	0	0	3	**05/03/13**	0	0	0	6	**30/07/13**	0	0	0	0
8	**31/07/12**	1	0	3	18	**05/03/13**	2	0	2	9	**30/07/13**	2	0	1	11
9	**31/07/12**	0	0	0	0	**05/03/13**	0	0	0	0	**30/07/13**	0	0	0	12
10	**31/07/12**	0	0	0	12	**05/03/13**	0	0	0	0	**30/07/13**	0	0	0	23
11	**31/07/12**	2	0	3	66	**05/03/13**	2	0	1	18	**30/07/13**	0	0	2	39
12	**31/07/12**	0	0	0	3	**05/03/13**	0	0	0	0	**30/07/13**	0	0	0	0
7	**29/08/12**	0	0	0	2	**03/04/13**	0	0	0	0	**06/08/13**	0	0	0	0
8	**29/08/12**	5	1	6	71	**03/04/13**	0	0	1	10	**06/08/13**	10	1	1	12
9	**29/08/12**	2	0	0	9	**03/04/13**	0	0	0	0	**06/08/13**	0	0	0	11
10	**29/08/12**	0	0	0	0	**03/04/13**	0	0	0	0	**06/08/13**	0	0	0	0
11	**29/08/12**	1	0	6	46	**03/04/13**	0	1	2	13	**06/08/13**	0	0	1	25
12	**29/08/12**	0	0	0	4	**03/04/13**	0	0	0	0	**06/08/13**	0	0	0	0
7	**26/09/12**	0	0	0	0	**30/04/13**	0	0	0	0	**17/08/13**	0	0	0	8
8	**26/09/12**	4	1	4	33	**30/04/13**	4	2	1	16	**17/08/13**	2	0	1	51
9	**26/09/12**	0	0	0	0	**30/04/13**	0	0	0	0	**17/08/13**	0	0	0	0
10	**26/09/12**	0	0	0	0	**30/04/13**	0	0	0	0	**17/08/13**	0	0	0	2
11	**26/09/12**	9	1	3	29	**30/04/13**	4	0	1	21	**17/08/13**	0	0	0	8
12	**26/09/12**	0	0	0	0	**30/04/13**	0	0	0	4	**17/08/13**	0	0	0	0
7	**23/10/12**	0	0	0	0	**27/05/13**	0	0	0	0					
8	**23/10/12**	9	2.5	3	18	**27/05/13**	3	2	2	43					
9	**23/10/12**	0	0	0	2	**27/05/13**	0	0	0	0					
10	**23/10/12**	0	0	0	0	**27/05/13**	0	0	0	0					
11	**23/10/12**	21	2	3	26	**27/05/13**	6	0	5	33					
12	**23/10/12**	0	0	0	0	**27/05/13**	0	0	0	0					

Table B3: Recorded mite abundance (kg of dry soil) in Culmore SRC Willow Plantation

		Site				Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
7	**29/8/12**	137.3	303.2	364.3	**03/04/13**	20.3	55.2	59.5	**30/07/13**	5.9	12.8	89.8
8	**29/8/12**	54.8	455.2	27.8	**03/04/13**	18.2	21.4	8.5	**30/07/13**	9.8	22.4	20.6
9	**29/8/12**	374.4	114.4	538.5	**03/04/13**	10.0	15.8	5.2	**30/07/13**	31.3	51.1	6.2
10	**29/8/12**	58.3	163.0	107.4	**03/04/13**	153.3	157.0	90.7	**30/07/13**	37.2	13.3	12.4
11	**29/8/12**	116.3	31.3	0.0	**03/04/13**	23.6	4.7	25.5	**30/07/13**	85.9	32.3	24.2
12	**29/8/12**	18.0	29.5	0.0	**03/04/13**	7.5	30.8	173	**30/07/13**	85.6	80.0	0.0
7	**26/9/12**	586.9	173.5	217.3	**30/04/13**	489.7	52.6	0.0	**06/08/13**	24.4	9.1	94.1
8	**26/9/12**	298.9	25.2	35.9	**30/04/13**	20.4	14.4	0.0	**06/08/13**	62.6	17.6	29.1
9	**26/9/12**	37.1	50.6	44.0	**30/04/13**	112.2	61.1	0.0	**06/08/13**	101.3	95.4	0.0
10	**26/9/12**	89.4	60.8	280.8	**30/04/13**	67.5	5.7	0.0	**06/08/13**	28.9	59.4	36.3
11	**26/9/12**	132.4	111.7	96.5	**30/04/13**	65.4	40.6	0.0	**06/08/13**	84.4	15.3	86.9
12	**26/9/12**	104.8	72.4	2.9	**30/04/13**	0.0	15.7	0.0	**06/08/13**	32.5	12.3	106.1
7	**23/10/12**	106.5	135.5	31.5	**27/05/13**	13.9	47.6	120	**17/08/13**	81.2	72.2	14.3
8	**23/10/12**	10.4	59.3	92.0	**27/05/13**	0.0	16.1	9.5	**17/08/13**	109.6	43.8	0.0
9	**23/10/12**	25.5	104.1	3.7	**27/05/13**	17.2	13.0	32.5	**17/08/13**	45.4	0.0	56.7
10	**23/10/12**	18.5	87.4	29.7	**27/05/13**	233.3	5.3	18.7	**17/08/13**	45.4	65.2	53.7
11	**23/10/12**	3.3	17.9	7.4	**27/05/13**	61.4	22.6	0.0	**17/08/13**	46.5	109.7	57.0
12	**23/10/12**	65.1	106.1	149.4	**27/05/13**	9.2	23.2	19.0	**17/08/13**	189.2	58.9	72.3
7	**20/11/12**	155.0	994.6	35.6	**25/06/13**	96.6	98.1	81.3				
8	**20/11/12**	11.9	16.0	26.0	**25/06/13**	39.1	6.4	31.5				
9	**20/11/12**	135.7	10.0	104.6	**25/06/13**	47.5	37.2	106				
10	**20/11/12**	0.0	38.9	54.3	**25/06/13**	47.0	19.0	12.1				
11	**20/11/12**	3.2	0.0	0.0	**25/06/13**	31.6	0.0	9.6				
12	**20/11/12**	100.8	36.7	0.0	**25/06/13**	23.6	26.5	0.0				
7	**05/03/13**	189.5	125.5	70.2	**16/07/13**	49.7	39.2	4.7				
8	**05/03/13**	30.7	51.6	5.5	**16/07/13**	26.4	12.7	18.7				
9	**05/03/13**	0.0	0.0	0.0	**16/07/13**	0.0	3.9	12.9				
10	**05/03/13**	160.5	113.3	142.9	**16/07/13**	3.5	31.9	28.6				
11	**05/03/13**	125.9	94.7	5.2	**16/07/13**	41.0	46.6	0.0				
12	**05/03/13**	392.4	0.0	0.0	**16/07/13**	82.4	46.4	37.8				

Table B4: Recorded springtail abundance (kg of dry soil) in Culmore SRC Willow Plantation

		Site				Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
7	29/8/12	393.3	238.2	178.6	03/04/13	10.1	10.2	170	30/07/13	24.9	130.9	271.8
8	29/8/12	94.0	183.8	34.7	03/04/13	0.0	0.0	0.0	30/07/13	59.7	147.1	73.1
9	29/8/12	73.5	42.9	52.8	03/04/13	0.0	42.6	0.0	30/07/13	75.6	633.4	31.2
10	29/8/12	41.7	49.0	46.7	03/04/13	24.8	157.0	23.	30/07/13	85.8	33.3	61.1
11	29/8/12	29.1	31.3	0.0	03/04/13	13.3	0.0	15.3	30/07/13	231.7	291.6	108.9
12	29/8/12	18.0	22.1	0.0	03/04/13	18.9	11.8	27.9	30/07/13	31.7	0.0	0.0
7	26/9/12	215.7	58.8	202.0	30/04/13	12.0	2.6	0.0	06/08/13	42.2	38.8	58.5
8	26/9/12	106.7	3.6	15.3	30/04/13	0.0	0.0	0.0	06/08/13	172.7	0.0	119.8
9	26/9/12	148.4	86.0	34.2	30/04/13	0.0	0.0	0.0	06/08/13	88.0	28.9	0.0
10	26/9/12	6.5	49.6	47.3	30/04/13	12.3	18.7	0.0	06/08/13	212.8	199.8	99.8
11	26/9/12	252.8	61.4	91.6	30/04/13	58.9	65.8	0.0	06/08/13	0.0	92.6	88.9
12	26/9/12	31.4	106.4	38.9	30/04/13	6.4	0.0	0.0	06/08/13	32.5	24.5	75.0
7	23/10/12	53.5	21.8	0.0	27/05/13	17.1	9.4	9.5	17/08/13	39.9	162.4	49.4
8	23/10/12	36.4	43.5	51.1	27/05/13	0.0	30.0	0.0	17/08/13	234.9	59.4	78.6
9	23/10/12	38.3	69.4	0.0	27/05/13	37.4	28.2	10.6	17/08/13	2.7	4.2	42.2
10	23/10/12	0.0	36.4	3.7	27/05/13	0.0	69.4	74.6	17/08/13	206.4	34.1	73.9
11	23/10/12	6.5	31.4	14.8	27/05/13	79.6	89.9	0.0	17/08/13	92.0	101.4	113.1
12	23/10/12	70.0	66.4	150.6	27/05/13	4.1	42.2	33.1	17/08/13	90.1	22.1	20.2
7	20/11/12	8.7	48.2	3.6	25/06/13	75.5	87.1	62.4				
8	20/11/12	0.0	0.0	0.0	25/06/13	46.0	60.8	83.4				
9	20/11/12	49.8	5.0	44.8	25/06/13	40.2	60.5	47.4				
10	20/11/12	3.9	11.7	8.2	25/06/13	1.2	7.8	16.1				
11	20/11/12	38.5	4.9	00	25/06/13	69.7	0.0	69.9				
12	20/11/12	15.3	0.0	0.0	25/06/13	18.9	18.8	0.0				
7	05/03/13	44.8	40.1	49.0	16/07/13	345.9	53.8	233				
8	05/03/13	0.0	22.0	10.3	16/07/13	59.03	0.0	48.7				
9	05/03/13	0.0	0.0	10.1	16/07/13	81.3	0.0	81.2				
10	05/03/13	26.0	41.2	81.2	16/07/13	25.9	3.0	0.0				
11	05/03/13	22.6	14.9	0.0	16/07/13	69.7	0.0	518				
12	05/03/13	0.0	0.0	132.1	16/07/13	40.9	4.8	4.7				

Table B5: Recorded earthworm abundance per m^2 in mixed genotype plots, Hillsborough

		Site			Site	
Plot	**Date**	**A**	**B**	**Date**	**A**	**B**
Control	**20/07/12**	142	0	**16/04/13**	32	24
T2	**20/07/12**	206	90	**16/04/13**	50	34
T3	**20/07/12**	20	0	**16/04/13**	0	0
T4	**20/07/12**	6	8	**16/04/13**	0	0
Control	**13/08/12**	42	176	**14/05/13**	14	50
T2	**13/08/12**	94	112	**14/05/13**	12	62
T3	**13/08/12**	2	0	**14/05/13**	7	1
T4	**13/08/12**	0	8	**14/05/13**	4	2
Control	**11/09/12**	102	0	**11/06/13**	10	20
T2	**11/09/12**	62	0	**11/06/13**	24	10
T3	**11/09/12**	38	0	**11/06/13**	0	0
T4	**11/09/12**	36	0	**11/06/13**	0	0
Control	**09/10/12**	2	96	**09/07/13**	24	18
T2	**09/10/12**	56	40	**09/07/13**	14	8
T3	**09/10/12**	0	0	**09/07/13**	0	0
T4	**09/10/12**	6	4	**09/07/13**	0	0
Control	**06/11/12**	18	26	**23/07/13**	0	0
T2	**06/11/12**	12	2	**23/07/13**	0	0
T3	**06/11/12**	0	0	**23/07/13**	0	0
T4	**06/11/12**	0	0	**23/07/13**	0	0
Control	**04/12/12**	20	10	**13/08/13**	6	56
T2	**04/12/12**	4	20	**13/08/13**	16	20
T3	**04/12/12**	0	0	**13/08/13**	0	0
T4	**04/12/12**	0	0	**13/08/13**	0	0
Control	**20/03/13**	0	0	**28/08/13**	4	0
T2	**20/03/13**	2	0	**28/08/13**	20	74
T3	**20/03/13**	0	0	**28/08/13**	0	14
T4	**20/03/13**	2	6	**28/08/13**	0	0

Table B6: Recorded earthworm abundance per m^2 in single genotype plots, Hillsborough

		Site			Site	
Plot	**Date**	**A**	**B**	**Date**	**A**	**B**
Control	**17/07/12**	n.d	n.d	**16/04/13**	34	48
T2	**17/07/12**	n.d	n.d	**16/04/13**	52	50
T3	**17/07/12**	n.d	n.d	**16/04/13**	0	0
T4	**17/07/12**	n.d	n.d	**16/04/13**	0	0
Control	**14/08/12**	104	74	**14/05/13**	48	66
T2	**14/08/12**	0	0	**14/05/13**	36	62
T3	**14/08/12**	0	0	**14/05/13**	0	0
T4	**14/08/12**	0	0	**14/05/13**	0	0
Control	**11/09/12**	114	0	**11/06/13**	12	10
T2	**11/09/12**	52	46	**11/06/13**	10	20
T3	**11/09/12**	16	0	**11/06/13**	0	0
T4	**11/09/12**	0	0	**11/06/13**	2	6
Control	**09/10/12**	66	68	**09/07/13**	4	42
T2	**09/10/12**	48	52	**09/07/13**	6	34
T3	**09/10/12**	6	0	**09/07/13**	0	0
T4	**09/10/12**	0	0	**09/07/13**	0	0
Control	**06/11/12**	14	20	**23/07/13**	12	34
T2	**06/11/12**	16	6	**23/07/13**	2	12
T3	**06/11/12**	0	0	**23/07/13**	0	0
T4	**06/11/12**	0	4	**23/07/13**	0	0
Control	**04/12/12**	10	8	**13/08/13**	52	14
T2	**04/12/12**	4	2	**13/08/13**	130	0
T3	**04/12/12**	0	0	**13/08/13**	0	0
T4	**04/12/12**	0	0	**13/08/13**	0	0
Control	**20/03/13**	0	0	**28/08/13**	56	38
T2	**20/03/13**	0	0	**28/08/13**	41	55
T3	**20/03/13**	0	0	**28/08/13**	0	0
T4	**20/03/13**	0	0	**28/08/13**	0	0

Table B7: Breakdown of epigeic (EP), endogeic (EN), anecic (An) and non-clitellate (NC) earthworms (m^2) in Hillsborough mixed genotype plots

Plot	Date	EP	EN	AN	NC	Date	EP	EN	AN	NC
Control	18/07/12	3	4	2	62	16/04/13	4	1	1	22
T2	18/07/12	17	16	5	109	16/04/13	12	4	2	24
T3	18/07/12	1	2	0	7	16/04/13	0	0	0	0
T4	18/07/12	0	0	0	7	16/04/13	0	0	0	0
Control	13/08/12	7	3	1	98	14/05/13	1	0	3	28
T2	13/08/12	18	11	5	69	14/05/13	1	0	5	31
T3	13/08/12	0	0	0	1	14/05/13	1	0	0	3
T4	13/08/12	0	0	0	4	14/05/13	1	0	0	2
Control	11/09/12	19	0	0	32	11/06/13	1	1	1	12
T2	11/09/12	6	4	2	18	11/06/13	3	1	2	11
T3	11/09/12	4	1	1	12	11/06/13	0	0	0	0
T4	11/09/12	4	2	1	11	11/06/13	0	0	0	0
Control	09/10/12	0	0	3	46	09/07/13	7	0	0	5
T2	09/10/12	10	2	4	32	09/07/13	2	0	0	8
T3	09/10/12	0	0	0	0	09/07/13	0	0	0	0
T4	09/10/12	1	0	0	4	09/07/13	0	0	0	0
Control	06/11/12	4	1	2	15	23/07/13	0	0	0	0
T2	06/11/12	2	0	0	5	23/07/13	0	0	0	0
T3	06/11/12	0	0	0	0	23/07/13	0	0	0	0
T4	06/11/12	0	0	0	0	23/07/13	0	0	0	0
Control	04/12/12	0	0	0	15	13/08/13	0	0	0	31
T2	04/12/12	0.5	0	0	11.5	13/08/13	0	0	0	18
T3	04/12/12	0	0	0	0	13/08/13	0	0	0	0
T4	04/12/12	0	0	0	4	13/08/13	0	0	0	0
Control	20/03/13	0	0	0	0	28/08/13	0	0	0	2
T2	20/03/13	0	0	0	1	28/08/13	0	0	0	47
T3	20/03/13	0	0	0	0	28/08/13	0	0	0	0
T4	20/03/13	0	0	0	4	28/08/13	0	0	0	0

Table B8: Breakdown of epigeic (EP), endogeic (EN), anecic (AN) and non-clitellum (NC) earthworms (m^2) in Hillsborough single genotype plots

Plot	Date	EP	EN	AN	NC	Date	EP	EN	AN	NC
Control	17/07/12	n.d	n.d	n.d	n.d	16/04/13	13	0	4	24
T2	17/07/12	n.d	n.d	n.d	n.d	16/04/13	30	2	3	19
T3	17/07/12	n.d	n.d	n.d	n.d	16/04/13	0	0	0	0
T4	17/07/12	n.d	n.d	n.d	n.d	16/04/13	0	0	0	0
Control	14/08/12	22	12	6	49	14/05/13	17	2	5	33
T2	14/08/12	0	0	0	0	14/05/13	14	10	4	21
T3	14/08/12	0	0	0	0	14/05/13	0	0	0	0
T4	14/08/12	0	0	0	0	14/05/13	0	0	0	0
Control	11/09/12	17	10	3	27	11/06/13	5	0	0	6
T2	11/09/12	4	1	1	43	11/06/13	3	0	1	11
T3	11/09/12	0	1	1	6	11/06/13	0	0	0	0
T4	11/09/12	0	0	0	3	11/06/13	1	1	0	2
Control	09/10/12	7.5	3.5	1	55	09/07/13	8	0	0	15
T2	09/10/12	22	0	4	24	09/07/13	3	0	0	17
T3	09/10/12	1	0	0	2	09/07/13	0	0	0	0
T4	09/10/12	0	0	0	0	09/07/13	0	0	0	0
Control	06/11/12	4.5	3	0.5	9	25/07/13	5	0	0	18
T2	06/11/12	2	0	0	9	25/07/13	6	1	0	13
T3	06/11/12	0	0	0	0	25/07/13	0	0	0	0
T4	06/11/12	0	0	0	2	25/07/13	0	0	0	0
Control	04/12/12	0	0	0	9	13/08/13	2	0	0	31
T2	04/12/12	0	0	0	3	13/08/13	2	1	2	60
T3	04/12/12	0	0	0	0	13/08/13	0	0	0	0
T4	04/12/12	0	0	0	0	13/08/13	0	0	0	0
Control	20/03/13	0	0	0	0	28/08/13	0	1	0	46
T2	20/03/13	0	0	0	0	28/08/13	0	0	0	48
T3	20/03/13	0	0	0	0	28/08/13	0	0	0	0
T4	20/03/13	0	0	0	0	28/08/13	0	0	0	0

Table B9: Recorded mite abundance (kg of dry soil) in mixed genotype plots, Hillsborough

		Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
Control	**11/09/12**	8.0	12.0	3.4	**11/06/13**	0.0	2.9	2.9
T2	**11/09/12**	12.6	0.0	6.8	**11/06/13**	0.0	0.0	6.6
T3	**11/09/12**	90.0	25.4	112.7	**11/06/13**	15.7	9.8	6.8
T4	**11/09/12**	51.1	51.1	24.9	**11/06/13**	6.8	0.0	0.0
Control	**09/10/12**	12.8	29.4	9.0	**09/07/13**	10.3	23.2	8.5
T2	**09/10/12**	5.4	0.0	38.5	**09/07/13**	29.8	58.4	12.9
T3	**09/10/12**	16.4	23.0	0.0	**09/07/13**	7.1	7.4	6.5
T4	**09/10/12**	14.7	14.2	3.4	**09/07/13**	29.0	10.2	30.2
Control	**06/11/12**	0.0	0.0	0.0	**25/07/13**	0.0	8.3	0.0
T2	**06/11/12**	0.0	0.0	0.0	**25/07/13**	7.4	0.0	0.0
T3	**06/11/12**	2.6	0.0	0.0	**25/07/13**	0.0	0.0	7.9
T4	**06/11/12**	0.0	0.0	3.3	**25/07/13**	0.0	0.0	0.0
Control	**04/12/12**	0.0	0.0	0.0	**13/08/13**	31.6	5.4	30.2
T2	**04/12/12**	4.3	0.0	5.4	**13/08/13**	33.7	25.6	7.0
T3	**04/12/12**	0.0	5.7	13.9	**13/08/13**	44.2	15.7	37.0
T4	**04/12/12**	42.0	5.5	4.1	**13/08/13**	41.7	25.8	16.5
Control	**20/03/13**	0.0	6.4	0.0	**28/08/13**	52.7	44.1	43.8
T2	**20/03/13**	10.1	0.0	0.0	**28/08/13**	16.0	46.5	71.2
T3	**20/03/13**	49.3	11.5	0.0	**28/08/13**	20.8	48.4	15.4
T4	**20/03/13**	0.0	410.6	0.0	**28/08/13**	686.9	45.5	51.1
Control	**16/04/13**	14.6	0.0	7.2				
T2	**16/04/13**	0.0	0.0	0.0				
T3	**16/04/13**	6.6	18.5	16.4				
T4	**16/04/13**	25.4	0.0	0.0				
Control	**14/05/13**	3.0	0.0	0.0				
T2	**14/05/13**	3.2	0.0	0.0				
T3	**14/05/13**	23.7	6.4	0.0				
T4	**14/05/13**	3.5	0.0	4.2				

Table B10: Recorded mite abundance (kg of dry soil) in single genotype plots, Hillsborough

		Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
Control	**11/09/12**	9.9	0.0	7.0	**11/06/13**	8.5	2.8	0.0
T2	**11/09/12**	10.5	7.8	0.0	**11/06/13**	0.0	0.0	2.9
T3	**11/09/12**	63.2	48.7	6.8	**11/06/13**	0.0	0.0	2.9
T4	**11/09/12**	18.0	47.7	23.3	**11/06/13**	0.0	6.0	8.9
Control	**09/10/12**	9.0	66.3	29.1	**09/07/13**	24.9	9.3	97.7
T2	**09/10/12**	0.0	0.0	0.0	**09/07/13**	24.3	21.9	0.0
T3	**09/10/12**	9.8	14.2	54.9	**09/07/13**	0.0	3.4	0.0
T4	**09/10/12**	4.6	5.9	3.0	**09/07/13**	3.8	27.1	0.0
Control	**06/11/12**	7.5	2.5	3.7	**25/07/13**	44.8	0.0	0.0
T2	**06/11/12**	3.1	0.0	3.3	**25/07/13**	0.0	1.2	0.0
T3	**06/11/12**	0.0	8.6	44.1	**25/07/13**	33.6	0.0	0.0
T4	**06/11/12**	34.8	7.6	9.2	**25/07/13**	0.0	0.0	0.0
Control	**04/12/12**	19.4	3.9	0.0	**13/08/13**	64.0	42.6	82.5
T2	**04/12/12**	0.0	0.0	0.0	**13/08/13**	46.8	34.5	86.3
T3	**04/12/12**	28.0	29.7	0.0	**13/08/13**	14.7	10.4	22.4
T4	**04/12/12**	0.0	0.0	0.0	**13/08/13**	51.0	27.7	30.1
Control	**20/03/13**	7.8	0.0	3.2	**28/08/13**	0.0	4.7	25.5
T2	**20/03/13**	0.0	10.2	7.3	**28/08/13**	56.5	34.0	27.2
T3	**20/03/13**	39.6	3.2	55.8	**28/08/13**	0.0	5.5	5.2
T4	**20/03/13**	9.3	0.0	6.4	**28/08/13**	7.0	27.7	0.0
Control	**16/04/13**	0.0	11.9	7.9				
T2	**16/04/13**	3.8	0.0	3.4				
T3	**16/04/13**	3.8	6.4	4.3				
T4	**16/04/13**	0.0	0.0	0.0				
Control	**14/05/13**	0.0	0.0	0.0				
T2	**14/05/13**	0.0	0.0	0.0				
T3	**14/05/13**	0.0	0.0	0.0				
T4	**14/05/13**	0.0	0.0	0.0				

Table B11: Springtail abundance (kg of dry soil) in mixed genotype plots, Hillsborough

		Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
Control	**11/09/12**	4.0	28.0	6.7	**11/06/13**	0.0	8.8	2.9
T2	**11/09/12**	6.3	15.6	0.0	**11/06/13**	5.7	0.0	6.6
T3	**11/09/12**	30.0	39.8	50.1	**11/06/13**	7.8	13.0	3.4
T4	**11/09/12**	3.9	71.6	49.8	**11/06/13**	3.1	0.0	5.9
Control	**09/10/12**	15.3	84.1	3.0	**09/07/13**	226.6	188.8	34.0
T2	**09/10/12**	19.0	6.4	65.9	**09/07/13**	72.7	129.8	116.5
T3	**09/10/12**	27.4	26.3	0.0	**09/07/13**	24.8	43.5	9.8
T4	**09/10/12**	5.9	14.2	0.0	**09/07/13**	16.1	10.2	4.3
Control	**06/11/12**	0.0	6.1	0.0	**25/07/13**	0.0	24.8	0.0
T2	**06/11/12**	0.0	0.0	0.0	**25/07/13**	7.4	0.0	0.0
T3	**06/11/12**	0.0	0.0	4.7	**25/07/13**	15.9	0.0	0.0
T4	**06/11/12**	3.4	0.0	000	**25/07/13**	0.0	0.0	0.0
Control	**04/12/12**	0.0	4.1	0.0	**13/08/13**	13.5	27.2	5.0
T2	**04/12/12**	0.0	11.2	8.0	**13/08/13**	117.9	12.8	14.1
T3	**04/12/12**	12.4	17.0	20.9	**13/08/13**	51.5	36.7	15.9
T4	**04/12/12**	37.4	5.5	16.4	**13/08/13**	31.3	28.5	16.5
Control	**20/03/13**	0.0	6.4	0.0	**28/08/13**	0.0	36.8	56.3
T2	**20/03/13**	5.1	0.0	0.0	**28/08/13**	5.3	17.4	21.9
T3	**20/03/13**	16.4	5.7	0.0	**28/08/13**	5.2	48.4	46.3
T4	**20/03/13**	123.0	51.3	0.0	**28/08/13**	7.6	71.6	17.0
Control	**16/04/13**	5.8	2.8	7.2				
T2	**16/04/13**	7.4	0.0	4.1				
T3	**16/04/13**	16.6	40.0	13.1				
T4	**16/04/13**	0.0	0.0	0.0				
Control	**14/05/13**	0.0	3.2	8.7				
T2	**14/05/13**	0.0	0.0	7.3				
T3	**14/05/13**	43.4	3.2	7.0				
T4	**14/05/13**	3.5	0.0	4.2				

Table B12: Springtail abundance (kg of dry soil) in single genotype plots, Hillsborough

		Site				Site		
Plot	**Date**	**A**	**B**	**C**	**Date**	**A**	**B**	**C**
Control	**11/09/12**	6.6	27.4	7.0	**11/06/13**	8.5	7.1	0.0
T2	**11/09/12**	38.4	3.9	27.4	**11/06/13**	3.6	11.2	0.0
T3	**11/09/12**	72.2	6.3	27.4	**11/06/13**	0.0	0.0	5.9
T4	**11/09/12**	17.1	27.0	9.5	**11/06/13**	0.0	10.5	0.0
Control	**09/10/12**	42.2	91.7	29.1	**09/07/13**	141.9	61.1	397.0
T2	**09/10/12**	13.5	11.5	0.0	**09/07/13**	7.3	7.3	17.9
T3	**09/10/12**	130.8	22.8	56.9	**09/07/13**	3.5	0.0	4.1
T4	**09/10/12**	0.0	18.4	0.0	**09/07/13**	0.0	9.0	0.0
Control	**06/11/12**	32.4	8.8	3.7	**25/07/13**	84.9	0.0	0.0
T2	**06/11/12**	3.1	3.3	2.7	**25/07/13**	7.1	0.0	0.0
T3	**06/11/12**	12.0	11.4	36.1	**25/07/13**	26.9	0.0	0.0
T4	**06/11/12**	46.4	0.0	4.6	**25/07/13**	0.0	6.3	0.0
Control	**04/12/12**	11.6	10.0	0.0	**13/08/13**	103.8	41.3	33.4
T2	**04/12/12**	0.0	0.0	0.0	**13/08/13**	97.8	113.3	137.2
T3	**04/12/12**	28.0	6.6	0.0	**13/08/13**	10.4	22.4	11.3
T4	**04/12/12**	0.0	0.0	0.0	**13/08/13**	34.0	166.4	48.1
Control	**20/03/13**	11.7	0.0	0.0	**28/08/13**	7.6	14.2	45.2
T2	**20/03/13**	0.0	25.6	3.6	**28/08/13**	17.0	149.7	6.8
T3	**20/03/13**	15.2	6.3	38.6	**28/08/13**	14.1	23.4	11.0
T4	**20/03/13**	0.0	0.0	12.8	**28/08/13**	5.2	7.1	6.9
Control	**16/04/13**	0.0	57.9	3.9				
T2	**16/04/13**	0.0	0.0	0.0				
T3	**16/04/13**	0.0	127.	0.0				
T4	**16/04/13**	0.0	0.0	0.0				
Control	**14/05/13**	0.0	0.0	0.0				
T2	**14/05/13**	0.0	0.0	0.0				
T3	**14/05/13**	0.0	0.0	0.0				
T4	**14/05/13**	0.0	0.0	0.0				

Table B13: Average soil pH in each plot at Culmore SRC willow plantation on specified sampling dates

Plot	Date	pH	Date	pH	Date	pH
7	**30/7/12**	5.31	**05/03/13**	5.31	**16/07/13**	4.55
8	**30/7/12**	5.84	**05/03/13**	5.84	**16/07/13**	4.47
9	**30/7/12**	5.71	**05/03/13**	5.71	**16/07/13**	4.43
10	**30/7/12**	5.61	**05/03/13**	5.61	**16/07/13**	4.23
11	**30/7/12**	5.69	**05/03/13**	5.69	**16/07/13**	4.53
12	**30/7/12**	5.60	**05/03/13**	4.04	**16/07/13**	4.51
7	**29/8/12**	4.60	**03/04/13**	4.16	**30/07/13**	4.37
8	**29/8/12**	4.74	**03/04/13**	4.64	**30/07/13**	4.31
9	**29/8/12**	4.56	**03/04/13**	4.45	**30/07/13**	4.44
10	**29/8/12**	4.86	**03/04/13**	3.74	**30/07/13**	4.47
11	**29/8/12**	4.81	**03/04/13**	4.39	**30/07/13**	4.52
12	**29/8/12**	4.97	**03/04/13**	4.31	**30/07/13**	4.63
7	**26/9/12**	4.90	**30/04/13**	4.45	**17/08/13**	4.37
8	**26/9/12**	4.10	**30/04/13**	4.15	**17/08/13**	4.40
9	**26/9/12**	4.97	**30/04/13**	4.23	**17/08/13**	4.61
10	**26/9/12**	4.76	**30/04/13**	4.25	**17/08/13**	4.40
11	**26/9/12**	4.67	**30/04/13**	4.55	**17/08/13**	4.47
12	**26/9/12**	5.14	**30/04/13**	4.33	**17/08/13**	4.96
7	**23/10/12**	4.80	**27/05/13**	4.46		
8	**23/10/12**	4.86	**27/05/13**	4.59		
9	**23/10/12**	5.07	**27/05/13**	4.06		
10	**23/10/12**	4.95	**27/05/13**	4.25		
11	**23/10/12**	4.85	**27/05/13**	4.82		
12	**23/10/12**	4.80	**27/05/13**	4.65		
7	**20/11/12**	4.68	**25/06/13**	4.70		
8	**20/11/12**	4.65	**25/06/13**	4.29		
9	**20/11/12**	4.67	**25/06/13**	4.31		
10	**20/11/12**	4.74	**25/06/13**	4.67		
11	**20/11/12**	4.76	**25/06/13**	4.59		
12	**20/11/12**	5.14	**25/06/13**	4.43		

Table B14: Average soil temperature in each plot at Culmore SRC willow plantation on specified sampling dates

Plot	Date	Temp (oC)	Date	Temp (oC)	Date	Temp (oC)
7	30/7/12	19	05/03/13	6	16/07/13	17
8	30/7/12	19	05/03/13	6	16/07/13	17
9	30/7/12	19	05/03/13	6	16/07/13	17
10	30/7/12	19	05/03/13	6	16/07/13	17
11	30/7/12	19	05/03/13	6	16/07/13	17
12	30/7/12	19	05/03/13	6	16/07/13	17
7	29/8/12	17	03/04/13	5	30/07/13	18
8	29/8/12	17	03/04/13	5	30/07/13	18
9	29/8/12	17	03/04/13	5	30/07/13	18
10	29/8/12	17	03/04/13	5	30/07/13	18
11	29/8/12	17	03/04/13	5	30/07/13	18
12	29/8/12	17	03/04/13	5	30/07/13	18
7	26/9/12	12	30/04/13	10	17/08/13	16
8	26/9/12	12	30/04/13	10	17/08/13	16
9	26/9/12	12	30/04/13	10	17/08/13	16
10	26/9/12	12	30/04/13	10	17/08/13	16
11	26/9/12	12	30/04/13	10	17/08/13	16
12	26/9/12	12	30/04/13	10	17/08/13	16
7	23/10/12	11	27/05/13	13		
8	23/10/12	11	27/05/13	13		
9	23/10/12	11	27/05/13	13		
10	23/10/12	11	27/05/13	13		
11	23/10/12	11	27/05/13	13		
12	23/10/12	11	27/05/13	13		
7	20/11/12	9	25/06/13	16		
8	20/11/12	9	25/06/13	16		
9	20/11/12	9	25/06/13	16		
10	20/11/12	9	25/06/13	16		
11	20/11/12	9	25/06/13	16		
12	20/11/12	9	25/06/13	16		

Table B15: Average %SMC in each plot at Culmore SRC willow plantation on specified sampling dates

Plot	Date	%SMC	Date	%SMC	Date	%SMC
7	**30/7/12**	52.4	**05/03/13**	46.6	**16/07/13**	44.2
8	**30/7/12**	42.6	**05/03/13**	49.7	**16/07/13**	40.7
9	**30/7/12**	34.8	**05/03/13**	45.6	**16/07/13**	38.8
10	**30/7/12**	44.0	**05/03/13**	42.4	**16/07/13**	39.9
11	**30/7/12**	49.5	**05/03/13**	53.5	**16/07/13**	30.1
12	**30/7/12**	39.5	**05/03/13**	61.4	**16/07/13**	46.3
7	**29/8/12**	43.4	**03/04/13**	41.5	**30/07/13**	38.5
8	**29/8/12**	51.0	**03/04/13**	42.8	**30/07/13**	42.0
9	**29/8/12**	39.1	**03/04/13**	45.2	**30/07/13**	45.9
10	**29/8/12**	43.9	**03/04/13**	43.2	**30/07/13**	48.1
11	**29/8/12**	47.6	**03/04/13**	47.3	**30/07/13**	38.8
12	**29/8/12**	38.3	**03/04/13**	52.2	**30/07/13**	49.6
7	**26/9/12**	46.9	**30/04/13**	47.6	**17/08/13**	36.0
8	**26/9/12**	53.3	**30/04/13**	45.3	**17/08/13**	38.7
9	**26/9/12**	50.2	**30/04/13**	44.5	**17/08/13**	32.2
10	**26/9/12**	28.1	**30/04/13**	38.4	**17/08/13**	29.9
11	**26/9/12**	45.2	**30/04/13**	48.5	**17/08/13**	39.4
12	**26/9/12**	39.8	**30/04/13**	35.8	**17/08/13**	45.3
7	**23/10/12**	42.9	**27/05/13**	47.4		
8	**23/10/12**	49.9	**27/05/13**	45.6		
9	**23/10/12**	37.4	**27/05/13**	50.9		
10	**23/10/12**	52.6	**27/05/13**	50.9		
11	**23/10/12**	42.2	**27/05/13**	37.6		
12	**23/10/12**	36.4	**27/05/13**	28.6		
7	**20/11/12**	46.4	**25/06/13**	47.0		
8	**20/11/12**	33.8	**25/06/13**	51.0		
9	**20/11/12**	45.8	**25/06/13**	43.6		
10	**20/11/12**	40.6	**25/06/13**	49.8		
11	**20/11/12**	46.5	**25/06/13**	50.7		
12	**20/11/12**	46.3	**25/06/13**	36.1		

Table B16: Average soil pH in each plot at Hillsborough SRC willow plantation on specified sampling dates

Plot	Date	pH	Date	pH
Control	**17/07/12**	5.32	**16/04/13**	4.98
T2	**17/07/12**	5.39	**16/04/13**	4.48
T3	**17/07/12**	5.66	**16/04/13**	4.27
T4	**17/07/12**	5.31	**16/04/13**	4.82
Control	**14/08/12**	5.28	**14/05/13**	4.62
T2	**14/08/12**	5.37	**14/05/13**	4.70
T3	**14/08/12**	5.34	**14/05/13**	4.50
T4	**14/08/12**	5.10	**14/05/13**	4.62
Control	**11/09/12**	5.06	**11/06/13**	4.57
T2	**11/09/12**	4.87	**11/06/13**	4.40
T3	**11/09/12**	4.71	**11/06/13**	4.41
T4	**11/09/12**	5.06	**11/06/13**	4.68
Control	**09/10/12**	4.61	**09/07/13**	4.50
T2	**09/10/12**	5.12	**09/07/13**	4.77
T3	**09/10/12**	4.71	**09/07/13**	4.41
T4	**09/10/12**	4.83	**09/07/13**	4.26
Control	**06/11/12**	4.60	**25/07/13**	4.40
T2	**06/11/12**	4.79	**25/07/13**	4.48
T3	**06/11/12**	5.01	**25/07/13**	4.88
T4	**06/11/12**	4.75	**25/07/13**	4.72
Control	**04/12/12**	4.44	**13/08/13**	4.73
T2	**04/12/12**	4.70	**13/08/13**	4.31
T3	**04/12/12**	4.71	**13/08/13**	4.39
T4	**04/12/12**	4.75	**13/08/13**	4.44
Control	**20/03/13**	5.32	**28/08/13**	4.52
T2	**20/03/13**	5.39	**28/08/13**	4.52
T3	**20/03/13**	5.66	**28/08/13**	4.43
T4	**20/03/13**	5.43	**28/08/13**	4.78

Table B17: Average soil temperature in each plot at Hillsborough SRC willow plantation on specified sampling dates

Plot	Date	Temp (oC)	Date	Temp (oC)
Control	17/07/12	21	16/04/13	9
T2	17/07/12	21	16/04/13	9
T3	17/07/12	21	16/04/13	9
T4	17/07/12	21	16/04/13	9
Control	14/08/12	18	14/05/13	13
T2	14/08/12	18	14/05/13	13
T3	14/08/12	18	14/05/13	13
T4	14/08/12	18	14/05/13	13
Control	11/09/12	14	11/06/13	12
T2	11/09/12	14	11/06/13	12
T3	11/09/12	14	11/06/13	12
T4	11/09/12	14	11/06/13	12
Control	09/10/12	11	09/07/13	18
T2	09/10/12	11	09/07/13	18
T3	09/10/12	11	09/07/13	18
T4	09/10/12	11	09/07/13	18
Control	06/11/12	8	25/07/13	18
T2	06/11/12	8	25/07/13	18
T3	06/11/12	8	25/07/13	18
T4	06/11/12	8	25/07/13	18
Control	04/12/12	4	13/08/13	18
T2	04/12/12	4	13/08/13	18
T3	04/12/12	4	13/08/13	18
T4	04/12/12	4	13/08/13	18
Control	20/03/13	5	28/08/13	16
T2	20/03/13	5	28/08/13	16
T3	20/03/13	5	28/08/13	16
T4	20/03/13	5	28/08/13	16

Table B18: Average %SMC in SGP and MGP in various plots at Hillsborough SRC Willow Plantation on specified sampling dates

%SMC						
Plot	**Date**	**MGP**	**SGP**	**Date**	**MGP**	**SGP**
Control	**17/07/12**	37.51	36.73	**16/04/13**	48.15	44.07
T2	**17/07/12**	38.9	31.99	**16/04/13**	37.58	39.05
T3	**17/07/12**	30.68	33.89	**16/04/13**	38.88	36.95
T4	**17/07/12**	27.65	30.68	**16/04/13**	42.50	46.73
Control	**14/08/12**	31.44	31.71	**14/05/13**	43.74	37.79
T2	**14/08/12**	34.58	36.46	**14/05/13**	42.66	40.84
T3	**14/08/12**	29.33	34.60	**14/05/13**	25.24	36.59
T4	**14/08/12**	32.78	32.17	**14/05/13**	34.56	31.37
Control	**11/09/12**	46.91	43.28	**11/06/13**	22.33	22.73
T2	**11/09/12**	50.58	49.22	**11/06/13**	23.41	24.66
T3	**11/09/12**	37.65	31.55	**11/06/13**	21.94	17.93
T4	**11/09/12**	37.88	39.19	**11/06/13**	12.44	15.64
Control	**09/10/12**	32.92	37.40	**09/07/13**	35.31	39.29
T2	**09/10/12**	43.39	43.23	**09/07/13**	41.52	45.37
T3	**09/10/12**	30.99	33.27	**09/07/13**	21.33	32.86
T4	**09/10/12**	27.32	31.54	**09/07/13**	29.13	33.26
Control	**06/11/12**	45.67	33.35	**25/07/13**	25.00	33.34
T2	**06/11/12**	43.34	42.77	**25/07/13**	26.00	26.88
T3	**06/11/12**	45.66	45.60	**25/07/13**	24.16	57.71
T4	**06/11/12**	30.59	26.85	**25/07/13**	41.92	15.57
Control	**04/12/12**	47.48	40.17	**13/08/13**	33.85	36.83
T2	**04/12/12**	45.32	40.36	**13/08/13**	29.73	32.48
T3	**04/12/12**	33.63	32.41	**13/08/13**	37.25	38.05
T4	**04/12/12**	45.89	44.17	**13/08/13**	42.29	40.57
Control	**20/03/13**	48.11	48.61	**28/08/13**	34.84	35.67
T2	**20/03/13**	37.55	31.19	**28/08/13**	33.88	43.60
T3	**20/03/13**	40.49	58.21	**28/08/13**	40.05	35.20
T4	**20/03/13**	44.96	47.61	**28/08/13**	39.28	31.91

Appendix C: Meteorological Information for Culmore and Hillsborough

Table C1: Record of weather conditions for Culmore SRC plantation information obtained from nearest weather station at Malin Head, Co. Donegal

	Precipitation (mm)		Ambient temperature	
	Mean	% of 1981-2010 average	Mean	Difference from 1981-2010 average
July 2012	91.5	113%	13.2	-1.3
August 2012	86.6	91%	15.3	+0.06
September 2012	139.7	146%	12.9	-0.4
October 2012	123.2	102%	9.8	-1.0
November 2012	87.6	81%	7.4	-0.8
December 2012	150.1	129%	6.2	-0.2
March 2013	61.6	71%	4.3	-2.6
April 2013	62.8	97%	7.1	-1.2
May 2013	103.2	177%	9.8	-0.7
June 2013	83.8	119%	12.9	+0.2
July 2013	56.5	70%	15.6	+1.3
August 2013	92.6	97%	14.8	+0.4
September2013	69.7	72%	12.6	+0.3

Table C2: Recorded weather conditions for Hillsborough SRC plantation

	Rain	Ambient temperature	
	Precipitation (mm)	Min Temperature	Mean
July-2012	92.2	10.1	13.2
August-2012	74.1	12.1	14.1
September-2012	139.6	8.6	11.2
October-2012	115.5	5.2	7.3
November-2012	66.5	3.0	5.4
December-2012	100.7	1.9	4.2
March-2013	126.9	0.2	2.5
April-2013	59.5	2.7	5.8
May-2013	90	5.8	9.7
June-2013	95.8	9.2	13.0
July-2013	45.1	12.7	16.9
August-2013	51.5	11.8	14.4
September-2013	46.7	9.3	12.1

Appendix D: *p* Values for Statistical Testing in Culmore and Hillsborough SRC Willow Plantation

Table D1: *p* values for statistical testing of significance of earthworm abundance in various datasets recorded at Culmore SRC plantation using Kruskal Wallis Also shown are the interaction values obtained between irrigation and planting history using PerMANOVA

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 & 3 only
	Irrigation	Planting History	Irrigation	Planting History	Interaction between irrigation & planting history	Interaction between irrigation & planting history
All plots	0.585	0.001	0.792	0.001	0.247	0.480
All Previous Poplar (P) plots only	0.915	n/a	0.855	n/a	n/a	n/a
All Previous Grass (G) plots only	0.265	n/a	0.533	n/a	n/a	n/a
All Previous Willow (W) plots only	0.790	n/a	0.560	n/a	n/a	n/a
Irrigated Plots Only						
Previous Poplar vs. Previous Grass	n/a	0.001	n/a	0.001	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.599	n/a	0.873	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.001	n/a	0.001	n/a	n/a
Non-Irrigated Only						
Previous Poplar vs. Previous Grass	n/a	0.001	n/a	0.001	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.918	n/a	0.875	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.001	n/a	0.001	n/a	n/a

Table D2: *p* values for statistical testing of significance of mite abundance in various datasets recorded at Culmore SRC plantation using Kruskal Wallis Also shown are the interaction values obtained between irrigation and planting history using PerMANOVA

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 and 3 only
	Irrigation	Planting History	Irrigation	Planting History	Interaction between irrigation & planting history	Interaction between irrigation & planting history
All plots	0.198	0.001	0.091	0.006	0.251	0.369
All Previous Poplar (P) plots only	0.208	n/a	0.345	n/a	n/a	n/a
All Previous Grass (G) plots only	0.808	n/a	0.294	n/a	n/a	n/a
All Previous Willow (W) plots only	0.973	n/a	0.689	n/a	n/a	n/a
Irrigated Plots Only						
Previous Poplar vs. Previous Grass	n/a	0.107	n/a	0.103	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.323	n/a	0.191	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.580	n/a	0.702	n/a	n/a
Non-Irrigated Only						
Previous Poplar vs. Previous Grass	n/a	0.001	n/a	0.001	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.001	n/a	0.005	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.877	n/a	0.884	n/a	n/a

Table D3: *p* values for statistical testing of significance of springtail abundance in various datasets recorded at Culmore SRC plantation using Kruskal Wallis Also shown are the interaction values obtained between irrigation and planting history using PerMANOVA

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 & 3
	Irrigation	Planting History	Irrigation	Planting History	Interaction (irrigation & planting history)	Interaction (irrigation & planting history)
All plots	0.065	0.055	0.06	0.043	0.754	0.848
All Previous Poplar (P) plots only	0.175	n/a	0.232	n/a	n/a	n/a
All Previous Grass (G) plots only	0.296	n/a	0.280	n/a	n/a	n/a
All Previous Willow (W) plots only	0.417	n/a	0.312	n/a	n/a	n/a
Irrigated Plots Only	n/a	0.331	n/a	0.290	n/a	n/a
Previous Poplar vs. Previous Grass	n/a	0.263	n/a	0.290	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.158	n/a	0.114	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.979	n/a	0.943	n/a	n/a
Non-Irrigated Only	n/a	0.126	n/a	0.133	n/a	n/a
Previous Poplar vs. Previous Grass	n/a	0.300	n/a	0.433	n/a	n/a
Previous Poplar vs Previous Willow	n/a	0.054	n/a	0.043	n/a	n/a
Previous Willow vs. Previous Grass	n/a	0.772	n/a	0.724	n/a	n/a

Table D4: *p* values for statistical testing of significance of earthworm abundance in various datasets recorded at Hillsborough SRC plantation using Kruskal Wallis and PerMANOVA. Also shown are the interaction values obtained between irrigation and planting regime

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 and 3 only
	Irrigation	Planting Regime	Irrigation	Planting Regime	Interaction	Interaction
All plots	n/a	0.669	n/a	0.769	0.721	0.712
Control plot	n/a	0.629	n/a	0.648	n/a	n/a
Treatment 2 (T2)	n/a	0.636	n/a	0.614	n/a	n/a
Treatment 3 (T3)	n/a	0.513	n/a	0.251	n/a	n/a
Treatment 4 (T4)	n/a	0.126	n/a	0.423	n/a	n/a
Control vs. T2	0.930	0.997	0.832	0.975	0.408	0.424
Control vs. T3	0.001	0.961	0.001	0.953	0.711	0.721
Control vs. T4	0.001	0.954	0.001	0.993	0.665	0.742
MGP only	0.001	n/a	0.000	n/a	n/a	n/a
Control vs. T2	0.469	n/a	0.370	n/a	n/a	n/a
Control vs. T3	0.001	n/a	0.001	n/a	n/a	n/a
Control vs. T4	0.001	n/a	0.001	n/a	n/a	n/a
SGP only						
Control vs. T2	0.339	n/a	0.488	n/a	n/a	n/a
Control vs. T3	0.001	n/a	0.001	n/a	n/a	n/a
Control vs. T4	0.001	n/a	0.001	n/a	n/a	n/a

Table D5: *p* values for statistical testing of significance of mite abundance in various datasets recorded at Hillsborough SRC plantation using Kruskal Wallis and PerMANOVA. Also shown are the interaction values obtained between irrigation and planting regime

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 and 3 only
	Irrigation	Planting Regime	Irrigation	Planting Regime	Interaction	Interaction
All plots	n/a	0.101	n/a	0.003	0.108	0.094
Control plot	n/a	0.556	n/a	0.266	n/a	n/a
Treatment 2 (T2)	n/a	0.259	n/a	0.329	n/a	n/a
Treatment 3 (T3)	n/a	0.586	n/a	0.017	n/a	n/a
Treatment 4 (T4)	n/a	0.258	n/a	0.178	n/a	n/a
Control vs. T2	0.550	0.257	0.753	0.146	0.065	0.121
Control vs. T3	0.448	0.645	0.874	0.064	0.156	0.058
Control vs. T4	0.240	0.691	0.102	0.488	0.028	0.092
MGP only						
Control vs. T2	0.981	n/a	0.933	n/a	n/a	n/a
Control vs. T3	0.206	n/a	0.459	n/a	n/a	n/a
Control vs. T4	0.598	n/a	0.826	n/a	n/a	n/a
SGP only						
Control vs. T2	0.128	n/a	0.714	n/a	n/a	n/a
Control vs. T3	0.867	n/a	0.417	n/a	n/a	n/a
Control vs. T4	0.295	n/a	0.476	n/a	n/a	n/a

Table D6: *p* values for statistical testing of significance of springtail abundance in various datasets recorded at Hillsborough SRC plantation using Kruskal Wallis and PerMANOVA. Also shown are the interaction values obtained between irrigation and planting regime

	Kruskal-Wallis				**PerMANOVA**	
	Phase 1, 2 and 3		Phase 1 and 3 only		Phase 1, 2 and 3	Phase 1 and 3 only
	Irrigation	Planting Regime	Irrigation	Planting Regime	Interaction	Interaction
All plots	n/a	0.258	n/a	0.083	0.627	0.785
Control plot	n/a	0.684	n/a	0.506	n/a	n/a
Treatment 2 (T2)	n/a	0.639	n/a	0.773	n/a	n/a
Treatment 3 (T3)	n/a	0.334	n/a	0.045	n/a	n/a
Treatment 4 (T4)	n/a	0.144	n/a	0.451	n/a	n/a
Control vs. T2	0.087	0.949	0.179	0.577	0.4842	0.3772
Control vs. T3	0.823	0.725	0.594	0.076	0.5520	0.4142
Control vs. T4	0.473	0.018	0.395	0.021	0.2828	0.5496
MGP only						
Control vs. T2	0.426	n/a	0.211	n/a	n/a	n/a
Control vs. T3	0.379	n/a	0.649	n/a	n/a	n/a
Control vs. T4	0.390	n/a	0.094	n/a	n/a	n/a
SGP only						
Control vs. T2	0.129	n/a	0.449	n/a	n/a	n/a
Control vs. T3	0.639	n/a	0.300	n/a	n/a	n/a
Control vs. T4	0.015	n/a	0.104	n/a	n/a	n/a

Table D7: *p* Values for statistical testing of significance differences in pH, %SMC and temperature in various datasets at Culmore SRC willow plantation

	pH		%SMC		Soil temperature	
	Phase 1, 2 and 3	Phase 1 and 3 only	Phase 1, 2 and 3	Phase 1 and 3 only	Phase 1, 2 and 3	Phase 1 and 3 only
Irrigated plots vs non-irrigated plots	0.209	0.409	0.718	0.950	1.000	1.000
Irrigated plots only	0.001	0.001	0.487	0.527	1.000	1.000
Non irrigated plots only	0.242	0.686	0.913	0.652	1.000	1.000
Plots with different planting history	0.001	0.001	0.944	0.474	1.000	1.000
Previous poplar plots only	0.901	0.178	0.360	0.386	1.000	1.000
Previous willow plots only	0.003	0.001	0.272	0.386	1.000	1.000
Previous grass plots only	0.631	0.176	0.442	0.829	1.000	1.000
Previous grass vs. previous poplar	0.662	0.146	0.897	0.644	1.000	1.000
Previous grass vs. previous willow	0.119	0.102	0.252	0.564	1.000	1.000
Previous poplar vs. previous willow	0.060	0.043	0.990	0.878	1.000	1.000
Phase 1 vs. Phase 3	n/a	0.001	n/a	0.001	n/a	1.000

Table D8: *p* Values for statistical testing of significance differences in pH, %SMC and temperature in various datasets at Hillsborough SRC willow plantation

	pH		%SMC		Soil temperature	
	Phase 1, 2 and 3	Phase 1 and 3 only	Phase 1, 2 and 3	Phase 1 and 3 only	Phase 1, 2 and 3	Phase 1 and 3 only
All treatment plots	n/a	0.162	n/a	0.113	1.000	1.000
Control vs T2	0.305	0.939	0.022	0.071	1.000	1.000
Control vs T3	0.545	0.371	0.027	0.259	1.000	1.000
Control vs T4	0.015	0.123	0.027	0.114	1.000	1.000
MGP vs SGP			0.683	0.778	1.000	1.000
Phase 1 vs. Phase 3	n/a	0.001	n/a	0.309	n/a	0.001

Table D9: *p* values for statistical testing (Kruskal Wallis) of significance of earthworm abundance vs. phase in various datasets recorded at Culmore SRC plantation

	Earthworms
Phase 1 vs. Phase 3 (Irrigated and Non-Irrigated plots)	0.179
Phase 1 vs. Phase 3 (Irrigated plots only)	0.185
Phase 1 vs. Phase 3 (Non-Irrigated plots only)	0.611
Previous Poplar Only	
Phase 1 vs. Phase 3 (Irrigated and Non-Irrigated plots)	0.748
Phase 1 vs. Phase 3 (Irrigated plots only)	1.000
Phase 1 vs. Phase 3 (Non-Irrigated plots only)	0.619
Previous Grass Only	
Phase 1 vs. Phase 3 (Irrigated and Non-Irrigated plots)	0.003
Phase 1 vs. Phase 3 (Irrigated plots only)	0.004
Phase 1 vs. Phase 3 (Non-Irrigated plots only)	0.034
Previous Willow Only	
Phase 1 vs. Phase 3 (Irrigated and Non-Irrigated plots)	0.931
Phase 1 vs. Phase 3 (Irrigated plots only)	0.484
Phase 1 vs. Phase 3 (Non-Irrigated plots only)	0.732

Table D10: *p* values for statistical testing (Kruskal Wallis) of significance of earthworm abundance vs. phase in various datasets recorded at Hillsborough SRC plantation

	Earthworms
Phase 1 vs. Phase 3 (Control)	0.407
Phase 1 vs. Phase 3 (T2)	0.689
Phase 1 vs. Phase 3 (T3)	0.138
Phase 1 vs. Phase 3 (T4)	0.194

Appendix E: Correlation Results for Abundance of Soil Invertebrates and Soil pH, Temperature and %SMC in SRC willow plantations

Table E1: Correlation results for earthworm, mite and springtail abundance vs soil pH, temperature and %SMC in Culmore SRC willow plantation

	Moisture Content (Range 30.1-70%)	**pH** (Range 4.1-5.8)	**Temperature** (Range 5-19^{o}C)
Earthworms	0.087	-0.13	0.095
Mites	-0.032	-0.171	-0.181
Springtails	0.091	-0.136	-0.004

Table E2: Correlation results for earthworm, mite and springtail abundance vs soil pH, temperature and %SMC in Hillsborough SRC willow plantation

	Moisture Content (Range 12.4-58.2%)	**pH** (Range 4.3-5.7)	**Temperature** (Range 4-21^{o}C)
Earthworms	-0.129	0.039	0.006
Mites	-0.107	-0.146	0.045
Springtails	-0.144	-0.156	0.009