Impacts of Rewilding at Knepp Castle Estate on Abundance and Species Richness of Carabidae and Scarabaeidae



Claire Murphy 177346 MRes Conservation Biology (17/18) Dr. Alan Stewart Acknowledgements I'd like to acknowledge and thank the following people who supported me and the creation of this dissertation. Firstly, I'd like to thank my supervisor Alan Stewart for his support and guidance throughout the year. Secondly, Chris Sandom who also offered his help and advice during this project. Thirdly, my fellow masters students Betsy Gorman, Claire Wallace, Tom Dando, Georgina Pashler for their help in the field. And finally, the Knepp estate for their hospitality during our stay.

#### ABSTRACT

Rewilding has recently gained popularity as a possible means of conservation in Europe and the UK. However, there are few studies regarding the impact rewilding might have on invertebrates. This study focuses on the impact that various measures of rewilding at Knepp Estate, UK has had on abundance and species richness of local dung and carabid beetles. Vegetation structure variation, year since fields were fallowed (rewilded) and historical pesticide use were analysed to see whether they had impacts on local dung and carabid beetle populations. Vegetation species richness was also analysed. The impact of bare ground cover on beetle size was also investigated. Overall, the data doesn't appear to be sufficient to draw strong conclusions regarding the impacts of vegetation structure on dung and carabid beetles at Knepp, although drones may not be sufficient for analysing vegetation structure on a microhabitat scale required for carabids. It appears that increased bare ground cover is beneficial for smaller beetles, perhaps allowing ease of movement. Both year of fallowing and historical pesticide usage doesn't appear to have retained impacts between the fields and had no significant effect on beetles sampled. Management of Knepp's large herbivores appear to have benefitted dung beetles, although high dominance of species such as fleabane may be limiting microhabitats for carabid beetles. Long term monitoring is required to observe species responses to rewilding schemes. Rewilding projects are also limited by their surrounding environments, which limits colonization by species with poorer dispersal abilities. It's possible that some changes in the management of grazers at Knepp could benefit invertebrates, such as 'pulsed grazing', and is possible that this could be attained without deviating from the core principles of rewilding.

## INTRODUCTION

The increase in abandoned agricultural land offers new opportunities for habitat restoration, but the question of how conservationists should proceed with the task is unclear (Navarro and Pereira, 2015). It is becoming increasingly evident that traditional restoration techniques which use past ecosystems as targets need updating in the face of a changing world. Rewilding restores lost ecological processes using minimal human intervention, but its traditional use of historical baselines is threatened by environmental and climatic change. Rewilding projects are found worldwide, and share the aim of maintaining or increasing biodiversity, whilst reducing human impact through restoration of ecological processes and species. Land abandonment in Europe (Rey Benayas *et al.*, 2007), and potential farming subsidy changes following Brexit may potentially benefit biodiversity and offers rewilding opportunities, but succession will have 'winners' and 'losers', creating an urgent need to identify which groups may be at risk from a hands-off conservation approach.

There is a particular lack of data regarding how rewilding may impact smaller taxa (Clark and May, 2002), such as invertebrates, which make up a bulk of biodiversity, and carry out a wide

range of ecosystem services. Invertebrates are an ideal group to study rewilding systems as they respond rapidly to environmental changes and disturbances, compared to larger longer lived vertebrates (Desender *et al.*, 1991). Invertebrates also play functional roles in many ecosystem processes including pollination, decomposition, predation, trophic interactions and as prey (Samways, 2007).

It is possible that a decrease in pesticide presence and opening of available habitat would be beneficial overall, but impacts on individual taxonomic groups are relatively unknown. An increase in vegetation results in increased organic matter content and water holding capacity, which may lead to greater densities of invertebrate families (Arbelo *et al.*, 2006; Navarro and Pereira, 2015). Close monitoring of invertebrate populations during succession and vegetation structure changes may help predict how populations could be affected by a hands-off conservation approach.

Without a hands-on management approach, vegetation structure will likely change and become more variable as succession occurs. Vegetation structure strongly impacts many invertebrate groups (Merckx, 2015), and popular management tools are one of the key drivers of vegetation structure, including grazing and burning. This requires careful planning as application too often, too intensely, at unsuitable times of the year and on too large a scale, may destroy a large proportion of invertebrate fauna (New, 2009). However, management is still favoured by many conservationists, whilst rewilding is perceived as a potential threat by some, as abandonment may pose a threat to species adapted to semi natural biotopes during later stages of succession (Merckx *et al.*, 2013).

As well as vegetation structure change, the impact of large herbivores on invertebrates needs to be taken into consideration when undergoing a rewilding project. Large grazing herbivores have large influences on their habitat and play an important role in all grassland systems (Olff *et al.*, 2002) and are a popular tool for maintaining various semi natural grasslands to preserve open landscapes (WallisDeVries, 1998). The effects of grazing on plant biodiversity are variable as both positive and negative effects are present in literature (van Klink *et al.*, 2015). In comparison, the impacts of grazing on arthropods are also variable but generally the trends are negative, therefore monitoring plant biodiversity may not be a reliable indicator of invertebrate biodiversity (van Klink *et al.*, 2015).

Habitat abandonment will likely benefit forest specialists dependent on old growth forests (Bengtsson *et al.*, 2000), but total abandonment could cause habitat homogenisation and loss of species dependent on disturbance (Taboada *et al.*, 2006, 2008).

Most studies done on grazing impacts on invertebrates focus on a small group of taxa, so studies which focus on overall impacts on invertebrates are lacking. Rewilding studies may need to monitor grazing levels as well as vegetation structure changes to assess impact on overall invertebrate diversity. To prevent excessive grazing, grazing refuga could be considered. In ecosystems with top predators, these can be maintained through a landscape of fear (Laundré *et al.*, 2010), but the question of how these refuga exist in a grazing based system is open to debate.

Grazing refuga in a prominent rewilding project Oostvaardersplassen, found that grazing enclosure edges appear to support higher arthropod and plant diversity, in comparison to grazed areas (van Klink *et al.*, 2016). Fencing off areas from grazers, and culling of excess herbivores could help prevent overgrazing, and maintain habitat heterogeneity and therefore invertebrate biodiversity (Klaver *et al.*, 2002; Lorimer and Driessen, 2014). However it's arguable that either of these operations may not be compatible with the true concept of rewilding.

Of all the invertebrate taxa, beetles make up the largest groups with the greatest number of species. Some beetle groups aren't well represented in literature, but groups such as Carabidae (ground beetles), which are highly responsive to vegetation structure changes, and Scarabaeidae (dung and scarab beetles) will likely be impacted by the presence of large herbivores and dung, could be good initial candidates for rewilding studies.

The ecology of Carabidae has been well studied, making them good candidates to research impacts of changing landscapes, in comparison to other beetle taxonomic groups. Studies have found that Carabidae species abundance are strongly linked to habitat due to larvae requirements (Lovei and Sunderland, 1996). Carabids are already used to indicate habitat alteration, therefore they are a useful bioindicator, but should be used with caution as our understanding of their relationship with other species is incomplete (Raino and Niemela, 2003). Some ground beetle species hunt more effectively in short vegetation and bare ground (Morris, 2000), thus tall vegetation may protect arthropods from predation. Carabids are also known to respond negatively to increased soil moisture and positively to increased ground litter (Sitzia et al., 2015). Studies on shrub encroachment have found increased shrub to have initial positive effects on carabid species richness, but generalist species mainly occur in dense shrub sites (Schirmel et al., 2014), whereas shrub encroachment above 60% risks losing smaller species and unique carabid assemblages of open dry grasslands (Schirmel et al., 2014). Rewilding will likely see the increase of shrub and forest cover as active management is decreased, and with humans being a key moderator in the modern landscape of the UK and much of the world, which will likely cause changes in carabid communities. Some may benefit from the reduction of pesticides, but changes in vegetation structure may have initial positive effects, but result in decline of some species.

Carabids in the UK are currently facing declines, with average population reductions of >30% over 10 year periods in agricultural areas (Brooks *et al.*, 2012) which covers 69% of land area in the UK (DEFRA, 2015). It's also possible that climatic change may further threaten carabid communities, although studies are lacking (Telfer, 2016). It's likely that carabids that reside in the cooler climates within the UK, such as *Nebria nivalis* could suffer from declines as a result of mean annual temperature rise (Telfer, 2016). Climatic change will also likely result in changes to seasonality and predictability of weather, such as storms which could pose a threat to some carabids (Brooks *et al.*, 2012). It's possible that these more extreme weather events may threaten

carabids that specialise on coastal and river habitats, although predicting outcomes is difficult (Telfer, 2016). Carabids have also suffered as a result of urbanisation and agricultural intensification, as has much of the UK's wildlife, and will likely continue to threaten carabids into the future.

Lack of understanding surrounding distribution, habitat requirements and ecology threatens rare carabids, and often where there's a good understanding of a species, this often hasn't been distilled into management recommendations for land managers. Furthermore, the lack of popularity of beetles compared to other taxonomic groups, has resulted in less surveying, conservation effort and funding (Telfer, 2016). As consequence, rewilding may have hard to predict and variable impacts on carabid populations, highlighting the importance to monitor carabids not only as bioindicators but as a conservation concern.

A large quantity of research has also been published on Scarabaeidae due to their wide distribution and relevance to disposal of dung. These beetles provide ecosystem services such as trophic regulation, nutrient cycling, seed dispersal and plant growth enhancement (Nichols *et al.*, 2008). However, there is still a lack of information regarding the biology of some groups, including Aphodiinae. It's possible that food is the chief environmental factor affecting beetles, as food is one of the most important considerations for both larval and adult biology (Ritcher, 1958). For example, the removal of tropical and temperate large herbivores and their dung, negatively impacts the diversity, abundance, and feeding behaviour of dung beetles (Nichols *et al.*, 2009; Halffter and Halffter, 2009). Therefore, the restoration of large herbivores would likely benefit Scarabaeidae populations.

Many dung beetles have been regarded important ecological indicators, and more refined than plants due to their reliance on factors such as vegetation structure, microclimate and substrate (Lane and Mann, 2016). They are also found in a far more diverse range of habitats than many popular invertebrate taxa such as butterflies and bumblebees. Therefore monitoring their abundance can be a useful indicator of ecological 'health' in a way other taxonomic groups may not.

Dung beetles can be an important prey source for some birds and mammals, and provide important ecosystem services which hold strong economical benefits. At present it is believed that they save the UK cattle industry about £365 million each year due to reduced pest flies and gastrointestinal parasites, dung removal and increased soil nutrients (Beynon *et al.*, 2015). Habitat decline values can be used as a proxy for population declines for species that are strongly associated with specific habitat types. However, it should be acknowledged that evidence of habitat fidelity in most Scarabaeidae is generally anecdotal. Even where such fidelity exists quantitative data on habitat declines are rarely available (Lane and Mann, 2016).

Habitat data, such as vegetation structure has been known to be vastly important to invertebrates, however, for many dung beetle species data is poor or incomplete, resulting in difficulties ascertaining species-level habitat preferences. Many dung beetles are also capable of dispersing over large distances, so the recorded capture site may not be the breeding site. There is also

insufficient research regarding how the flight and dispersal ability of dung beetles enable them to utilise habitat mosaics, impacts of habitat fragmentation and colonisation (Roslin, 2000).

Many dung beetles flight activities have been found to be directly correlated with higher air temperatures, high humidity and little to no air movement (Lane and Mann, 2016). As consequence, dung beetle mobility will likely be higher in southern and eastern Britain than in the cooler north and west, which may impact how well beetles can colonise available habitat across the UK. It is possible that rewilding sites in these regions of the UK may see a higher species richness and abundance of dung beetles colonizing their site, compared to projects in the cooler west and north of the UK. The degree of habitat fragmentation across these areas will also have an impact. Rare species usually have a more limited dispersal ability or require a more specific range of environmental conditions, and in some cases a combination of these factors (Lane and Mann, 2016) ergo are more susceptible to fragmentation.

Loss of permanent pasture through conversion to other uses, habitat degradation and changes in grazing which limits dung supply, and the use of avermectins to treat livestock are all factors which pose major threats for coprophilous Scarabaeoidea (e.g. Beynon *et al.*, 2012a; Beynon *et al.*, 2012b; O'Hea *et al.*, 2010).

The loss of permanent pasture through pasture improvement (Lane and Mann, 2016), which involves application of fertilizers, conversion to grass monocultures and reseeding have been linked to dung beetle declines (Hutton and Giller, 2003). Pasture improvement may also result in higher water content in the soil, which can cause reduced dung quality, making it less suitable for most dung beetles.

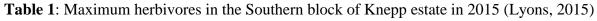
Grazing land abandonment is a key factor affecting dung beetle conservation. Due to their dependence on dung, pasture abandonment leads to an ecological cascade stemming from the loss of trophic resources, with negative effects on dung beetle communities (Nichols *et al.*, 2009). Dung beetles have depended on wild megafauna dung for millions of years (Ahrens *et al.*, 2014), but due to extinction and reductions of herbivore populations, beetles became more reliant on dung of domestic herbivores (Barnosky, 2008; Sandom *et al.*, 2014). However, livestock management has changed dramatically in the last 50 years, following a period of approximately 10,000 years of traditional low intensity grazing, to intensive management, and the abandonment of pasture (Tonelli *et al.*, 2017). Pasture abandonment has been found to cause changes in plant composition (Peco *et al.*, 2006) and lead to decreases in avian species richness (Suarez-Seoane *et al.*, 2002), butterfly species evenness (Poyry *et al.*, 2004) and Orthoptera species richness (Marini *et al.*, 2009).

The recolonization of sites are only possible when another source population exists within flight range and large herbivores are reintroduced during the beetles activity period (Lane and Mann, 2016). If there are no local source populations, or grazing ceases at a wider landscape level, colonization of dung beetles into rewilding sites may be difficult. However, once colonisation

can occur, it is likely that projects with herbivore management strategies such as leaving cattle out over winter, minimal use of medication, absence of pasture improvement schemes and near constant levels of herbivore numbers, could result in a strong population of dung beetles.

To attempt to study the impacts that rewilding may have on carabid and dung beetle populations within the UK, a 3 week study was carried out in the Southern Block of Knepp Castle Estate, Horsham, East Sussex. Knepp Castle Estate is a rewilding project in the South of the UK which aims to follow a 'process-led' naturalistic grazing system by restoring large herbivores (Table 1). The whole estate is 1400 ha with 990 ha dedicated to the grazing project, and 473.17 ha in the Southern block. Positive impacts on biodiversity have been recorded since the start of the project, which has allowed Knepp to gain a large amount of funding through ecotourism (Tree, 2017). However, there is relatively little data available regarding the impact that the project has had on invertebrate groups. A baseline study (Greenaway, 2006) and some collected data by various enthusiasts are available, and can provide some insight into invertebrate populations. However seeing that a variety of methods and levels of expertise were used to collect the data, it is likely that they cannot be used in a study to accurately assess change in invertebrate abundance and species at Knepp.

Herbivores in Southern Block	Max. no.
Longhorn Cattle	130
Exmoor Ponies	11
Tamworth Pigs	22
Red Deer	30
Fallow Deer	235



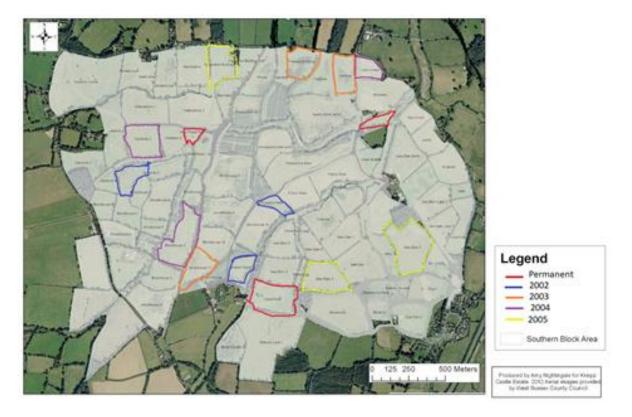
The aim of this study was to investigate how rewilding impacted species richness and abundance of carabid and dung beetles. This was done by measuring the following variables; vegetation height variation, date since abandonment and historical pesticide use. Bare ground cover was measured against species richness, abundance and mean length (as a proxy for overall body size) of beetles sampled .

The study hypothesised that vegetation height variation may have a positive impact on carabid beetles, and possibly on dung beetles due to increased microhabitat availability. The earlier the field followed, the greater the species richness and abundance of dung beetles. Pesticide use will likely have no impact on carabid and dung beetles due to the absence of use for 17 years. Finally, areas with higher percentage bare ground cover may hold species with a smaller mean length.

#### METHOD

#### Study site

The study sites were located in the Southern block (Knepp Wildlands) at Knepp Castle Estate, Horsham, West Sussex. The data collection was carried out between the 25<sup>st</sup> May and 15<sup>th</sup> June 2018. The study investigated 15 fields which were allowed to fallow at different time periods (2002, 2003, 2004, 2005) and permanent pasture, which had not been rewilded and were mown periodically, but roaming herbivores still had access to (fig. 1). For each field type, 3 fields were selected, resulting in a total of 15 study sites.10 pitfall traps were set up per field, creating a total of 30 pitfall traps within each category, and 150 pitfall traps across the whole project. To minimise possible effects from other factors, study sites were selected at random and were all originally open fields, woodland sites were avoided. All fields were located in the southern block of Knepp Castle estate to minimise effects of variation in numbers and types of large herbivores, as different blocks have different large herbivores present. Due to close proximity of study sites, factors such as weather, altitude, slope should not have resulted in differences between the study sites.



**Figure 1:** Map of Southern Block at Knepp Castle Estate. Coloured key represents dates that fields where taken out of agricultural production. 3 randomly selected fields that have each of the

5 categories or years set to fallow (Permanent, 2002, 2003, 2004 and 2005) have been randomly selected, making a total of 15 fields.

## Experimental methods

Prior to data collection fresh dung was sourced from the resident longhorn cattle, which were highly abundant within the study area. The collected dung was then frozen to remove any dung beetles that may already be in the dung, and to preserve freshness.

The beetle sampling was carried out by using a set of 5 replicate pitfall traps for carabid beetles and 5 replicate baited pitfall traps for dung beetles. For carabid beetles, a plastic coffee cup was buried with the rim in alignment with the ground, filled to a third with a solution of water and washing up liquid, then left for 5 days. A pilot study was performed before the study to determine how long pitfall traps will need to be left in the ground, and 5 days was determined an adequate time to sample enough carabid beetles without compromising the limited study time. In comparison, the dung beetle traps were similar to the carabid traps, but with the addition of chicken wire used as a mesh to cover the top of the cup. In the centre of the mesh, a bait of dung was placed on a 3cm radius/diameter cardboard circle. Each dung beetle trap was baited with approximately 10g of longhorn cattle dung. This was left for 2 days. A solution of washing up liquid and water was used to fill the traps, at 1 and 4 parts washing up liquid and water. Ideally a chloral hydrate solution as a preserving fluid would have been used, but presence of free roaming herbivores meant there was a risk of fluid consumption. Traps were marked with a wooden skewer and flagging tape with the trap number written on it. The contents of the traps were collected in standard plastic collection pots with the date, time collected, trap number and field recorded for each pitfall trap.

Each of the traps were located in a 20m diameter circle from the centerpoint of each field, which was located and marked using GPS. The pitfall traps were located in the centrepoint of each field to avoid any potential edge effects of the surrounding environments and hedgerows. Within the circle, a total of 10 pitfall traps were randomly located. Around each pitfall trap a 2mx2m quadrat was marked to record the vegetation present and estimated percentage cover. Date, time and weather was also recorded. Different types of pitfall traps were used for the two beetle groups. Trapping commenced between the 28th May and 15th June. Due to the distance between the sites, and time taken to set up the traps, study sites were divided over 3 consecutive weeks i.e. five fields a week. Biases resulting from trapping in the same field types was minimised by having at least 4 of each field type per week. After separating adult dung and carabid beetles from other specimens within the traps, adults were identified down to species level, dried and stored accordingly to the trap and field type they were found in. Carabids were identified using a handbook (Luff, 2007) and dung beetles were identified using various resources from the Dung Beetle UK Mapping Project (DUMP, 2018).

Vegetation structure data was collected separately from the project using a drone. The drone was used to calculate the standard deviation of vegetation height variation at a 20m diameter circle around each centrepoint. Drone imagery was used to measure vegetation structure by calculating a mean of the vegetation height through calculating distance from a cumulative average. Vegetation with a high variation in structure would have a higher value e.g. mixture of grass and shrub, whereas vegetation with a low variation would have a lower value e.g. grazed grassland.

#### Data analysis

Rewilding was measured using the vegetation height variation, year since fallowing, and historical pesticide use to analyse effects on the abundance and species richness of dung and carabid beetles. Percentage bare ground cover was calculated from quadrat data and compared against mean beetle length, species richness and abundance of collected beetles. To calculate species richness and abundance, samples were analysed to species level. Beetle length was used as a proxy for body size and was calculated by finding the median beetle length of each species in literature. The normality of the data was tested using a Shapiro-Wilk test. Vegetation structural mean, vegetation species richness and year of fallowing on species richness and total individuals of dung and carabid beetles as data was found to be non parametric. A Spearman's rank test was used to test for correlation on vegetation structure, year fallowed on the species richness and abundance of dung and carabid beetles. For the four species that appeared most frequently, their abundance was also tested against these factors to analyse response of individual species to Knepp's rewilding practices. A one sample t-test was used to compare abundances and species richness of dung and carabid beetles between sites to test whether there was a significant difference between the samples from each site.

To analyse other factors that might be impacting beetle communities, mean percentage bareground cover was compared against beetle length, species richness and abundance using a Spearman's rank test.

Pesticide data was found to be parametric using the Shapiro-Wilk test, so a one way ANOVA was used to compare Cymetherin, Hallmark Zeon and no pesticide treatment of 8 fields with their last recorded usage in 2001 on abundance and species richness of beetles. Plant improvement products including fertilizers and herbicides were used in all the fields.

All data analysis was performed in R 3.5.1.

## RESULTS

Dung beetle species

Over the entire sampling period a total of 391 adult dung beetles of 10 species were collected and identified. Of all identified trapped individuals, *Onthophagus similis* was the dominant species, making up 66.1% (n=295) of all captured individuals. This was followed by

*Melinopterus consputus* at 19.7% (n=77), *Onthophagus coenobita* 10% (n=39), *Colobopterus erraticus* 3.8% (n=15) *Agrinus ater* at 1.3% (n=5). The occurrence of *Acrosus luridus*, *Melinopterus sphacelatus*, *Nimbus obliteratus*, *Sigorus porcus* and *Violinus sticticus* was very low at 0.3% (n=1). Only in field Brookhouse 6, dung beetles were absent from traps. Overall, 22.5% (n=88) were captured in permanent pasture, 13.3% (n=52) in 2002 fields, 44.8% (n=175) in 2003, 9.5% (n=37) in 2004 and 10.0% (n=39) in 2005. One 2005 field, Hampshire Buildings Big, was removed from the data due to errors in data collection, so data for 2005 fields are only from 2, rather than 3 fields.

## Carabid beetles species

Over the entire sampling period a total of 109 adult carabid beetles of 10 species were collected and identified. *Poecilus cupreus* was the dominant species, making up 68.4% (n=68) of all captured individuals. This was followed by *Nebria brevicollis* at 8.3% (n=9), *Sphodrus leucophthalmus* at 6.4% (n=7), *Elaphrus riparius* 3.7% (n=4), *Nebria rufescens* 1.8% (n=2), and *Notiophilus quadripunctatus*, *Pterostichus melanarius* and *Pterostichus vernalis* at 0.9% (n=1). Overall, 23.9% (n=26) were captured in permanent pasture, 25.7% (n=28) in 2002 fields, 23.9% (n=26) in 2003, 19.3% (n=21) in 2004 and 6.4% (n=7) in 2005. One 2005 field, Hampshire Buildings Big, was removed from the data due to errors in data collection, so data for 2005 fields are only from 2, rather than 3 fields.

## Between fields

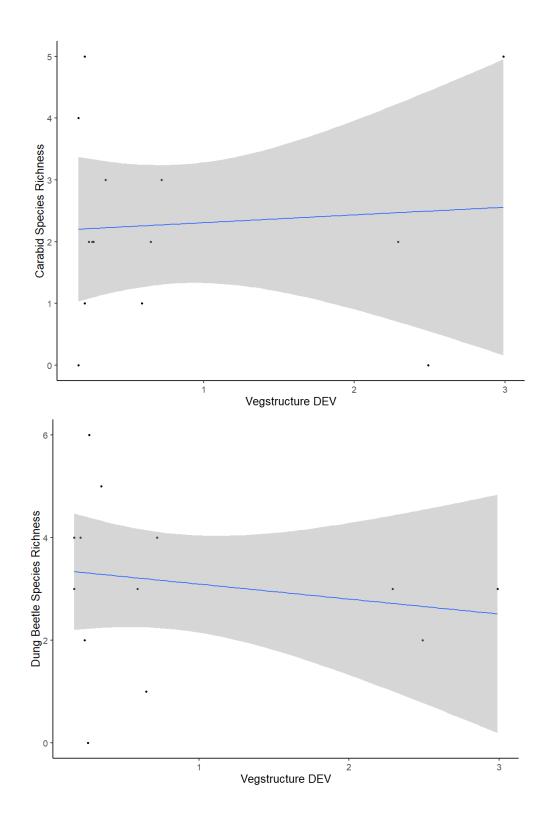
The species richness were significantly different across fields for both dung (t = 7.5289, p-value < 0.05) and carabid (t= 5.3795, p-value < 0.005) beetles.

# .Vegetation

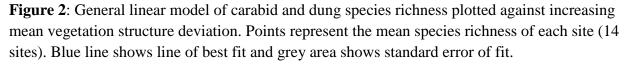
In total 29 plant species were described in the quadrats. A vegetation mean species richness of 11.1 was recorded in the fields. All fields had common ryegrass *Lolium perenne*, present. 10 species (fleabane *Pulicaria dysenterica*, white clover *Trifolium repens*, red clover *Trifolium pratense*, ragwort *Senecio jacobaea*, creeping thistle *Cirsium arvense*, curly dock *Rumex crispus*, creeping buttercup *Ranunculus repens*, common vetch *Vicia sativa*, and bramble *Rubus fruticosus*) appearing in half of the fields.

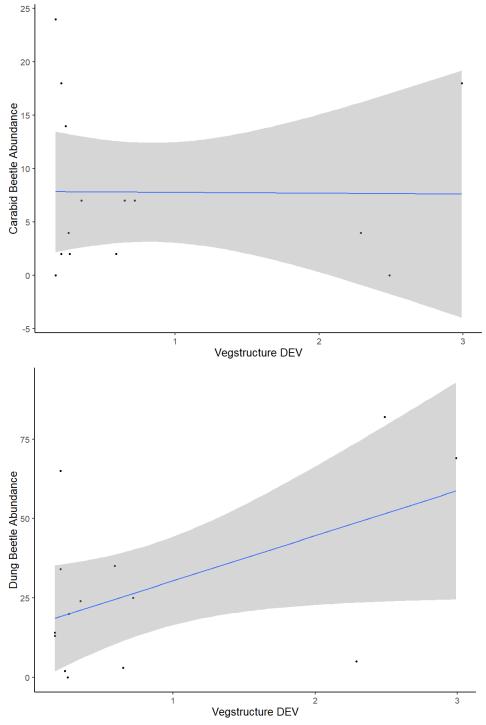
## Vegetation structure variation

Spearman's rank test found vegetation structure had no significant effect on mean species richness of dung (S = 566.85, p-value = 0.3969) and carabid beetles (S = 430.8, p-value = 0.5716)(fig.2).Vegetation structure had no significant effect on the abundance of dung (S = 304.67, p-value = 0.2486) and carabid beetles (S = 485.44, p-value = 0.4101)(fig.3). Vegetation structure variation also had no impact on abundance of dung beetles *Onthophagus similis* (S = 477.1, p-value = 0.5655), *Onthophagus coenobita* (S = 448.86, p-value = 0.4818),



*Melinopterus consputus* (S = 470.28, p-value = 0.5454), *Colobopterus erraticus* (S = 477.1, p-value = 0.5655) and carabid beetles *Poecilus cupreus* (S = 590.45, p-value = 0.8494).



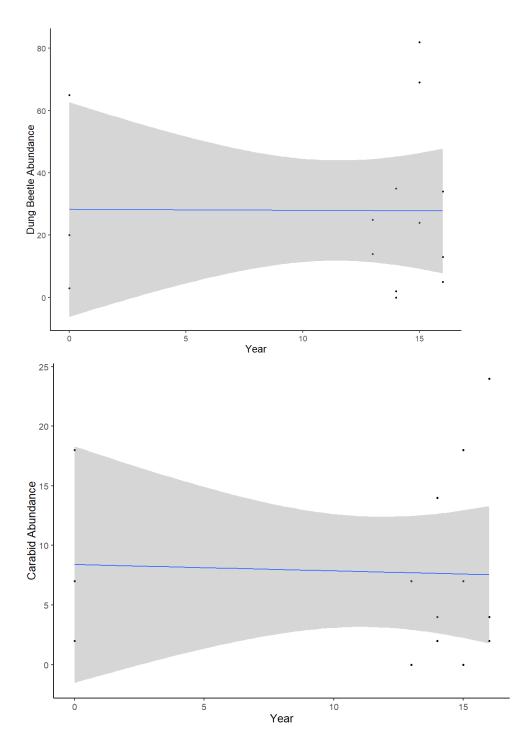


**Figure 3**: General linear model of carabid and dung abundance plotted against increasing mean vegetation structure deviation. Points represent the mean species richness of each site (14 sites). Blue line shows line of best fit and grey area shows standard error of fit.

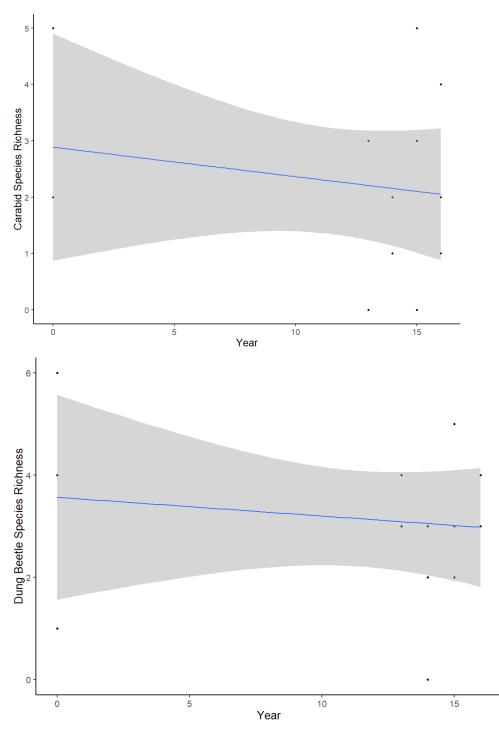
#### Year Fallowed

Spearman's rank found year fallowed had no significant impact on abundance of dung (S = 441.43, p-value = 0.9194) and carabid beetles (S = 472.8, p-value = 0.4472)(fig. 4), and species richness of dung (S = 416.27, p-value = 0.7723) and carabid beetles (S = 434.88, p-value = 0.8807) (fig. 5). Year fallowed also had no significant effect on abundance of dung beetles *Onthophagus similis* 

(S = 475.94, p-value = 0.5621), Onthophagus coenobita (S = 525.2, p-value = 0.7008), Melinopterus consputus (S = 269.78, p-value = 0.07428), Colobopterus erraticus (S = 475.94, p-value = 0.5621) and carabid beetles*Poecilus cupreus*(S = 501.96, p-value = 0.6373).



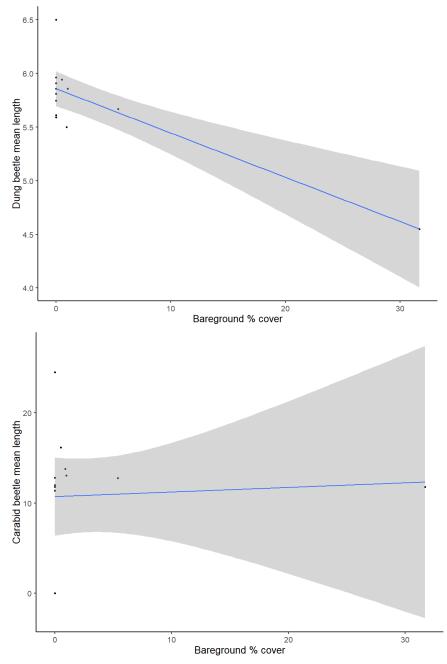
**Figure 4**: Linear regression model of dung and carabid beetles abundance plotted against increasing years since field fallowing. Points represent the mean species richness of each site (14 sites). The blue line shows line of best fit and grey area shows standard error of fit.



**Figure 5**: Linear regression model of dung and carabid beetles species richness plotted against increasing years since field fallowing. Points represent the mean species richness of each site (14 sites). The blue line shows line of best fit and grey area shows standard error of fit.

Bare ground

Spearman's rank test found bare ground cover to be negatively correlated with dung beetle mean body length (S = 514.14, p-value <0.05). However, bare ground cover was not found to be correlated with mean carabid beetle size (S = 273.36, p-value = 0.9213) (fig 6).

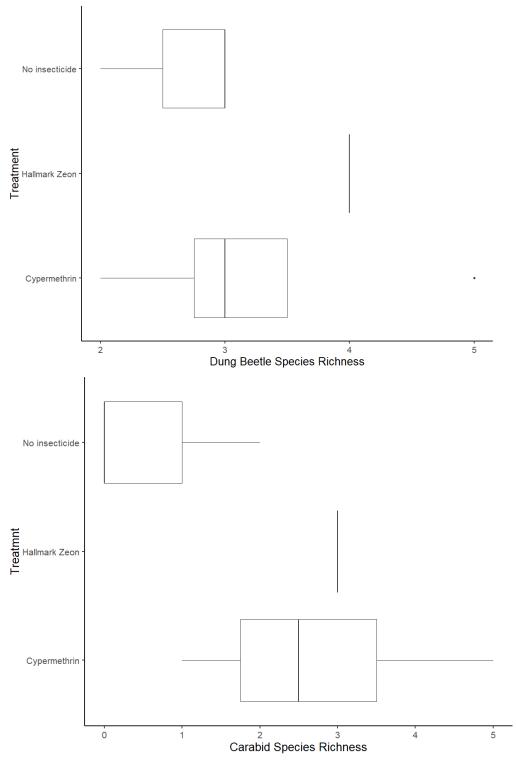


**Figure 6**: Linear regression model of mean length of dung and carabid beetles plotted against increasing mean bareground percentage cover. Points represent the mean species richness of each site (14 sites). The blue line shows line of best fit and grey area shows standard error of fit. Spearman's rank found that mean percentage bare ground cover was found to have no significant impact on dung species richness (S = 376.23, p-value = 0.4566) and abundance (S = 364, p-value

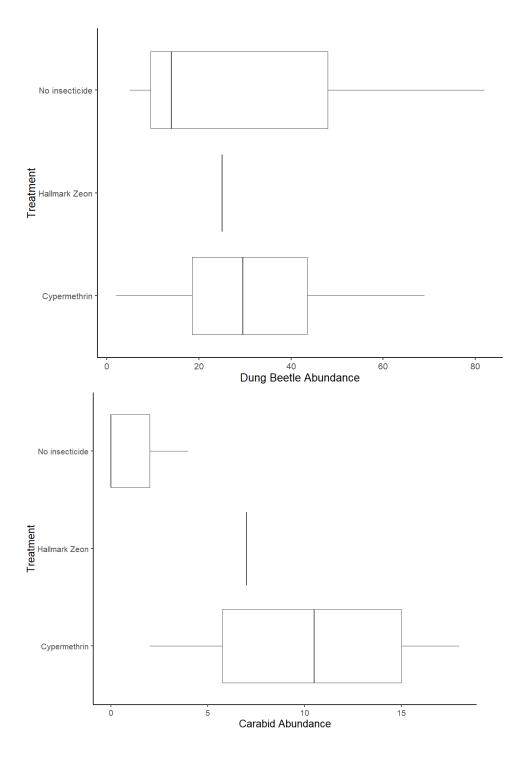
= 0.5), carabid species richness S = 266.23, p-value = 0.8126) and abundance (S = 300.01, p-value = 0.7172)

### Pesticide use

Last recorded pesticide use was in 2001, prior to rewilding of site. 8 fields and the type of insecticide used; Cymetherin, Hallmark Zeon and No insecticide where compared again dung and carabid species richness and abundance. Differences in fields that had insecticide applied (Cymetherin and Hallmark Zeon), compared to no insecticide, showed no significant difference in species richness and abundance of dung and carabid beetles. There was no significant difference difference found between insecticide application type and dung (t= 0.6731, p-value= 0.551) and carabid species richness (t=1.8796 p=0.2462) (fig.7). There was also no significant impact on dung (t= 0.0248, p-value=0.9756) and carabid abundance (t=2.0895, p-value =0.219) (fig.8).



**Figure 7**: Boxplot representation of Dung and carabid species richness plotted against three 2001 field applications; no insecticide, Hallmark Zone and Cymetherin from records of 8 fields. Bars represent range, lines are the means and boxes the lower and high interquartile range.



**Figure 8**: Boxplot representation of dung and carabid beetle abundance plotted against three 2001 field applications; no insecticide, Hallmark Zone and Cymetherin from records of 8 fields. Bars represent range, lines are the means and boxes the lower and high interquartile range.

DISCUSSION

#### Impacts of vegetation variation and structure

The impacts of vegetation on dung and carabids was not clear, with results for vegetation structure variation at the study's spatial scale showing no significant impact on the species richness and abundance of beetles sampled. The lack of evidence supporting changes in beetle species richness and abundance to vegetation structure was surprising, as ground beetle assemblages are known to be sensitive to habitat structure, microclimate and management (Luff and Rushton, 1989, McFerran *et al.*, 1994, Ekschmitt *et al.*, 1997, Dennis *et al.*, 1997, Wardle *et al.*, 1999). Soil water moisture content also has a large impact on many carabid beetles (e.g. Rykken *et al.*, 1997), this abiotic factor may have caused the differences in carabid species richness seen across the fields.

Vegetative structure, especially frequency and time of disturbances, would have resulted in significant microclimatic and soil surface temperature differences. However, the lack of pattern seen by vegetation structure could have been due to the study being on too large of a scale. The Southern block has a recorded high dominance of fleabane (Lyons, 2015). It's possible that reduced dominance of fleabane may benefit vegetation structure variation. Lack of rare species could be due to colonization ability as most species with conservation status recorded were associated with wetlands and old growth habitats which were present on the site prior to the start of the rewilding project (Lyons, 2015). Grasslands should have species rich vegetation with variation in structure and good flower abundance to gain optimal benefits for insects when using grazers. However, grassland like this is sparse outside of Knepp, limiting species colonisation (Lyons, 2015).

Insufficient browsing at Knepp, and indeed in the UK, could provide future problems for species reliant on grasslands if the grazers at Knepp aren't sufficient to keep shrub from spreading. Past large herbivores had their own feeding strategies, creating a complex landscape, but are now extinct. At present, deer provide a mixture of grazing and browsing (Abbas *et al.*, 2012), but an increase of shrubland may result in heavier grazing of the grasslands by cattle, which may be beneficial to carabid beetles, which prefer shorter grassland, but problematic for other invertebrate species such as butterflies and bees. The impact on dung beetles is uncertain as vegetation preferences of dung beetles aren't well recorded and often speculated (Lane and Mann, 2016). It's possible that lack of variation seen amongst dung beetles could also be due to their good dispersal abilities.

Vegetation also appeared to be species poor, with a total of 29 species recorded in quadrats across 15 different sites, with a high overlap of shared species. This may have accounted for the lower species richness of carabid beetles, and possibly dung beetles. Good soil fertility could account for the low species richness of plants, as applications of fertilizers would have been frequent during Knepp's time as arable farmland, and have been found to be detrimental to some species even at low levels (Silvertown *et al.*, 1994). Lack of reseeding in the Southern block could also account for lower species richness. Both the Northern and Middle Blocks of Knepp

received re-seeding with native seed mixes, whilst the Southern block was allowed to regenerate naturally.

The development of species rich vegetation is often constrained on abandoned arable land due to a depleted seed bank, establishment of competitive weeds already present in arable seed banks, and poor dispersal of late succession species, even when natural abiotic conditions have been restored (Bakker and Berendse 1999; Hansson and Fogelfors, 1998).

But, whilst plant composition may show little difference between the sites, beetle groups can still show differences. Ground-dwelling beetles mainly respond to changes in the microclimate and the soil-moisture and therefore allow an efficient indication of restoration management after 3–5 years. (Perner and Malt, 2003). Ideally studies on carabids should have been carried out prior and soon after rewilding was attempted, rather than 17 years post start of the project.

## Bareground cover

Of all the blocks, the Southern block is reported to have the most bare ground cover, dung and carrion, providing good habitat for dung beetles (Lyons, 2015). Increased presence of bare ground was found to contain beetles of smaller sizes, with mean length of dung beetles present as a proxy for overall body size. The highest area of bare ground cover was found in Honeypool house field, which had a patch of woodland in the centre of the field. Studies were carried out at the border of the woodland, but presence of bare ground was still high as a result of the tree cover. Competition for light amongst the trees and ground flora likely resulted in a poor ground coverage. Presence of bare ground or woodland may strongly impact what species are available. *Melinopterus consputus* dung beetle appeared in huge numbers in the traps, and far less beetles of *Onthophagus* species and may benefit from the presence of bare ground, which may enable easier movement. Alternatively, the larger presence of bare ground may deter cattle, resulting in lower presences of cattle dung. Less competition for resources may allow *M.consputus* to exist in large quantities.

Body size can also be determined using biomass, and bare ground cover is not the only factor that has shown evidence of impacting. In a study which compared dung beetle biomass in various grazing intensities, it was found that areas with low and moderate intensity grazing lands had higher biomass and larger-sized dung beetles, whereas an abandoned site showed up to 78% loss of dung beetle biomass, as larger dung beetles need more dung for food (Tonelli *et al.*, 2017). Therefore, areas with poorer dung availability may host smaller dung beetles.

Studies have reported that larger dung beetles are highly important contributors to ecological processes like dung removal (Larsen *et al.*, 2005; Braga *et al.*, 2013; Slade *et al.*, 2007; Nervo *et al.*, 2014; Ortega-Martinez *et al.*, 2016). Therefore losses in larger beetles due to abandonment may have negative impacts on the functioning of grassland ecosystems. Providing abandonment is short term, this could be be a minor problem if there is small amounts of dung provided by wild animals, although little is known about dung beetle recovery ability after grazing abandonment (Tonelli *et al.*, 2017).

#### Year fallowed

Year in which fields were allowed to fallow had no significant impact on species richness and abundance of dung and carabid beetles. Year fallowed may have had no significant impact because many ecological processes are influenced by time such as colonization rates and depletion of plant improvement products. It's possible that in the initial early stages of the project, the difference between dung and carabid beetles may have been more obvious due to those factors. However, at this stage of the project, year fallowed doesn't appear to have an impact. However, if vegetation structure changes due to shrub encroachment occur in the future, a significant difference between the permanent pasture, which is still managed, and rewilded fields may become evident.

#### Pesticide use

Historical pesticide use was found to have no significant impact on dung and carabid beetle abundance and species richness when 2 pesticides, Cymetherin and Hallmark zeon were compared to fields with no recorded pesticide use. As the last recorded application of pesticides and other plant growth and defence products was in 2001, it's likely that the time period of 17 years has diminished the impacts of the insecticides. Cypermethrin and Hallmark Zeon are types of pyrethroids used commonly as insecticides (Metcalf, 2000) which are normally broken down by sunlight and atmosphere in a few days, but can persist longer in sediment (Luo and Zhang, 2011). In separate laboratory studies, some invertebrate communities have been found to recover after 24 weeks (Van Brink et al., 1996). For carabids, this is primarily due to individuals colonising the area from surrounding untreated locations (Thacker and Dixon, 1996). Field carabids are a species rich group found in arable fields, but affected by intensive agriculture (Kromp, 1999), so it's likely some were already present at Knepp whilst it was arable land, and could have colonised from surrounding arable areas. A percentage of dung beetles may have originated from dairy cattle kept originally pre-rewilding at Knepp, whilst others colonised from surrounding pastures, although species richness would have likely been poorer due to avermectin usage on the dairy cattles, abundance would likely have been poorer although the number of dairy cattle kept, compared to longhorn cattle in the present, would have affected this as some dung beetles can survive on intensively managed fields (Hutton and Giller, 2003). Good dispersal abilities of dung beetles would have aided this movement. It is likely that during the early years of the rewilding project the impacts of insecticides may have been more prominent, but with increased time, the breakdown of the insecticides and colonisation of beetles into the area would have reduced the impact.

#### Pitfall trapping and limitations

Pitfall trapping formed the entirety of the data this study is based on. However, there are limitations regarding pitfall trapping and data interpretation (see Gittings, 1994). It is possible that factors such as weather conditions and disturbance by large grazing herbivores affected the relationship between pitfall captures and absolute population size. For example, *Sphaeridium* 

species, members of coprophagus Hydrophillidae (Hanski, 1980), are sampled less efficiently by pitfall trapping, when compared with pat sampling (Finn *et al.*, 1999). However, in logistical terms pitfall trapping is still a relatively effective method of sampling many invertebrate populations. To minimise the impact of habitat heterogeneity, pitfall traps were placed in fields that where all previously open fields prior to the rewilding project, and were located at the centrepoint of each field to minimise impacts of field boundaries, surrounding environments and roads. However, one field, Honeypool House had a woods located in the centre, so impacts of the surrounding woodland may have had influenced data collection. For example, the presence of trees likely caused an increase in bare ground cover, which has shown evidence to impact dung beetle body size. Therefore it's possible that the presence of woodland benefited smaller species such as *M.consputus*. Roads have also been found to impact beetles, with significantly lower abundances of Staphylinidae captured close to roads than in the centre of the pasture (Barth *et al.*, 1994).

Due to constraints on both time and effort, only 10 small pitfall traps were set up per field. Ideally a greater quantity, larger traps and a longer study period would have benefitted the study data, enabling further assessment regarding the potential impacts of vegetation structure, pesticide use and year fallowed might have on species within the Knepp estate project. The time period in which the study was carried out may have impacted the data collected. It has been found in a previous study across southern Ireland that the greatest variability in dung beetle assemblage structure occurred in the late summer, with equivalent variability among sites. Whereas in spring and early summer, when this study was carried out, the overall variability was less, but with a differing variability among sites (Finn *et al.*, 1999).

Size of the samples may have contributed to the selection of species seen. The abundance and richness of species found in a cow pat, compared to a 10g dung sample used in this study would most likely be far greater. Tree (2018) claims that 22 species of dung beetles were found in a single cow pat at Knepp, compared to the 10 identified with this study.

Moreover, only longhorn cattle dung was used in this study, and may have provided some limitation as to what dung beetle species were present. Normally, dung beetles are believed to be generalists, enabling survival post megafauna extinction, but they are known to prefer certain dung types (Gittings and Giller, 1998). The high abundance of dung available at Knepp from numerous herbivore species may allow beetles to show a preference, not just from the free roaming herbivores, but also from the horses present at the stable within the Knepp Wildlands campsite area. Crows have also demonstrated foraging preferences between dung types, opting for horse dung to that of cattle (Horgan and Berrow, 2004). These predation preferences may influence which species are present in different dung types.

The species richness of the carabid and dung beetles sampled were surprisingly low, however data collected within the pitfall traps may not necessarily be a true representation of the beetle community. Traps were also small and limited to 5 per beetle type per field, limiting data

collection. Ideally the experiment would be repeated in an area where assemblages of beetles were better understood to understand how pitfall traps reflected communities.

Data represented by this study is a snapshot of Knepp at its present state, ideally experiments should be repeated over numerous years to assess changes in species communities over time and as vegetation changes. This study would have been compared to earlier studies. But such are lacking, or methodology was not clear enough to replicate (e.g. Greenaway, 2006). Amatuer data may be available as Knepp is becoming increasingly popular with wildlife enthusiasts, but the variation in collection methods and reliability of identification would make building an accurate picture of historical population changes at Knepp difficult.

This is data which is lacking within rewilding studies, which still stands as a fairly new discipline. Whilst references to rewilding is increasing in scientific literature (Lorimer *et al.*, 2015), most are commentary, rather than research based (Svenning *et al.*, 2015). Many rewilding projects focus on carrying out project itself and but neglect to monitor or publish studies detailing the ecological impact and changing communities.

# Rewilding at Knepp

Overall, it is difficult to assess the community assemblage of both dung and carabid beetles only using pitfall trap data, a larger study over a period of time with controls outside of Knepp would enable better assessment regarding impacts of rewilding. The blocks at Knepp also differ, so results for the Southern block may not be representative of the entire Knepp estate. The Southern block has been found to have the lowest number of species, especially Coleoptera. The Southern block also has the lowest numbers of herbivores, detritivores and specialists, least woody vegetation and least species associated with grasses and legumes of all the blocks (Lyons, 2015).

*Onthophagus similis* (n=295) made up a majority of dung beetles collected, and have been recorded in cattle dung of organic farms, but absent from intensively farmed cattle (Hutton and Giller, 2003). *Poecilus cupreus* (n=68) made up a large proportion of the carabid beetles present, and is found widespread across southern Britain in dry habitats and fields. One rare dung beetle species was identified, *Sigornis porcus* (n=1). During a baseline study of Knepp, 6.2% of invertebrate species found had a conservation status. Typically nature reserves have 10%, but Knepp has until recently been arable land. It's also important to consider that whilst conservation status is a useful method for site assessment, it is flawed as numerous groups have not been updated for years and can use slightly different criteria for assessment (Lyons, 2015).

As a conservation project, Knepp likely has a good population of dung beetles due to minimal avermectins useage unless needed on individual cattle. Knepp also keeps the number of herbivores fairly constant, which results in a good dung supply throughout the year, but arguably doesn't replicate natural conditions which will have fluctuating populations due to factors such as disease and predation. In general, the abandonment of land and decrease in grazing animals could pose further threats to dung beetles, so rewilding projects should seek to quickly restore

the presence of large grazers, or have another site nearby to which dung beetles can colonise. Dung quantity is also important to assemblages of dung beetles locally (Lobo *et al.*, 2006) and due to loss of large herbivores and widespread use of domestic livestock, it's argued that dung beetles are not able to maintain viable communities on wild herbivore dung alone (Jay-Robert *et al.*, 2008) in agro-ecosystems or from domestic animals in suburban areas (Carpaneto *et al.*, 2005).

Dung beetles have shown the ability to make a quick recovery in species richness and abundance with an increase in fresh dung availability. (Lumaret *et al.*, 1992). However, dung beetle recovery can also take decades, as was found in a study in central Europe where "colonization of new pastures or restored habitats by dung beetles is strongly time-dependent", taking decades before large portions of specialist species are able to colonize these areas (Buse *et al.*, 2015). Global studies have also found dung beetle recovery in forest restoration to take a significant amount of time, from 15 (Quintero and Roslin, 2005) to 18 years (Audino *et al.*, 2014). Recovery time can be a slow process, and with current rates of species reductions, land abandonment may pose a threat to threatened dung beetle species. But if grazing is maintained to a low to moderate intensity then rewilding could benefit the conservation of dung beetle communities (Tonelli *et al.*, 2017).

Rewilding will result in a decline of pesticide use which should be beneficial for taxa, but the changing vegetation structure will greatly alter microhabitats, which are important to carabid beetles. It is difficult to predict how rewilding will change the landscape, but such factors should be monitored closely to attempt to understand and predict impact on invertebrates. A large increase in shrub will likely have negative consequences for smaller carabid beetle species which are more adapted to open grassland. Unless a method of restricting shrub and plants such as bramble (Lyons, 2017) is installed, it's possible that Knepp may see an increase in shrub cover. Bramble also appears to be acting as a protective barrier for trees to establish at Knepp, and whilst deer both browse and graze, whether that will be enough to restrict encroachment is uncertain. Shrub could be controlled through occasional active management, although may not be in line with the definition of rewilding.

Previous reviews of Knepp have recommended a slight reduction of grazing and including intensive 'pulsed grazing'. In 'pulsed grazing', some areas are grazed heavily in parts of the year to maintain pasture and reduce takeover by competitive species by forcing grazers to graze on less favorable plants, whilst other areas are allowed to recover (Lyons, 2015). It's possible that reducing grazing around spring and summer and not continuously grazing as heavily every year, could improve grassland species richness at Knepp (Lyons, 2015). Pulse grazing is naturally replicated by a presence of predators through a landscape of fear which influences prey movement, but at present, sites like Knepp lack the space and legal support to ethically maintain a viable population of predators. Some non peer reviewed evidence of a rewilding site known as 'Butcherlands and Ebernoe Commons' currently maintains 'pulsed grazing' through a fenced system and has been found to benefit invertebrate taxa by allowing the development of structural

types in the sward which cannot be provided by continuous grazing (Lyons, 2017). At present, Knepp's herbivores are free roaming and lack any predator or stimulus attempting to replicate a landscape of fear. Herbivores may have preferred fields, but this may not be enough to maintain a structural diversity needed for maintaining high biodiversity amongst invertebrates. As well as smaller carabids, an increase of shrub may also negatively impact butterflies (Merckx, 2015) as they are thermoregulators and require open sunny spaces, but would benefit moths, which make up 95% of Lepidoptera, and are reliant on woody food plants or wooded biotopes (Mercx, 2015). Naturally, succession will result in benefits and losses for different taxa, and if it is to be viewed as a 'process led' approach with no particular end goal, then the change in species will be expected. However, Knepp may lose some of it's rare species to which tourism has benefitted. It's also important to consider that processes such as predation are still absent from the UK, so rewilding sites may not necessarily replicate a natural environment, and human intervention, which has been in use for the past few millennia may be required in some cases. Continuous monitoring and feedback is required in rewilding projects as with nature reserves, as they are still a novel conservation concept with few rigorous monitoring schemes, and species responses to a changing environment are still uncertain. Lyons (2015) also argues that our own confirmation biases should not dictate the outcomes of rewilding projects, and constant fine-tuning by observation and changes in management is required. Therefore, whilst this study provides some insight into the impacts of rewilding on carabid and dung beetles at Knepp, further work is still required.

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