IMPERIAL COLLEGE LONDON
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CENTRE FOR ENVIRONMENTAL POLICY

THE EFFECT OF PIG ROOTING ON EARTHWORM ABUNDANCE AND SPECIES DIVERSITY IN WEST SUSSEX, UK

BY

JOSEPH COLE

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Executive Summary:
The effect of pig rooting on earthworm abundance and species diversity in West Sussex, UK.

By: Joseph Cole, Centre for Environmental Policy, Imperial College London
Academic Year: 2012/13
Supervisor: Dr Tilly Collins, CEP, Imperial College London

Aims and Objectives:

The aim of this project was to contribute to research in rewilding. Specific objectives focussed on understanding the effect pig rooting is having on earthworm community structure within the Knepp Wildland Project at the Knepp Castle Estate in West Sussex, UK. The premise was to:

- Determine if pig rooting is having an effect on earthworm abundance.
- Determine if pig rooting is having an effect on earthworm species diversity.
- Determine the effect of pig rooting on soil moisture content, soil temperature, soil pH and soil infiltration rate.

I was able to outline the importance of earthworms in the context of rewilding and to demonstrate the effects of large mammals on earthworm communities. This was done in the hope that consideration for our soils and associated below ground fauna is accounted for within rewilding projects and, where possible, wider policy commitments.

Introduction:

Governance for rewilding is growing as losses to ecosystems and biodiversity become increasingly unacceptable as a greater focus is placed on the conservation of our native flora and fauna (Millennium Ecosystem Assessment, 2005). Conservation aimed at reversing the negative effects on landscapes is growing in popularity. Earthworms, as the most important component of soil fauna (Lee & Foster, 1992) and ecosystem engineers (Jones, Lawton & Shachak, 1997), are fundamentally important to the concept of rewilding and for us to succeed in land regeneration and reclamation (Butt, Frederickson & Morris, 1995).

Pigs root the ground as important exploratory behaviour in the hunt for food (Jensen & Toates, 1993) and are regular predators of earthworms (Granval & Muys, 1995). Considering the effect of rooting and a pig's ability to alter the conditions in the soil, together with active predation of earthworms, it is believed pigs will have a negative effect on earthworm abundance and species diversity. Considering the ecosystem services (Turbé et al, 2010) and an earthworm's input in the provision of such services (Edwards, 2004), it was deemed important to quantify the effects on earthworm populations of pigs in a rewilded landscape.

Methodology:

Research took place at the Knepp Wildland Project in the grounds of Knepp Castle Estate in West Sussex, UK. Study sites were selected at random to ensure good spatial representation and to avoid bias in the results. 31 fields were selected and in each field 3 pits were sampled, one disturbed site and two undisturbed sites for comparisons to be made, giving a total of 93 sampled pits. At each sample site earthworms were sampled, water infiltration rate was measured, relevant soil characteristics were observed and the number of years the field had been in rewilding was noted.

At each pit a 0.25m X 0.25m X 0.10m monolith was extracted for hand sorting earthworms (Sherlock, 2012). In each pit a vermifuge of hot mustard was used to extract deep burrowing anecic earthworms (Sherlock, 2012), following the procedure described by Lawrence & Bowers

(2002). Extracted earthworms were immediately placed in preserving alcohol and returned to the laboratory for analysis. Juvenile and adult earthworms were counted for abundance. Adult earthworms, easily identifiable due to the presence of a clitellum, were identified to species level for analysis of species diversity using Shannon's Diversity Index (Begon, Townsend & Harper, 2006).

Water infiltration was measured with a single ring infiltrometer. 100ml of water was poured into the tube and measurements were taken at 30 second intervals. An upper limit of 10 minutes was selected to ensure a consistent timeframe between sites and in order for good comparisons to be made. Soil moisture content, temperature and pH were recorded with digital meters.

All data analysis was conducted using the statistical software R: version 3.0.1. Kruskal-Wallis tests were performed to explore the distribution between the different ecological groups and between juvenile and adult earthworm abundance. Generalised linear models (GLMs) were used to explore the relationships between the measured explanatory variables (EV) (water infiltration, soil moisture content, soil temperature, soil pH and years in rewilding) and a variety of response variables (RV) (anecic, epigeic and endogeic earthworms; juvenile, adult and total earthworm abundance).

Regarding species diversity, species richness was first calculated for each sample pit and converted into an index value for diversity following Shannon's Diversity Index calculations. A GLM was then used to investigate the impact of measured EVs on diversity. For the six most abundant species ($n \geq 15$) a GLM was used to explore the impact of the main effects on each individual species. For all GLMs stepwise model simplification was used to determine any significant relationships (that is, one which yielded a significance level $p<0.05$). Analysis of variance (ANOVA) was used to determine whether removing an EV from the model reduced the models explanatory power. Where removing an EV yielded a significant p-value ($p<0.01$) it was left in the model. Model simplification continued until only significant EVs remained in the model.

Results:

A total of 1289 earthworms (909 juveniles and 380 adults) were sampled across the 93 sample sites with a total of 19 individual species being identified. Species diversity analysis was confined to using only 29.48% of the total sample population because only adult earthworms could be identified to species level. Abundance was found to be greater at undisturbed sites for RVs mentioned previously. More species were found at undisturbed sites and 7 species were found only at undisturbed sites. *Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea rosea, Lumbricus castaneus, Lumbricus rubellus and Octolasion lacteum* were found to be the most abundant species ($n \geq 15$).

The distribution in each ecological group varied, with the total number of endogeic earthworms being significantly greater than the number of anecic and epigeic earthworms. Anecic earthworm abundance was significantly affected by the number of years in rewilding, epigeic earthworm abundance was significantly affected by soil pH and endogeic earthworm abundance was significantly affected by both site type and soil pH.

Regardless of species, juvenile abundance was significantly greater than adult abundance. Total earthworm abundance and juvenile earthworm abundance were both significantly affected by site type and the number of years in rewilding, while adult earthworm abundance was significantly affected by site type and soil pH.

The median species richness was similar at both disturbed and undisturbed sites; however the distribution was markedly different with a greater proportion of undisturbed sites having a higher number of species, which was confirmed by Shannon's Diversity Index values for both undisturbed and disturbed sites. Species diversity was positively affected by soil pH. With respect to the six most abundant species; *Allolobophora chlorotica* was significantly affected by site type and the number of years in rewilding; *Aporrectodea caliginosa* was significantly affected by soil temperature; *Lumbricus castaneus* was significantly impacted by years in rewilding; *Lumbricus rubellus* was significantly affected by soil moisture; *Octolasion lacteum* was significantly affected by site type, soil moisture and soil temperature; and *Aporrectodea rosea* was not significantly affected by any of the measured EVs.

Discussion and Conclusions:

Earthworm data and soil properties largely followed the expected trajectories as a result of exploratory rooting behaviour of the pigs. As in previous studies of earthworm populations it was shown that soil pH, soil moisture, soil temperature, site type and the number of years a field had been in rewilding have varying degrees of impact on earthworm abundance and species diversity. Soil pH had an effect on certain earthworm categories because the neutral pH was within their tolerance thresholds. Had soil pH been less favourable then abundance and diversity would have appeared more dispersed and both would have been reduced across the study area. The results of this study imply soil pH is affecting Shannon's Diversity Index. However pH did not vary considerably across the Knepp Wildland Project, implying even slight alterations in pH can change earthworm species diversity. Soil moisture was shown to significantly affect *Lumbricus rubellus* although considering this species thrives in moist soils and soil moisture content was low this is expected. The lack of soil moisture being significant in any other models is also expected because a lack of soil moisture does not necessarily equate to a lack of earthworm presence as earthworms remain in-situ in a state of aestivation. Similarly soil temperature does not equate to a lack of earthworm presence as they will enter aestivation in situ if temperature conditions become unfavourable. The site type, either disturbed or undisturbed, was shown to have a significant impact on many of the earthworm categories analysed. Since rooting pigs change localised soil conditions, creating an unfavourable habitat, this was expected.

Rooting in locations where pigs had been searching for food was shown to reduce earthworm abundance, suggesting predation by pigs reduces the incidence of earthworm presence. On the other hand, considering the relative proximity of undisturbed sites to disturbed sites it can be argued that pig rooting has a localised effect on earthworm populations and so if excess rooting is controlled by limiting stock size then rooting would not appear to have a profound impact in the larger area.

Earthworm abundance was shown to decrease with time in rewilding. This was an unexpected result as literature suggests that rewilded landscapes can benefit earthworm populations through increased quantity of surface litter available both as a food source and as a means of naturally controlling temperature and moisture content in the soil, thus reducing the negative impacts of major variations in soil conditions on earthworm communities.

Species diversity was significantly affected by pig rooting, and whether it is through predation or other means, pigs are lowering the important diversity of earthworm populations within the soil profile. Even the small change in soil pH caused by rooting had an effect on species diversity and so inhibits the soil from receiving the full benefits of having a broad range of earthworm species engineering it. This study showed a greater species richness and species diversity at undisturbed sites, confirming the negative effect of pig rooting on earthworm populations.

Limitation of the study and suggestions for further work:

The mustard extraction technique was largely ineffective in the dense impervious clay soils at the Knepp Estate. Because of poor infiltration rates the solution did not enter the soil and could not carry out its intended purpose. Hand sorting was an effective method for earthworm extraction and I would suggest that if the project was repeated, or a similar study conducted at a different location with the same soil type, then this preferred technique should be used. It would be advised that larger monoliths are considered for sampling where a vermifuge is expected to be unsuccessful. A simple infiltration test could be used to establish the potential of a vermifuge.

Climatic conditions varied from day to day and could have impacted earthworm behaviour. As soils dried out considerably towards the end of the field sampling period, with an increase in ambient temperatures, earthworms could have retreated to deeper locations in the soil profile and would not have been sampled due to issues encountered with the mustard extraction technique.

It should also be noted that the results from this study very much portray a snapshot in time and cannot be used for conclusive results on the effects of pig rooting on earthworm behaviour. If repeated a future study should include samples taken over a greater time frame to ensure natural population dynamics are accounted for.

Further studies should aim to quantify the effects of rewilding on earthworm populations and to assess the impacts of pigs on earthworm population dynamics over greater spatial and temporal scales. Improving the spatial scale and resolution could be achieved by increasing the number of sites sampled and providing a more complete picture of the effect of pig rooting on earthworm population dynamics within the study site. Improvements to the temporal scale could be achieved through sampling regularly over a long time frame. I recommend these improvements are carried out both at the same sample site and at other rewilding projects which use pigs as a means of creating natural disturbances throughout the UK. In so doing more decisive results could be achieved in order to present more conclusive evidence to policy makers for the adoption of rewilding with concern for earthworm population effectiveness and longevity into governmental policy commitments.

Implications for Rewilding Management Practices:

From the reviewed literature it becomes clear that earthworms could play a vital role in future land management practices within agro-ecosystems and rewilded land because of their abilities in soil reclamation, formation and maintenance, and in nutrient cycling. It is unreasonable to expect immediate results. However with time earthworm populations could establish optimal functioning levels and improve the ecological performance of such environments.

In the present day, with a lack of policy directly aimed at the conservation of soil ecosystems, we are in danger of losing the essential workings of these delicate and indispensable ecosystems. Due to the importance of earthworms in their functioning and engineering qualities within these environments it would seem pertinent to implement rewilding policies that aim to enforce the longevity of such landscapes. In so doing it becomes increasingly likely that healthy earthworm abundance and species diversity is upheld where it is needed most, with thriving and diverse ecosystems at the heart of it.

With relevance to the use of pigs in rewilding projects it should be noted that they are undoubtedly effective in creating favourable habitat niches for a range of invertebrates and germinating seedlings. However considering earthworm predation, reducing both abundance and species diversity, pig stock size becomes an important consideration with the need for a controlled population level that is not too destructive. Further research into optimal stock size would be needed to ensure earthworm populations do not become subject to overly destructive pig rooting.

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I declare that this thesis

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is entirely my own work and that where any material could be construed as the work of others, it is fully cited and referenced, and/or with appropriate acknowledgement given.

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Name of student (Please print): Joseph Cole

Name of supervisor: Dr Tilly Collins

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Abstract

Rewilding articles are becoming increasingly prevalent in literature concerning the conservation of natural resources and biological diversity. Situated in West Sussex, UK, the Knepp Wildland Project is attempting to rewild 1000 acres of land with the help of large mammals to create natural disturbances and drive habitat changes. Tamworth pigs are one of six species being used with the purpose of creating bare patches of ground through their rooting behaviour. This study aims to assess the effect of pig rooting on earthworm abundance, on earthworm species richness, and on earthworm species diversity. Earthworm populations, soil moisture content, soil temperature, soil pH, infiltration rate and the number of years the land had been in rewilding were recorded at 31 locations across the Wildland Project. At each location an area affected by pig rooting was compared to two undisturbed patches of ground for comparisons to be made and to establish if pig rooting was in fact having a significant effect on earthworm population structure. Land rooted by pigs was shown to have significantly lower abundance, lower species richness and lower species diversity than unaffected areas. The measured soil properties were shown to have varying degrees of impact on earthworm community structure. Since pig rooting has been shown to affect earthworm populations and soil properties and taking into account that pigs are known to predate earthworms due to their high protein content, it is predicted that a greater stock size of pigs will have a greater impact of earthworm communities. Given the importance of earthworms in soil formation and maintenance it is essential that populations remain strong in rewilding projects where land is being restored to their state prior to degradation.

Contents

Figures and Tables

Figures

Tables

1. Introduction

Currently descriptions of wilderness in the United Kingdom are largely attributed to the highlands of Scotland and the northern counties (Carver, 2007). Conversely, to a well trained eye this land is not 'wild'; rather it is the product of centuries of human activity and landscaping. The assemblage of differing land uses has, in time, carved out these landscapes which boast an air of wilderness, where the remoteness and tranquillity of nature can be enjoyed and observed. Although in truth, these landscapes are not the full article: they are not truly 'wild' (Carver, 2007).

1.1. Governance for Rewilding

Re-wilding is fundamentally important to the ecological components of our planet as we are in danger of losing these components through unsustainable growth and development. With an estimated human population of 9.1 billion by the year 2050 (UNDESA, 2006) issues of conservation and sustainable development are very much in the forefront of international governance; for with the intensive land use required to support growing human populations, we can expect a greater decline in many populations of native species of flora and fauna throughout the United Kingdom (Millennium Ecosystem Assessment, 2005).

Global policy commitments are becoming increasingly common. Although very much in their infancy, policy initiatives generally adhere to the framework set out by the Brundtland Report (United Nations, 1987) which advocates strong sustainability in the course of accounting for future generations through legacy and sacrifice. Further to the Brundtland Report, Agenda 21 from the 1992 Rio conference (United Nations, 1992) represents a shift in the context of concerns declaring we should "live in harmony with nature, not at natures' expense". This change in context signifies the end of conservation aimed at the protection of individual species and instead highlights the need to protect entire ecosystems to effectively conserve our valuable biodiversity and to safe guard the vital services we receive from our natural environments.

The Convention on Biological Diversity (CBD) (1992a) focuses on governance with an ecosystem approach. The convention aims to regulate and conserve our natural resources through practices which encompass the management of land, water and natural resources equitably and sustainably (Convention on Biological Diversity, 1992b). Under Article 6 of the CBD contracting states are required to develop national strategies for the conservation and sustainable use of biodiversity (Convention on Biological Diversity, 1992c; DEFRA, 2008). Further evidence to suggest that a large scale ecosystem approach to conservation is being implemented can be seen in the European Commission's Habitats Directive (European Commission, 2013a). In total the Habitats Directive protects over 1000 animals and plant species and over 200 habitat types of European importance with Article 3 requiring the implementation of high quality conservation sites which make significant contributions to the conservation of species (Joint Nature Conservation Committee, 2010). Under the Habitats Directive the Natura 2000 Network (European Commission, 2013b) is at the heart of European Union nature and biodiversity policy. The Network attempts to protect habitats, and subsequently species, through the implementation of Special Areas of Conservation (SACs) and by Special Protection Areas (SPAs) under the Birds' Directive. These sites, and similar sites, are also often designated as Sites of Special Scientific Interest (SSSIs) , preserving the finest wildlife and geological biodiversity of these beautifully stunning sites and habitats, and most importantly heightening the profile and promoting the importance of these sites in the public's awareness (Natural England, n.d.).

Another convention paving the way for the conservation of ecosystems is the RAMSAR convention (1971), uniquely focussing on just one particular ecosystem, wetlands. Using a very broad definition of wetlands encompassed in its mission, the philosophy of the "wise use" focuses on the conservation and sustainable use of wetlands and their resources contributing to sustainable development. By recognising the importance of ecosystem services and the importance of regulation within our natural ecosystems the convention sets a high marker for which we can aim in our search to ensure the longevity of other ecosystems within our landscapes.

Restoration projects are a modern approach to improving desecrated areas of biodiversity through improvements to agricultural and forestry management practices. In 2010, the CBDs 'Global Biodiversity Outlook 3' (SCBD, 2010) recognised the importance of restoring and rewilding abandoned landscapes, estimating that Europe has the potential to free up approximately 200000 square kilometres of land for such projects by 2050, thus recognising the importance of conservation and sustainable land use as identified by the policy commitments discussed above.

The importance of rewilding degraded landscapes and conserving ecology is again highlighted by Article 8 of the Convention on Biodiversity (1992d) which specifies that nature should be conserved in its natural environment.

An example of such management changes can be seen in the Knepp Wildland Project (Knepp Castle Estate, n.d.) where large grazing and browsing animals have been introduced to the land and are managed and conserved in their natural environment, thus allowing biodiversity to respond to the low intensity management regime. By creating land which is able to support richer and more diverse populations of invertebrates, mammals and fauna we can support the growth and development of populations of insectivorous, carnivorous, omnivorous and herbivorous species respectively – all within their natural environments.

1.2. Knepp Wildland Project

The Knepp Wildland Project is the setting for this investigation. The Wildland Project at Knepp targets the potential to support the recovery and future growth of Biodiversity Action Plan (BAP) (HMSO, 1994) species and environmental restoration. At the time of implementation the Knepp Estate project contained populations of 22 species currently present on the BAP list (HMSO, 1994); the expectation being that the impact of the re-wilding project was likely to be a positive one, increasing both the number and variety of important (BAP) species. The creation of pasture woodland, marshy and dry grasslands, and riverside habitats is believed to bring added benefits as the ecosystems evolve. An example of this is the emergence of a red listed species (IUCN, 2008), the barbastelle bat, since the beginning of the Wildland project on the estate. Further aims involve the restoration of the River Adur through the reinstatement of historic features which will reduce downstream flooding whilst benefitting local habitats and ecology. The project is supported (as are many others) by Natural England through Environmental Stewardship grants (currently through the higher tier scheme (HLS)) and their continued support with annual operations by the way of an independent advisory group (Knepp Castle Estate, n.d.).

Prior to the rewilding project at the Knepp Castle Estate, the land was in arable agriculture for some considerable time .The net effect on earthworm community structure by the intensive management of the land (for example: tillage and pesticide/herbicide use) is likely to have been negative.

1.3. Study Purpose

Earthworms are an important component of the biodiversity of an ecosystem, not only because of their important role in ecosystem management but also due to their central position in the diets of many mammals and birds. As 'ecosystem engineers' (Jones, Lawton & Shachak, 1994; Jones, Lawton & Shachak, 1997; Lavelle et al, 1997) earthworms have been selected for this small scale study due to their prominent position within the community structure of soils and due to their function with respect to soil formation and maintenance (Edwards, 2004). With this in mind the effect of pig rooting on earthworm community structure and whether or not this behavioural trait (rooting) is affecting earthworm abundance and species diversity becomes interesting.

The study was designed to contribute to research on the topic in the United Kingdom (UK) and investigated the effect of pig rooting on earthworm abundance and earthworm species diversity within a temperate environment in South East England. Rewilding of degraded landscapes is a current and pertinent part of UK environmental governance. Tamworth pigs are a fundamental aspect of the Knepp Wildland Project and after considering their impact, through foraging activity, on the creation of natural disturbances it was decided to measure and analyse this impact. Comparisons between disturbed and undisturbed areas were considered, and measurements on both types of sites were made.

Soil characteristics were recorded and the impact these had on earthworm abundance was investigated and comparisons made between areas of rooting (disturbed) and areas not rooted by pigs (undisturbed). The soil characteristics considered were soil moisture content, soil temperature, soil pH, and infiltration rate. The number of years a field had been in rewilding was also taken into account.

2. Literature Review

2.1. Agriculture and Rewilding

The Green Revolution of the mid-1960s (Barrow, 1995) focussed on intensifying agriculture to create higher yields in an effort to feed growing populations (Adams, 2001). Little concern was given to the physical environment which was being degraded, often beyond repair, at an alarming rate. One very important species adversely affected by this intensification was the earthworm.

Studies have found that earthworm populations in England, in long established arable land, were significantly smaller than populations in comparable soil types (Low, 1972; Low, 1976). It has also been suggested that repeated, heavy tilling can have an accumulative negative effect on earthworm populations Edwards (2004), suggesting zero-tillage methods can help to increase total abundance in an area (Ehlers, 1975). According to Lee (1985) this phenomenon can also be seen in no fewer than 8 other countries worldwide; including the USA (Hopp, 1946) and Australia (Barley 1959; Barley 1961). Though they do not have many direct effects it is believed pesticides used in arable farming, including insecticides, herbicides, fungicides and nematicides, exert some indirect effects on earthworm populations through the reduction of food sources caused by removing microorganisms which provide food by breaking down dead organic matter (Edwards, 2004). The upshot of these negative effects on earthworms is that during the course of the arable cropping regime at Knepp earthworm populations will have been devastated, rendering them low at the beginning of the rewilding project.

Rewilding is the large scale restoration of ecosystems (The Rewilding Institute, n.d.) focussing on large central areas that increase connectivity and house specific keystone species (Sandom, Svenning & Ejrnaes, 2012). The concept, in its broadest sense, relates to restoration ecology; however it separates itself from the more traditional view of habitat restoration and species reintroduction (Sandom et al, 2013) in that less intensive anthropogenic management techniques are favoured to allow for the natural regeneration of habitats (Navarro & Pereira, 2012; Sandom, Svenning & Ejrnaes, 2012). Indeed, Hughes et al (2011) imply that in open ended restoration projects, targets of increased ecosysytem services and improved habitat mosaics can be attained through recovering connectivity in time and space.

Human societies depend on and benefit from ecosystem services (Turbé et al, 2010); such services are provisioning services (e.g. Food, fibre and freshwater), regulating services (e.g. flood and climate regulation) and supporting services (e.g. soil formation and nutrient recycling) (Millennium Ecosystem Assessment, 2005). Biological diversity within soil, of which earthworms are an important aspect (Edwards, 2004), is responsible for providing and regulating certain elements of these ecosystem services (Barrios, 2007). Rewilding increases incidence of the provision of ecosystem services from the environment. Through rewilding a degraded landscape it is possible to: increase ecosystem service provision through soil and nutrient protection; to provide habitats for biodiversity; to regulate water courses; to sequester carbon; and to increase the recreation value of the land in question (Navarro & Pereira, 2012).

In practice rewilding has received mixed reviews with many contrasting opinions being presented; some from a biocentric standpoint whereby the natural world is considered to have an inherent value, others from an ecocentric angle deeming nature to hold value for the human species (Adams, 2001). Adams (2001) goes on to recognise the importance of work by philosopher Arne Naess whose terminology "Deep Ecology" has been likened to the application of wilderness in remote areas. The positive opinions associated with rewilding stem from the fact that rewilding does not aim to conserve specific species, habitats or landscapes. Rather it allows for an environment to develop continuously both temporally and spatially, depending on the limits of the rewilding project in question. This continuous development allows for new habitats and new species to evolve (Sandom, Svenning & Ejrnaes, 2012). In contrast, many members of both the public (Enserink & Vogel, 2006; Bauer, Wallner & Hunziker, 2009) and of the scientific community (Conti & Fagarazzi, 2005; Moreira & Russo, 2007) have spoken out against such management programmes. This negative outlook regarding rewilding stems from concerns relating to the loss of traditional agricultural landscapes, whereby cultural history rather than natural history is of greater importance. This view follows the idea of positive environmentalism with technology, innovation and economic development being key components.

As shown by Fonte, Winsome & Six, (2009) management of residues on the soil surface can benefit earthworm populations. If left in situ residues can reduce the extent of temperature and moisture variations, whilst simultaneously providing an uninterrupted food supply. Furthermore, Traore et al (2004) found that greater quantities of fallen leaf litter can increase the concentrations of carbon and nitrogen in the soil, which as highlighted by Fonte, Winsome & Six, (2009) has a positive correlation with earthworm abundance.

In the present day, private capital and personal initiatives are the biggest drivers in the push for rewilded landscapes, primarily due to the negative attitudes hindering rewilding's inclusion in land management policy (Navarro & Pereira, 2012). Sandom, Svenning & Ejrnaes (2012) go on to note that public sector scientists and government policy are struggling to keep up with private stakeholders. A great example of this pattern can be seen at the Knepp Castle Estate where, over time, scientists have come to be involved with the implementation of the 'Wildland Advisory Group', after the success of the project had been cemented by the land owner, who ultimately took the greatest risk in the first instance.

2.2. Earthworms

2.2.1 Earthworms as Engineers

Earthworms have been described as ecosystem engineers (Jones, Lawton & Shachak, 1994; Jones, Lawton & Shachak, 1997; Lavelle et al, 1997), such is the importance of the service they provide in soil formation and maintenance through their burrowing, mixing and casting behaviour (Edwards, 2004). The ecological effects of such modification are both varied and important (Lawton, 2000), because the soils in which they ply their trade are used directly and indirectly by almost every other species present in that environment. Much like the engineers' of bridges, buildings and roads, earthworms adapt the structure of their environments. An earthworms' ability to condition the soil it inhabits is so great they are essential in soil reclamation projects subsequent to soil degrading activities (Butt, 2008). For example the Earthworm Inoculation Unit technique cultivates earthworms and introduces them to industrially degraded land with the intent of reclaiming desecrated soils (Butt, Frederickson & Morris, 1995).

Earthworms are the most important component of soil fauna in relation to soil modification (Lee & Foster, 1992; Römbke, Jänsch & Didden, 2005). Their importance is so great it was recognised as early as the 4th century BC by Aristotle who labelled them "the intestines of the earth" (OPAL, n.d.) and Charles Darwin was so fascinated in earthworms he said, "It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creature" (Darwin, 1882). This being said it is important that a reasonable level of species diversity is maintained for the full effect of each species to be seen within a soil profile (Verhulst et al, 2010) because the specific ways in which earthworms affect their environment is largely determined by their ecological classification (Earthworm Society of Britain, n.d.).

2.2.2 Ecological Classification

Earthworms were divided into three ecological groups (anecic, endogeic, epigeic) by Bouché (1982). *Table 1* outlines the three ecological groups and which species fit into each group:

Ecological Group	Ecological Group Description	British Species include
Anecic	Deep burrowing earthworms that create and use vertical burrows. They feed on dead organic matter that they drag into their burrows. Generally cast on the surface at their burrow entrance.	*Appoectodea longa* *Lumbricus castaneus* *Lumbricus friendi* *Lumbricus terrestris*
Endogeic	Soil dwelling earthworms that burrow horizontally. Rarely come to the surface and they consume the soil itself.	*Allolobophora chlorotica* *Apporectodea caliginosa* *Apporectodea cupulifera* *Apporectodea icterica* *Apporectodea limicola* *Apporectodea rosea* *Eiseniella tetraedra* *Helodrilus oculatus* *Lumbicus rubellus* *Microscolex phosphoreus* *Murchieona muldali* *Octolasion cyneum* *Octolasion lacteum* *Spargano temaesis*
Epigeic	Generally do not create burrows but are surface dwelling earthworms found under leaf litter and in rotting logs. They feed directly on decaying organic matter and consume little or no soil.	*Allolobophora eiseni* *Dendrobaena attemsi* *Dendrobaena hortensis* *Dendrobaena pygmaea* *Dendrobaena octaedra* *Dendrobaena veneta* *Dendrobaena rubidus* *Eisenia fetida* *Lumbricus festivus* *Satchellius mammalis*

Table 1: Earthworm ecological groups with a description of how each species operates and a list containing which of the 27 British species are within each ecological group (Sherlock, 2012).

2.2.3. Influence on the Environment

The drilosphere is a dynamic zone within a soil profile which unwaveringly changes in both space and time (Brown, Edwards & Brussaard, 2004), due to the influences of earthworms and their casting and burrowing behaviour (Lavelle, 1988). Earthworms affect soil structure, which in turn affects microbial and invertebrate communities and thus organic matter decomposition and nutrient cycling (Brown, Edwards & Brussaard, 2004; Lavelle et al, 2004).

Burrowing is one form of engineering undertaken by earthworms, with different ecological groups burrowing with alternating methods; some vertically and some horizontally (Edwards, 2004; Lee, 1985). Burrows can create pores in the structure of the soil which allows for oxygen to enter the soil and carbon dioxide to exit (Edwards, 2004). Moreover, earthworms' burrows act to increase surface water infiltration, and without earthworms, infiltration decreases, and runoff increases; thus increasing surface erosion (Sharpley, Syers & Springett, 1979). Further to their burrowing activity, earthworms play an important role in breaking down dead organic matter. The process of decomposition allows for nutrient rich organic matter to make available its nutrients for use by living organisms. Earthworms are particularly adept at this as when they consume organic matter and break it into smaller pieces, it allows for bacteria and fungi to feed on it and release the locked in nutrients (Earthworm Society of Britain, n.d.). Through their search for organic matter, earthworms naturally mix the soil through which they travel, and because of this attribute, Darwin described them as 'nature's ploughs' (Darwin, 1882). By mixing organic matter into the soil and increasing the dispersal of nutrients earthworms improve the fertility throughout the soil profile.

Earthworm casts are particularly important to soil profile development and the structure of soils and it has been estimated that up to 50% of aggregates in the top layers of a soil profile are earthworm casts (Lee, 1985). The physical properties of these aggregates influences, among other things, aeration, infiltration and water holding capacity and are therefore particularly important for the poor quality degraded soils found in areas of rewilding. Casts are thought to be responsible for some of the fine crumb structure of soils (Edwards, 2004) and it has been shown there is a positive correlation between the quantity of casts and soil stability (Lee, 1985; Shipitalo & Le Bayon, 2004). Further to stability improvements, casts enhance the chemical composition of soils by increasing the nitrogen and carbon content in excreted casts (Lee, 1985) and so contribute to plant growth; another key feature of casting important for rewilding projects.

2.2.4. Earthworm Community Structure and Impacts from the Environment

Earthworm populations follow a similar structure to other soil inhabiting invertebrates and tend to have a pyramidal age structure, generally with a higher proportion of juveniles (Edwards & Bohlen, 1996). Population size is determined by many factors, largely outside the realms of their control and especially in agricultural soils. One of the main factors in this respect are the properties of the soil in which the earthworms live, key indicators are soil moisture, soil temperature, soil pH and soil type (Edwards, 2004).

As discussed by Lee (1985), studies have shown that earthworm populations are critically affected by variations in soil moisture. Moisture tolerance levels are best expressed in relation to matric potential (pF). This is a measure of the free energy of water held in a soil and it relates to the energy required to extract water from the soil. *Aporrectodea caliginosa* and *Aporrectodea rosea* are two species which have been shown to reduce in population size when soil moisture levels fall below their tolerance level, although it should be noted that different species prefer different levels of soil moisture content. Bouché (1982) showed these differences to exist between general ecological groups; anecic earthworms have low tolerances (pF 2.06 to 3.08), endogeic tolerances are higher (pF 2.31 to 3.33), and epigeic tolerances are not clearly defined. As soil moisture levels decrease earthworms will seek wetter soils but if moist soils are not available earthworms will enter a state of aestivation; in Europe lumbricids are reported to go into aestivation when moisture content falls below 25-30% (Lee, 1985). However, if moisture levels are too low mortality rates within populations will increase (Lee, 1985; Edwards, 2004).

Aestivation is a state where earthworms lack or slow their levels of activity and metabolism during temperature extremes. Depending on the species earthworms either go into diapause or quiescence. In diapause earthworms create a mucus lined chamber whereas in quiescence they simply go into a torpid state and do not create a chamber. In quiescence earthworms often suffer from tissue dehydration.

Soil temperatures vary seasonally, diurnally and with depth in the soil profile. It is recognised that seasonal extremes of high and low temperatures create high levels of adult mortality (Edwards & Bohlen, 1996) although it is not easy to define specific temperature preferences of earthworms. Despite high adult mortality earthworm populations can recover quickly once favourable conditions return due to embryos surviving in cocoons within the soil profile (Edwards, 2004). It was discussed by Edwards & Bohlen (1996) that some studies have suggested a link between cocoons and earthworm population survival, where cocoons act as the main survival method for populations of earthworm species living in soils with unfavourable conditions.

Drought (associated with high temperatures) and low temperatures induce a response mechanism in earthworms that helps to increase survival rate. Individuals retreat deep into the soil profile where temperatures are more stable; they remain motionless and do not feed in a state of aestivation (Lee, 1985) as when soil moisture content drops.

Earthworm burrows help to increase water infiltration. Burrows are known to be a significant drainage system; by allowing surface water to dissipate earthworm engineering helps to create more fertile, non-waterlogged soils. In so doing earthworms also regulate soil moisture content by creating conditions more favourable to their tolerance thresholds for soil moisture content.

Soil porosity, aeration and water infiltration rates have been shown to be positively influenced by earthworm burrows and in turn run-off rates are reduced (Kretzschmar, 1998; Roth & Joschko, 1991). Soil porosity is affected by earthworm burrowing and by the deposition of loosely packed casts on the soil surface (Lee, 1985). Increasing porosity allows for enhanced gaseous exchange (Edwards, 2004) between the drilosphere and the atmosphere, thus improving conditions within the soil profile. A further benefit of superior porosity and surface reaching burrows is in faster infiltration rates. Carter, Heinonen & Vries (1982) found that the presence of earthworms can enable water infiltration to occur at a rate two to ten times faster than in soils deprived of earthworm activity.

Soil pH is an important factor affecting earthworm populations. pH extremes have been demonstrated to create unfavourable conditions for earthworm habitation with earthworm presence being rare when pH falls below 4.0 and generally nonexistent when pH falls below 3.5. Different earthworm species are known to favour different soil pH ranges. It has been demonstrated by (Laverack, 1961) that certain species will not burrow at all in soils with a pH lower than their preference threshold and that unfavourable pH can have an effect on the total abundance of earthworms in a region (Baker et al, 1992; Baker et al, 2006)

Fewer earthworms in a soil profile can not only act to reduce the abundance of decomposing bacteria and fungi but these decomposers are also less active. Therefore with no earthworms present in an area, one can expect to see a build up in organic matter. Such a phenomenon could be seen in a small woodland 3km from the Avonmouth Smelter, where a standing crop of leaf litter equivalent to ca. 25 years of leaf fall was found. This was attributed to the loss of major detrivores and the ensuing reduction in rates of decomposition (Coughtrey et al, 1979).

2.3. Pigs and Rooting

2.3.1. Exploration

Depending on the extent of environmental heterogeneity (Brown, 2000) of the habitat in question, pigs will display a variety of foraging behaviours ranging between extrinsic exploration (behavioural traits involving the search for food or a nice place to lie) and intrinsic exploration (concerning the search for novelty and to gather information on their surroundings to increase chances of survival) (Studnitz, Jensen, & Pedersen, 2007). Stolba & Wood-Gush (1989), as cited by Studnitz, Jensen & Pedersen (2007), found that domestic pigs living in a semi-natural environment spent 52% of the daylight period foraging (rooting and grazing) and another 23% in locomotion and direct investigation of environmental features. Exploratory behaviour is important in pigs (Jenson & Toates, 1993) with rooting being of high priority (Studnitz, Jensen & Pedersen, 2007). Rooting has been categorised as a preferable activity (Brown, 2000), due to it providing a variety of food sources and nutrients from one single foraging activity. Due to these complementary fitness inputs rooting becomes the most profitable foraging technique in terms of fitness gained from time spent foraging (by arbitrage) (Brown, 2000). Further evidence of preference for rooting was shown by Day, Kyriazakis & Lawrence (1995) and Studnitz et al (2003) who found that pigs which had had rooting behaviour inhibited began to root immediately once restrictions were removed. This being said, if rooting is prevented then other exploratory behavioural elements will be exposed (Studnitz et al, 2003; Studnitz, Jensen & Pedersen, 2007).

In contrast, studies by Studnitz, Jensen & Pedersen, 2007 (2007) and Haskell et al (1996) suggest rooting appears to be novel and will diminish over time with prolonged access to rooting material.

2.3.2. Feeding Behaviour

Pigs are monogastric, non-ruminant omnivorous animals. They are generalist feeders and will eat what is available (Nogueira-Filho, Nogueira & Fragoso, 2009). It has been suggested (Edwards, 2003) that pigs will root (below ground foraging) when available nutrients from above ground food stuffs becomes unavailable. Roots and invertebrates (Cushman, Tierney & Hinds, 2004) are believed to contribute to the diet significantly in the short term at times when herbage is unavailable.

2.3.3. Land Disturbance

As cited by Cushman, Tierney & Hinds (2004); Bratton (1975) and Barrett (1978) found that pigs significantly increase disturbance levels by overturning large quantities of soil and organic matter whilst foraging for below ground plant foods and invertebrates. Consequently within an environment where the purpose of pig presence is to create disturbance they are reliable (Cushman, Tierney & Hinds, 2004) and can even provide germination niches for seedlings and invertebrates on disturbed soil patches (Sandom, Hughes & Macdonald, 2013 and Carpenter et al, 2012). Sandom, Hughes & Macdonald (2013) found that rooting increases with stock size and rooting noticeably increased at times of increased precipitation suggesting wetter soil is preferred for rooting. These findings are considered to indicate seasonal behavioural changes.

2.4. Earthworms and Pigs

Earthworm populations have the potential to be influenced by populations of grazing animals. This is not so much from competition especially in its true ecological sense as the effects are largely indirect. Grazing animals remove organic matter, and do not consume resources in the same manner as earthworms. Hone (2002) reports that rooting causes a major disturbance to plant communities. The extent of these disturbances has the potential to reduce herbaceous cover by up to 80-95% (Howe, Singer & Ackerman, 1981). This deduction in organic matter lessens the amount of available food sources for earthworm populations (Fonte, Winsome & Six, 2009) and reduces interactions between surface litter and the microorganisms responsible for providing alternate food sources for earthworms (Curry, 2004).

As mentioned previously, pigs are known to forage for invertebrates (Cushman, Tierney & Hinds, 2004) with many studies (Edwards, 1995; Mitchell & Mayer, 1997) associating earthworm consumption with rooting activity and as such pigs will have a negative effect on earthworm populations when organic matter and other above ground food sources have been exhausted. Baubet, Ropert-Courdert & Brandt (2003) also suggest earthworms may be ingested during the process of rooting for bulbs or roots. Rose & Williams (1983) suggest a link between weight gain in young pigs and earthworm consumption and it has also been proposed that earthworms can be actively sought after to aid the development of piglets and young pigs during the growing stage (Henry, 1987; Choquenot et al 1996). It is thought this is due to the high levels of dietary protein found in earthworms.

As shown by Baubet, Ropert-Courdert & Brandt (2003) wild boars consume earthworms in all seasons of the year and earthworms account for 92% of the diet by frequency of occurrence. In addition, earthworm reduction from feral pig predation in Queensland, Australia was found to vary from 62-93% (Pavlov & Edwards, 1995). From the classifications proposed by Granval & Muys (1995) wild boars can be categorised as regular predators of earthworms. Conversely Baubet, Ropert-Courdert & Brandt (2003) suggest earthworm consumption is opportunistic and they become a food source when climatic conditions favour earthworms to be on the soil surface, such as when excess rainfall increases earthworm abundance and accessibility on the ground surface (Macdonald, 1980; Bouché, 1982).

Furthermore, as reported by Chan (2001) disturbances in soils often create changes in the abundance and diversity of earthworm populations. Anecic earthworms tend to decline and endogeics populations can increase if their food supply increase from the disturbance, this may be the case with pig rooting considering the rooted material will often remain as dead organic matter on the soil surface at the disturbed site.

3. Materials and Methods

3.1 Study Sites

Research took place within the Knepp Wildland Project at the Knepp Castle Estate. The estate is situated in West Sussex (*Figure 1*) and comprises 3,500 acres. Land use practices were traditionally concerned with arable and dairy farming. However, in 2001 the estate owner made the decision to move away from these agricultural land uses and to move towards a series of regeneration and restoration projects due to his keen interest in nature and conservation and his wish to pass on the land in a better state than he inherited it. The new projects were aimed at nature conservation and a means of producing meat for market in a more sustainable and extensive way. Knepp Estate is compartmentalised into three distinct regions; the Northern Block, The Park (the central section containing the Castle and much of the Estate's infrastructure) and the Southern Block.

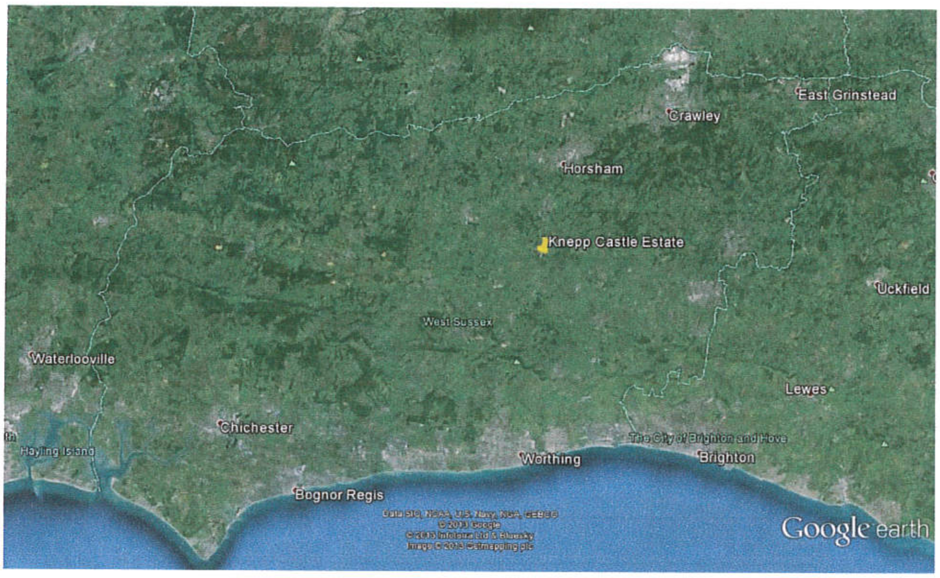

Figure 1: Overview of Knepp Castle Estate situated in West Sussex (Google Inc., 2013a).

The Southern Block, comprising approximately 1000 acres, is devoted to the 'Knepp Wildland Project' and is the area used for the purpose of this investigation. The 'Knepp Wildland Project' aims to explore the processes of farmland reversion and natural regeneration. The re-wilding in the Southern Block uses large herbivores to drive habitat changes through the ways in which the various herbivores interact with the vegetation structure. At the Knepp Estate, the large herbivores used are Longhorn Cattle, Tamworth Pigs, Exmoor Ponies, Red Deer, Roe Deer and Fallow Deer. They are helping to create a matrix of habitats including open grassland, scrub, forested groves, and of specific interest to this project, bare ground. Disturbances are created in different ways by each species. Tamworth Pigs turn over ground in the search of food (Brown, 2000), creating bare patches of ground through rooting.

3.2 Background

3.2.1. Soil Types

The soils types within the Knepp Wildland Project consist of Wickham 1 and Wickham 5. The soils are slowly permeable, seasonally waterlogged soils. Loamy topsoils over a parent material of impermeable Cretaceous clay substrates with no storage capacity (National Soil Resources Institute, 2013). *Figure 2* outlines the soil profile series of Wickham soils.

Figure 2: Wickham component series profile. Slowly permeable seasonally waterlogged fine silty over clayey, fine loamy over clayey and clayey soils (National Soil Resources Institute, 2013).

3.2.2. Climatic Conditions

Climatic conditions for the region are typical of a temperate region with mean daily temperatures in winter (Dec-Feb) ranging between 0.3°C and 5.5°C and in summer (Jun-Aug) ranging between 8.8°C and 19.1°C. In winter the annual average days of rainfall (rainfall >1mm) for the region is 14.2 days and is 11.1 days in summer (Met Office, n.d.).

3.3. Hypotheses

The study followed the hypotheses that;

(a) Rooting will have a significant effect on soil characteristics,
(b) Earthworm abundance will be lower at disturbed sites,
(c) Earthworm species richness and species diversity will be lower at disturbed sites.

3.4. Data Collection

Earthworm sampling was conducted between 21st May 2013 and 21st June2013. Water infiltration was conducted 20th June 2013. The infiltration tests were conducted on a single day to ensure the timeframe was minimal to safeguard against external influences, such as climatic variation.

3.4.1. Site Selection

Once all areas of disturbance had been identified across the Southern Block of the Knepp Estate they were entered into a database and 31 fields were selected at random with the use of random number generators; this ensured the experiment contained a good representation of the whole area through a fair distribution. It also meant there were enough replicates for the statistical analysis (Crawley, 2005). For each disturbed site, two undisturbed areas were needed for comparisons to be made. Random number generators were also used to assign random compass bearings (degrees) and random distances (meters) in order to determine where sampling for undisturbed areas would take place. In both instances randomization of site selection helped to avoid bias in my population (Crawley, 2005). Sample locations are outlined in *Figure 3*, with further detail given in *Appendix 1*.

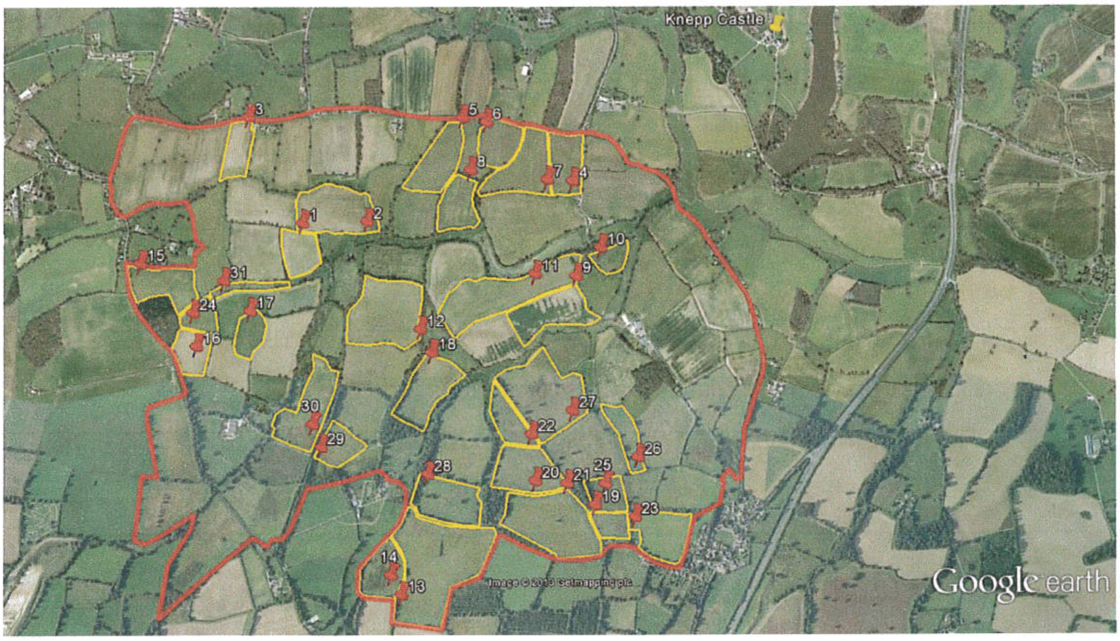

Figure 3: Knepp Estate rewilding project with sampled fields at disturbance locations labelled (Google Inc., 2013b).

3.4.2. Earthworm Sampling

Two methods of extraction were used for sampling earthworms. Firstly a 0.25m X 0.25m X 0.10m monolith was extracted from the site and hand sorted for earthworms (Römbke, 2005; Sherlock, 2012). The second technique used was a vermifuge to extract deep burrowing anecic earthworms as suggested by Sherlock (2012). The most effective solution is formalin but not recommended due to its hazardous nature and so the hot mustard extraction technique was used (Lawrence & Bowers, 2002; Römbke, 2005; Sherlock, 2012). This technique was undertaken within the hole from which the monolith was extracted. A mustard paste was mixed from hot mustard powder and water at a ratio of two parts water to one part mustard powder. For every pit 28 ml of mustard paste was made into solution with 1250 ml of water, following the procedure described by Lawrence & Bowers (2002). An example of the monolith and pit can be seen in *Figure 4*.

Any earthworms found were immediately placed into alcohol for preservation (Sherlock, 2012) and specimens were retained and returned to the laboratory for analysis. Juvenile and adult earthworms were counted for abundance; the difference between mature and immature earthworms easily identifiable due to the presence of a clitellum of adult specimens. Any adult earthworms were identified to species level in conjunction with Sherlock's (2012) identification key. A standard optical microscope was used for the purpose of identification.

3.4.3. Water Infiltration

Infiltration is the process by which water enters into a soil profile (Whittow, 2000). The rate of infiltration is the maximum rate at which water can enter the soil (Whittow, 2000) and is determined by the properties of the soil being tested, the initial rate of saturation at the time when rainfall (or in my case testing) occurs, and by the ways in which the landscape has been modified by human influences.

For the purpose of this experiment a Ring Infiltrometer was used. The ring infiltrometer (Surface Area = $0.38cm^2$ and Volume = $4.43cm^3$) was inserted in the soil at the desired site to a depth of 2cm in order to create a seal. 100mm of water was poured into a tube and a stopwatch was started. Measurements were taken at 30 second intervals and recorded. I decided to place an upper time limit of ten minutes on the test and measurements were not taken after this maximum time limit was reached in order that good comparisons could be made and to ensure a consistent time frame between sites.

These rules allowed for a rate of infiltration to be calculated from the results for comparison between disturbed and undisturbed sites.

Calculations include (University of Vermont, n.d.):

$$A = \pi r^2 \qquad V = A * h \qquad H = \frac{V}{A} \qquad I = \frac{H}{t}$$

Where:

A = surface area	*r = radius*	*V = volume*
h = height of water	*I = Infiltration Rate*	*t = time*
H = Depth of Water Infiltrated		

3.4.4. Soil Characteristics

The soil characteristics measured were soil moisture, soil temperature and soil pH, because all are known to have an effect on earthworm populations. For each characteristic three readings were taken at each sampling location and averaged. This helped to minimise error when only using a single value for each sample site during data analysis. Soil moisture ($m^{-3}.m^3$) was recorded using a digital soil moisture probe meter; soil temperature was measured with a digital soil temperature probe; and soil pH was assessed by means of a digital soil pH probe. All three digital probes are shown in *Figure 4*.

Figure 2: Field tools used to conduct soil characteristic sampling, soil monolith prepared for hand sorting of earthworms, excavated pit for hot mustard extraction technique.

3.5 Data Analysis

All data analysis was conducted using the statistical software R: version 3.0.1 (R-Project, 2013).

For all generalised linear models (GLM) stepwise model simplification was used to determine any significant relationships between explanatory and response variables. A significant relationship being one which yielded a significance level: $p<0.05$. Analysis of variance (ANOVA) used to determine whether removing an explanatory variable reduces the model's explanatory power (Collins, 2013; Crawley, 2005). Where removing an explanatory variable from the model yielded a significant p-value ($p<0.01$) it was left in the model. Model simplification continued until only significant explanatory variables remained.

Explanatory variables used were: soil infiltration, soil temperature, soil moisture, years in rewilding, soil pH and site type (either disturbed or undisturbed).

3.5.1 Ecological Groups

A Kruskal-Wallis test was performed to explore if the distribution of earthworm abundance differed between ecological groups with the null hypothesis that distribution within each ecological group is the same.

Relationships between each of the three ecological groups and the measured explanatory variables were explored using GLMs. These models take into account only adult earthworm abundance because juvenile earthworms could not be identified to species level. Where data was over dispersed and displayed non-normal distribution, quasi-Poisson family regression was used (Collins, 2013; Crawley, 2005).

3.5.2 Earthworm Abundance

A Kruskal-Wallis test was performed to determine if juvenile and adult earthworm abundance differed. The test was conducted using the null hypothesis that distributions are the same.

The relationships between measured explanatory variables and 3 separate response variables (adult earthworm abundance, juvenile earthworm abundance and total earthworm abundance) were explored using GLMs. Where data was over dispersed and displayed non-normal distribution, quasi-Poisson family regression was used (Collins, 2013; Crawley, 2005).

3.5.3 Species Diversity

Species diversity analysis was conducted using data only from adult earthworms because juvenile individuals could not be identified to species level.

The total number of species (species richness) calculated for each site was subsequently converted into an individual Shannon's Diversity Index value (H). A GLM was used to investigate the impact of the measured explanatory variables on the diversity at each site. Where data was over dispersed quasi-Poisson was considered (Collins, 2013; Crawley, 2005).

Shannon's Diversity Index (H) was calculated using the following formula (Begon, Townsend & Harper, 2006):

$$H = -\sum_{i=1}^{S} p_i \, lnp_i$$

Where:

S= number of species

p = *the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N)*

For species where more than 15 individuals were sampled (i.e. the most abundant species) the distribution of these species was explored in a GLM to establish which of the measured explanatory variables had an effect on the distribution on individual species. Once again, where data was over dispersed and non-normal a quasi-Poisson family regression was used (Collins, 2013; Crawley, 2005). There were 6 dominant species explored in this manner. These include *Allolobophora chlorotica,* *Apporectodea caliginosa, Apporectodea rosea, Lumbricus castaneus, Lumbricus rubellus, and Octolasion lacteum.*

4. Results

A total of 1289 earthworms (925 juveniles and 380 adults (*Figure 5*) were sampled across the 93 sample sites with a total of 19 individual species being identified *(Figure 6)*. It should be noted that species diversity analysis was restricted to using only 29.48% of the total sample population because only adult earthworms could be identified to species level. Abundance was greater at undisturbed sites for all classifications categorised in Table 1.

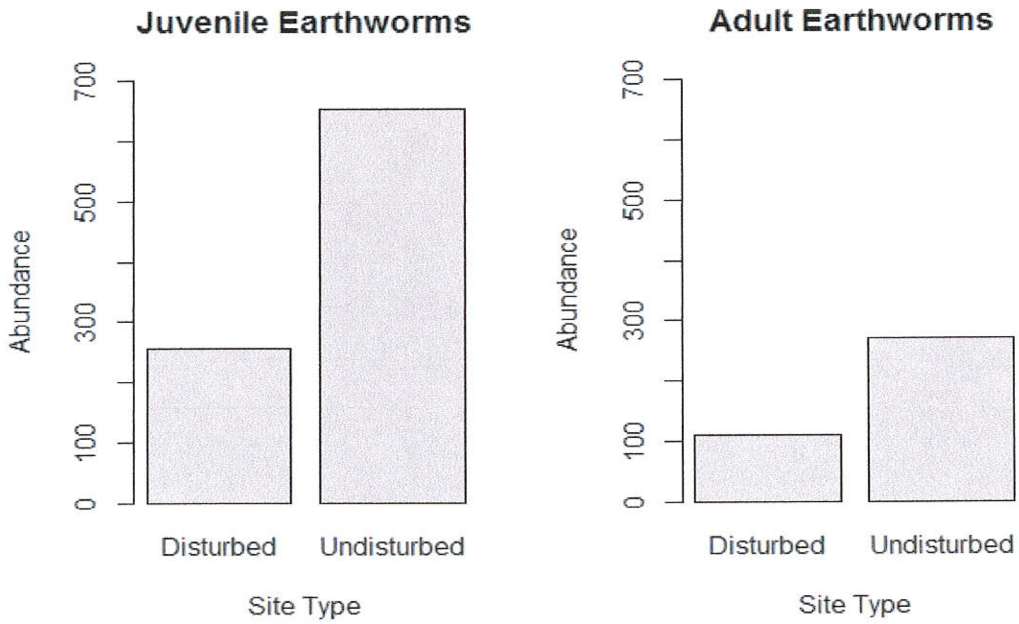

Figure 5: Distribution of total juvenile abundance and total adult abundance with respect to site type.

The numbers of species found at disturbed and undisturbed sites were 12 and 18 respectively *(Table 2)*. There were 7 individual species found only at undisturbed sites. The six most abundant species (total abundance >15) were *Allolobophora chlorotica* (135 total), *Aporrectodea caliginosa* (19 total), *Aporrectodea rosea* (62 total), *Lumbricus castaneus* (35 total), *Lumbricus rubellus* (44 total), and *Octolasion lacteum* (17 total). *Figure 6* shows the abundance of each species found.

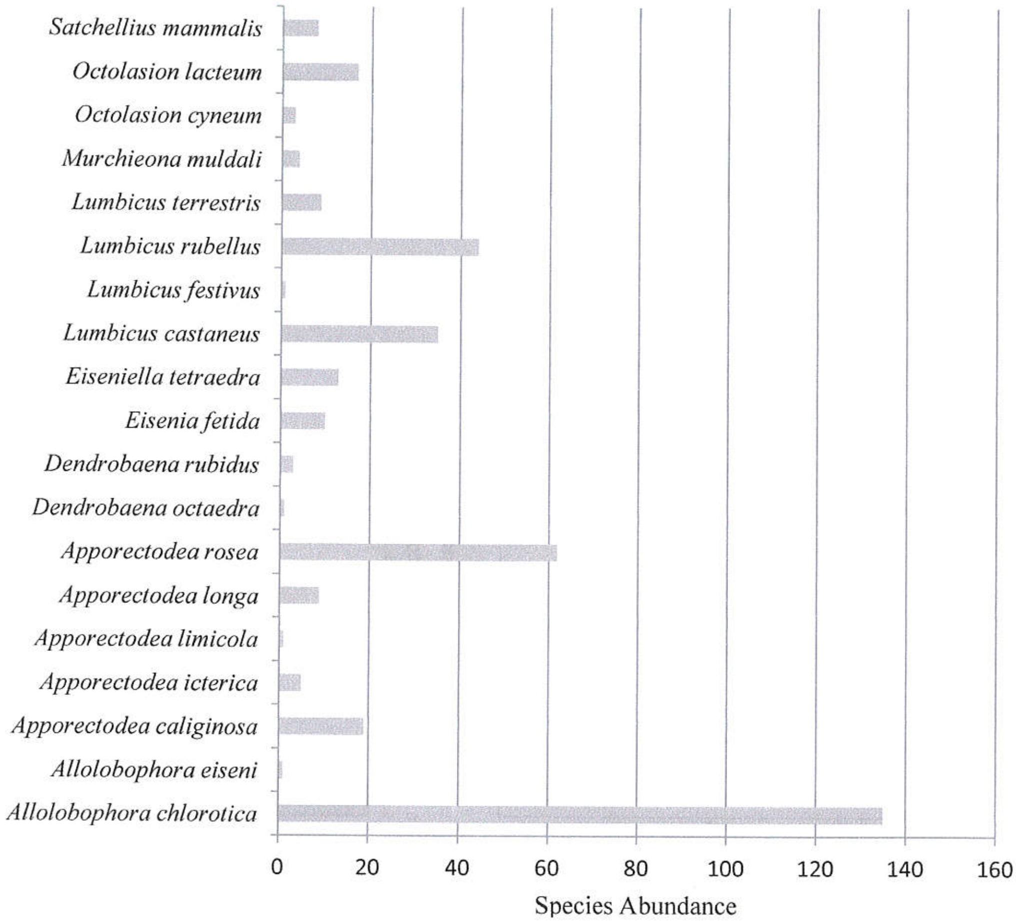

Figure 6: Species abundance of adult earthworms.

	Disturbed	Undisturbed	Total
Total Earthworms	364	925	1289
Juvenile Earthworms	255	654	909
Adult Earthworms	109	271	380
Anecic Earthworms	9	44	53
Endogeic Earthworms	94	209	303
Epigeic Earthworms	6	18	24
Number of Species	12	18	19

Table 2: Earthworm abundance and species richness across site types.

4.1. Ecological Groups

The distribution within each of the 3 ecological groups varies (X^2=130.68, D.F. =2, p=2.2e^{-16}, $p<0.01$) (Figure 3). The total number of endogeic earthworms was significantly greater than the number of anecic and epigeic earthworm ($p<0.05$) *(Figure 7)*.

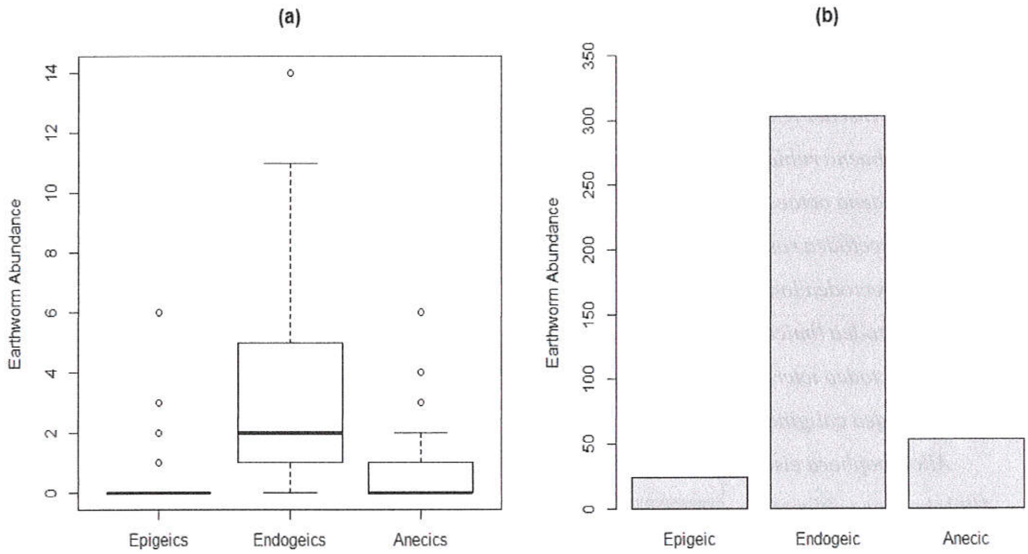

Figure 7: (a) The variation in distribution of the three ecological groups across all sample sites, and (b) the total abundance of each of the three ecological groups across all sample sites.

4.1.1. Anecic Earthworms

Anecic earthworm abundance was significantly affected by the number of years in rewilding ($t=-3.32$, $p=0.001$) *(Figure 8).*

Anecic abundance was not affected by soil moisture ($F=3e^{-04}$, d.f=86,87, $p=0.99$, $p>0.01$), site type ($F=1.65$, d.f=87,89, $p=0.20$, $p>0.05$), soil pH ($F=0.50$, d.f=89,90, $p=0.48$, $p>0.05$), infiltration rate ($F=0.41$, d.f=90,91, $p=0.14$, $p>0.05$), and soil temperature ($F=2.17$, d.f=91,92, $p=0.14$, $p>0.05$).

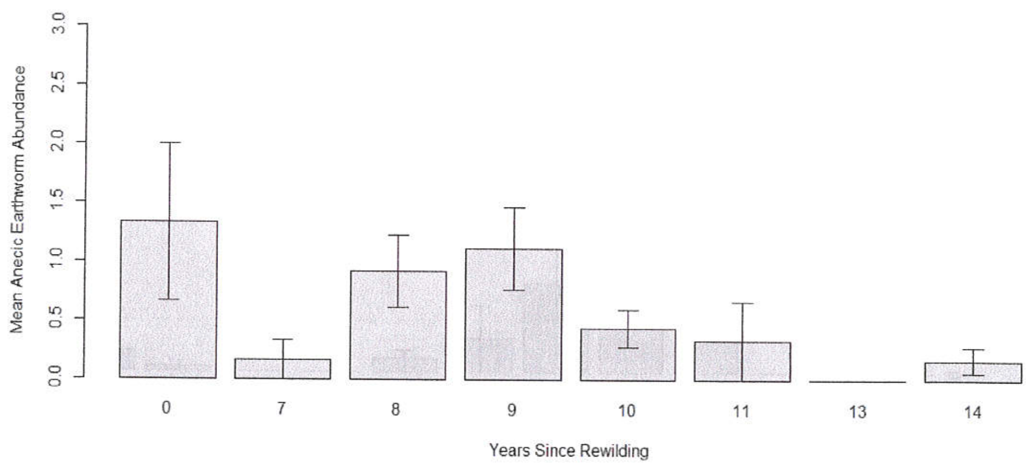

Figure 8: The effect of years in rewilding on mean anecic earthworm abundance.

4.1.2. Epigeic Earthworms

Epigeic earthworm abundance was significantly affected by soil pH ($t=-4.23$, $p=5.51e^{-05}$) *(Figure 9).*

Epigeic abundance was not affected by infiltration rate ($F=0.09$, d.f=87,88, $p=0.76$, $p>0.05$), soil moisture ($F=0.39$, d.f=87,88, $p=0.53$, $p>0.05$), number of years in rewilding ($F=0.76$, d.f=88,89, $p=0.39$, $p>0.05$), soil temperature ($F=0.57$, d.f=89,90, $p=0.45$, $p>0.05$), and site type ($F=0.91$, d.f=90,92, $p=0.41$, $p>0.05$).

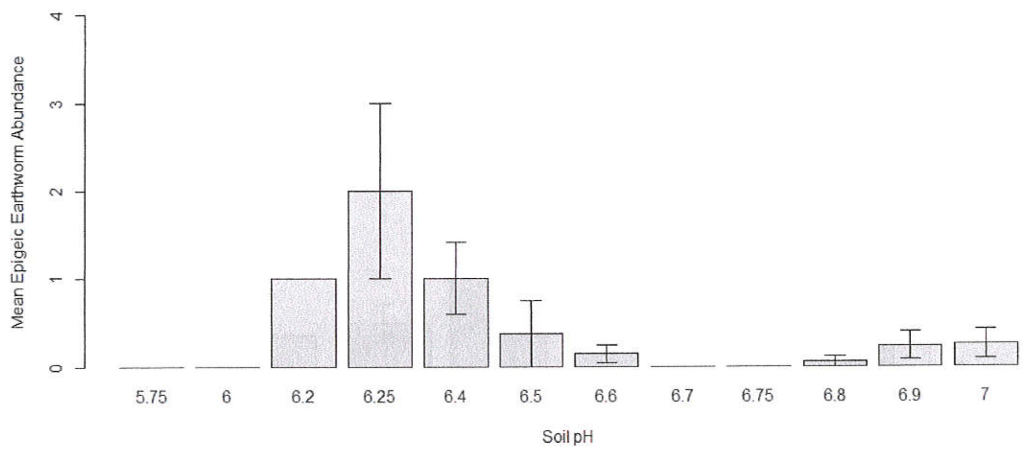

Figure 9: The effect of soil pH on mean epigeic earthworm abundance.

4.1.3. Endogeic Earthworms

In contrast to Anecic and Epigeic earthworms, Endogeic earthworm abundance was significantly affected by site type (Disturbed t=2.66, p=0.009 and Undisturbed t=2.71, p=0.008) and also soil pH (t=-2.14, p=0.03) *(Figure 10).*

Endogeic abundance was not affected by soil temperature (F=0.27, d.f=86, 87, p=0.60), number of years in rewilding (F=0.83, d.f=87, 88, p=0.36), infiltration rate (F=1.06, d.f=88, 89, p=0.31), and soil moisture (F=1.59, d.f=89, 90, p=0.21).

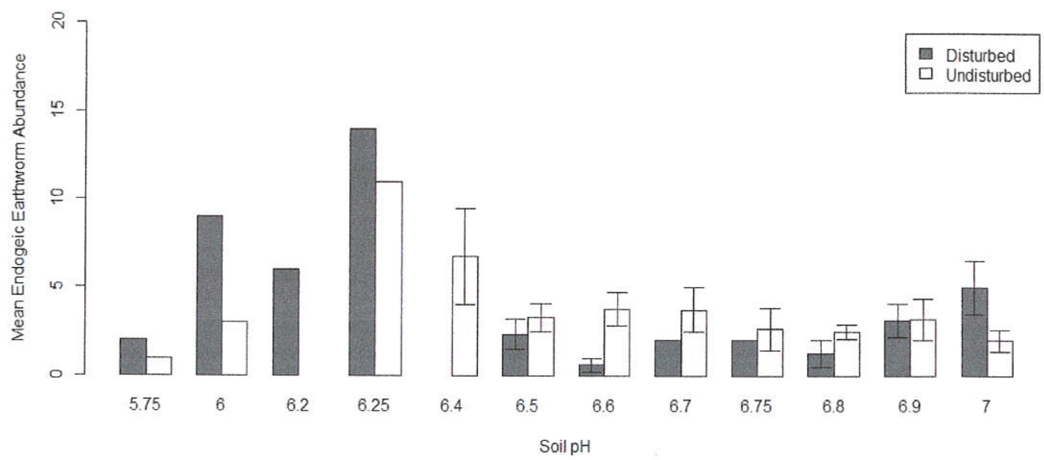

Figure 10: The effect of soil pH with respect to site type on mean endogeic earthworm abundance.

4.2. Earthworm Abundance

Regardless of species, juvenile abundance was found to be significantly greater than adult abundance $X^2=57.55$, d.f=1, $p=3.3e^{-14}$, $p<0.01$) (*Figure 11).*

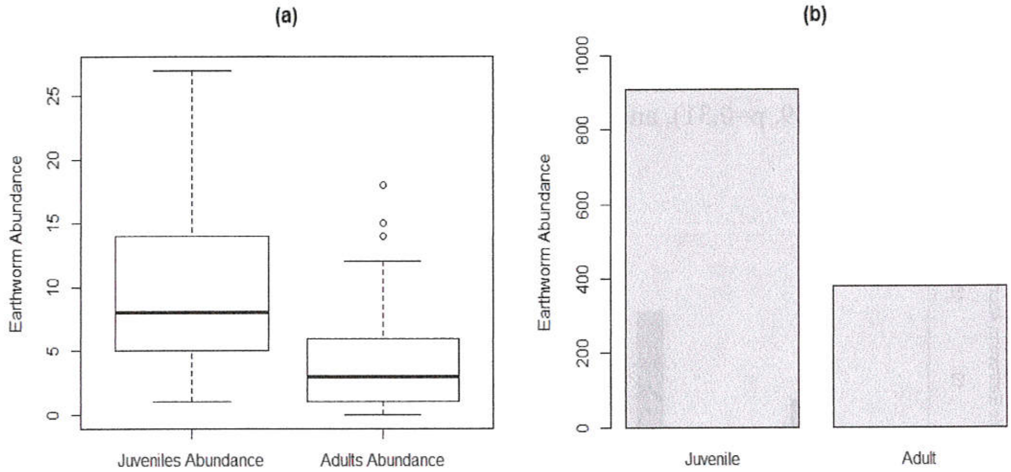

Figure 11: (a) The variation in distribution of juvenile and adult earthworm abundance across all sample sites, and (b) the total abundance of juvenile and adult earthworms across all sample sites.

4.2.1. Total Earthworms Abundance

Total earthworm abundance was significantly affected by site type (Disturbed $t=14.32$, $p<0.001$ and Undisturbed $t=16.92$, $p<0.001$) and number of years in rewilding ($t=-3.043$, $p<0.05$) *(Figure 12).*

Total earthworm abundance was not affected by soil temperature ($F=0.09$, d.f=86, 87, $p=0.77$), infiltration rate ($F=0.47$, d.f=87, 88, $p=0.50$), soil pH ($F=0.66$, d.f=88, 89, $p=0.42$), and soil moisture ($F=1.99$, d.f=89, 90, $p=0.16$).

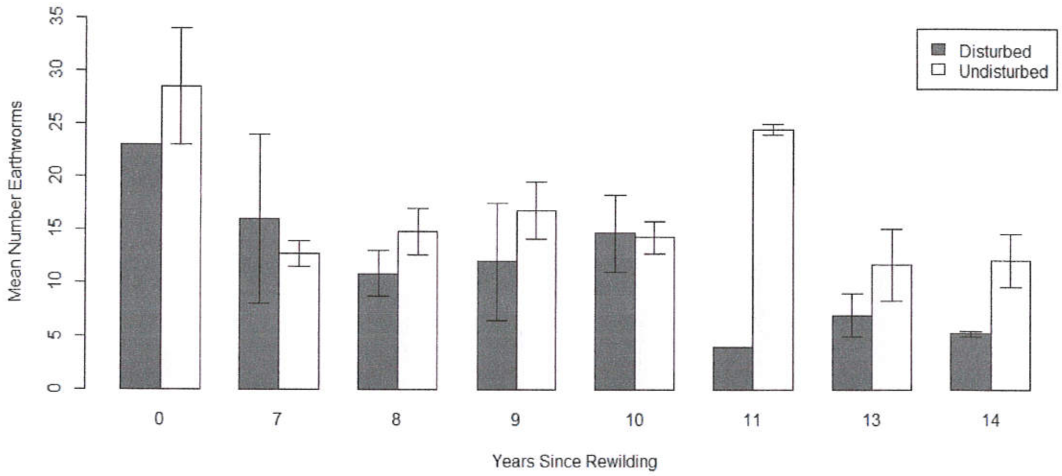

Figure 12: The effect of years in rewilding with respect to site type on mean earthworm abundance.

4.2.2. Juvenile Earthworm Abundance

Juvenile earthworm abundance was significantly affected by site type (Disturbed $t=14.16$, $p<0.001$ and Undisturbed $t= 16.98$, $p<0.001$) and number of years in rewilding ($t=-3.762$, $p<0.001$) *(Figure 13).*

Juvenile earthworm abundance was not significantly affected by soil pH ($F=0.01$, d.f=86, 87, $p=0.90$), soil temperature ($F=0.09$, d.f=87, 88, $p=0.71$), infiltration rate ($F=0.34$, d.f=88, 89, $p=0.56$), and soil moisture ($F=0.79$, d.f=89, 90, $p=0.38$).

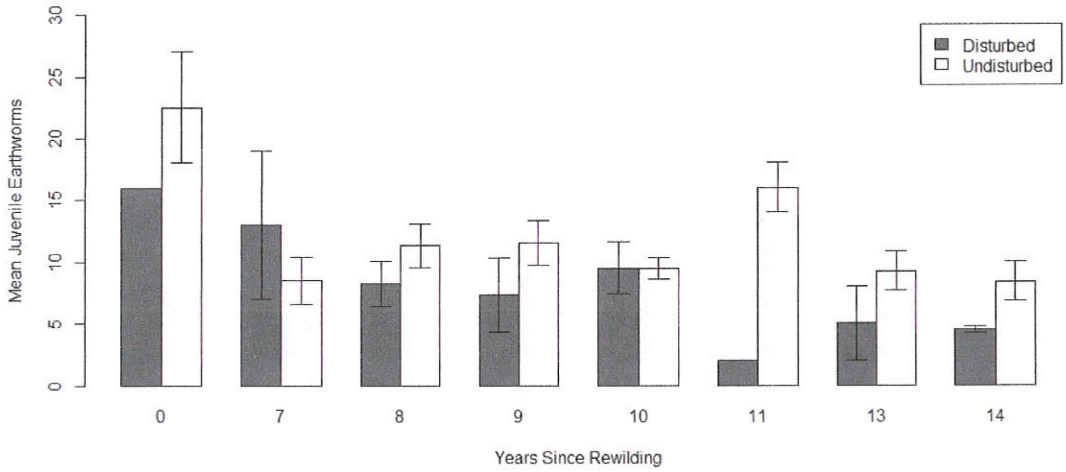

Figure 13: The effect of years in rewilding with respect to site type on mean juvenile earthworm abundance.

4.2.3. Adult Earthworm Abundance

Adult earthworm abundance was significantly affected by site type (Disturbed $t=2.800$, $p<0.01$ and Undisturbed $t=2.898$, $p<0.01$) and soil pH ($t=-2.202$, $p<0.05$) *(Figure 14).*

Adult earthworm abundance was not affected by soil temperature ($F=0.003$, d.f=86, 87, $p=0.95$), infiltration rate ($F=0.44$, d.f=87, 88, $p=0.51$), years in rewilding ($F=1.40$, d.f=88, 89, $p=0.24$), and soil moisture ($F=1.30$, d.f=89, 90, $p=0.26$).

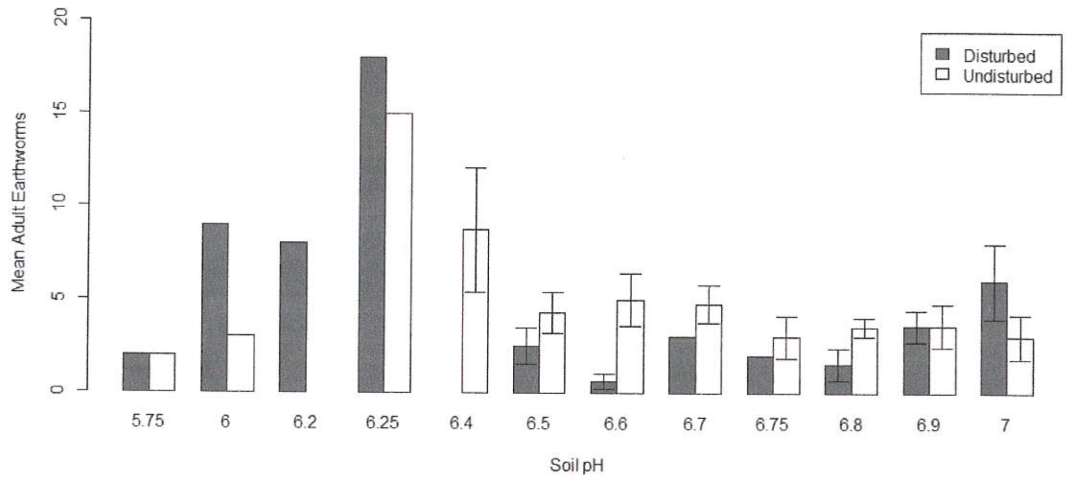

Figure 14: The effect of soil pH with respect to site type on mean adult earthworm abundance.

4.3. Species Analysis

4.3.1. Species Richness and Species Diversity

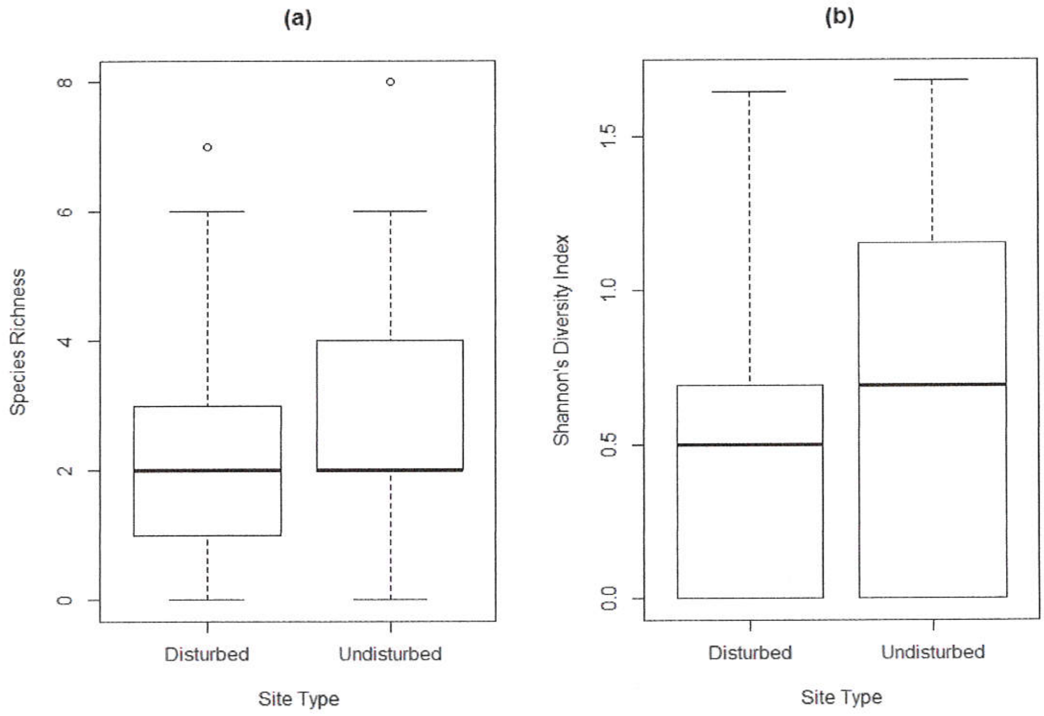

Figure 15: (a) Species richness with respect to site type, and (b) Shannon's Diversity Index with respect to site type.

The median species richness was similar at both disturbed sites and undisturbed sites. However the distribution was markedly different with a greater proportion of undisturbed sites having a higher number of species sampled than at disturbed sites *Figure 15 (a)*.

By Shannon's Diversity Index there was higher species diversity at undisturbed sites (Hs=3.93) than at disturbed sites (Hs=3.09). The median Shannon's Diversity Index was higher at the undisturbed sites than at disturbed sites *Figure 15 (b)*.

Species diversity was positively affected by soil pH (t=-4.888, p<0.001) (*Figure 16*).

Species diversity was not affected by infiltration rate (F=0.002, d.f=86,87, p=0.96), site type (F=1.97, d.f=87,89, p=0.15), soil temperature (F=0.16, d.f=89,90, p=0.69), number of years in rewilding (F=0.89, d.f=90,91, p=0.35), and soil moisture (F=0.84, d.f=91,92, p=0.36).

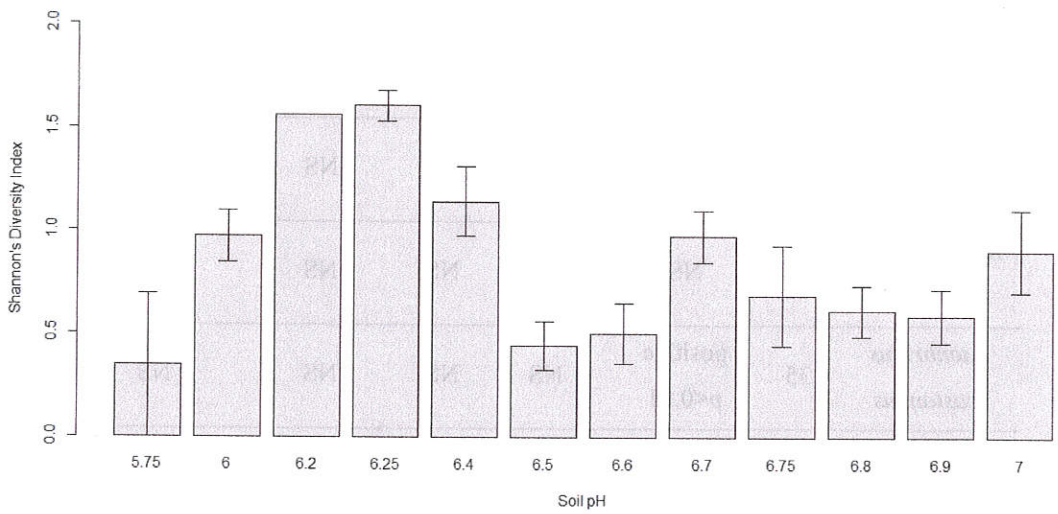

Figure 16: The effect of soil pH on Shannon's Diversity Index.

4.3.2. Most Abundant Species

For species which had at least fifteen individuals sampled, the distribution was explored with respect to the main effects measured. Table 2 summarises the findings:

Table 3: The effects of measured explanatory variables on the six most abundant species (NB: NS = No Significance)

Species	N	Years in Rewilding	Site Type	pH	Soil Moisture	Soil Temperature	Infiltration Rate
Allolobophora chlorotica	135	positive $p<0.01$	positive $p<0.01$	NS	NS	NS	NS
Aporrectodea caliginosa	19	NS	NS	NS	NS	positive $p<0.01$	NS
Aporrectodea rosea	62	NS	NS	NS	NS	NS	NS
Lumbricus castaneus	35	positive $p<0.01$	NS	NS	NS	NS	NS
Lumbricus rubellus	44	NS	NS	NS	positive $p<0.01$	NS	NS
Octolasion lacteum	17	NS	positive $p<0.01$	NS	positive $p<0.01$	positive $p<0.01$	NS

5. Discussion

On the whole, earthworm data and soil properties largely followed expected trajectories by means of the exploratory rooting behaviour displayed by the pigs. It was initially hypothesised that abundance and species diversity of earthworm communities would be significantly reduced in areas where pigs had rooted which was largely based on the changes in favourable soil conditions when pigs exert their influence on the land.

5.1 Soil Characteristics

As earthworms have been shown to play an important role in soil conditioning through their formation and maintenance behaviour (Edwards, 2004) and by their key role in the provision of ecosystem services (Barrios, 2007) they could hold the key in rewilding projects to making good the damage caused by agriculture and other management practices. In conjunction with previous studies (Baker et al, 1992; Baker et al, 2006; Lee, 1985) of earthworm populations it was shown that soil pH, soil moisture, soil temperature, site type and the number of years in rewilding have varying degrees of impact on earthworm abundance and species diversity.

5.1.1. Soil pH

In tandem with Baker et al (1992), Baker et al (2006), and Laverack (1961) soil pH had an effect on certain earthworm categories, in particular epigeic, endogeic and adult earthworms. I suggest this is because the pH of the soils at the Knepp Wildland Project is generally neutral and within these species tolerance ranges. If soil pH had been less favourable in some areas then earthworm distributions would have been dispersed (Lee, 1985) and both abundance and diversity would have been reduced across the study area. Species richness and diversity is affected when species are absent. The results from this study imply that soil pH is affecting Shannon's DI, so we must assume that soil pH within the rewilding project is affecting the abundance of some species. Within the study, pH levels did not vary considerably, however even slight alterations in pH have been shown to change earthworm species diversity.

5.1.2. Soil Moisture

Lumbricus rubellus has been shown to favour soils on the wetter end of the spectrum, and in many cases it thrives in moist soils (Costello & Lamberti, 2008). Soils were not unusually moist within Knepp's rewilding project but soil moisture was shown to have a significant effect on this species. Despite *Lumbricus rubellus* being one of the most abundant species within the Knepp Wildland Project, it could be inferred that their abundance would be greater if soils had had greater moisture content.

The lack of presence of soil moisture in any of the other models is largely understandable because the lack of moisture in the soil does not necessarily equate to a lack of earthworm presence (Lee, 1985). The soil moisture content throughout the rewilding project was consistent. Therefore earthworms could not feasibly find moister soils. Consequently individuals would remain in situ after entering a state of aestivation. Even when in aestivation individual earthworms would still be sampled if aestivation occurred in the top section of the soil profile and within the extracted and hand sorted monolith.

5.1.3. Soil Temperature

Similarly, soil temperature was not too low for earthworm habitation and so did not force earthworms into a state of aestivation. Therefore, provided burrows were not blocked then earthworms will seek a more favourable soil temperature in a different location. Soil temperature was shown to have an effect on *Apporectodea caliginosa* and *Octolasion lacteum*. It must be assumed that soil temperatures were within tolerance thresholds for these species and so their relatively high abundance, in comparison to other species, can be associated with preferable habitation conditions. Furthermore, as is the case with soil moisture, low temperatures would not necessarily effect earthworm presence or abundance because if temperatures were outside tolerance thresholds then earthworms would enter a state of aestivation and remain in situ (Lee, 1985).