WHAT ROLE DO CONSERVATION GRAZING ANIMALS AND WORMING REGIMES PLAY IN DETERMINING SOIL INVERTEBRATE COMMUNITIES ON A LOWLAND HEATH SYSTEM?

A technical report prepared from an MRes Wildlife Conservation project, Marwell Wildlife & University of Southampton

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What role do conservation grazing animals and worming regimes play in determining soil invertebrate communities on a lowland heath system?

Abstract

This study looks at some of the effects large herbivores can have on the macrofauna on a managed site. Large herbivores are often used to graze areas of conservation concern. They are usually employed in low densities to keep vegetation at a specific desired height or to maintain specific habitat requirements for particular species to survive. Livestock used for conservation grazing purposes are not often on a regular worming regime as it is widely believed that, once excreted, the medication will have negative impacts on the flora and fauna.

The effects of dunging, ivermectin (currently the most common anthelmintic) and the species of herbivore are considered on the abundance, richness and diversity of soil invertebrate communities on a managed site. The site is a 78.72ha lowland heathland grazed by five Przewalski's horses and eight Highland cattle. Ten exclusion zones were erected on the site and dung of four combinations (horse treated, horse untreated, cow treated and cow untreated) was applied to each plot. These were compared against each other and against controls with no dung applied. Anthelmintics are shown to have a significantly negative effect on the abundance of soil invertebrate communities ($F_{2,4.91}$=6.870, p=0.038). Although this effect is significant, it is also weak and the presence of dung is shown to have a far greater positive effect on abundance. The type of herbivore dung also has a significant effect on the abundance of invertebrates ($F_{1,5.47}$=15.87, p=0.009), with cattle dung showing a higher mean abundance (9.06) than dung from horses (7.78). Richness and diversity of soil invertebrate communities are also considered and this study shows that they are not significantly affected by the presence or absence of dung and anthelmintics and the species of herbivore also does not affect them. Management recommendations are made based on these findings for graziers and managers of lowland heathlands or similar systems.

Target Journal

This research project can be adapted for submission to 'Basic and Applied Ecology'.

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Introduction

Literature Review

Heathlands

The study site for this project is a lowland heathland. This is a diverse habitat with unique characteristics. Many rare and varied flora and fauna have come to rely on heathland, with some species, such as the smooth snake (*Coronella austriaca*), Britain's rareset reptile, almost exclusively found on heathlands (Forestry Commission, 2003). The UK Biodiversity Action Plan (BAP) for heathlands (Maddock, 2008) describes lowland heathland as a "broadly open landscape on impoverished, acidic mineral and shallow peat soil, which is characterised by the presence of plants such as heathers and dwarf gorses". It is usually at an altitude of less than 300m above sea level, giving it a unique vegetation composition. Lowland heath is a man-made habitat (Webb, 1986), much of which was created by Bronze Age settlers clearing forests to make way for agriculture around 4000 years ago (Forestry Commission, 2003). The habitat requires a temperate climate and mild winters and is found throughout north-west Europe (Gimingham, 1972). There is less than 5000km^2 of lowland heathland left worldwide and about twenty per cent of this is found in the UK (JNCC, 2014). Its rapid decline and unique characteristics make lowland heathland a habitat of conservation concern throughout its distribution. Soil types and climate can affect the flora and fauna found on heathlands and their location within the country will have an impact on this (Price, 2003). The Tertiary soils of the Hampshire and London Basins (in which Eelmoor sits) are highly acidic and typically have the driest plant species of any heathlands in the country (Webb, 1986). Lowland heathlands vary in wetness from very dry, sandy soils to wet and boggy with peaty soil. The characteristic poor nutritional value of the soil gives a diverse range of vegetation, the make-up of which is unique to heathlands.

Heathlands are specifically managed to avoid increased nutrient loading, prevent fast-growing vegetation from dominating and prevent succession to acid grasslands (Gimingham, 1992). With the development of agricultural practices, heathland is in severe decline and many areas are being put under threat from housing development, modern agriculture and abandonment. This habitat is only surviving where it is being conserved and restored for conservation purposes. Without intervention and management, this man-made habitat will quickly succeed into woodland, altering the soil structure and vegetation composition (Crofts and Jefferson, 1999). Heathland plant seeds can remain viable in the soil for many years, enabling potential restoration of a heathland habitat after severe degradation. Eelmoor Marsh is a prime example of where restoration has enabled these species to take hold again. The most successful practice to restore and manage lowland heaths is to graze them with large herbivores; machinery is also sometimes used in the restoration process.

Herbivores and Grazing

Eelmoor Marsh, the study site for this project, is grazed by large herbivores as part of its management plan. Large herbivores are often used for conservation purposes (Barbero et al., 1999). They are employed usually in low densities (Fricke and VonNordheim, 1992) to graze an area and keep vegetation at a specific desired height or to maintain specific habitat requirements for particular species to survive (Bullock and Pakeman, 1997; DeBonte et al., 1999). Conservation grazing is livestock grazing that meets nature conservation objectives (Grazing Animals Project, 2009) and has increased in popularity as a habitat management tool over the last decade (Delescaille, 2002). Lowland heath systems, such as Eelmoor Marsh, are man-made habitats that would revert to woodland if left unmanaged (Bullock and Pakeman, 1997); therefore they are usually extensively managed, with grazing as a key tool in keeping the vegetation down (Bakker et al., 1983). Some types of grazing also prevent a build-up of nutrients in the soil and continually remove new growth; thus fast growing competitive species cannot achieve dominance (Price, 2003).

Large herbivores can impact the landscape and habitats within it in many different ways, the three main ways are by grazing, trampling and defecating (Grayson and Swanson, 2008). By grazing, they keep vegetation low and prevent young shoots from growing (Price, 2003). Trampling flattens vegetation and creates gaps and on softer ground can create dents in the soil - poaching - which provide micro-habitats for many plant, invertebrate and reptile species (Tilman, 1997; Mulholland & Fullen, 2007). Dunging, a focus of this study, can increase the nutrient availability in the soil and can provide new micro-habitats for plant and animal species (Bull et al., 1998). Cow dung is host to a particularly diverse and extensive range of invertebrates (Skidmore, 1991). It is usually distributed throughout the habitat, however is sometimes more concentrated in resting areas (Ausden, 2007). This is very different to the dunging regimes of horses, which make territorial dung heaps - middens - in certain areas, this puts a disproportionately large amount of dung in particular areas and over a long period of time can cause local nutrient increase in the soil (Ausden, 2007).

The need to worm

Parasites, including helminths, are a huge issue for farmers and the welfare of livestock throughout the year (Steelman, 1976). They can impair health, growth and reproduction of animals and cause severe infections, sometimes resulting in death of the animal (Waller, 1999). Almost all livestock will have a parasite burden and anthelmintics can help to manage this by significantly reducing the worm burden. Common important internal bovine parasites include brown stomach worm (*Ostertagia*), the coccidia (*Eimeria bovis*) the lungworm (*Dictyocaulus*) and liver flukes (Corwin and Randle, 1993; Paz-Silva et al., 2010). Common equine parasites include strongyles and cestodes, with cyathostomins often making up 95% of the worm burden in horses (Neilsen et al., 2012). Parasites, particularly worms, can infect almost any part of the body; each parasite will have a particular niche, many usually infest the intestines of their host animal (Kennedy & Guégan, 1996). If left untreated, numbers can rise and they can cause extensive and irreparable damage to internal organs. Most commercial livestock will be on a regular worming regime to minimise the impact of these parasites, however even with the best anthelmintics and the perfect deworming schedule, animals will most likely still have a worm burden (Waller, 2006). Even though the parasite burden often cannot be completely removed, it is important to treat animas with anthelmintics to reduce their parasite burden and maintain their health and wellbeing.

Parasites & Helminths

Parasites can be classed as micro or macro parasites (Elliott, 2003). Macroparasites multiply within their definitive (final) host, whereas microparasites do not, they may still reproduce within their final host though (Dinoverm, 2013). Macroparasites only spend part of their life cycle in a single host and then move to another host species; they may also have a free-living life stage where they do not rely on a host. Microparasites complete their whole life cycle within a single host (AMRITA, 2014). Helminths, a study subject in this project, are macroparasites. Macroparasites can have a simple or complex life cycle, involving no or many intermediate hosts. Reproduction occurs in the definitive host and commonly produces eggs, though some viviparous species generate larvae (Grenfell et al., 1995). These eggs are usually deposited in the host's faeces, where they will either remain free living in the soil or will be ingested by another host grazing the land (Anderson, 1980). They may then take hold within a species-specific area of that individual's body and develop within it. Parasites, particularly species of worms, can infect any part of the body. With helminths, this second host could be the definitive host or could be one of potentially multiple intermediate hosts. It is within the intermediate host that asexual reproduction can occur (Dinoverm, 2013). Helminths are parasitic worms with elongated flat or round bodies; they are invertebrates and there are many species of them. Helminths can be categorised into three major groups: trematodes (flukes), nematodes (roundworms) and cestodes (tapeworms) (Barrett, 1981). The helminths are all macroparasites so do not multiply within their hosts. They infect many host species, including bovines and equines which are studied in this project.

Anthelmintics

The animals in this study are treated with ivermectin which is the most frequently used anthelmintic in the UK at present. Ivermectin (22,23-dihydroavermectin B_1) is the generic name given to two types of modified avermectins that are mixed together (Jackson, 1989) and is arguably the most effective anti-parasitic medication on the market at this time. It is thought to paralyse invertebrates by block the signal transmissions of nerves and muscles by interfering with glutamate-gated chloride channels, causing paralysis and death to the organism (Martin et al., 2002; Wolstenholme and Rogers, 2005). It has very low toxicity to vertebrates, bacteria and fungus though (Campbell & Benz, 1984; Wardhaugh & Beckmann, 1996). Ivermectin is made via the fermentation of the soil-dwelling actinomycete *Streptomyces avermitilis* and is a macrocyclic lactone (Campbell et al., 1983). It undergoes little metabolism whilst in the animal and Campbell et al. (1983) suggest that at least 98% of the medication is excreted unaltered. Ivermectin helps to control external and internal parasites by leaving a residue in the dung of treated animals that prevents the development of fly and beetle larvae and have been found to have significant negative effects on dung fauna (Strong et al, 1996; Jensen & Scotts-Fordsmand, 2012) and thus the degradation of dung. Dung from treated Przewalski's horses came from animals treated with invermectin, mainly to treat against onchocerciasis but it is also effective against a number of more common equine parasites (Dourmishev et al., 2005). Dung from treated highland cattle was sourced from a herd treated with a type of ivermectin called Ivomec. This is a broad spectrum cattle and sheep worming medication that treats against gastro-intestinal roundworms, lungworms, eyeworms, mange, mites and sucking lice (Hyperdrug, 2013). FlyPor is used on cattle to treat against flies and lice. It is particularly effective against horn flies and biting flies and as a preventative for chorioptic mange (Hyperdrug, 2013). The treated cattle for this project were given FlyPor at the end of the summer.

Introduction to Study

Many cattle and horses are used as habitat managers and it is known and documented that this has positive effects on habitats (Kirkpatrick et al., 2011; Diehl et al., 2013), mainly by keeping vegetation low and preventing succession towards woodland (Bullock and Pakeman, 1997; Schaich and Barthelmes, 2012). However other more subtle effects, such as nutrient and chemical input from the animals into the soil, have not been the subject of much research. Most conservation grazers are not wormed as it is believed that anthelmintics will have negative effects on the environment (O'Hea et al., 2010; Jensen & Scotts-Fordsmand, 2012). Very little research has been conducted to quantify these effects and still less focusing on the effects of anthelmintics on invertebrates in the soil. This will be the focus of this research project.

This eight month research project forms part of a research masters in Wildlife Conservation associated with The University of Southampton and Marwell Wildlife. It addresses a topic identified by Marwell as needing further research and has the potential to inform management decisions both within Marwell Wildlife and the wider conservation community. This project begins to address the gaps in knowledge with grazing livestock as a habitat management tool for conservation purposes.

Hypotheses

The hypotheses that this project will investigate are shown below.

Hypothesis 1: The presence of horse or cattle dung or the presence of anthelmintics will have significantly negative effects on the abundance of soil invertebrate communities.
Null Hypothesis 1: The abundance of invertebrates will not be negatively affected by the presence of horse and cattle dung or of anthelmintics.

Hypothesis 2: The presence of horse or cattle dung or the presence of anthelmintics will have significantly negative effects on the richness of soil invertebrate communities.
Null Hypothesis 2: The richness of invertebrates will not be negatively affected by the presence of horse and cattle dung or of anthelmintics.

Hypothesis 3: The presence of horse or cattle dung or the presence of anthelmintics will have significantly negative effects on the diversity of soil invertebrate communities.
Null Hypothesis 3: The diversity of invertebrates will not be negatively affected by the presence of horse and cattle dung or of anthelmintics.

Aims and Objectives

This project aims to assess whether anthelmintics have an effect on soil invertebrate communities and to quantify the extent of any effects. To do this, the abundance, richness and diversity of invertebrates will be measured through the evaluation of soil invertebrates from areas treated with four different combinations of dung and anthelmintics (horse dung, cattle dung, treated and untreated with ivermectin). It also aims to look at whether soil invertebrate communities are affected by the type of herbivores (horses and cattle) grazing and dunging on the soil. An additional output from the study is to provide recommendations to graziers on lowland heathlands to manage faecal parasite load in their livestock.

Methodology

Study Site

This study was undertaken on Eelmoor Marsh, Farnborough, UK (Figure 1). An aerial photograph of this site can be seen in Appendix 1. It is a lowland heath which was formerly part of a larger lowland peat moor system that is now fragmented and the marsh is partly isolated (Wilkie, 2013). This site is a prime example of where restoration has enabled seeds stored in the soil from typical heathland vegetation to take hold again; it now has populations of over 400 species of conservation concern as well as many other more common species. The 78.72ha site was designated as a Site of Special Scientific Interest (SSSI) by Natural England in 1993; it is also became a Site of Interest for Nature Conservation (SINC) in 2000 and has been part of the Thames Basin Heaths Special Protected Area (SPA) since 2005, partly due to breeding pairs of nightjar (*Caprimulgus europaeus*), woodlark (*Lullula arborea*) and Dartford warblers (*Sylvia undata*) (Wilkie, 2013). The heath is actively managed to restore the lowland heath system. This is mainly achieved by grazing large herbivores across the site and undertaking mechanical management on a yearly basis over the winter (Hall et al., 2009) (Figure 2).

Figure 1: Eelmoor Marsh, a 78.72ha lowland heath designated as a SSSI, SPA and SINC.
E. Rendells, 2014

Figure 2: Eelmoor Marsh is managed partly by grazing horses (seen here) and cattle.
E. Rendells, 2014

Herbivores

Eelmoor Marsh is currently grazed by five Przewalski's Horses (*Equus ferus przewalskii*) (Figure 3) and eight Highland Cattle (*Bos taurus*) (Figure 4). The horses are all part of a European Endangered Species Program (EEP) and are a bachelor herd. The cattle herd comprises of bulls and cows. The animals are free to range across Eelmoor marsh, with a fence separating three cattle to the north of the site. None of these animals are wormed at present and faecal parasite monitoring indicates a higher parasite load than domesticated conspecifics. The untreated horse and cattle dung for this project came from these animals.

Dung from treated Przewalski's Horses came from the captive herd at Marwell Zoo. Their diet is very different to the horses on Eelmoor, they are fed 550g of pony nuts each per day and have access to an unlimited supply of hay; they also have access to a grassed paddock with few shrubs. They are wormed with Ivermectin over a six day period every eight weeks (they are given 750g of pony nuts for those days); their treatments corresponding with this study finished on February 1st and March 29th, 2014. This information was collected from personal communication with Phil Robbins, the Przewalski's Horse keeper at Marwell, on 24/02/2014.

Dung from treated Highland Cattle was sourced from a local cattle farmer. The herd is made up of thirteen individuals and consists of young bulls and cows. They are grazed on pastures and fed hay in the winter and are also given mineral licks as supplements, making their diets comparable to the cattle at Eelmoor. They are treated with Ivomec twice yearly; the last time they were treated was October 2013. They are also treated with Flypor when needed (when coats are moulting and they're likely to scratch themselves). The last Flypor treatment was late summer 2013. This information was collected by personal communication with Tim McLeod-Clarke, the owner of these cows, on 17/02/2014.

Figure 3: Przewalski's horse stallions. There are five horses that graze Eelmoor Marsh.
E. Rendells, 2014

Figure 4: Highland Cow. Six cows and two bulls graze Eelmoor Marsh, with three cows fenced off to the north of the site.
E. Rendells, 2014

Project Methodology

This project begins to look at the potential effects anthelmintics are having on invertebrates within the soil. It compared dung from animals treated with anthelmintics with that of untreated animals. It looked at the diversity and composition of beneath-soil invertebrates under both conditions and compared them to areas with no dung. Dung was used from horses and cattle, the two major herbivores used for conservation grazing management. The model for this experiment can be seen below in Figure 5.

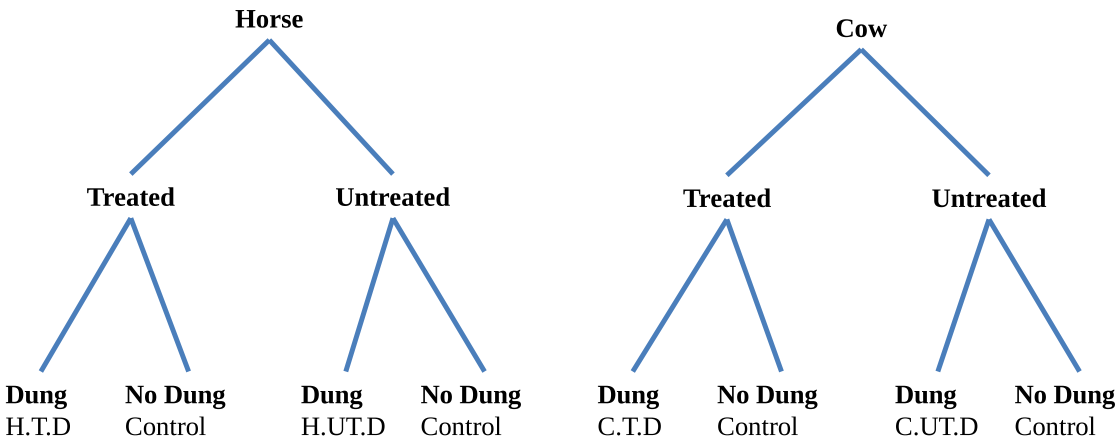

Figure 5: The model behind the experimental design for this project. It shows the three factors in a nested design and demonstrates the four factor combinations along with the four control groups.

Treatment

Ten areas were set up across Eelmoor Marsh and fenced off so the livestock cannot graze, trample or defecate in them; these form the treatment plots for this project. A map in Appendix 1 shows the location of the plots within the study site. The plots are all south facing. Each plot is 14.2m by 5.6m and is divided into 32 sub-plots of 1m^2. There is at least a 0.4m buffer between each sub-plot to help to minimise leaching between them. The 32 subplots have been sectioned into four groups to enable all four types of dung (treated horse, treated cattle, untreated horse and untreated cattle) to be put on each plot. The design of the plots leads to a paired design, with controls also being sectioned within the four areas. Each group of eight sub-plots is buffered from the others with a 1m strip ensuring the other treatments are less likely to be affected if leaching does occur. The plots have had livestock excluded since 2010. This means that the current composition of invertebrates in the soil may be different to those expected if the areas had been naturally grazed and defecated in. Some subplots tested in this project will therefore have no dung inputs so any impacts of the dung can be measured against a control. It is important to test whether putting dung on the soil affects the number of insects because it can help to quantify the effects of the treated dung. Four subplots in each group were treated with dung; the other four were not treated with dung and remain as controls. A complete subplot is represented in Figure 6.

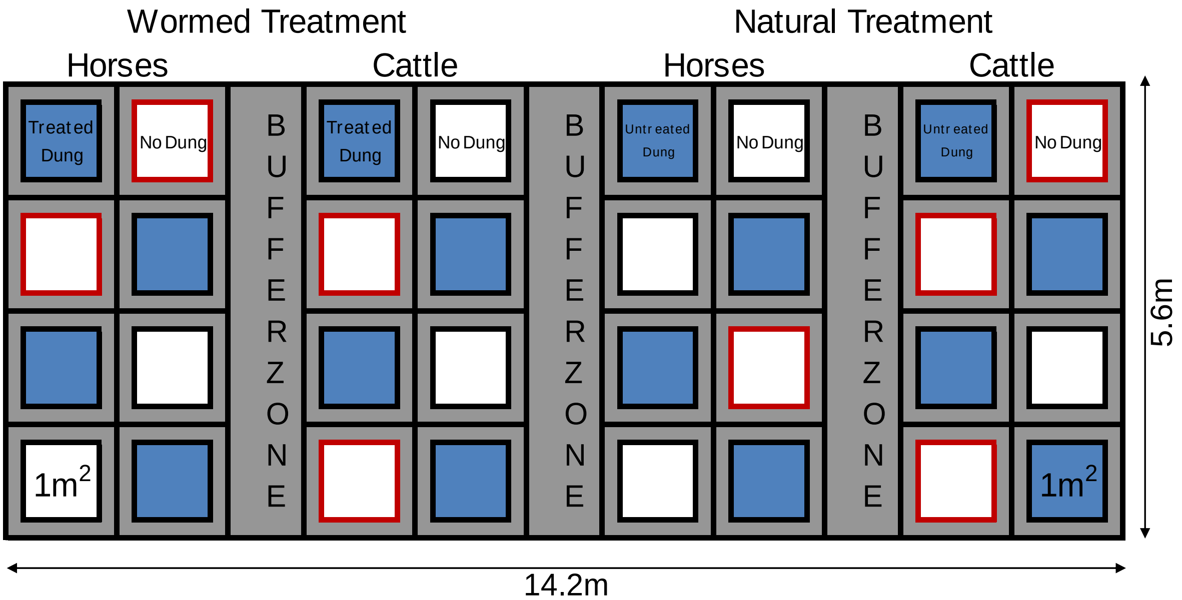

Figure 6: Diagram of a treatment plot, showing 32 subplots split into four areas of different factor combinations and the controls associated with them. 24 soil cores are taken from each of the sixteen subplots with dung applied (blue) and eight randomly selected control plots (red borders).

The plots were treated with dung every four weeks between February and May, four times in total. Care was taken when walking around the site and in the plots, minimal damage was done to vegetation and, where possible, walking was done on paths to minimise disturbance. The amount of dung used reflects the ideal stocking densities of the marsh: 0.08 livestock units per hectare for horses and 0.12 livestock units per hectare for cattle (Wilkie, 2013); the current density, 0.06lu/ha for horses and 0.10lu/ha for cattle, is slightly lower than ideal due to loss of animals. The dunging intensity at this ideal stocking density was calculated by Wilkie (2013) and was used to calculate how much dung should be put on the soil. 0.70kg/m2 of horse dung or 0.45kg/m2 of cow dung were put on each relevant treatment plot. This dung was diluted according to the methodology in Kohler, 2004. This dilution means that 3kg/m2 of horse dung solution (0.70kg of dung, 2.3l of water) or 2kg/m2 of cow dung solution (0.45kg of dung, 1.55l of water) were put on each relevant subplot. Control subplots had no dung applied to them. Excess dung was discarded in one area near to the study hut to minimise the increase in nutrient load on undisturbed areas.

Sampling

Data collection commenced at the end of May. Invertebrates were collected according to the code of conducted written by the Joint Committee for the Conservation of British Insects (JCCBI) (2002). Due to the nature of the project, live specimens of invertebrates had to be collected. A single soil core was taken from the centre of each treated subplot within each of the ten treatment plots. Soil cores were taken from eight control subplots; Microsoft Excel's random number generator was used to select the eight control plots from within the four different treatment combinations. In total, 24 soil cores were taken from each plot. The soil cores taken on each day were from six subplots (one of each treatment type and two controls) within four randomly selected - where possible - plots. This minimised any temporal variation in invertebrates found within each plot. All soil cores were collected over a 35 day period.

All species collected required extraction from the soil cores and identification under a microscope. This necessitated the killing of the specimens. This was done according to Edwards, 1991, and McSorely & Walter, 1991. After collection, the cores were immediately run through Tullgren funnels (Tullgren, 1918) to extract the invertebrates (Figure 7), with specimens falling into an alcohol bath (70% ethanol) to be preserved (Moreau et al., 2013). To prepare the cores, any vegetation and leaf litter was removed from the core and the top

10cm of soil was cut to use in the funnels; the remainder of the core was discarded (Figure 8). The soil was broken up by hand into small pieces and ran through a funnel for approximately 48 hours. To encourage the invertebrates to move through the funnels, black card was wrapped around the lower portion of each funnel to make it darker and foil was placed over the top of the frames to reflect the light from the bulbs back onto the soil. Mesh was placed in the bottom of each funnel to prevent soil particles from falling into the alcohol pots. When the extraction process was complete, invertebrates were stored in 100% ethanol and identified as soon as possible. No more dung treatments were applied to the soil during the data collection process. A small number of sample cores were taken from areas around the plots beforehand to give an estimate of how many invertebrates and of what species could be expected. From this, a suitable method of counting the total number of insects was selected. The quantity of invertebrates and the rate at which cores could be run through the funnels (24 every two days) meant that identification was completed after data collection was finished. Invertebrates were identified down to taxonomically identifiable groups; however there was not time to identify the groups down to species.

Figure 7: Tullgren Funnels with soil cores running through. The black card helped ensure the invertebrates moved downwards into the collection pots containing 70% ethanol.
E. Rendells, 2014

Figure 8: A soil core being prepared for running through a Tullgren funnel. The vegetation is removed and the top 10cm of the core are cut off and broken into regular sizes for the funnels.
E. Rendells, 2014

Data Analysis

In total, data were collected from 205 soil cores correctly run through the Tullgren funnels. These data were invertebrates identified down to a taxonomically identifiable group and the number of specimens in each group for each core. An analysis of variance was conducted on the control data to check for leaching of the dung between plots. Data analysis was then conducted in IBM SSS Statistics 21. Normality tests were conducted and if data were transformed using the natural logarithm or a Box-Cox transformation (Osborne, 2010). Three factor nested analyses of variance were then undertaken on abundance and richness data. Further analysis was conducted on any significant results to discover where the differences lie. Simpson's Diversity Indices were conducted on data for each factor to give an estimate of how herbivore, dunging and anthelmintics affect the diversity of the invertebrate communities.

Results

In total, 205 cores were correctly run through the Tullgren funnels and 173 of these contained at least one invertebrate. Plots treated with dung from medicated horses had the highest proportion of empty soil cores. A total of 1,335 invertebrate specimens were collected overall. 41.57% of all specimens collected were mites, they accounted for 29.18% of all occurrences and 16 different 'types' were identified.

Controls

Before any comparisons were made with the controls, a one-way analysis of variance was conducted to check for any differences in the controls from the four treatment areas. Because of the experimental plot design, the controls were split into four sections within the four treatment types (see Figures 5 & 6). The results from this analysis show that no significant differences were found between the control groups ($F_{1,58}$=2.192, p=0.144). This suggests that any dung put on the subplots had not leached into any of the controls and enabled the controls to be treated as one group for analysis against the herbivore, treatment and dung factors.

Abundance

The abundance data are the total number of invertebrate specimens found in each soil core; the relative abundance of each taxonomically identifiable group for each of the factor combinations is shown in Figure 9. The mite taxonomic group constituted the largest proportion of the soil macrofauna sampled. Mites, worms and larvae together accounted for seventy eight per cent of the total specimens collected, despite being only three of sixteen taxonomically identifiable groups. The abundance data were transformed to give a normal distribution and the SPSS results for the three factor nested analysis of variance are shown in Table 1. A significant interaction was observed with herbivores ($F_{1,5.47}$=15.87, p=0.009) and also with treatment nested in herbivores ($F_{2,4.91}$=6.870, p=0.038).

Post-hoc testing could not be performed on the herbivore data as there were only two factors. A graph is shown in Figure 10 which looks at the mean abundance for each factor combination. The error bars were calculated from the standard error of the data. This graph suggests the interaction with the herbivores could have been between untreated horse dung and untreated cattle dung (two dark bars), with significantly more invertebrates being found in the soil cores from cow dung areas than from horse dung areas, with means of 9.06 and 6.50 respectively. The graph also suggests there may have been a significantly higher abundance of invertebrates in the untreated cow dung (mean of 9.06) than in both treated and untreated horse dung (means of 6.12 and 6.39 respectively). This is shown by the bars with borders and correlated with the findings of the analysis of variance. The graph suggests there was a significantly higher abundance of invertebrates in the cores from plots with dung added to them (total mean of 7.015) than the control plots (total mean of 5.381), despite some of the dung containing anthelmintics.

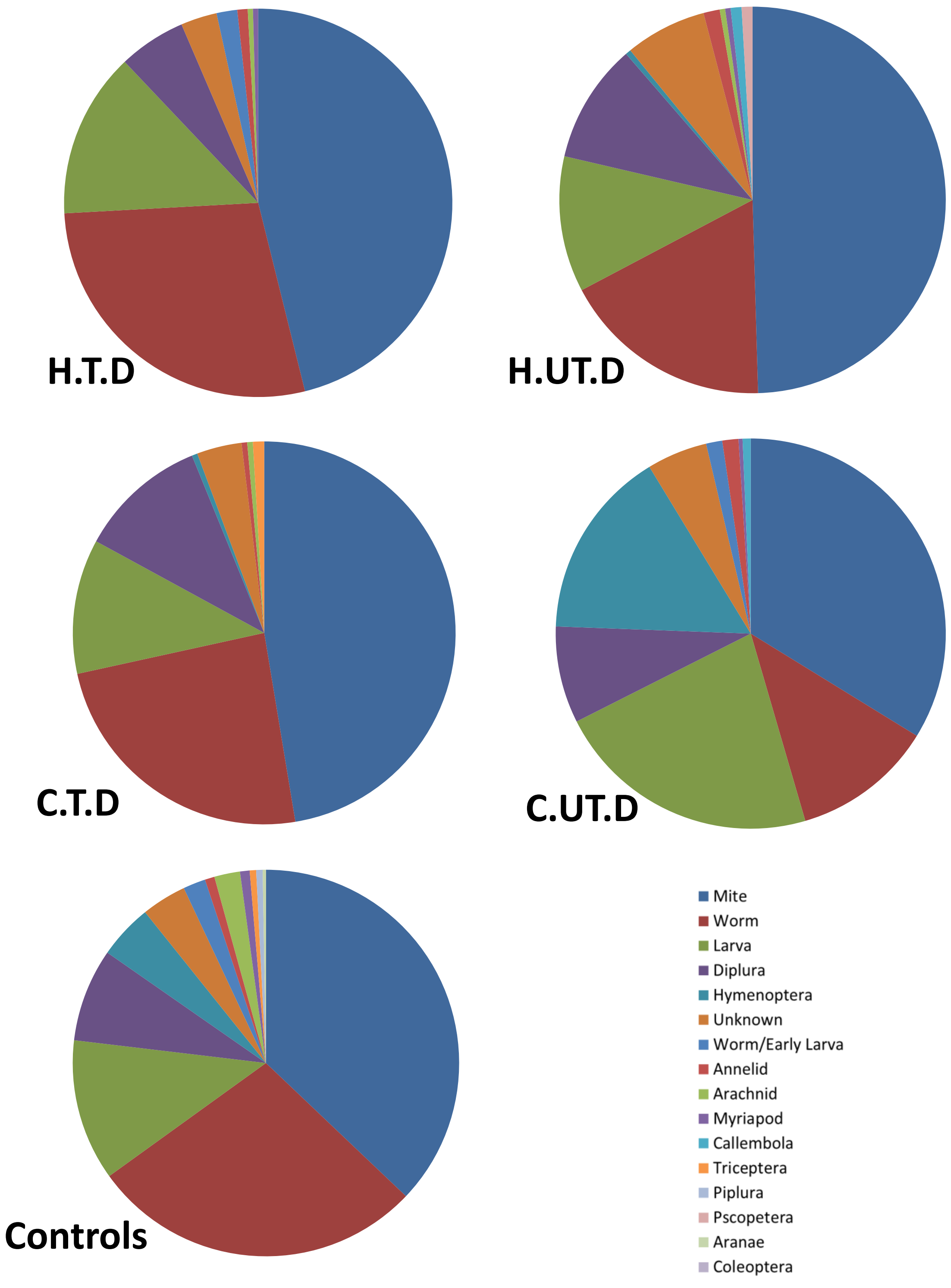

Figure 9: The abundances of the sixteen identified taxonomic groups in each factor combination and in the control plots. H=Horse, C=Cattle, T=Treated with anthelmintics, UT=Untreated with anthelmintics, D=Dunged. It is clear from this that mites are the most represented macrofauna overall.

Table 1: The results for a three factor nested analysis of variance on the abundance data. Significant interactions are shown with herbivore and treatment nested in herbivore.

Factor	Degrees of Freedom		*F* value	Significance
	Hypothesis	Error		
Herbivore	1	5.472	15.87	0.009
Treatment(Herbivore)	2	4.909	6.87	0.038
Dung(Treatment(Herbivore))	4	164	0.125	0.973

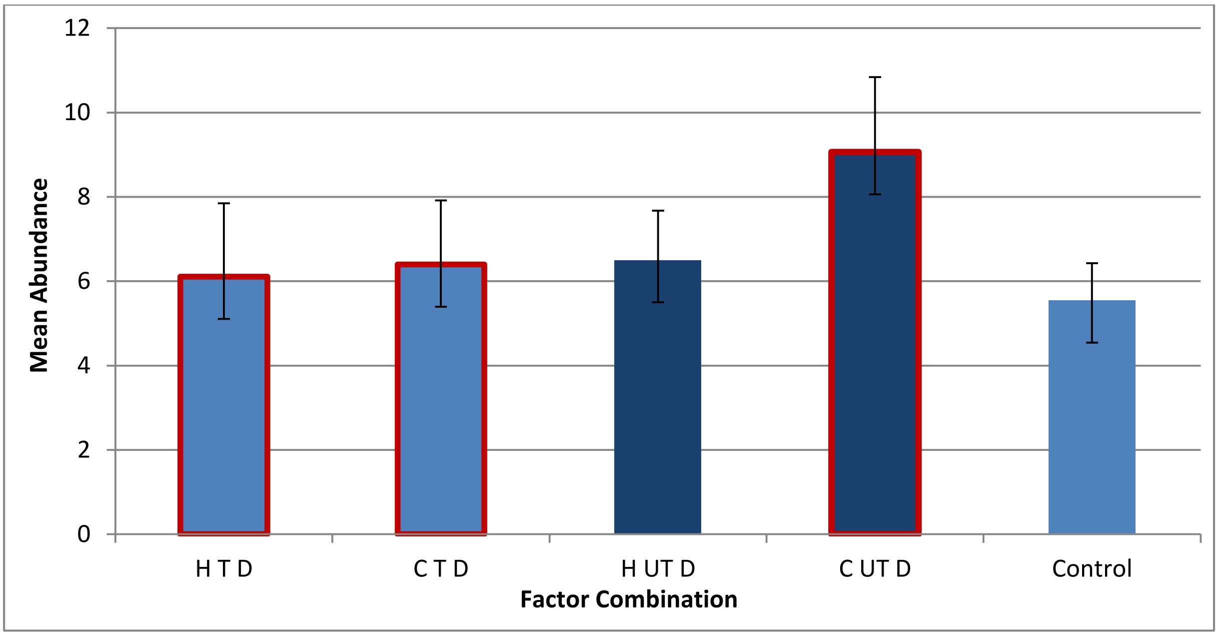

Figure 10: The mean abundance of specimens for each factor combination. H=Horse, C=Cattle, T=Treated with anthelmintics, UT=Untreated with anthelmintics, D=Dunged. The error bars are calculated from the standard error of the abundance data.

Richness

The richness data is the number of taxonomically identifiable groups found in each soil core. The invertebrates were identified down to groups of the same or similar species; there were 16 taxonomically identifiable groups. The richness data was analysed in SPSS and found to have a not-normal distribution. A Box-Cox transformation was conducted on the data and the best transformation was used for analysis. A three-factor nested analysis of variance was then performed on the transformed richness data, the results from which are shown in Table 2. This analysis of variance found no significant interactions between any of the factors and the richness of the soil cores. A graph showing the mean richness for each factor combination is shown below in Figure 11. This also suggests there are no significant factors affecting the richness of the 16 invertebrate groups as the error bars indicate a high variance for each factor and are overlapping. However, it suggests untreated cattle dung has a slightly higher mean richness than other factor combinations and treated horse dung has the least number of taxonomically identifiable groups within it.

Table 2: The results for a three factor nested analysis of variance on the richness data. This shows there is no significance within the factors.

Factor	Degrees of Freedom		*F* value	Significance
	Hypothesis	Error		
Herbivore	1	14.53	0.905	0.357
Treatment(Herbivore)	2	6.603	1.403	0.311
Dung(Treatment(Herbivore))	5	197	1.423	0.218

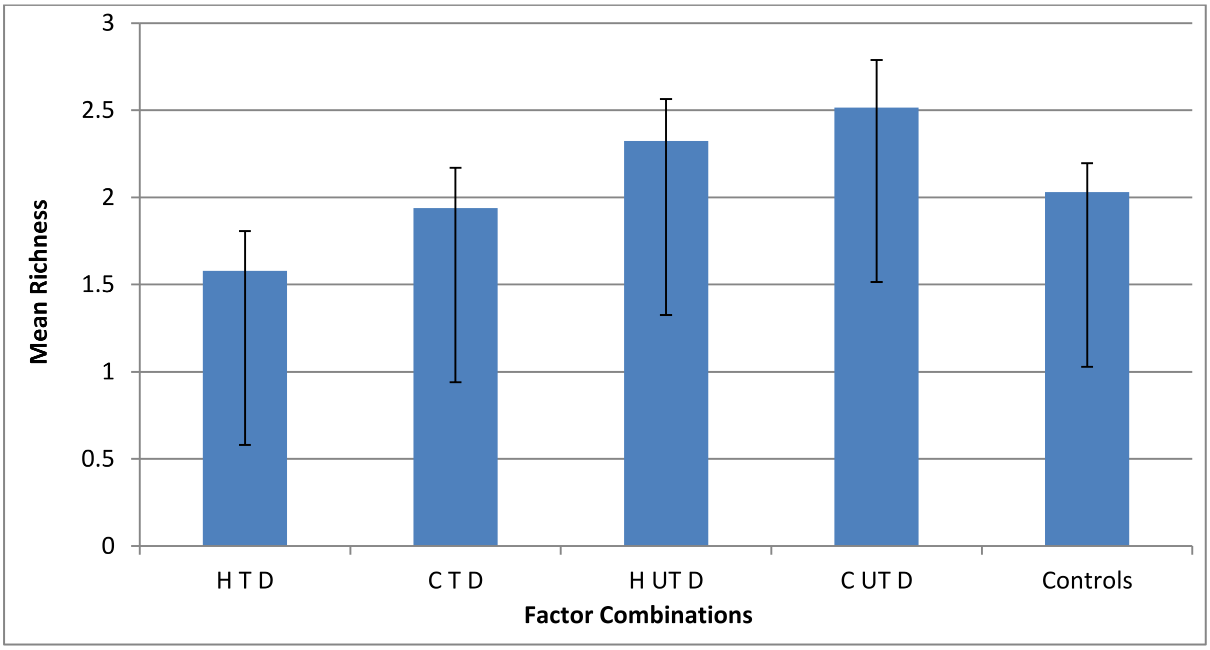

Figure 11: The mean richness of the taxonomically identifiable groups for each factor combination. H=Horse, C=Cattle, T=Treated with anthelmintics, UT=Untreated with anthelmintics, D=Dunged. The error bars are calculated from the standard error of the abundance data.

Diversity

Simpson's Diversity Indices were calculated on the data to quantify the overall biodiversity of the study site and to show whether there are any differences in the diversity of the invertebrate communities under different treatment types. Simpson's Index of Diversity accounts for both the abundance and evenness of data to calculate the diversity and shows the probability of two individuals randomly selected from a population belonging to different taxonomically identifiable groups. The diversity counts for the four different factor combinations and the controls are shown below in Table 3. This suggests the diversity does not differ greatly between the factor combinations; however Simpson's gives more weight to the more abundant groups, such as mites. Since they are abundant in every sample, this may have skewed the data. For this reason, more indices of diversity were done on the data excluding mites to see how this affected the scores. These can be seen below in Table 4. This has impacted the diversity index scores for the factors involving horse dung, but has had little effect on the factors or differences between them overall. The lowest diversity is in the plots with treated horse dung (0.69) and the highest diversity in the plots with the untreated cattle dung (0.79).

Table 3: The Simpson's Indices of Diversity (1-D) for each factor combination and the controls. H=Horse, C=Cattle, T=Treated with anthelmintics, UT=Untreated with anthelmintics, D=Dunged.

H T D	H UT D	C T D	C UT D	Controls
0.688274	0.701028	0.693839	0.792036	0.761687

Table 4: The Simpson's Indices of Diversity (1-D) not accounting for mites, for each factor combination and the controls. H=Horse, C=Cattle, T=Treated with anthelmintics, UT=Untreated with anthelmintics, D=Dunged.

H T D	H UT D	C T D	C UT D	Controls
0.653935	0.777671	0.699754	0.783879	0.743516

Discussion

Overall, this project has met the aims set out in the introduction. It has, with some success, assessed whether anthelmintics - specifically ivermectin - have an effect on soil invertebrate abundance, richness and diversity. It has also looked at the effects of the two herbivore types (horses and cattle) on the invertebrate communities. Through this, this project is able to provide management recommendations to managers of lowland heaths and wider systems that utilise large herbivores for conservation purposes. The key findings, limitations, recommendations and extensions for this project are set out below.

Key Findings

It is important with any management interventions that side-effects are kept to a minimum and, as vegetation managers, it could be said that effects of herbivores on invertebrates should be kept as close to ‘normal’ - absence - as possible. However the very nature and composition of lowland heath requires the presence of large herbivores. A common aim of management techniques for this habitat is to maintain or increase biodiversity and abundance. The reintroduction of these herbivores to undertake the role of their historic counterparts could be viewed as increasing the biodiversity of the habitat and any effects they have be natural to the system. The natural dunging intensity of these animals at their ideal stocking densities was calculated and was reflected in the amount of dung put down on the soil to give a truer representation of the natural conditions. For these reason, this study will report any increases from the controls in abundance, richness and diversity as positive results and any decreases as negative.

In total, 1,335 specimens were collected and approximately forty two per cent of these were mites. Römbke et al. (2010) found that ivermectin did not affect mites or collembolans in the soil. They also recorded that mites were attracted to dung pats that had been spiked with ivermectin; this could partially explain why such a large number and high proportion of invertebrates recorded were mites. The cores taken are representative of the entire study site, however they are only a sample and it is possible that mites have been over-represented in these cores. At the time of year that the soil cores for this study were taken (May), there were expected to be many invertebrates in larval form (Scriber, 1977; Owens, 2010). The findings conformed with the literature as 205 larvae were identified; many of these were coleoptera, however only two mature coleoptera were collected.

The most noticeable finding in this study is the difference in invertebrate abundance between the untreated cattle and horse dung, thus Null Hypothesis 1 can be rejected. The abundance is significantly higher in soil cores taken from plots treated with dung from untreated cattle ($F_{1,5.47}$=15.87, p=0.009). Cattle dung has a high water content and less undigested vegetation than horse dung, which is drier and harder to break down (Xin-min, 2011). This could begin to explain the findings as the invertebrates researched in this study are soil invertebrates which may not be well adapted to dealing with dung. The wetter, more digested dung could have leached into the soil more readily and attracted more invertebrates than the horse dung, which may have to be actively sought out. This result conforms to many previous studies and literature which may explain the findings. For example Dormont et al. (2010) suggest the species already present in the dung may affect preferences of other species, noticeably dung beetles. They also noted a significant attraction to cow dung over horse dung in dung beetles. Dormont et al. (2004) conducted a similar study using scarab beetles and found cattle dung attracted significantly more invertebrates than horse dung (2,570 and 1,706 specimens respectively); marginally more species preferred feeding on cow dung but none were exclusive to one type of dung. These two studies explained the findings using the invertebrates' olfactory senses. Analysis of dung violates showed distinct differences between dung types and allowed discrimination by invertebrates. Conversely, Tesarik and Waitzbauer (2008) found that dung beetles preferred horse dung, with five times as many recorded on it than on cow dung; however there was no significant difference in the species composition of the two dung types. Xin-min (2011) studied the dung preferences of dung beetles and found the richness was highest in horse dung; whereas the diversity of species was highest in cow dung. A study conducted on the abundance of earthworms by Scown & Baker (2006) found the preferred dung type was horse dung, however the age of the dung and the length of exposure to it were also important and there were differences in the preferences of the various species.

This study showed that there was a significant decrease ($F_{2,4.91}$=6.870, p=0.038) in the abundance of invertebrates in plots with dung from herbivores treated with anthelmintics (mean of 6.25) compared to plots with dung from untreated herbivores (7.78). This corresponds with Jensen & Scotts-Fordsmand (2012) who looked at the effects of ivermectin on a soil multi-species system. They documented a decrease in the community abundance for all species which corresponded well with an increase in the exposure concentrations of the anti-parasitic medication. They document that the medication can leach out of the dung into the soil and that it is known to affect the early developmental stages of invertebrates. It is possible that any ivermectin in the dung could have leached in to the soil, this was not something that could be tested in this project due to lack of resources. The soil cores were collected in May; this is during the developmental stages for many invertebrates so they could have been particularly susceptible to low concentrations of ivermectin. Other literature looks at the effects of anthelmintics specifically on one or a group of species and many have recorded no effects on these species when exposed to ivermectin. Grønvold et al. (2004) found that ivermectin in its pure form as a chemical compound could cause death in populations of the soil nematode *Pristionchus maupasi* in laboratory experiments. However in the concentrations excreted from cattle treated with a bolus, ivermectin is not toxic to this nematode. Kryger et al. (2004) found that Cevamec (an ivermectin injection given to cattle) caused no apparent negative ecotoxicological effects on the structure of dung beetle communities in South Africa. Svendsen et al. (2005) experimented with the earthworm *Lumbricus terrestris* and looked at how naturally excreted ivermectin affected them in a laboratory environment. They also documented no adverse effect to the individual worm's survival and growth. An experiment by Isaksson and Vessby (2006) observed how worming medications affected dung beetles in horse and cattle dung; they obtained no significant results for differences in abundance of dung beetles in wormed and non-wormed dung.

This study documented that although ivermectin does have a significantly negative affect on the abundance of invertebrates, it is only a weak effect and the presence or absence of dung - regardless of anthelmintics - is a more important factor. This could be due to the persistence of the anthelmintics and the fact there was potentially very little left in the dung by the time it was spread on the ground and even less by the time the cores were collected. It is shown that Ivomec, the pour-on ivermectin given to the cattle in this project, has a peak faecal concentration just two days after the medication is administered (Herd et el., 1996) followed by a gradual decline until 28 days post-administration. Schmidt (1983) also noted a negative effect of ivermectin in cattle dung on the emergence of adult horn flies for 28 days post-treatment. The last dose of treatment for the highland cattle in this study was October 2013. The dung was not able to be analysed as there was no access to the equipment necessary; however the literature suggests there may have been very little anthelmintics remaining in the dung at the time of this experiment. This could not be mitigated against as the cattle dung used was from the only available herd of highland cattle in the area that were treated with worming medication and their worming schedule could not, and should not, be altered for the purposes of this study. The horses from Marwell Zoo are given anti-parasitic medication on an eight-weekly schedule and were treated on January 27th and March 24th, 2014. Dung was collected shortly after these two dates. Again though, the literature suggests that ivermectin given orally may only remain at a measurable concentration in the dung for a few days. Gokbulut et al. (2005) conducted a study with donkeys (*equus asinus*) and found that excreted ivermectin concentrations were at their highest two days after the drug was administered and could only measure the ivermectin levels excreted in dung for nine days post-treatment. Perez et al. (2001) conducted a similar study with horses and discovered approximately the same maximum concentration time (2.5 days post-treatment); however they detected excreted ivermectin for forty days after administering the medication. This study did collect cores within forty days of the horses' last worming treatment; however literature suggests that the concentration of ivermectin present in the soil may have been insufficient to have a significant effect on the invertebrate communities.

What these papers do all show is that however the anthelmintics affect the invertebrate communities, it is likely only to be for a short duration as the concentration of medication excreted in the dung becomes undetectable after forty days or less (Herd et al., 1996; Perez et al., 2001). The excretion concentrations peak at around two to three days, this would be the best time to observe the effects of anthelmintics on the invertebrates, although it would clearly not give a complete picture. The exception to this is with the sustained-release bolus which delivers a low dosage of ivermectin for a prolonged period of time.

The presence of dung was shown to have a positive effect on invertebrates, regardless of whether the dung was from animals treated with ivermectin or not. It is documented by Gullan & Cranston (1994) that dung produced by herbivores can form an important nutrient source for many invertebrates. It can also provide an environment to lay eggs or deposit larvae in and can add nutrients to the soil surrounding it.

This study found that none of the factors had any significant effects on the richness or diversity of invertebrates so Null Hypotheses 2 and 3 cannot be rejected. This may be due to the invertebrates only being identified down to sixteen taxonomically identifiable groups.

There are many species within these groups; in 'mites' alone sixteen different 'types' were observed and this was only based on obvious morphology under a 40x magnification. It is certain that there are many more than sixteen species in the 1,335 invertebrates collected and it may be that the richness and/or diversity are significantly affected at a lower level of taxonomy than this study considers. This is a limitation of this study and would be an interesting area of research to expand into. Although the results are insignificant, the highest richness and diversity was noted in the dung from untreated cattle and the lowest richness and diversity in the dung from treated horses. This shows the same pattern as the significant results in abundance.

Project Limitations

Tullgren funnel storage

The Tullgren funnels were stored in a greenhouse at Marwell Wildlife. Disturbance in passing caused some soil cores to become unusable as the invertebrates were not collected according to the methodology. The greenhouse was also very hot during the day, with temperatures ranging up to 39^{o}C. This is likely to have negatively impacted the findings. It may have reduced the effectiveness of the light bulbs at driving insects down through the soil cores by lessening the difference in temperature between the top and bottom of the cores. This may have reduced the overall number of invertebrates collected from the soil cores; the temperatures varied from day to day, causing any effects on the invertebrates to also vary. At the start of the project, the extreme and unexpected heat caused all of the alcohol solution to evaporate from the pots, so the specimens could either escape and those already dead went mouldy. These soil cores were disregarded. The greenhouse is in direct sunlight for a large proportion of the day; this will again reduce the effectiveness of the bulbs. To partially counteract against these external effects, foil was placed above the bulbs to reflect heat and light back down on the soil cores.

Plots

Despite fencing, the livestock on Eelmoor Marsh entered two of the plots, defecating and trampling within them. Excess dung was removed from plots where possible and this disturbance is not thought to have significantly impacted the results. This was prevented from happening again by installing electric fence batteries on the problem plots and increasing the height of fences where possible; if this study were to be repeated, these measures should be put in place immediately. Plot 9 flooded and soil cores were not able to be collected from all subplots; two plots contained petrol from the surrounding industry. This could not be controlled for and it is unknown whether it had an effect on the invertebrates collected.

Time

Another limitation to this study was time. Because of the nature of this project, it was difficult to schedule time to allow for identifying the invertebrates as it was unclear until data collection had finished just how many specimens there would be. 1,486 specimens were collected in total, which is more than expected from preliminary cores. For this reason, invertebrates were only able to be identified down to order or a taxonomically identifiable group, rather than down to species. Although this reduces the detail in the data, it was a satisfactory compromise to ensure data analysis could be completed in the available time. Soil cores were collected over 35 days (longer than initially planned). This temporal variation could have affected the total number of invertebrates collected. It was mitigated against as much as possible by taking cores from subplots within each treatment type and from controls on each occasion. If this study were to be repeated, more Tullgren funnels should be set up so the cores could be taken in a shorter time period.

Implications on wider ecology

This study has shown that anthelmintics may not have such negative effects on invertebrate macrofauna than common perception suggests. It has shown that the presence of dung itself has a stronger positive effect on the soil invertebrate communities than the anthelmintics do negative. However, this study has still found a lower abundance of invertebrates in plots with dung from treated animals than in that from untreated animals. For this reason, it is still wise to withhold a regular worming regime until further research of this nature has been conducted; however this study has found that worming individuals on a necessity basis may have fairly small and short-term effects on the soil invertebrate communities.

This study has also shown that grazing herbivores can have wider benefits than just managing vegetation. Heathlands should contain large herbivores as part of their ecosystem and this study shows the dung from these herbivores can increase the abundance of invertebrate communities within the soil and potentially elsewhere. This highlights the importance of these conservation grazers.

Recommendations for site management

Eelmoor Marsh currently employs a mixed grazing regime of horses and cattle. This project indicates that to maximise the abundance of invertebrates, only cattle should be used; this may not be practical for management purposes though and a mixed grazing regime may have other benefits not accounted for in this study. The management team also monitor the levels of faecal parasites and only treat the livestock when the worm burden is high. This practice is backed up by the research from this study as anthelmintics were shown to have a negative effect on the invertebrate communities which suggests worming should be avoided where possible. Literature shows that ivermectin is found in particularly high quantities in the dung up to three days post-treatment. If livestock are being treated with anthelmintics, it would be beneficial to keep them off of the management site for the three days post-treatment if possible.

Project extensions

This study provides a firm basis for further research and gives many opportunities to expand the work.

An obvious extension would be to conduct a similar study using this methodology and identify the specimens down to species level. This will enable the results of this study to be validated and give a more comprehensive understanding of the invertebrate communities in the different treatment areas. A longer time frame would be required for the study and/or a researcher(s) with specialist invertebrate identification knowledge. This study was conducted by a primary researcher and an assistant; identification skills and inter-recorder reliability were gained quickly, however the lack of knowledge slowed the identification process down and hindered its completeness.

Soil cores for this study were collected over May and June. This is a very limited time period and will only have given a snapshot of the invertebrates likely to be found on the heathland. If dung continued to be applied on a monthly basis whilst soil cores were taken, the data collection phase could be extended and seasonal variation of invertebrates could be taken into account and considered. The disturbance to the invertebrate communities and the wider fauna and flora should be carefully considered and appropriately mitigated against before major extensions take place.

The effects of horse and cattle dung on invertebrate communities were discussed in this project, due to the study site being grazed by these animals. Sheep are also commonly used as a vegetation management tool for conservation grazing, but were not considered in this study. Literature suggests sheep dung could play an important role in invertebrate species evenness (Xin-min, 2011). Including dung from treated and untreated sheep would build a more complete picture. If these species are all considered in one study, it may be possible to look into the best combinations of herbivores to use to minimise the impact of anthelmintics on invertebrates. Careful consideration should take place before conclusions are written up though to ensure any recommendations are specific to the conditions of the study, for example on lowland heaths with herbivores treated with ivermectins.

Another extension might be to look at the effects of and compare different worming medications; this study only looked at pour-on and ingested ivermectin. The effects on invertebrates could then be associated with the effectiveness of each medication to give an idea of the best medication for reducing the parasite burden and having the least impact on the environment. Again, any recommendations would be specific to the conditions of the study.

In this study, the anthelmintics were in dung which was placed on the soil. It was not possible to measure the levels of medication in the dung. Further studies on this topic could look at placing the anthelmintics directly on the soil which will enable the quantity to be regulated. Invertebrates could also be exposed to the medication in a laboratory environment, enabling control of all other factors.

The design for this study was optimal for the time and space available for this project. However a nested design is not necessarily the best practice for this study. If more space were available, a completely randomised design should be employed with enough space between each sub plot to guarantee no leaching occurs between subplots. Each subplot should be randomly allocated a factor combination, preventing the design from being nested. This will allow for the factors to be assessed independently as well as in combination.

Conclusions

The findings of this research show that anthelmintics have a weak, but significant, negative affect on the abundance of soil invertebrate communities. This research also shows a significantly stronger positive effect of dung on invertebrate communities, with cow dung having a higher abundance overall. Richness and diversity of invertebrate communities were not significantly affected by the factors explored in this study. These results have validated the conservation management practices undertaken on Eelmoor Marsh and provide evidence for the continual grazing on lowland heaths, with future prospects of dosing animals with worming medication on a case-by-case basis. Further research is required before this can confidently be adopted as part of the grazing regime.

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Appendix 1 – Site Map

The red border shows the boundary for Eelmoor Marsh. The site is 78.72ha. The ten treatment plots used in this project are shown here by orange points.