RESPONSE OF INVERTEBRATE COMMUNITIES TO INTENSIVE MANAGEMENT OF IMPROVED PASTURE ECOSYSTEMS

Paul Manning

Department of Zoology, University of Oxford
Magdalen College

Thesis submitted for the degree of Doctor of Philosophy, Michaelmas Term 2016

To my family, friends, and especially Janey. Thank-you all for your support, love, and patience during this adventure.

Contents

Paul Manning **Magdalen College**
Thesis submitted for DPhil in Zoology **Michaelmas Term 2016**

RESPONSE OF INVERTEBRATE COMMUNITIES TO INTENSIVE MANAGEMENT OF IMPROVED PASTURE ECOSYSTEMS

Abstract

The number of people on our planet is projected to rise to between 9.4 and 10 billion by 2050. Some estimates suggest that current levels of food production will need to double to feed this population. Increasing the intensity of food production on existing agricultural land will be a crucial element in meeting this goal, but practices associated with intensive management can cause biodiversity declines and erode the ecosystem functions that underpin production. My work explores agricultural intensification within the context of pasture-based cattle production. I focus on a variety of ecosystem functions supported by dung beetles (Coleoptera: Scarabaeoidea) and other dung-associated invertebrates. I investigate how variations in diversity, and chemical perturbations of veterinary anthelmintics affect the delivery of multiple ecosystem functions. I show that maintaining species-rich dung beetle assemblages has inconsistent benefits in providing multiple ecosystem functions. While dung beetles play an important role in supporting functioning in the short term, my work also reveals that their contributions may be less evident when considered over longer periods. Chemical perturbations caused by anthelmintic residues represent a significant threat to some invertebrate groups, but my experiments show that exposure does not always translate into an immediate reduction in ecosystem functioning. While use of anthelmintic products with relatively low toxicological risk did not cause obvious reductions in function, my work shows exposure can have significant consequences for the conservation of sensitive species. Overall, my work highlights the need for multigenerational studies, mathematical modelling, and careful consideration of sublethal effects to assess fully the risks of anthelmintic residues in the pasture environment. Furthermore, the emphasis on dung beetles (rather than other dung-associated invertebrates) in the existing literature neglects potentially important functional benefits provided by other taxa, such as earthworms. As the global human population continues to expand, it is important that we find sustainable ways to produce food while simultaneously conserving biodiversity. As loss of biodiversity in agricultural ecosystems does not always have functional consequences, it is important that wider justifications for conservation remain integrated into agricultural policy and practice.

Chapter 1: General Introduction

A growing human population and the future of farming

Our capacity to feed the world sustainably will be faced with numerous challenges in the coming decades. By the year 2050, the global human population is projected to reach between 9.4 and 10 billion people (United Nations, 2015). Simultaneously, the daily average per capita caloric intake is predicted to reach 3130 kcal, representing an increase of 12% relative to 2001 levels (Alexandratos and Bruinsma, 2012). To fulfil these demands, some estimates suggest that current food production must be doubled by 2050 (Godfray *et al.*, 2010).

An obvious solution to this problem would be to increase the area of land under agricultural production. However, expanding agriculture into natural habitats comes at a significant cost to the environment (Tilman, 1999) often characterised by a loss of biodiversity: the variability within species, between species, and amongst ecosystems. For example, deforestation associated with agricultural production is one of the most significant threats to global biodiversity (Gibbs *et al.*, 2010). Converting natural ecosystems to agricultural land use will have both local costs to biodiversity (Wright and Muller-Landau, 2006), and cause significant emissions of greenhouse gases into the atmosphere (Stern *et al.*, 2006).

Two principal strategies have been suggested to balance the need to conserve biodiversity and produce food: land sharing and land sparing (Green *et al.*, 2005). Land sharing is a strategy that aims to use sensitive agricultural practices to simultaneously maintain conservation and production within the same land area (Kleijn *et al.*, 2006).

Such practices include low chemical inputs (Geiger *et al.*, 2010), and maintaining high proportions of non-crop habitat (Bhagwat *et al.*, 2008), allowing natural communities to thrive alongside agricultural production (Phalan *et al.*, 2011). A prominent example of land-sharing is the use of agri-environment schemes within the European Union, which have had varying levels of success in meeting conservation targets (Kleijn *et al.*, 2006).

In contrast, land sparing is the process by which land used for agriculture and land used for conserving biodiversity are decoupled (Phalan *et al.*, 2011). This is achieved by managing homogeneous areas of farmland at high intensity thus maintaining high yields, and limiting agricultural expansion into natural ecosystems. This acts to 'spare' natural ecosystems from human disturbance associated with production practices (Green *et al.*, 2005). While also showing promise, land that is set aside during planning must be adequately protected for this strategy to succeed (Fischer *et al.*, 2011). As large remote areas managed for wildlife are difficult to monitor (Rauset *et al.*, 2016), ensuring adequate protection of spared land remains a significant challenge in conserving biodiversity.

While these strategies in approaching the complex challenge of conserving biodiversity and agricultural production appear at odds, their relative benefits are closely associated with the specifics of the particular taxa or taxon of interest, and the wider environmental and social contexts (Egan and Mortensen, 2012). In the challenge of feeding a growing human population, it is likely that both land sharing (e.g. Clough *et al.*, 2011) and land sparing (e.g. Egan and Mortensen, 2012) will play important roles in conserving biodiversity.

Regardless of whether biodiversity will benefit most from land-sharing or land-sparing,

intensification of production will undoubtedly play an important role in meeting human needs in the future, just as it has in the past. For example, in the last four decades of the 20th Century, the global human population has more than doubled, and yet per capita cereal consumption grew by 20%, with only a 7% increase in the area of land devoted to cereal production (Byerlee *et al.*, 2014). This was achieved through technological advances such as higher-yielding crop varieties (Evenson and Gollin, 2003), pesticides (Carvalho, 2006) and mineral fertilisers (Cordell *et al.*, 2009). Inevitably, these advances in yields and productivity have not been achieved without significant detrimental consequences for the environment (Tilman *et al.*, 2001).

Environmental impacts of intensive agriculture

The consequences of agricultural intensification might best be exemplified by the use of mineral nitrogen fertilisers. Nitrogen is typically the most limiting nutrient across terrestrial ecosystems (LeBauer and Treseder, 2008) and the development of mineral fertilisers has been one of the most significant drivers underpinning improvements in agricultural productivity (Keeler *et al.*, 2016). Use of these fertilisers however has, however, resulted in current measures of reactive nitrogen in the environment reaching more than double pre-industrial levels (Galloway *et al.*, 2008). While instrumental in achieving yield targets, additional nitrogen in the environment has been linked to a number of environmental problems including algal blooms (Anderson *et al.*, 2002) and steep increases in greenhouse gas emissions (Matson *et al.*, 1997).

Although the impacts of intensification on biodiversity are perhaps not as stark as changes associated with agricultural expansion, a number of practices associated with routine agricultural management contribute to biodiversity loss (Bengtsson *et al.*, 2005).

Though the exact drivers of biodiversity loss are difficult to tease apart, more intensive management has been linked to a mean 40% decline in the species richness of taxa living in agricultural landscapes (Tuck *et al.*, 2014). While the simplification of ecological communities living in agroecosystems is first and foremost an issue of conservation, a growing body of evidence suggests that declining biodiversity can also erode levels of ecosystem functioning (Allan *et al.*, 2015).

Ecosystem functions support agricultural production

Ecosystem functions are defined as biological, physical and geochemical processes which give ecosystems capacity to provide goods and services that directly or indirectly satisfy human needs (de Groot, 1992). In agricultural systems, these include functions that have important roles in underpinning production, for instance: pollination (Kremen *et al.*, 2002), and pest control (Snyder *et al.*, 2006).

Loss of functioning caused by declines in biodiversity may be counteracted by human intervention (i.e. chemical or energetic inputs) to compensate for insufficient delivery of ecosystem functions. From an ecological perspective, these interventions may act as perturbations with the potential ability to further disrupt biodiversity-ecosystem functioning relationships. Perturbations that impair or reduce biodiversity can have short-term consequences for functioning (Beynon *et al.*, 2012a) and, when repeated over time and grouped with additional perturbations, may cause chronic negative effects for biodiversity (Hutton and Giller, 2003; Tuck *et al.*, 2014). Loss of functioning associated with biodiversity declines, and compensation of lost function provided by inputs, may create a positive feedback loop where the economic and environmental sustainability of agricultural production are gradually eroded.

Livestock production and waste management

Land used for grazing livestock covers more than two billion hectares, representing almost 80% of global agricultural land use (Steinfeld *et al.*, 2006). Predicted dietary trends favouring more frequent meat and dairy consumption in developing countries will undoubtedly lead to a continued increase in the global number of cattle (Steinfeld *et al*., 2006): where over the past 50 years the number of cattle has grown by approximately 150% (Godfray *et al.*, 2010).

Cattle produce significant quantities of dung. A single cow can produce enough dung to cover an area of 0.8 m^2 each day (Floate, 2011). If the dung produced by 20 cattle over a five month grazing season was not decomposed, roughly 0.25 hectares of land would be covered in dung during the grazing season. As dung represents a relatively nutrient-rich resource (Holter, 2016), it is colonised quickly by a diverse community of invertebrates which consume, bury, and fragment the dung (Kaartinen *et al.*, 2013). This collective activity facilitates microbial decomposition, and is known as dung removal (MacQueen, 1973). Alongside the direct action of removing dung from the pasture surface, invertebrate activity supports a number of related functions which include reducing greenhouse gas fluxes from dung (Slade *et al.*, 2016), promoting higher forage productivity (Chapter 3) limiting the spread of faecal pathogens (Jones *et al.*, 2015), reducing the abundance of dung-developing pest flies (Wallace and Tyndale-Biscoe, 1983), promoting higher biological activity in soils (Chapter 2), and improving the hydrological properties of soil (Brown *et al.*, 2010).

Dung-associated invertebrates.

In temperate coprophagous invertebrate communities, most functioning is associated with feeding activity, which is varied both within (Doube, 1990) and amongst (Holter, 2016) taxa. The dominant invertebrate groups which support functioning in the dung environment are flies, dung beetles, and earthworms (Skidmore, 1991; O'Hea *et al.*, 2010a).

Flies (Diptera) are typically the first invertebrates to colonise cow dung (Skidmore, 1991). Some larvae found within dung (e.g. *Muscina* spp. (Hanski and Cambefort, 1991)) are predatory, although the majority are coprophagous with the details of their feeding behaviours poorly understood (Holter, 2016). Although fly larvae are often the most abundant invertebrates developing in cattle dung (Floate, 1998), their importance for ecosystem functioning is rarely considered (but see O'Hea *et al*. (2010)). Some flies which develop in dung, including the face fly (*Musca autumnalis* L.) and the horn fly (*Haematobia irritans* L.), are haematophagous as adults, and are significant pests of cattle and other livestock (McLintock *et al.*, 1954; Pickens and Miller, 1980).

A number of different families of beetles are found in cattle dung (Skidmore, 1991). Dung beetles (Coleoptera: Scarabaeoidea) feed on dung throughout their lives: adults feed on the liquid fraction of the dung pats, while bulk-feeding larvae feed indiscriminately on the undigested plant material and liquid components (Holter, 2016). In temperate systems, beetles that dwell and feed within the dung pat (known as endocoprids) are typically the most abundant (Hutton and Giller, 2003). Tunnelling dung beetles (known as paracoprids) often contribute disproportionately to measures of dung removal by partitioning dung into subterranean tunnels (Kaartinen *et al.*, 2013). Rolling

beetles (known as telecoprids) pack dung into spheres and bury the dung in the soil some distance away from the dung pat. These latter strategies allow larvae to develop under less competitive conditions, and are more commonly observed in highly competitive environments (Hanski and Cambefort, 1991). Cattle dung also supports a number of water beetle species (Coleoptera: Hydrophilidae) which are coprophagous as adults and predatory as larvae. Rove (Coleoptera: Staphylinidae) and hister (Coleoptera: Histeridae) beetles are also frequently present in cow dung, and are predatory both as larva and adults (Skidmore, 1991).

Earthworms are the final dominant group which are involved with dung removal and associated functions. Earthworms tend to colonise cattle dung later in succession and can contribute significantly to dung removal (Holter, 1979a). They provide numerous beneficial roles in improving the physical (Shipitalo and Le Bayon, 2004) and chemical (Cortez *et al.*, 2000) properties of soil. After feeding on dung, vertical movements of earthworms through the soil have been shown to increase pasture productivity by translocating dung to plant roots at lower horizons (Zhao *et al.*, 2013).

The relative importance of these invertebrate groups in providing functioning will depend on environmental factors (Wu *et al.*, 2011), the composition of local invertebrate assemblages (Roslin and Koivunen, 2001; Zhao *et al.*, 2013) and the particular function(s) under consideration (Slade and Roslin, 2016). Although dung-associated invertebrates are well established to support numerous ecosystem functions (Nichols *et al.*, 2008), little research has considered their simultaneous delivery – a concept known as ecosystem multifunctionality (Hector and Bagchi, 2007). As pasture-based systems rely heavily on the simultaneous delivery of multiple functions to underpin production,

understanding the particular benefits which various invertebrate groups provide is important in informing management to effectively capitalise on ecosystem functioning.

Dung-associated invertebrates and perturbation

Invertebrates supporting ecosystem functioning in dung are sensitive to a number of perturbations associated with intensive management of cattle raised on pasture. For instance, removing hedgerows to accommodate larger field sizes can lead to local extinctions of shade-loving species (Horgan, 2002), and soil cultivation associated with ley-based systems negatively affects both scarab larvae (Huiting *et al.*, 2006) and earthworms (Curry *et al.*, 2002). A perturbation of particular concern in pasture-based production systems is the presence of veterinary anthelmintic residues in cattle dung (Wall and Beynon, 2012).

Cattle suffer from a variety of internal and external parasites which, alongside being a welfare issue (Sutherland and Leathwick, 2011), can cause significant declines in farm productivity by limiting weight gain (Soutello *et al.*, 2007) and milk yield (Gross *et al.*, 1999). A number of formulations (parasiticides) are available to treat parasites of livestock, with the most commonly used class being the macrocyclic lactones (MLs) (Boxall *et al.*, 2007; Sutherland and Leathwick, 2011). This class of treatment works by preventing the transmission of electrical activity in nervous tissue of parasites by enhancing the efficacy of the neurotransmitter glutamate at glutamate-gated ion channels (Bloomquist, 1996). This causes nervous cells to undergo hyperpolarisation which leads to paralysis or death of the parasite (Wolstenholme, 2012). Macrocyclic lactones are administered to cattle through a number of different formulations which include topical applications, subcutaneous injections, and inter-ruminal boluses (Boxall

et al., 2007). Regardless of administration route, MLs undergo little metabolism (González Canga *et al.*, 2009), and more than 95% of active compounds are passed in the faeces (Steel, 1998). The same mechanism which is effective in controlling parasites also acts on beneficial insects colonising and feeding on dung, causing a range of lethal (Lumaret *et al.*, 2012) and sublethal effects which include impaired chemoreception (Verdú *et al.*, 2015), lower fecundity (Pérez-Cogollo *et al.*, 2015), reduced muscle strength (Verdú *et al.*, 2015), and prolonged development (Krüger and Scholtz, 1997).

Macrocyclic lactones can reduce levels of ecosystem functioning provided by dung insects via direct short-term impairment (Wall and Strong, 1987), or by longer-term chronic depletion of population diversity and abundance (Hutton and Giller, 2003). While experimental evidence shows that loss of functionally dominant groups (Kaartinen *et al.*, 2013), and exposure to anthelmintics (Beynon *et al.*, 2012a) can limit dung removal, it is unlikely that dung removal is in all cases positively correlated with other beneficial functions. For instance, while dung beetles translocating dung deep into subterranean tunnels can provide significant reductions in the abundance of infective parasite larvae (Gregory *et al.*, 2015), the functional benefits of dwelling dung beetles are less clear-cut (Sands and Wall, 2016). Furthermore, earthworms are common within livestock production systems and exhibit little sensitivity to MLs (Kaneda *et al.*, 2006).

Thesis plan and outline

In this thesis, I use field and semi-field experiments to explore the ways that declining insect diversity, and perturbations associated with macrocyclic lactone use, affect various ecosystem functions associated with dung removal. My work aims to

demonstrate the consequences that continued intensification might have for ecosystem functioning.

In Chapter 2, using three dung beetle species, I consider how the presence of different functional groups affects the delivery of three functions which underpin productive pastures. I find that different functional groups are better suited to support particular functions over others, and that a three-species assemblage performs well across multiple functions.

Building on this evidence that higher dung beetle species richness could provide multiple ecosystem functions, in Chapter 3, I use four common dung beetle species to test whether higher species richness affects four individual functions and multifunctionality both in the presence, and absence, of a chemical perturbation. I find weak evidence of enhanced species richness supporting individual functions, and some evidence suggesting chemical perturbation negatively affects functioning. I find no compelling evidence to suggest higher levels of species richness is a strong driver of multifunctionality. However, I suggest that ecosystem multifunctionality may decline under chemical perturbation.

In Chapter 4, I consider how exposing a single species to a chemical perturbation influences the concurrent and eventual delivery of individual ecosystem functions and multifunctionality. I find one individual function was negatively impacted by concurrent chemical perturbation, but no evidence that other functions were affected by concurrent or persistent exposure. I find no evidence that chemical perturbations affect ecosystem multifunctionality.

In Chapter 5, I ask how impairing coprophagous insects through a potent chemical perturbation affects pasture productivity, both directly and indirectly (by facilitating weed establishment). I find that weed establishment was erratic, and did not allow for a meaningful comparison amongst treatments. Additionally, I find that while the presence of dung significantly increases forage growth in the long-term, the presence of a chemical perturbation has no effect. Earthworm casts were abundant on site, suggesting that earthworm activity might compensate for insect impairment.

Given that parasiticide use is unlikely to wain under conventional production practices, in Chapter 6, I investigate how an ML with relatively low non-target toxicity affects survival and functioning of two dung beetle species. Each species exhibits contrasting sensitivity to agricultural intensification. I find that exposure to residues has a strong lethal effect on the species sensitive to intensification, and the non-sensitive species is unaffected. In the short-term, the two species have equal importance in supporting functioning. Over the long-term, available evidence suggests again that earthworm activity may compensate for insect impairment.

In Chapter 7, I explore a fundamental question about the biology of a common dung beetle species (*Aphodius fossor* L.). Individuals are normally found in male-female pairs in cattle dung, but how individuals find one another at low densities in patchy environments is unknown. Using a simple olfactometer experiment, I show that the sex of previously colonising beetles can influence successive colonisation patterns.

Finally, in Chapter 8, I discuss my findings more generally and offer recommendations for future research. I summarise that conserving invertebrate diversity in pasture may offer some benefit for ecosystem functioning and subsequent agricultural productivity. I

suggest that while MLs pose a serious risk to coprophagous insects, exposure does not always translate into loss of ecosystem functioning. I emphasise the need for ML toxicological research to appreciate the effects of sublethal exposure, particularly as it relates to insect dispersal. Lastly, I recommend that future research could benefit by considering a wider range of invertebrate diversity associated with dung, and its role in supporting ecosystem multifunctionality in pasture.

Chapter 2: Functionally rich dung beetle assemblages are required to provide multiple ecosystem services

Paul Manning[1,2], Eleanor M. Slade[1,3], Sarah A. Beynon[1,2], Owen T. Lewis[1]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

3. Lancaster Environment Centre, Lancaster University, Balrigg, Lancaster LA1 4YQ, UK

Author Contributions: PM and SAB designed and set-up the experiment. PM collected the data. EMS helped design models describing feeding depth. PM performed all analyses and wrote the manuscript. EMS, SAB, and OTL provided multiple rounds of feedback on earlier versions of this manuscript.

Chapter Status: Published
Manning, P., Slade, E.M., Beynon, S.A. and Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems and Environment*, **218**, 87–94.

Abstract:

Dung beetles mediate a variety of important ecosystem services in both natural and human-modified habitats. These services are associated with the exploitation of dung by beetles for breeding and feeding, with different functional groups using dung in different ways. While many studies have considered how individual ecosystem functions and services (primarily dung removal and seed dispersal) are affected by changes in dung beetle diversity, fewer studies have considered the consequences for multiple functions and services. We used manipulative experiments to evaluate the functional efficiency of three species of dung beetles, each representing one of the three functional groups present in temperate Europe. Standardising beetle biomass, we compared single-species treatments to a three-species mixture containing each of the species in equal biomass. We then measured three ecosystem services relevant in supporting pasture-based livestock production systems: dung removal, soil fauna activity, and soil aeration. The presence of dung beetles significantly elevated all three ecosystem services. However, delivery of each service peaked under different treatments, indicating that no single-species assemblage can provide maximum functioning across multiple services. For all three services, the three-species polyculture provided a level of functioning indistinguishable from the most efficient single-species treatment. Our results highlight the importance of considering multiple functions and services when assessing the relationship between biodiversity and ecosystem functioning, and suggest that the conservation of functional richness within dung beetle communities could play an important role in securing the delivery of multiple ecosystem services.

Introduction

Rapid changes in land use, driven largely by the intensification of agriculture over the past century, have resulted in widespread declines in species associated with agricultural landscapes (Butler *et al.*, 2007). Many of these species help maintain agricultural productivity by providing ecosystem services: biological, physical, and geochemical processes occurring within an ecosystem that benefit humankind (de Groot *et al.*, 2002). These services may become diminished or lost as beneficial populations decline, threatening capacity for sustainable food production (Kremen *et al.*, 2002; Menalled *et al.*, 2007). While individual functions and services can be maximised at relatively low levels of diversity (Perkins *et al.*, 2014), the full importance of species-rich ecological communities in delivery of ecosystem services may become more apparent when multiple ecosystem functions are considered simultaneously (Lefcheck *et al.*, 2015). This is the concept of ecosystem multifunctionality (Hector and Bagchi, 2007).

Pasture-based livestock production systems represent a large proportion of global agricultural land use, corresponding to more than 64% of the United Kingdom's agricultural area (DEFRA, 2013). In these systems, dung beetles play an important role in mediating the ecosystem service of dung decomposition through direct consumption, by incorporating dung into the soil, and by aerating the dung via tunnelling (Hanski and Cambefort, 1991). While the action of removing dung from the pasture surface has the direct benefit of allowing continued growth of the pasture sward, supporting services linked to dung removal have far-reaching benefits for the sustainability of

agroecosystems (Nichols *et al.*, 2008; Beynon *et al.*, 2015). These include increased mineralisation of organic nitrogen contained in dung (Gillard, 1967; Yokoyama *et al.*, 1991); enhanced hydrological properties of soils (Brown *et al.*, 2010); reduced transmission of gastrointestinal parasites (Gregory *et al.*, 2015); and reduced greenhouse gas fluxes (Penttilä *et al.*, 2013). Dung removal (the rate at which dung is cleared from the pasture surface) is often used as the sole measure of dung beetle-mediated functioning (Beynon *et al*., 2012; Kaartinen *et al*., 2013). The wide variety of reproductive and behavioural strategies used by dung beetles does, however, mean that dung removal rates may not adequately reflect other functions and services. The effects of dung beetle functional diversity on multiple ecosystem services have not been explored previously for temperate species.

Dung beetles are an excellent model system to explore biodiversity-ecosystem functioning relationships, as they exploit discrete and ephemeral resources, and their diversity can be manipulated experimentally at a relevant spatial scale (Beynon *et al*., 2012; Lähteenmäki *et al*., 2015; Slade *et al*., 2007). To gain a better understanding of how the functional identity of dung beetles influences the provision of multiple ecosystem services, we manipulated dung beetle assemblages experimentally, holding beetle biomass constant, while varying species richness and functional diversity. In addition to the widely-studied service of dung removal, we focus on two additional ecosystem services: soil surface aeration and feeding activity by soil fauna leading to plant litter decomposition. Pasture soils are compacted by livestock and agricultural machinery, which may limit pasture productivity (Martínez and Zinck, 2004). Compaction may also lead to off-site impacts, where rainfall cannot percolate into the

soil, and runoff may enter nearby watercourses (Alderfer and Robinson, 1947). Plant litter decomposition is a critical component of nutrient cycling (Swift *et al.*, 1979), mediated by microbes, but accelerated by soil invertebrates (Hättenschwiler *et al.*, 2005). Although dung is a rich source of nutrients, it is a hypoxic environment (Holter and Spangenberg, 1997): dung persisting on the pasture surface may cause hypoxia in the underlying soil, a condition known to limit the activity of soil fauna (Qualls and Richardson, 2000). Through the action of dung beetles aerating the dung, and incorporating nutrient-rich material into the soil, soil invertebrate functioning could be enhanced by the presence of dung, when subject to dung beetle activity.

We predict that variation in feeding behaviour will result in certain functional groups being better suited to provide individual ecosystem services over others. Thus, while a single species may provide high levels of a single ecosystem service, it may trade-off with the provision of other services. We predict that a combination of multiple functional groups may be more effective in simultaneously supporting multiple ecosystem services (ecosystem multifunctionality).

Materials and Methods

Study site

The experiment was conducted on an improved pasture at Dr Beynon's Bug Farm, St. David's, Pembrokeshire, UK (51°53′22″, 5°14′06″). The dominant plant species was perennial rye grass *Lolium perenne* L., which was sown in 2006, with white clover *Trifolium repens* L. The underlying soil is part of the Moore Gate series; a well-drained, humose gritty loam in the England and Wales Soil Survey Classification (Clayden and Hollis, 1985). The field is managed under a rotational system, with land use over the

past 10 years including potatoes, spring barley and short-term (~4-6 year) *Lolium perenne*-based grazing leys. At the time of the experiment, a flock of 40 Welsh Mountain ewes and their lambs grazed the field in rotation with other fields on the farm. Immediately before the experiment, the sward in the immediate experimental area was cut to a length of *c.* 1 cm and cuttings were removed by hand. Sheep were excluded from the immediate experimental area with electric fencing.

Experimental enclosures and treatments

To manipulate dung beetle assemblages, enclosures were constructed using 14 L, 35 cm diameter black buckets with the bases removed, sunk into the soil to a depth of *c.* 4 cm (Figure 2.1a). Enclosures were arranged in a 5 m × 5 m grid, spaced at 1 m. One of four dung beetle assemblage treatments (three single-species treatments and one multi-species treatment) or two control treatments (beetle-free dung, and enclosures without dung or beetles) was assigned at random to each enclosure.

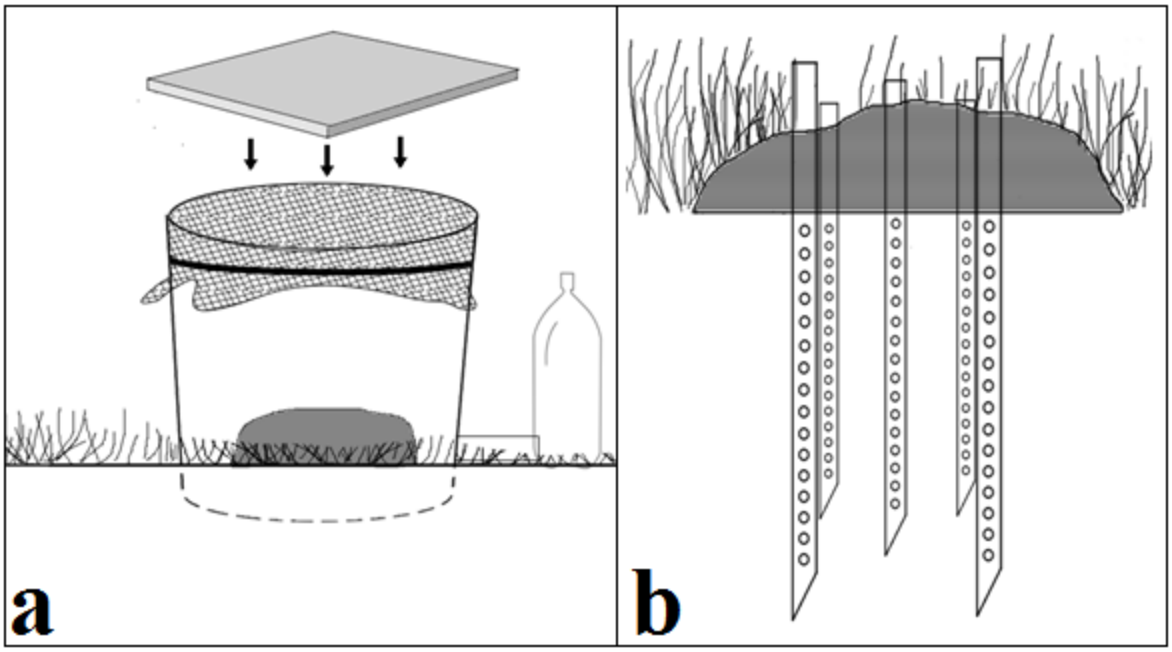

Figure 2.1 Experimental setup. (a) enclosure, with emergence bottle attached. Soil fauna are able to colonise naturally from surrounding soil. (b) Placement of bait lamina strips through a dung pat. Strips are inserted to same depth in dung free controls.

British dung beetles can be classified into three principal functional groups, based on their mode of exploiting dung (Doube, 1990). Dung-ovipositing endocoprids feed and lay eggs within dung, and larvae feed within dung or underlying soil. Soil-ovipositing endocoprids also feed within dung, but move to the dung-soil interface to lay their eggs, with larvae typically dispersing into the dung to feed. Paracoprid dung beetles dig tunnels in the soil below dung, creating brood chambers provisioned with dung, where eggs are laid and larvae feed. Each of the three functional groups was represented by a single species in monoculture: *Aphodius ater* De Geer (dung-ovipositing endocoprid), *Aphodius fossor* L. (soil-ovipositing endocoprid) and *Onthophagus joannae* Goljan (paracoprid). Live dung beetles were collected at various sites in western UK during June 2014 and identified using Jessop (1986). The fourth assemblage was a polyculture, with each of the three species contributing equally to overall dry biomass.

Logistical constraints prevented us from testing two-species combinations. In all treatments and replicates (except the controls), beetle biomass was standardised at a target mass of 156 mg (Table 2.1), following Beynon *et al*. (2012) and reflecting the natural biomass of beetles typically processing a single dung pat. As the sex of *A. fossor* is readily determined, we used equal numbers of males and females within enclosures to encourage natural dung-exploitation behaviours associated with breeding. We were unable to sex *Aphodius ater* and *O. joannae*, but treatments always included six or more individuals per species, making it highly likely that both sexes would be present in each replicate (Beynon *et al.* 2012). Each treatment and control was replicated six times, giving a total of 36 enclosures.

Table 2.1. Composition of dung beetle assemblages used within semi-field enclosures. Dung beetle dry biomass is approximately 156.10 mg in each treatment, based on biomass values from the literature. M1–M3 represent the three monocultures, while P represents the polyculture. Integer values represent the abundance of each species included within the experimental assemblages.

Species	Functional group	Individual Dry mass (mg)	Beetle treatment M1	M2	M3	P
Aphodius ater (De Geer)	Dung-ovipositing endocoprid	5.43[a]	29	—	—	10
Aphodius fossor (L.)	Soil-ovipositing endocoprid	26.10[a]	—	6	—	2
Onthophagus joannae (Goljan)	Paracoprid	9.47[b]	—	—	16	6

[a] Biomass values from Gittings and Giller (1997)
[b] Biomass values from Beynon *et al.* (2012)

The experiment ran for fifty-two days from June 18th 2014, corresponding to the six week period of egg-to-pupa development for the two endocoprid species included

(Stevenson and Dindal, 1985). Fresh (<6 h old) dung for the enclosures was collected from cattle grazing on a rye grass and white clover pasture at a neighbouring farm. These cattle had not been treated with anthelmintics or ectoparasiticides during the previous three months. Dung was frozen at -20 °C for >1 week, then thawed for 48 h before the start of the experiment. This procedure kills any invertebrates contaminating the dung, but does not affect dung beetle reproduction (Yamashita and Hayakawa, 1991). Dung was homogenised with a mortar mixer and 550 g pats were placed within the enclosures (except for the dung-free treatments), shaped using an 18 cm diameter circular mould and placed on a 2 cm aperture wire grid. Enclosures were covered with a fine (0.8 mm aperture diameter) nylon mesh, secured with elastic, following Beynon *et al. (2012)*. Beetles were added six hours after placing the dung within enclosures. Corresponding to a typical period of beetles being resident in a dung pat, emergence traps (clear plastic bottles connected to the enclosures by clear plastic, 2 cm diameter tubes) were fitted to the enclosures at Day 14 (Figure 2.1a). A porcelain tile was placed on top of each enclosure. When enclosures were darkened by the tile, beetles moved towards the light and were subsequently captured in the emergence trap. This allowed beetles to disperse from the dung while preventing further colonisation by other invertebrates (Beynon *et al*. 2012).

Measuring ecosystem services

Dung removal was measured at Day 42 by carefully lifting the dung from the soil surface using the wire grid, weighing the combined components on a portable balance accurate to 1 g, and subtracting the pre-recorded mass of the grid. The dung pat was immediately returned to its original position to allow measurements of other functions.

Soil fauna feeding activity was measured in the underlying soil over a 10-day period, starting at Day 42, using bait lamina (Terra Protecta GMbH, Berlin, Germany). Bait lamina are PVC strips (1 mm x 6 mm x 120 mm), with 16, 1.5 mm diameter perforations at 5 mm intervals, filled with a standardised bait of cellulose powder, ground wheat bran and activated charcoal (70:27:3). Strips are inserted vertically into the soil at a standardised depth, for a given period, and when removed, the activity of soil fauna is assessed by viewing strips against a light source. The status of each bait-filled perforation is classified as being either 'consumed' or 'intact' depending on whether light penetrates through the hole (Kratz, 1998). At the start of the experiment, six bait lamina strips were inserted through each dung pat and into the soil in a hexagonal pattern so that the top hole was positioned just below the soil surface (Figure 2.1b). Laboratory tests have shown that feeding activity is predominately driven by soil invertebrates, with limited contributions by microbes (Helling *et al.*, 1998). In dung-free controls, the bait lamina strip was inserted to an identical depth directly into the soil. One of the six strips served as an indicator to determine an appropriate exposure period (in this case 10 days, corresponding to 30-60 % consumption across treatments). The five remaining bait lamina strips per replicate were carefully removed from the soil at Day 52 and feeding activity was assessed immediately.

Also at Day 52, we took a measure of surface soil penetrability as a surrogate measure of surface soil compaction. We took five measures under the dung pat using a handheld penetrometer (Humboldt Manufacturing: Model G-118-H-4200), by placing a numbered grid where each dung pat had been positioned, and using a random number generator to select sampling points. The penetrometer was inserted to a depth of 7 mm, and the

mean of five measurements was used in analysis. To measure soil moisture, which has been demonstrated to influence the activity of soil fauna (Simpson *et al.*, 2012), a 2.5 cm diameter × 8 cm deep soil core was taken from under the dung in each enclosure. Samples were graded through a 1 mm sieve; 5.00 g was then weighed in an aluminium crucible, and samples were dried at 105 °C for 24 hours, and re-weighed to determine soil moisture gravimetrically.

Analysis

Measures of dung removal (mass of dung remaining), feeding activity (proportion of bait units consumed), and soil compaction (surface penetrability) were compared across treatments using analysis of variance. Histograms of model residuals and plots of residuals against fitted values demonstrated that assumptions of parametric tests were met, so in all cases data were left untransformed. Significant overall effects of treatment were further explored using a *post hoc* Tukey test.

To better understand how dung beetle treatments influenced feeding activity of soil fauna, we used smoothers derived from generalised additive models to describe changes in soil fauna feeding activity as a function of soil depth, following Simpson *et al*. (2012). Smoothers are approximating functions, that attempt to capture important patterns in the data. To create smoothers, treatment was modelled as a fixed factor, and treatment levels showing a similar relationship between feeding activity and depth were combined to create a simpler model. Models were compared using Akaike's Information Criterion (AIC): a widely-used metric that estimates the quality of a statistical model, relative to a second. A smaller AIC value indicates the model has lower residual error (i.e. a better fit) relative to second. Including additional predictor

variables penalises the AIC score, which helps balance explanatory power and parsimony. Through comparing AIC values, we selected our model based on an *a priori* threshold of two AIC units: if the difference was less than two, the simpler model (i.e. the model with fewer predictors) was preferred.

The statistical package R 3.1.1 (R Core Team, 2014) was used for all analyses, with the lme4 (Bates *et al.*, 2014), and gamm4 (Wood and Scheipl, 2014) packages used to visualise patterns in soil fauna feeding activity.

Results

Dung removal (measured as the mass of dung remaining at 42 days) differed significantly among treatments ($F_{4,25,} = 5.97$, $P<0.001$; Figure 2.2). *Post hoc* comparisons showed that final dung mass was significantly lower in enclosures containing monocultures of the soil ovipositing endocoprid (*A. fossor*) (79.00 ± 2.16 g; mean ± SE) and the three-species polyculture (81.67 ± 3.05 g) compared to the beetle-free controls (90.83 ± 3.05 g). Dung mass remaining in the dung ovipositing endocoprid (*A. ater*) (86.67 ± 1.22 g), and the paracoprid (*O. joannae*) (87.00 ± 1.46 g) monocultures did not differ significantly from the beetle free controls, or from either of the other monocultures.

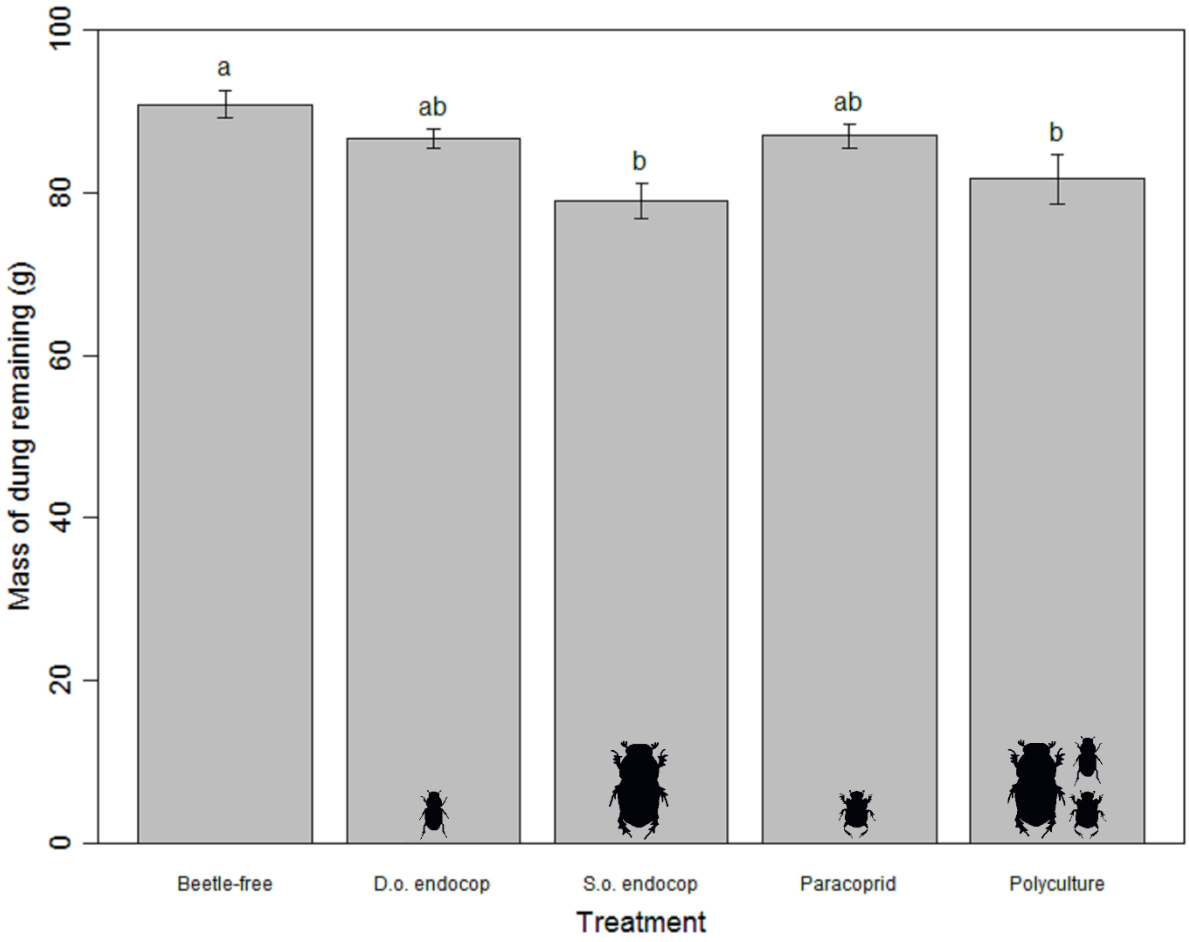

Figure 2.2. Effect of dung beetle assemblage on dung mass (g) remaining at six weeks. Bars are estimated means with standard error. *X*-axis labels refer to beetle-free dung, dung-ovipositing endocoprid (*A. ater*), soil-ovipositing endocoprid (*A. fossor*), paracoprid (*O. joannae)*, and the polyculture.

Feeding activity of soil fauna differed significantly among treatments ($F_{5,30} = 6.61$, $P<0.001$; Figure 2.3). Feeding activity was highest in enclosures containing the paracoprid (*O. joannae*) (51.45 ± 3.10% mean ± SE), and this was significantly greater than feeding activity in the dung-free (21.25 ± 4.04%), beetle-free (30.25 ± 5.43%), and dung ovipositing endocoprid (*A. ater*) (26.*88* ± 4.06%) enclosures. Feeding activity in the polyculture (40.63 ± 2.67%) was higher than that of dung-free controls, but did not

differ significantly from any other treatments. Variations in soil moisture across all treatments were small (81.4± 2.7%; mean ± SE) and non-significant ($F_{5,30}$ =0.984, *P*=0.44).

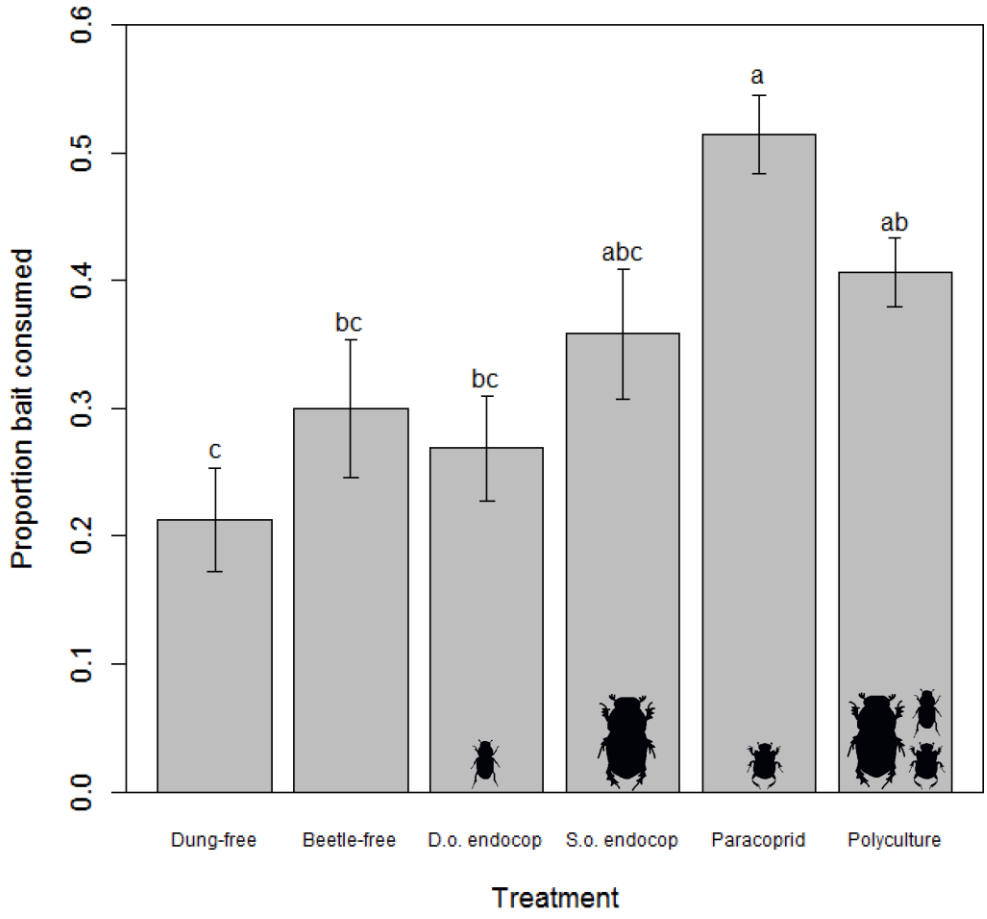

Figure 2.3. Effect of dung beetle assemblage on soil fauna feeding activity. Bars are estimated means with standard error. *X*-axis labels refer to dung-free control, beetle-free dung, dung-ovipositing endocoprid (*A. ater*), soil-ovipositing endocoprid (*A. fossor*), paracoprid (*O. joannae)*, and the polyculture.

Through use of additive mixed models, we found that treatment significantly influenced feeding activity. Using model simplification, and comparison of AIC we found that the optimal model used four smoothers to relate soil feeding activity with depth (Table 2.1).

Three were represented by individual factor levels: dung free control; paracoprid (*O. joannae*) and polyculture. A fourth smoother combined three levels: dung ovipositing endocoprid (*A. ater*), soil ovipositing endocoprid (*A. fossor*) and beetle-free dung.

Table 2.1. Comparison of four different models used to explain variation in soil fauna feeding activity as a function of dung beetle treatment. Models were selected by plotting each treatment level independently, and combining treatments showing similar trends. The optimal model (d) combined the dung and soil ovipositing endocoprids, alongside beetle-free dung. Dung-free controls, the paracoprid, and the polyculture were represented as individual factor levels.

Model	AIC Value
a) Dung-free + Beetle-free + D.o endocop + S.o endocop + Paracoprid + Polyculture	3441.9
b) Dung-free + Beetle-free + (D.o endocop + S.o endocop) + Paracoprid + Polyculture	3436.9
c) Dung-free + (D.o endocop + S.o endocop + Beetle-free) + (Paracoprid + Polyculture)	3429.2
d) Dung-free + (D.o endocop + S.o endocop + Beetle-free) + Paracoprid + Polyculture	3426.7

We demonstrated that feeding activity was highest near the soil surface and declined with depth (Figure 2.4). In most cases, these relationships were nonlinear (Table 2.2.). The two treatments containing the paracoprid dung beetle *O. joannae* (monoculture and polyculture) stimulated soil fauna feeding activity deeper in the soil. The introduction of dung stimulated higher soil fauna feeding activity at the soil surface (Figure 2.4).

Table 2.2. Optimal generalised additive mixed model: feeding as a function of beetle community and depth. Degrees of freedom increase as smoothers move further from linear relationships.

Explanatory variable	df	F	*P*
Assemblage	3	31.11	**< 0.001**
Smoother Control	2.42	10.542	**0.00895**
Smoother Endocoprids+ Dung	2.72	120.22	**< 0.001**
Smoother Paracoprid	1	6.528	**0.01062**
Smother Polyculture	2.3	6.839	**0.04418**

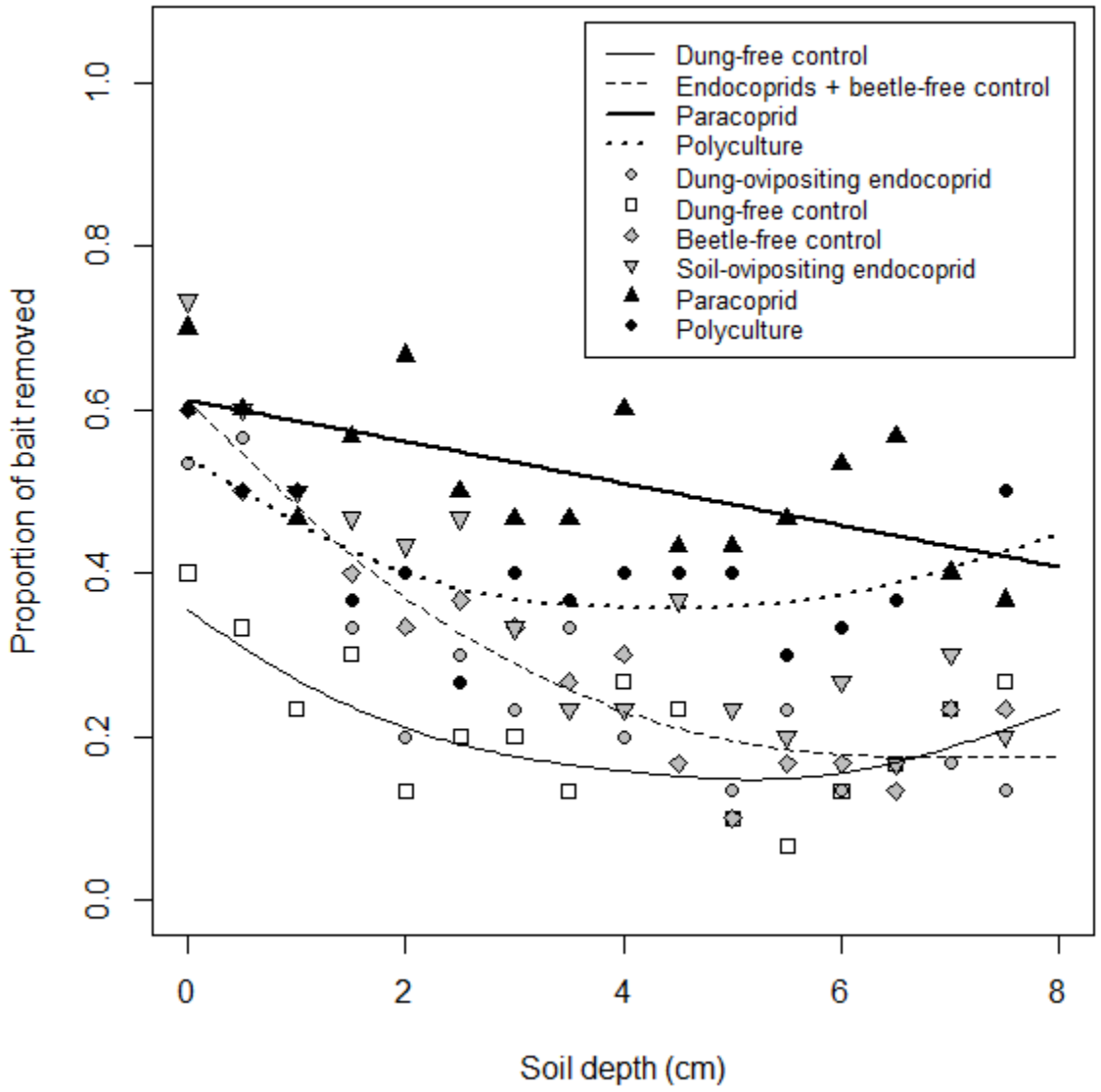

Figure 2.4. Effect of dung beetle assemblage on feeding activity across soil depth. Proportion of bait removed is the total number of holes perforated at each depth, averaged across replicates. Smoothers are derived from a generalised additive mixed model and are a continuous prediction explaining feeding activity as a function of soil depth. The model validation showed that monoculture communities of the soil ovipositing endocoprid (*A. fossor*) and the dung ovipositing endocoprid (*A. ater*) could be combined with the beetle free control and described using a single smoother (dashed line).

Based on our measures of surface penetrability, we found soil compaction under dung pats differed significantly amongst treatments ($F_{5,30} = 9.38$, $P<0.001$; Figure 2.5). *Post hoc* comparisons revealed that surface soil compaction was reduced significantly by the

soil ovipositing endocoprid (*A. fossor)* (0.98 ± 0.35 kg/cm^2) when compared to the dung free control (3.02 ± 0.32 kg/cm^2), and the beetle-free dung (2.59 ± 0.33 kg/cm^2). Surface soil compaction was significantly lower for the dung ovipositing endocoprid (*A. ater*) (1.40 ± 0.35 kg/cm^2) and the polyculture (1.68 ± 0.30 kg/cm^2) when compared to the dung-free controls, and was not significantly different from other treatments (including the *A. fossor* monoculture).

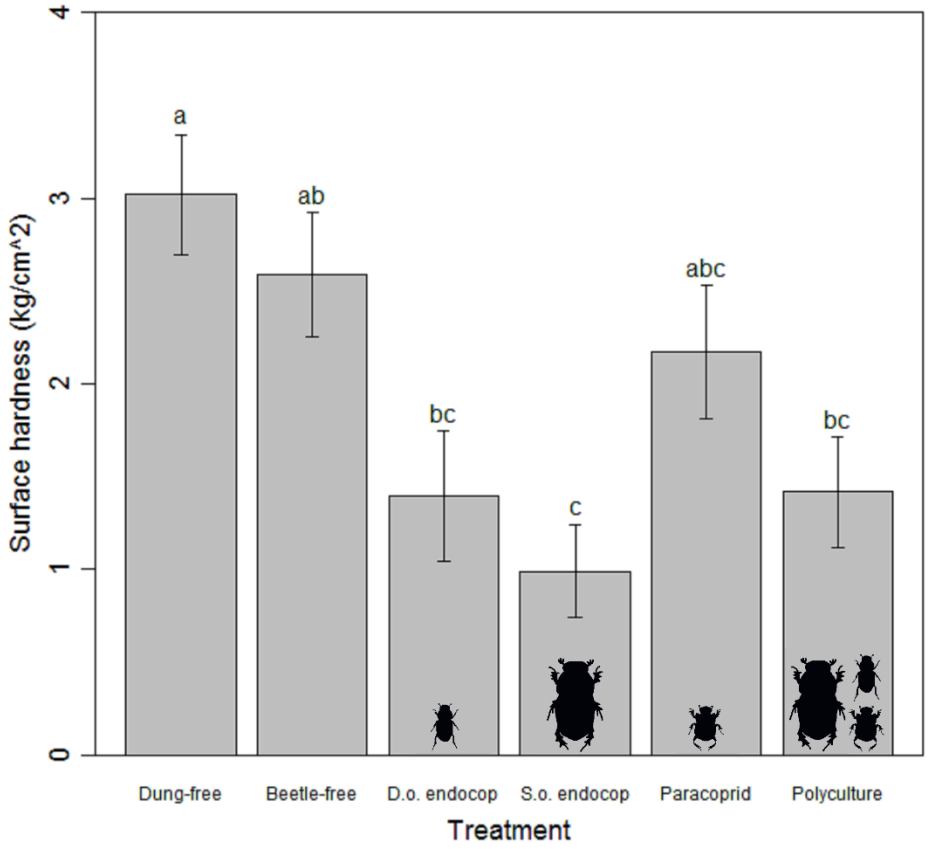

Figure 2.5. Effect of dung beetle assemblage on surface soil compaction. Bars are estimated means with standard error. *X*-axis labels refer to dung-free control, beetle-free dung, dung-ovipositing endocoprid (*A. ater*), soil-ovipositing endocoprid (*A. fossor*), paracoprid (*O. joannae)*, and the polyculture.

Discussion

Multifunctionality

Our results reveal that the functional importance of a dung beetle species (representing a particular functional group) depends on the function or service measured. No single-species assemblage was the most efficient for all services measured, suggesting that low-diversity assemblages are unlikely to maximise functioning across a range of ecosystem functions and services. Importantly, for each of the services considered here, the multi-species treatment (where three species were present, each at one-third of the biomass present in monocultures) performed as well as the most functionally efficient monoculture. This adds to evidence demonstrating that the importance of diversity to ecosystem functions and services may become more apparent when multiple ecosystem functions or services are considered (Hector and Bagchi, 2007; Byrnes *et al.*, 2014).

Although these results highlight the importance of functional group diversity in supporting multiple ecosystem services, it is worth noting that using a single species to represent an entire functional group might not be fully representative of a functional group's more general importance. This is due to significant variation in functioning amongst species within a functional group. For example, large-bodied paracoprid dung beetles (Scarabaeoidea: Geotrupidae) are often the most important in terms of functioning in temperate European ecosystems (Rosenlew and Roslin, 2008; Kaartinen *et al.*, 2013). While the benefits of functionally diverse assemblages are evident from our data, our experimental design confounds functional identity with species-specific attributes. Repeating this experiment with a greater number of species (including those

which are functionally dominant), would be a useful next step to better understand the importance of functionally diverse dung beetle communities in providing multiple ecosystem services.

Dung removal

Evaporation of water from dung pats played a strong role in explaining changes in dung mass (Figure 2.2), where levels of dung removal by the beetles were modest and only the *A. fossor* monoculture removed significantly more dung than the beetle-free control. The 6-week period of the experiment was atypically warm (mean high daily temperature 22 °C) and dry (42 mm of precipitation), which may have reduced dung beetle activity. *Aphodius fossor* is sensitive to exposed and dry conditions (Vessby, 2001) and it is likely that other closely related species will have similar responses. Furthermore, as enclosures were open-bottomed, dung beetle mediated dung removal may have been masked by earthworms, which are known to contribute to the service of dung removal but can interact with dung beetles to reduce net dung removal (O'Hea *et al*. 2010). The more pronounced treatment effects observed for the other functions suggests that dung removal, although the most easily and widely-utilised metric of dung beetle functioning, may not be the most sensitive or representative measure.

Positive effects of dung beetles on soil feeding activity

While dung is an important source of plant nutrients, it is a hypoxic environment (Holter and Spangenberg, 1997) which may influence soil invertebrate activity. To our knowledge, only one other study (Tixier *et al.*, 2015) has examined the link between dung beetles and plant litter decomposition. We found that the presence of dung stimulated soil fauna feeding activity, particularly in the upper soil horizon, suggesting

that any detrimental effects on soil fauna, (e.g. hypoxia), are outweighed by positive effects (e.g. additional resources). Higher rates of feeding activity could result from increased soil moisture under dung pats, as soil moistures has been shown to correlate with higher feeding activity (Simpson *et al.*, 2012). However, no significant differences in soil moisture were detected among treatments ($F_{5,30}$ =0.984, *P*=0.44). Instead, higher feeding activity may have been stimulated by increased recruitment of soil fauna from surrounding soil, perhaps via olfactory cues. The endogeic earthworm species *Allolobophora chlorotica* Savigny and *Lumbricus rubellus* Hoffmeister were regularly found feeding on bait lamina strips. Both species are known to congregate under cattle dung to feed and reproduce (Hendriksen, 1991). Microbial biomass leaching from the dung pat into the soil is a possible alternative explanation for enhanced feeding activity, although this is unlikely, as a laboratory study found that soil mesofauna removed twice as much bait as microbes alone, and that a significantly longer period of exposure was required for appreciable bait removal by microbes (Helling *et al.*, 1998).

At depths of 6-8cm, feeding activity in assemblages containing *O. joannae* was double that observed in other treatments (Figure 2.4), further supporting that paracoprid dung beetles may play an important role in stimulating nutrient cycling (Tixier *et al*., 2015). We hypothesise the increase of feeding activity at lower soil horizons (Figure 2.4) is caused by localised moisture, which prompts invertebrates from the surrounding soil to aggregate. Despite having a positive influence on soil fauna feeding activity, small-bodied paracoprid species contribute relatively little to dung removal in comparison to endocoprid dung beetles (Beynon *et al*., 2012; Nervo *et al*., 2014). Again, our results suggest that measures of dung removal may not adequately reflect species

contributions to other important ecosystem functions and services. Small-bodied paracoprid species have become locally and regionally extinct in parts of Europe in response to landscape fragmentation and agricultural intensification (Bistrom *et al.*, 1991; Roslin *et al.*, 2009). They also occur at low levels of abundance and richness and have are highly sensitive to the non-target effects of veterinary medicines (Beynon *et al*., 2012). Furthermore, the toxic effects of these veterinary medicines persist for longer in the absence of sunlight (Dubroca *et al.*, 2003), and paracoprid larvae feeding on buried dung may be exposed to particularly high concentrations of these chemicals. Thus, it is possible that an important ecosystem service provided by this component of the dung invertebrate community could be reduced or lost via agricultural intensification.

Reduction of surface soil compaction

Through measuring soil penetrability, we found that the soil ovipositing endocoprid *A. fossor* significantly reduced surface soil compaction in comparison to all treatments except the three species polyculture, and the monoculture of *A. ater*. While penetrability will vary widely amongst different soil types and management, our enclosures were placed within a small spatial scale. Therefore, the use of penetrability as a surrogate measure for compaction provides an insight into how the action of dung beetles may alleviate soil compaction. The efficacy of *A. fossor* in reducing soil compaction likely stems from the activity of both adult and larval beetles, which consume dung at the soil surface (Owen *et al.*, 2006). As *A. fossor* is an abundant and widespread species across unimproved and intensively-managed pastures (Hutton and Giller, 2003), soil aeration provided by *A. fossor* is likely an ecosystem service provided across many agricultural landscapes.

We found that *O. joannae* did not significantly reduce soil compaction when compared to a beetle-free control. This is consistent with previous work by Bang *et al*. (2005), who found that while larger bodied paracoprid beetles significantly improved soil permeability, smaller paracoprid beetles did not. The species of dung beetles we included are active only at shallow soil depths, where compaction tends to be quickly remediated by activity of soil fauna. However, we found the levels of functioning provided by *A fossor* to be greater than the contributions by background levels of colonising soil fauna (Figure 2.5), suggesting that dung beetles could play an important supplementary role in improving surface soil permeability. As the association between compacted soil and reduced forage yield is well established (e.g., Douglas and Crawford, 1991; O'Neil and Carrow, 1983), benefits of alleviated soil compaction mediated by dung-beetles merit further study. Furthermore, as large-bodied paracoprid dung beetles (e.g. Scarabaeoidea: Geotrupidae) excavate tunnels which may extend to depths reaching metres, these dung beetles may also play an important role in remediating compaction at lower horizons, where mechanical interventions are ineffective (Håkansson and Reeder, 1994).

Due to logistical constraints associated with our experimental design (e.g. destructive soil sampling and anticipated conflict with livestock), we were unable to determine the number of larvae within each enclosure. As endocoprid larvae provide a significant service of dung removal, and presumably other associated functions (Hanski and Cambefort, 1991), any species-specific variation in the number of eggs laid, and energetic requirements to develop from larva to pupa, may have complicated our efforts

to standardise dung beetle biomass across enclosures. Body size is also likely to be an important predictor of functioning. For example, in our experiment, smaller-bodied species such as *O. joannae* may not have been physically large enough to influence soil parameters, whereas larger bodied species, such as *A. fossor*, would potentially have been physically large enough to influence soil parameters and additionally have required greater energetic intake (increased dung removal) for development. Size differences are therefore likely to affect functioning at both larval and adult life-stages, and approaches which take this into consideration (e.g., Kaartinen *et al*., 2013, Nervo *et al*., 2014) are a useful step in achieving a better understanding of how functional identity influences ecosystem services provided by dung beetles.

Finally, we suggest that the entire suite of ecosystem services provided by invertebrate communities should be further recognised and valued as a complement to farm management. For example, while various mechanised solutions have been proposed to reduce soil compaction, benefits from these interventions are often short-lived (Curran Cournane *et al.*, 2011) highlighting the importance of regular biogenic aeration provided by dung beetles and other soil fauna. The role of enhanced nutrient cycling by soil communities will become increasingly important in pasture systems as the price of nitrogen fertiliser continues to rise (Van Grinsven *et al.*, 2013), and farming in many areas shifts towards organic production (Paull, 2011). However, farm management could also have the unintentional consequence of undermining ecosystem services provided by invertebrates. For example, chain harrowing pasture to speed the physical degradation of cattle dung (Weeda, 1967) seems likely to further compact soil, and physical disturbance of dung pats could have unintended negative effects on dung

beetle populations, compromising dung removal services. Experimental approaches that consider the multiple roles invertebrates play in providing ecosystem services are an important step towards recognising the value of invertebrate diversity in supporting productive and sustainable agricultural systems.

Chapter 3: Limited functional benefits achieved by conserving species rich north temperate dung beetle assemblages

Paul Manning[1,2], Eleanor M. Slade[1,3], Sarah A. Beynon[1,2], Owen T. Lewis[1]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

3. Lancaster Environment Centre, Lancaster University, Balrigg, Lancaster LA1 4YQ, UK

Author Contributions: PM, EMS, SAB and OTL designed the experiment. PM set-up the experiment, collected all the data, performed all analyses, and wrote the manuscript. EMS, SAB, and OTL provided multiple rounds of feedback on earlier versions of the manuscript.

Chapter Status: Submitted for publication

Abstract:

The relationship between biodiversity and ecosystem functioning is typically positive but saturating, suggesting functional redundancy often exists within ecological communities. However, theory predicts that perceived redundancy may be reduced or removed when systems are perturbed, or when multifunctionality (the simultaneous delivery of multiple functions) is considered. We investigated four ecosystem functions mediated by dung beetles with applied relevance for pasture-based livestock production: dung removal, primary productivity, soil faunal feeding activity, and reductions in soil bulk density. We used manipulative experiments to test whether higher levels of dung beetle species richness enhanced individual functions and multifunctionality, and whether these relationships were influenced by perturbation (in this case, non-target exposure to the veterinary anthelmintic ivermectin). For individual functions, perturbation had limited effects on functioning, with only dung removal significantly (negatively) affected. Species richness did not, on its own, explain variation in the delivery of individual functions. However, in the case of primary productivity, there was a significant interaction between richness and perturbation. At high species richness forage growth was enhanced in the control compared to the perturbed treatment. Using a composite 'multifunctionality index', we found that while species-rich dung beetle communities delivered marginally higher levels of multifunctionality in unperturbed conditions, this benefit was lost under perturbation. However, using a recently developed, robust method of assessing diversity-multifunctionality relationships across a range of thresholds, we found no significant benefit for higher species richness in underpinning multifunctionality. Our work provides some evidence that conserving species-rich dung beetle assemblages can support marginal benefits for ecosystem functioning, and suggests that benefits may be lost when systems are perturbed.

Introduction

Human activities alter the composition of communities by causing extinctions at local, regional, and global scales. These extinctions can have consequences for ecosystem functions; the biological, geochemical, and physical processes occurring within an ecosystem (de Groot *et al.*, 2002). Concern about these losses has prompted a wealth of research exploring the relationship between biodiversity and ecosystem functioning, with widespread evidence suggesting that more diverse communities deliver greater levels of ecosystem functioning when compared to less diverse communities (Hooper *et al.*, 2005). However, benefits of higher biodiversity are frequently saturating: Maximal functioning can be supported by a small proportion of the overall community (Perkins *et al.*, 2010). This suggests that from a purely functional perspective, some species within ecosystems can be considered 'functionally redundant'.

This notion of functional redundancy becomes weaker when approached from an applied perspective. Species exhibit differing sensitivities and responses to disturbance, meaning that the value of higher biodiversity in maintaining functioning may become more apparent when systems are perturbed (Hooper *et al.,* 2005). This phenomenon has been demonstrated experimentally in a wide variety of contexts, including chemical perturbation of decomposition facilitated by dung beetles (Beynon *et al.*, 2012), temperature perturbation of processes mediated by benthic invertebrates (Perkins *et al.*, 2014), and salinity and temperature perturbations of biomass production in algal communities (Steudel *et al.*, 2012).

Perceptions of widespread functional redundancy are further questioned when one considers that ecosystems typically provide numerous beneficial functions. When biodiversity-ecosystem functioning studies focus on single functional measures, the true value of biodiversity may be underestimated or even overlooked (Lefcheck *et al.*, 2015). We can build a stronger understanding of the importance of biodiversity using approaches that consider the simultaneous delivery of multiple ecosystem functions, a concept known as ecosystem multifunctionality (Hector and Bagchi, 2007).

While the value of biodiversity may become more evident when systems are perturbed, or when multifunctionality is considered, few studies have examined these questions simultaneously (but see Perkins *et al*., 2014). Understanding the consequences of species loss and perturbation for ecosystem multifunctionality is important in the context of rising extinction rates (Barnosky *et al.*, 2011), intensifying land use (Foley *et al.*, 2005), and a changing climate (Meehl and Tebaldi, 2004).

Biodiversity-multifunctionality relationships have particular applied relevance within agroecosystems. Agriculture represents approximately half of global land use (Lambin and Meyfroidt, 2011) and widespread intensification during the last century has resulted in considerable losses of farmland biodiversity (Robinson and Sutherland, 2002). Many taxa in decline play roles in providing functions which underpin agricultural production. For example, farmland birds (Donald *et al.*, 2001) and wild bees (Ollerton *et al.*, 2014) can support pest control and pollination, respectively. From an ecological perspective, elements of agricultural management (e.g. intensive soil cultivation, application of an insecticide, or the use of a synthetic fertiliser) can be considered as perturbations with the potential to disrupt biodiversity-multifunctionality relationships.

Dung beetles (Coleoptera: Scarabaeiodea) occurring in pastures provide an excellent model system for understanding how perturbation influences biodiversity-multifunctionality relationships within agroecosystems. Most dung beetle species feed on animal dung as larvae and adults, and play a key role in mediating dung removal (Menéndez *et al.*, 2016) through burying, directly consuming, and fragmenting livestock dung (Doube, 1990). Alongside the direct benefit of reducing fouling of pasture surfaces, dung beetle activity has numerous benefits for agroecosystems including: enhancing nutrient cycling (Yokoyama *et al.*, 1991), improving soil hydrological properties (Brown *et al.*, 2010), stimulating litter decomposition (Tixier *et al.*, 2015; Chapter 2), and reducing greenhouse gas emissions (Slade *et al.*, 2016). Dung beetles occurring in agricultural landscapes are sensitive to changes in land use intensity (Lobo, 2001; Hutton and Giller, 2003), with a perturbation of particular concern being the non-target consequences of veterinary anthelmintics entering the environment via livestock dung (Floate *et al.*, 2005). Many of these chemicals, which are used to control internal parasites of livestock, can have lethal and sublethal effects on dung beetles (O'Hea *et al.*, 2010b; Beynon *et al.*, 2012a; Verdú *et al.*, 2015), and some have been shown to impede dung decomposition (Wall and Strong, 1987).

Here, we investigate how perturbations influence biodiversity-ecosystem functioning relationships using manipulative experiments of dung beetle species richness in combination with a perturbation of ivermectin, a widely used veterinary anthelmintic. We measured four functions with applied relevance for pasture ecosystems: dung removal, primary productivity, feeding activity of soil fauna, and reduction of soil compaction. We predicted that increased biodiversity (higher species richness) would enhance individual

functions and ecosystem multifunctionality. As ivermectin has well-documented and species-specific negative effects on dung beetle survival and functioning (Beynon *et al.*, 2012a), we predicted that beetle survival, individual functions, and multifunctionality would be reduced by exposing dung beetles to ivermectin.

Methods

Experimental design

To manipulate dung beetle communities, we introduced known combinations of dung beetles into enclosures constructed by digging 14 L, baseless, black, plastic buckets (N=72) into the ground to a depth of 8 cm as described previously (Chapter 2). The experiment was conducted on a grassy margin in a courtyard at the John Krebs Field Station, Wytham, Oxfordshire, UK. Enclosures were separated by 1 m, and arranged into three parallel rows of 24, each row separated by *c.* 1 m. Enclosures were covered by a fine (2 mm aperture) mesh and secured with elastic.

We collected dung on June 30th 2015 from beef cattle under organic management at Wytham Farm, adjacent to the experimental site. Dung was collected immediately following defecation before invertebrates could colonise, and was thoroughly homogenised in a large container using a trowel. A standardised quantity of dung (550 g wet mass), which was within the size range of pats observed in the field, was used to form a pat within each enclosure. As livestock-administered ivermectin undergoes negligible metabolism (González Canga *et al.*, 2009), and we aimed to minimise differences in dung quality among replicates, ivermectin was added directly to dung using a standardised protocol described by Römbke *et al.* (2009). For the perturbation treatment, a 1.00 mL aliquot of a 275 ppm solution of ivermectin (5mg / mL

Molemec pour-on, Mole Valley Farmers, South Molton, Devon EX36 3LH, UK) in acetone was added to each dung pat. This resulted in a final ivermectin concentration of 0.5 ppm (wet weight), consistent with peak faecal concentrations following subcutaneous injection, and *c*. 50% of peak faecal concentrations following use of pour on formulations (Sommer *et al.*, 1992). In ivermectin-free controls, we used 1 mL aliquots of pure acetone in the place of the ivermectin solution (Römbke *et al.*, 2009). Each aliquot was thoroughly mixed through the dung, and dung pats were formed using a 12 cm diameter circular mould on a 2 cm aperture wire mesh which later facilitated separation of the dung pat from underlying herbage and soil. The experiment began when beetles were added 24 hours later, after allowing for evaporation of the acetone solvent (Römbke *et al.*, 2009).

Four native dung beetle species from the genus *Aphodius* were used in the experiment. Beetles were hand-collected from organically managed pastures in the vicinity of Oxford. All species had been observed in the pastures several weeks before collection, and were assumed to be sexually mature upon collection. Beetles were stored in a well-ventilated shed, separated into single-species, mixed-sex terraria and were fed anthelmintic-free cattle dung prior to beginning the experiment.

Beetle communities were standardised using dry mass (Beynon *et al.*, 2012a), where a target mass of 102 ± 0.5 mg of dung beetles was added to each dung pat (Table 2.1). All possible combinations of the four species were included, to generate Species Richness Levels (SRL) ranging from 1 to 4 (Table 3.1). Functioning observed during the experiment was mediated both by beetles introduced to the enclosures, any offspring they produced, and background levels of functioning provided by soil fauna. Because

our protocol for standardising communities was based on mass, our two heaviest species (*Aphodius fossor* L. and *Aphodius rufipes* L.) were each represented by a single individual at species richness levels 3 and 4. Since female beetles are likely to contribute more to functioning than males through the activity of their offspring, we used female *A. fossor* and *A. rufipes* in the SRL3 treatment, and males in the SRL4 treatment, to ensure that our tests of the effects of species richness on levels of functioning were conservative.

Table 3.1. Composition of beetle communities used within the experiment. All possible combinations of four species were used. Integer table values are the abundance of individuals of the corresponding species included within each replicate. Beetle communities are standardised based on mass. Shaded columns are used to separate the different assemblages based on their Species Richness Levels (SRL), which range from 1 to 4. Composition of assemblages can be determined by taking the sum of a column.

Species	Functional Group	Individual dry mass (mg)	SRL1				SRL2						SRL3				SRL4
***Aphodius ater* (De Geer)**	Dung-ovipositing endocoprid	5.4	18	-	-	-	9	9	10	-	-	-	8	7	6	-	4
***Aphodius fossor* (L).**	Soil ovipositing endocoprid	26.1	-	4	-	-	2	-	-	2	2	-	1	1	-	1	1
***Aphodius rufipes* (L).**	Soil-ovipositing endocoprid /Paracoprid	29.9	-	-	4	-	-	2	-	2	-	2	1	-	1	1	1
***Aphodius erraticus* (L).**	Paracoprid	9.0	-	-	-	11	-	-	5	-	5	5	-	4	5	5	3

Each combination of species was replicated twice, with the exception of the four-species community (Table 3.1, SRL4), which was replicated four times to generate a more balanced number of replicates at each level of species richness. For comparison, beetle-free controls (n = 4) were included to determine background levels of functioning provided by soil fauna. As the experiment was conducted under perturbed (dung

containing ivermectin) and control (dung without ivermectin) conditions, there was a grand total of 72 enclosures, 64 of which included dung beetles.

To estimate dung mass loss due to evaporation, we formed six additional reference dung pats from which all invertebrates were excluded: three with ivermectin and three without ivermectin, using the methods described above. These were placed on 2 cm aperture wire mesh, within 5 L plastic buckets (with the bottoms intact), filled with 20 cm of sandy loam soil. Buckets were covered with a 2 mm aperture mesh, as before, and placed adjacent to the main experiment.

The species included in our experiment spend varying periods in dung pats as adults (Holter, 1982), thus we monitored enclosures daily, attaching emergence traps when adult beetles were first observed walking or flying within enclosures during the day, night or crepuscular periods (Day 14). While the period that enclosures were sealed was longer than the periods reported in the literature for similar experiments (Holter, 1982), we assumed that our observations of above-ground beetle activity corresponded to the time when beetles would naturally emigrate to new dung pats. We fitted emergence traps to each enclosure using a short length of 2 cm clear plastic tubing. Emergence traps consisted of 750 mL translucent plastic containers filled with a 2 cm layer of sieved soil, and 50 g of fresh cattle dung. On Day 18, we added another 100 g of fresh cattle dung to each emergence trap to enhance attractiveness. On Day 21, the soil and dung in each emergence trap were hand-searched for beetles, before submerging the trap contents in water to remove additional beetles by flotation. Any beetles that did not emerge were assumed to have died in the enclosure, and we used the number of emigrated beetles as a proxy for adult survival. The experiment continued

uninterrupted until Day 42 (August 13th, 2015). At this point, beetle larvae were assumed to have pupated, concluding their feeding within the dung pat (Stevenson and Dindal, 1985).

Measurement of ecosystem functions

To measure feeding activity of soil invertebrates, we used the bait lamina test (Terra Protecta GMbH, Berlin, Germany). Bait lamina are PVC strips (1 mm x 6 mm x 120 mm) with 16 perforations (1.5 mm diameter) at 5 mm intervals. These perforations were filled with a standardised bait of cellulose powder, ground wheat bran, and activated charcoal (70:27:3). Following previous methods (Chapter 2), on Day 42, we inserted five bait lamina through each dung pat to a consistent depth so that the uppermost hole was located immediately below the soil surface. Test strips were placed alongside the experiment and a subset was checked daily until appreciable (30 - 50 %) feeding activity was observed. In our experiment, this occurred at seven days of exposure. Bait lamina were therefore removed from the ground on Day 49 (after seven days of exposure) and feeding activity was assessed by viewing strips against a light source, where each bait was classified as being ‘consumed’ or ‘intact’ depending on whether light penetrated through it or not (Kratz, 1998).

Remaining functions were measured on Day 50 (August 21st 2015). We removed dung pats and underlying wire mesh from the enclosures and took soil measures from the area directly under the former location of the dung pat. We measured soil compaction by taking a measure of bulk density. We hammered a segment of 5.5 cm diameter PVC pipe into the soil to a depth of 5 cm and carefully removed the intact sample with a trowel. Soil and dung samples were dried for 48 hours at 80°C. Dung removal (dry

mass) was measured to the nearest gram. Measurements were corrected by subtracting this figure from the mean dry mass of reference dung pats from which dung beetles and other soil fauna had been excluded. Soil bulk density (g / cm^3) was calculated as the dry mass of soil solids divided by the core volume.

We measured primary productivity by planting ryegrass (*Lolium perenne* L.) seeds into soils sampled from experimental enclosures (Slade and Roslin, 2016). Two additional 5.5 cm diameter x 5 cm deep soil samples were taken from the area beneath each dung pat. The soil samples were homogenised and sieved. A 185 mL sample of the soil from each enclosure was added to a 250 mL capacity plant pot and 1 mL of tetraploid perennial ryegrass seed (Cotswold Seeds, *Moreton-in-Marsh,* Gloucestershire, GL56 0JQ, UK) was scattered evenly across the pots surface. Seeds were then covered with an evenly-spread 15 mL soil sample from the same enclosure. All pots were placed in an unheated greenhouse, and watered regularly. After three weeks, we harvested all aboveground biomass by trimming the grass at the soil surface. We dried samples at 75 ºC for 48 hours (Zhao *et al.*, 2013) and weighed above ground biomass with a balance accurate to 0.01 g.

Analysis

We used linear models with Gaussian error structures (ANCOVA) to investigate the effects of species richness and perturbation for each ecosystem function. Examination of diagnostic plots demonstrated that residuals were homogenous and normally distributed, and thus all values were left untransformed. We fitted maximal models and dropped interactions until the minimum adequate model was obtained, selecting the

model with the smallest Akaike's Information Criterion (AIC) value: a measure of the quality of a statistical model, relative to a second.

To explore relationships between species richness and multifunctionality, we first employed the averaging approach to multifunctionality (Hooper and Vitousek, 1998). The averaging approach creates a 'multifunctional index' for each experimental unit by taking a mean estimate of all functions after standardising to a common scale. To standardise functions, the 'desirable' direction of each function must be consistent across measures. We considered higher values of dung removal, soil fauna feeding activity, and primary productivity to be beneficial, and hence desirable. As compact soils are known to limit plant growth, we also considered a lower bulk density to be more desirable. We reflected our values for soil bulk density (multiplied each observation by -1), and added the un-reflected maximum to each observation so that the lowest level of soil compaction was 0. We then standardised our functions to a common scale by expressing each function as a proportion of the maximum. Each maximum was defined using a mean of the three highest observations from all enclosures containing beetles. The multifunctionality index value was calculated by taking the mean of the four standardised functions. We used a linear ANCOVA model, as described above, for single functions in order to investigate the effects of perturbation and species richness on average multifunctionality.

While the averaging approach to multifunctionality is readily interpreted, a limitation of this method is that variability in responses amongst functions is not discernible (Byrnes *et al.*, 2014). For example, a strong negative relationship between biodiversity and functioning for a single process could obscure modest benefits of greater levels of

biodiversity in supporting all other functions. Thus, we also used the 'Exceeding Thresholds Approach' (Byrnes *et al.*, 2014) to explore how perturbation influences the relationship between species richness and ecosystem multifunctionality.

The Exceeding Thresholds Approach works by estimating a linear relationship between species richness, and the number of processes performing at or above a given threshold. The value of each threshold is calculated as a proportion of the function's maximum (as above, defined as the mean of the three highest observations). For example, if each function had a maximum value equal to 1, at a given threshold (e.g. 65%), we then determined how many of the measured functions (0 – 4) were equal to or exceeded the corresponding threshold value (0.65) for each replicate. As species richness within enclosures was experimentally manipulated, we could fit a linear model with a quasi-binomial error structure to estimate the relationship between species richness (1 – 4) and the number of functions (0 – 4) exceeding each threshold. For each threshold, the slope estimate represents the number of functions supported by the addition of a single species. This slope was calculated for all possible thresholds, in our case whole number increments between 1 % and 72 %. Above this upper threshold our models would not converge.

To visualise the relationship between species richness and multifunctionality, these slopes and their 95 % confidence intervals were plotted across a range of thresholds. Four metrics derived from these curves have been identified as being particularly informative in making comparisons amongst treatments (Byrnes *et al.*, 2014): 1) The minimum threshold (T_{min}), where multifunctionality is first significantly influenced by a change in species richness; 2) The maximum threshold (T_{max}), beyond which the

relationship between species richness and functioning first declines to be non-significantly different from zero; 3) The threshold of maximum diversity effect (T_{mde}), the threshold where species richness has its strongest positive or negative effect on functioning; and 4) The realised maximum effect of diversity (R_{mde}), the slope at T_{mde} which indicates the maximum observed effect of species richness on the number of functions surpassing the threshold. Low values of T_{min} and high values of T_{max}, T_{mde}, and R_{mde} indicate that species richness is a strong driver of multifunctionality (Byrnes *et al.*, 2014).

Finally, we tested the influence of ivermectin on beetle emigration (a proxy of survival) using generalised linear models with binomial errors. Emigration was calculated as the proportion of adult beetles entering the emergence traps. Where data were over-dispersed, a quasi-binomial error structure was used.

The statistical computing environment R 3.1.1(R Core Team, 2014) was used for all analyses. The multifunc package (Byrnes *et al.*, 2014) was used for analysis of multifunctionality data. Figures were created using the ggplot2 package (Wickham, 2009).

Results

Effects of species richness and perturbation on single functions and dung beetle survival

Perturbation significantly reduced dung removal (Figure 3.1a, Table 3.2) by approximately 17 % (Perturbed 27.5 ± 1.8 g, Control 32.1 ± 0.9 g, mean ± se). Species richness did not have a significant effect on dung removal (Table 3.2).

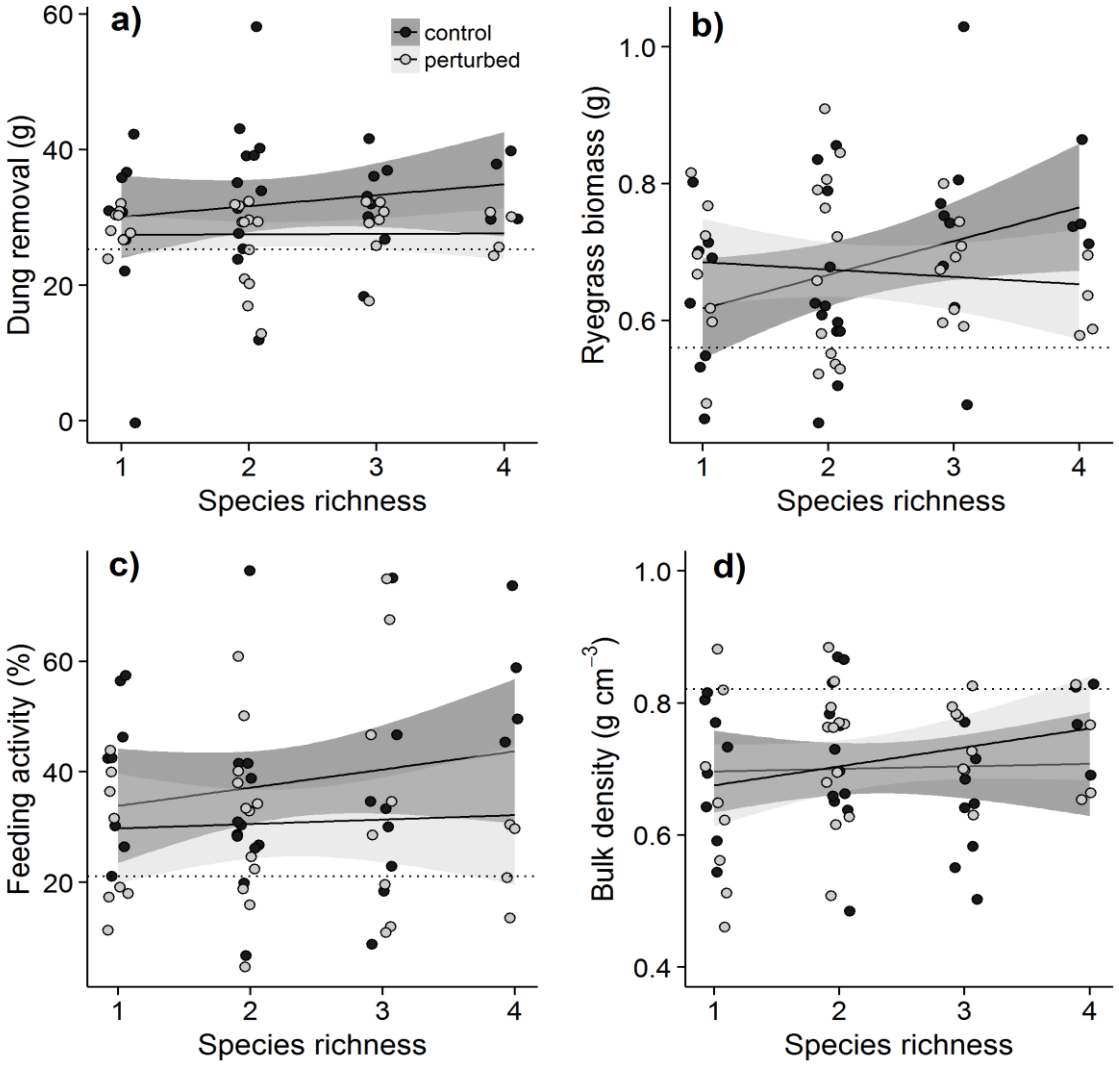

Figure 3.1. Individual ecosystem functions in response to perturbation and species richness levels: dung removal (a), forage growth (b), soil fauna feeding activity (c), soil bulk density (d). Shaded areas indicate 95% confidence intervals for the slopes. The dotted line represents the mean of beetle-free controls (enclosures solely containing dung), that show background levels of functioning provided by soil fauna.

Table 3.2. Results of analyses testing the effects of perturbation and species richness on individual functions and multifunctionality.

Term	F	*P*
a) Dung removal		
Perturbation	$F_{1,61}$=5.161	**0.027**
Species richness	$F_{1,61}$=0.643	0.426
b) Soil fauna activity		
Perturbation	$F_{1,61}$=2.857	0.357
Species richness	$F_{1,61}$=0.860	0.096
c) Bulk density		
Perturbation	$F_{1,61}$=1.430	0.237
Species richness	$F_{1,61}$=0.152	0.698
d) Primary productivity		
Perturbation	$F_{1,60}$=0.063	0.802
Species richness	$F_{1,60}$=1.649	0.204
Perturbation*Species richness	$F_{1,60}$=4.008	**0.050**
e) Multifunctionality		
Perturbation	$F_{1,60}$=5.688	**0.021**
Species richness	$F_{1,60}$=0.259	0.613
Perturbation*Species richness	$F_{1,60}$=3.849	0.054

For primary productivity, we found a marginally significant interaction between perturbation and species richness (Figure 3.1b, Table 3.2). In the absence of perturbation, primary productivity tended to be higher in more species-rich assemblages. Neither soil fauna feeding activity (Figure 3.1c), nor soil bulk density (Figure 3.1d) were significantly influenced by the presence of a perturbation or by species richness (Table 3.2).

Ivermectin significantly reduced adult emigration (a proxy for survival) for all four species (Figure 3.2), lowering the mean emigration of *A. ater* by approximately 15%, *A. erraticus* L. by 49%, *A. fossor* by 60%, and *A. rufipes* by 13%, relative to unperturbed controls.

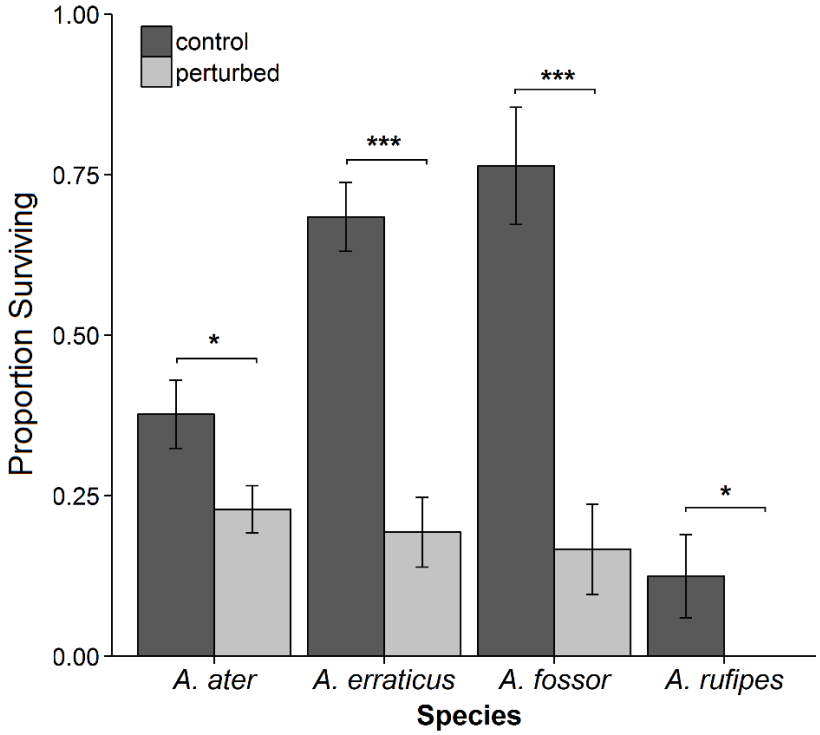

Figure 3.2. Estimated survival of different species in response to perturbation from ivermectin, based on recovery of adult beetles. Bars show means with standard errors. No *A. rufipes* were recovered in ivermectin treatment. Comparisons made between perturbation levels within species, with asterisks representing statistically significant differences in recovery.
* $P \leq 0.05$, *** $P \leq 0.001$

Effects of species richness and perturbation on average multifunctionality

We found weak evidence of an interaction between perturbation and species richness (Table 3.2) indicating that species rich communities tend to provide higher levels of average multifunctionality than species poor communities, but only under unperturbed

conditions (Figure 3.3). Although non-significant at conventional levels of statistical significance, the interaction was retained within our model because the main effects on their own explained little variation in average multifunctionality. At the lowest levels of species richness, perturbation with ivermectin did not affect average multifunctionality (Perturbed 0.57 ± 0.03, Control 0.57 ± 0.01, mean ± se). However, average multifunctionality was *c.* 30 % higher in control systems than in perturbed systems at the highest level of species richness (Perturbed = 0.49 ± 0.03, Control 0.64 ± 0.04). While perturbation significantly affected average multifunctionality (Table 3.2), effects were small when considering perturbation in isolation: Control systems had a mean multifunctionality index *c.* 10 % greater than perturbed systems (Perturbed 0.533 ± 0.016, Control 0.590 ± 0.018, mean ± se). We found no significant main effect of species richness on average multifunctionality (Table 3.2).

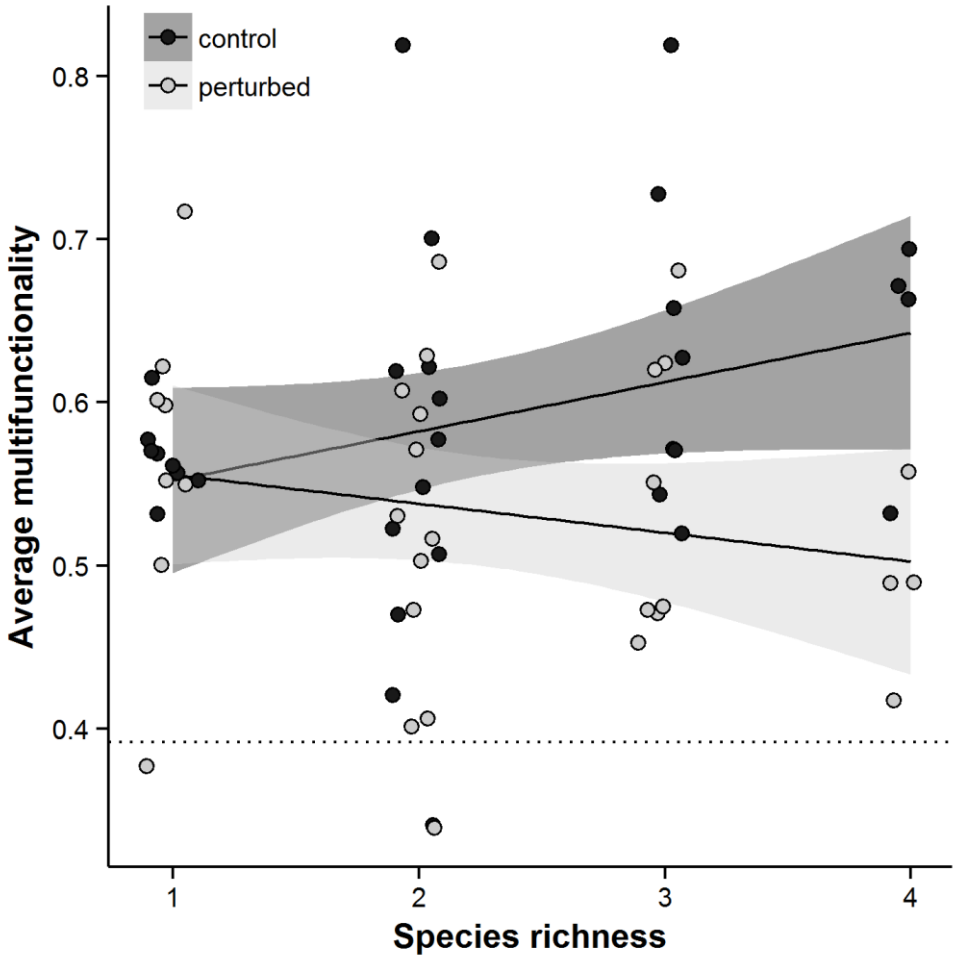

Figure 3.3. Average multifunctionality as a function of species richness and perturbation level. Shaded areas indicate 95% confidence intervals for the slopes. The dotted line is the mean of beetle-free controls.

Exceeding thresholds approach

When using the exceeding thresholds approach to test the effect of species richness in supporting multifunctionality across a range of thresholds, we found that, in the controls, species rich communities seemed to support higher levels of functioning than species-poor communities, but the benefits were not statistically significant (Figure 3.4a). Conversely, we found a generally negative (but never significantly so) relationship

between species richness and ecosystem multifunctionality in perturbed communities when moving from lower to higher thresholds (Figure 3.4b). As our model estimates were at no point significantly different from zero, no meaningful indices could be derived and hence are not reported.

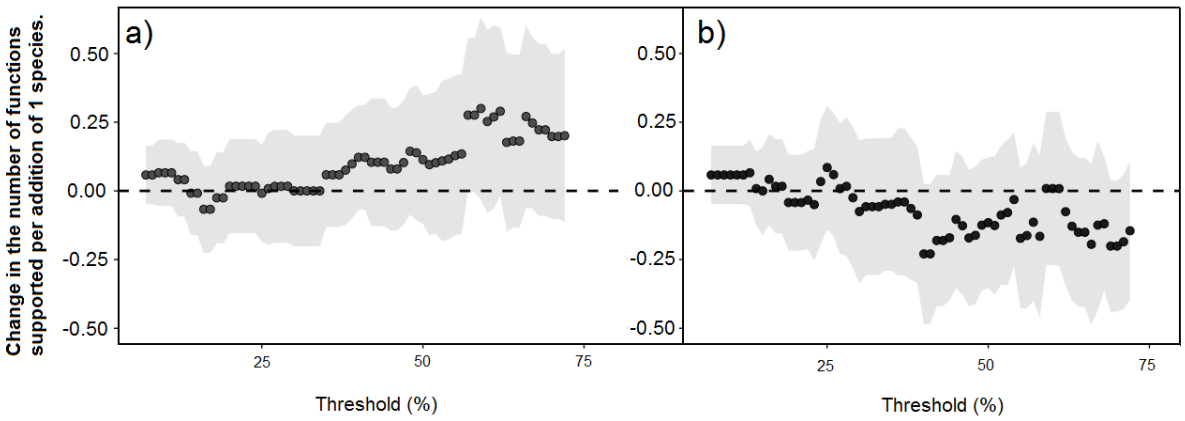

Figure 3.4. Relationships between species richness and multifunctionality in control (a) and perturbed (b) systems at thresholds from 1-72% of observed maxima. Confidence intervals (95%) around the estimated slopes (filled data points) indicate whether the intervals contain zero, providing a test of the threshold values at which diversity has no effect on multifunctionality.

Discussion

Higher levels of species richness may support multifunctionality, but benefits are context dependent

We found weak evidence that species rich dung beetle assemblages supported slightly higher levels of ecosystem multifunctionality than species poor communities (Figures 3.3, 3.4a). This means that taking measures to enhance the number of dung beetle species in agroecosystems could a role in supporting more productive pasture-based livestock production. However, these modest benefits were lost when the system was

perturbed with ivermectin (Figures 3.3, 3.4b). This suggests the benefits of maintaining dung beetle diversity, can be lost to routine management practices.

In the absence of perturbation, a positive correlation between ecosystem multifunctionality and species richness is expected based on both theory (Hooper *et al.*, 2005) and experimental evidence (Maestre *et al.*, 2012; Perkins *et al.*, 2014). As particular feeding traits seem to be closely affiliated with the delivery of specific ecosystem functions (Chapter 2), greater diversity in feeding traits (Table 3.1) is likely driving the increase in multifunctionality at higher species richness levels in the absence of chemical perturbation (Figures 3.3, 3.4a).

The benefit of maintaining high species richness in unperturbed conditions may have been relatively weak due to the fully factorial nature of this experiment. Under the constraints of this design, if a single species showed a disproportionately high role in supporting multiple functions this would undermine the importance of species richness in supporting multifunctionality. Past evidence suggesting the benefit of dung beetle species richness in explaining higher levels of dung removal (Beynon *et al.*, 2012a), could be explained by sampling effects, where functionally dominant species are more likely to be included in assemblages with higher species richness (Wardle, 1999).

A second possibility that explains why higher species richness provided only modest benefits to multifunctionality, stems from the limited range in functional trait diversity within our experiment. While the assemblages used represented a typical dung beetle community in our study area (P. Manning, pers. obs), higher trait variability may be present in less disturbed landscapes (Slade *et al.*, 2011). Where greater variety in

feeding trait diversity is observed (e.g. Palestrini *et al.*, 1998), the relationship between dung beetle species richness and multifunctionality is likely to be stronger than what we observed here (Figures 3.3, 3.4a).

We found the multifunctional benefits of increased species richness seemed to be lost under perturbation (Figures 3.3, 3.4b). The most parsimonious explanation is that the strength of the chemical perturbation pushed the system beyond the buffering capacity provided by higher species richness levels (Steudel *et al.*, 2012; Hofer *et al.*, 2016). Dung beetles in our experiment were exposed to an ivermectin concentration of 500 ppb (wet weight). Sublethal effects of ivermectin exposure have been detected at concentrations as low as 1 ppb (Verdú *et al.*, 2015). A combination of lethal and sublethal effects resulting from ivermectin exposure are the simplest explanation for observed losses of multifunctionality.

The influence of species richness and perturbation for individual functions

While dung removal was significantly reduced by ivermectin, the size of this effect was small (Figure 3.1a). Our results suggest that, despite its prevalence as a proxy for overall functioning (Rosenlew and Roslin, 2008; Beynon *et al.*, 2012a; Kaartinen *et al.*, 2013), this measure may not be sufficiently sensitive to detect more subtle changes in ecosystem functioning. As earthworms and dung beetles may introduce soil into dung (Owen *et al.*, 2006), removal rates may have been underestimated. Measuring organic matter loss (Holter, 1979a) may provide a better understanding of how species richness and perturbation influence dung removal.

We found a significant interaction between species richness and perturbation in explaining primary productivity (Figure 3.1b, Table 3.2). Under control conditions, species rich dung beetle assemblages contributed to higher ryegrass biomass than in low richness communities (Figure 3.1b). A similar benefit of higher species richness was found by Slade and Roslin (2016) who reported that higher levels of species richness supported enhanced primary productivity in experimentally warmed enclosures.

We found no significant effect of perturbation or dung beetle species richness on rates of feeding activity in soil underlying dung pats (Figure 3.1c). The influence of dung beetles on feeding activity or plant litter decomposition might be more strongly affected by tunnelling species (Tixier *et al.*, 2015). While our experiment included two tunnelling (paracoprid) species (*A. erraticus*, and *A. rufipes*), these species excavate relatively shallow burrows, and in the case of *A. rufipes*, show plasticity in reproductive strategies (Table 3.1). The activity of these dung beetles might not therefore sufficiently stimulate soil processes to the same extent as other larger bodied species (Tixier *et al.*, 2015), or deeper tunnelling species (Chapter 2). While we did not find any significant relationships between feeding activity and either species richness or perturbation of the system, the presence of dung beetles appeared to enhance feeding activity relative to beetle-free controls (Figure 3.1c), which may be caused by dung beetles promoting rapid aggregation of earthworms beneath dung pats, as reported by Holter (1979).

We found no significant effect of species richness, or perturbation, on soil bulk density (Figure 3.1d). Soils at our site were highly permeable and the importance of dung beetles in improving soil structure may be greater where soils are more compacted. Few studies have explored the benefits of dung beetles in improving the physical

properties of soils, but available data suggest tunnelling activity can improve soil porosity (Bang *et al.*, 2005; Brown *et al.*, 2010). The same tunnelling activity may also increase the quantities of soil in runoff, which may contribute to local soil erosion (Brown *et al.*, 2010). The species included in our experiment do not tunnel to the same extent as many larger-bodied species, and may thus be less likely to influence physical parameters of soil. Nevertheless, *A. fossor* has been shown to improve soil surface penetrability (Chapter 2).

Limitations of our experimental design

While adults of each species included in this experiment were affected by ivermectin exposure, we were unable to determine when beetles died, or to measure how our experimental perturbation influenced the development and survival of dung beetle larvae. Larvae play a key role in providing dung removal (Holter, 2016), and are more sensitive to perturbation than adult beetles (Beynon *et al.*, 2012a), so it is likely that our estimates of beetle survival did not fully capture the magnitude of the perturbation. A further inevitable limitation of our experiment is that we were able to measure only a subset of possible ecosystem functions. The relatively small benefits of enhanced species richness in supporting multifunctionality may be greater when considering different subsets of candidate ecosystem functions associated with dung beetle activity, which include: parasite suppression (Gregory *et al.*, 2015), and soil microbial respiration (Menéndez *et al.*, 2016).

Because survival in our experiment was variable amongst species, assemblages dominated by species that did not perform well in experimental enclosures (e.g. *A. ater, A. rufipes*) may have added further noise to our data. The benefit of species richness in

underpinning multifunctionality, may therefore be more evident if higher survival was found in the absence of perturbation.

Colonisation by soil fauna may have partly masked the benefits that species rich dung beetle assemblages provide in supporting ecosystem multifunctionality. While dung beetles have been described as key ecosystem providers of ecosystem functions (Nichols *et al.*, 2008), other soil invertebrates have a functional role comparable to dung beetles in supporting dung removal rates (O'Hea *et al.*, 2010a; Kaartinen *et al.*, 2013). Furthermore, when moving beyond dung removal to consider other functions that support production, benefits derived by conserving diversity of a single taxon may be limited in the context of a complex ecosystem.

Implications for agroecosystems and management

Intensive agricultural practices have been linked widely to biodiversity losses in taxa which provide ecosystem functions, including include bees (Holzschuh *et al.*, 2007) and dung beetles (Hutton and Giller, 2003). Efforts to stop, or reverse, these trends have been addressed through the use of agri-environment schemes, with varying levels of success (Wilson *et al.*, 2007; Perkins *et al.*, 2011). While the primary goal of agri-environment schemes is biodiversity conservation, ecosystem functions may also be provided as a further benefit. Our results suggest that maintaining species-rich dung beetle assemblages could have marginal benefits in this second regard.

The four species included in this experiment are widespread and common throughout Europe (Menéndez and Gutiérrez, 1996; Roslin and Koivunen, 2001; Beynon *et al.*, 2012a) and across a range of farming intensities (Hutton and Giller, 2003). This suggests that farms operating with markedly different management strategies could

experience a similar loss of function due to the non-target impacts of livestock parasite control chemicals. Furthermore, when the mid- and long-term consequences for dung beetles are considered (e.g. lower abundances (Hutton and Giller, 2003) and sublethal effects (Verdú *et al.*, 2015)), impacts may be even greater than those we report here, particularly when taking multifunctionality into consideration.

Despite the environmental risks associated with veterinary anthelmintic use, these products remain an important tool for optimising yield and livestock health under many current farming systems (Sanchez *et al.*, 2004; Charlier *et al.*, 2009). Nevertheless, there is scope for reducing non-target effects of anthelmintics on dung beetles through a number of practical management changes which can be promoted via knowledge transfer. For example, selectively treating livestock with known parasite burdens (Vercruysse and Claerebout, 2001), choosing anthelmintic groups that are less toxic to dung beetles (Floate *et al.*, 2005), and selecting products with more targeted administration routes (Laffont *et al.*, 2001) are simple ways of reducing eco-toxicological impacts of anthelmintic use. As dung beetles have been shown to respond to management at a farm scale (Hutton and Giller, 2003), communicating to farmers the functional benefits which can be achieved through sustainable parasite control could play an important role in encouraging more environmentally-sound livestock production.

Chapter 4: Quantifying immediate and delayed effects of anthelmintic exposure on dung beetle survival and ecosystem functioning

Paul Manning[1,2], Sarah A. Beynon[1,2], Owen T. Lewis[1]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

Author Contributions: PM and OTL designed the experiment. PM set-up the experiment, collected all the data, conducted all analyses, and wrote the manuscript. SAB and OTL provided multiple rounds of feedback on earlier versions of the manuscript.

Chapter Status: In preparation for submission

Abstract:

Dung beetles (Coleoptera: Scaraboidea) support a number of important ecosystem functions in pasture based livestock production systems. Exposure to veterinary anthelmintics in livestock dung can have lethal and sublethal effects on dung beetles, and can reduce rates of dung removal. However, the consequences for other dung beetle mediated functions have rarely been studied. We investigated how anthelmintic exposure affected survival in the dung beetle *Aphodius fossor*, and its delivery of four ecosystems functions that underpin pasture production: dung removal, soil fauna feeding activity, primary productivity, and reductions of soil compaction. We tested whether anthelmintic exposure had immediate, or delayed effects on these functions individually, and simultaneously (i.e. ecosystem multifunctionality). There was no evidence that ivermectin residues had a lethal effect on adult beetles. High levels of ivermectin exposure concurrent with measures of functioning, significantly reduced the amount of dung removed by beetles, but none of the other ecosystem functions investigated were significantly affected. For dung removal, we found a significant interaction between exposure-timing and functioning. While dung removal was impaired by concurrent exposure to high levels of ivermectin, functioning was unaffected when beetles that had been exposed previously to the same concentration of anthelmintic later interacted with untreated dung. There was no evidence to suggest any persistent impact of anthelmintic exposure on ecosystem multifunctionality. While anthelmintic residues remain a significant threat to dung beetle populations, for adult beetles we found no evidence that residues posed any functional consequences beyond the immediate point of exposure. Approaches that consider multiple generations of a diverse coprophagous insect community would be useful to better understand the demographic and functional consequences of anthelmintic residues in livestock dung.

Introduction

Globally, more than 80% of agricultural land is used for grazing livestock (Steinfeld *et al.*, 2006), a production method which depends heavily upon a number of ecosystem functions. While forage production (primary productivity) is the most directly relevant function in pasture ecosystems, it is underpinned by a number of supporting functions (e.g. nutrient cycling, improved physical properties of soil, and the control of herbivorous pests).

A number of ecosystem functions in pastures are supported by soil invertebrates, especially dung beetles (Coleoptera: Scarabaeoidea) (Rosenlew and Roslin, 2008; Kaartinen *et al.*, 2013). Dung beetles feed on livestock dung both as larvae and adults (Hanski and Cambefort, 1991), and bury dung in the soil (Sowig, 1995). This action limits the spread of gastrointestinal parasites (Bryan, 1973), improves soil permeability (Bang *et al.*, 2005), and stimulates more rapid decomposition of plant litter (Tixier *et al.*, 2015; Chapter 2).

Despite the functional importance of dung beetles within pasture ecosystems, functions they support can be readily lost due to environmental perturbations. For example, soft-bodied scarab larvae living in the soil are highly unlikely to survive cultivation practices associated with short-term ley production systems (Huiting *et al.*, 2006), and removal of hedgerows can lead to local extinctions of species with narrow thermal niches (Hutton and Giller, 2003).

A perturbation of particular concern is exposure to veterinary anthelmintic residues in livestock dung (Floate *et al.*, 2005; Sutherland and Leathwick, 2011). The most widely used class of anthelmintics is the macrocyclic lactones. After passing through the animal in dung, residues cause a suite of lethal (Beynon *et al.*, 2012a; Blanckenhorn *et al.*, 2013) and sublethal effects on dung beetles. These include reduced fecundity (Pérez-Cogollo *et al.*, 2015), lower muscle strength (Verdú *et al.*, 2015), and prolonged development (Pérez-Cogollo *et al.*, 2015).

These lethal and sublethal effects can also act to influence functioning: anthelmintic residues in dung have been linked repeatedly to lower dung removal rates (Wall and Strong, 1987; Beynon *et al.*, 2012a). Despite this, little is known about the effects of exposure on other dung beetle-mediated ecosystem functions in pastures. Understanding how anthelmintic residues affect both individual functions and multifunctionality (the simultaneous delivery of multiple functions - (Hector and Bagchi, 2007), would allow a better appreciation of the functional losses associated with anthelmintic use.

A second knowledge gap associated with anthelmintic residues, is that the impact of exposure on ecosystem functioning occurring after exposure (herein, ‘successive functioning’) has not been fully appreciated. Published literature on the effect of anthelmintic exposure on functioning considers impacts only at the source of exposure (the dung pat containing anthelmintic residues). However, beetles will typically disperse between multiple dung pats as adults (Roslin, 2000), and may encounter varying levels of exposure amongst movements. Understanding how prior exposure to anthelmintics

affects successive functioning, would be useful in better realising the functional consequences of anthelmintic use.

Given that anthelmintic residues often have lethal, and various sublethal consequences for dung beetles, we hypothesised that the negative impacts of anthelmintic residues would persist even when dung beetles later interacted with anthelmintic-free dung. Here, we investigate how exposure to residues of ivermectin (a widely used ML anthelmintic) affects four ecosystem functions mediated by the dung beetle *Aphodius fossor* L., a species known to play a key role in promoting multiple ecosystem functions (Slade and Roslin, 2016). In addition to quantifying the concurrent effect of residue exposure on functioning, our experiment allows us to investigate the delayed, cumulative lethal and sublethal effects of anthelmintic exposure on ecosystem functioning for two field-relevant exposures. We ask: **i)** Does exposure to anthelmintic residues have lethal effects on *A. fossor*? **ii)** Does exposure to anthelmintic residues affect the ability of *A. fossor* to support individual functions concurrently with exposure? **iii)** Does measuring functioning solely at the source of exposure underestimate or overestimate functional impairment associated with anthelmintic use? and **iv)** Do anthelmintic residues cause persistent declines in ecosystem multifunctionality beyond the source of initial exposure?

Methods

Overview

We conducted our experiment between June 7th and September 5th, 2016 in a grassy field at the John Krebs Field Station (Wytham, Oxfordshire, OX2 8QJ, UK). Our design was based on systematically moving groups of dung beetles through a series of three

enclosures over a three-week period. Adult dung beetles were allowed to feed in each enclosure for one week, with each enclosure being deemed a ‘Phase’. During one of the three Phases, dung beetles were exposed to dung containing: high anthelmintic (500 ppb ivermectin) residues, low anthelmintic (125 ppb ivermectin) residues, or anthelmintic-free controls. The high concentration corresponds to peak faecal concentration of ivermectin following cattle treatment with injectable formulations, and the low concentration corresponds to concentrations observed 10 days after treatment, when anthelmintic exposure is thought to have limited toxicological risk to dung beetles. Staggering the Phase when beetles were exposed (Figure 4.1), allowed us to compare the effect of anthelmintic residues on beetles exposed either two weeks before (Figure 4.1A), one week before (Figure 4.1B), or alongside our measures of functioning (Figure 4.1C). This delay between exposure and measures of functioning is herein referred to as ‘exposure-delay’. Each of the treatment combinations (3 exposure-delays x 3 residue concentrations) was replicated 8 times, giving 72 replicates in total.

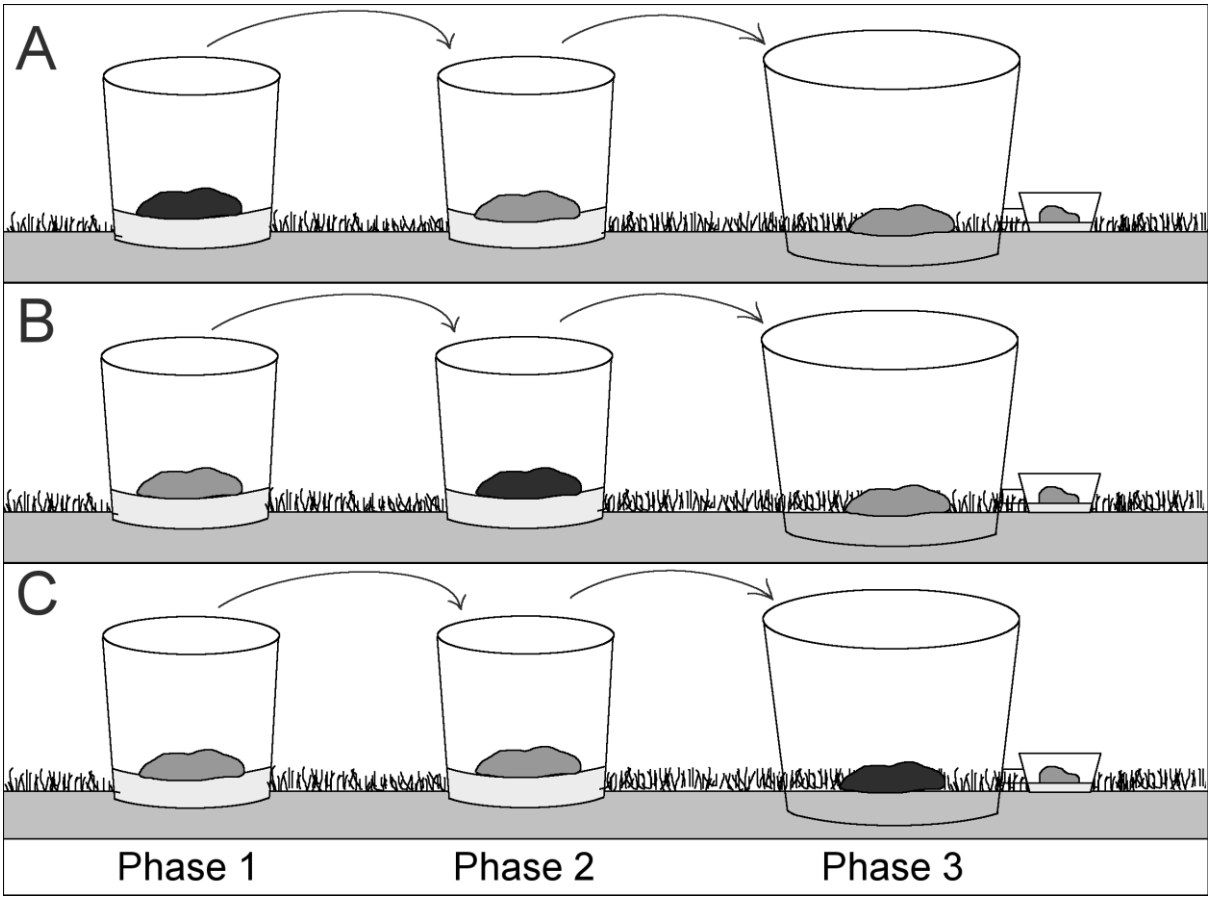

Figure 4.1. Schematic of the experimental design. All dung beetles used within the experiment were moved through a series of three enclosures, spending one week in each Phase. Black dung indicates the period of the experiment when the anthelmintic was added (or not added in the controls) to dung. All ecosystem functions were measured during the final part of the experiment (Phase 3). To manipulate the time between exposure and measurement of functioning, beetles were exposed to ivermectin (except in the controls) during either Phase 1, Phase 2, or Phase 3, which corresponded to 'exposure-delays' of two-weeks prior to (A), one-week prior to (B), or concurrently alongside (C) the enclosure where functions were measured (Phase 3). The experiment was replicated with: high anthelmintic residues (500 ppb ivermectin), low anthelmintic residues (125 ppb ivermectin), or anthelmintic-free controls. Enclosures used for Phase 1 and Phase 2 were fully sealed, while enclosures used for Phase 3 were open at the bottom, and dug into the soil to allow free colonisation by soil invertebrates.

Enclosure Design

Enclosures used for the Phases 1 and 2 of the experiment consisted of 5 L plastic buckets, filled with 5 cm of builders sand, and with six 3 mm holes cut in the bottom for drainage. Enclosures used for Phase 3 were 12 L black plastic buckets with the bottoms removed and dug into the ground to a depth of 8 cm (Chapters 2, 3). This allowed us to measure functions delivered by dung beetles and other soil fauna, which could colonise from the underlying soil.

We collected dung for the enclosures from organically managed beef cattle, raised on site at the Farm Animal Initiative (Wytham Farm, Oxfordshire, OX2 8QJ, UK). Cattle were grazing on semi-improved pasture dominated by perennial ryegrass (*Lolium perenne* L.*)* and white clover (*Trifolium repens* L.*)*. All dung was collected fresh (within 3 hours of defecation), frozen at -20°C for at least 48 hours, and thawed before use. Dung was homogenised for several minutes, so that dung pat consistency was standardised amongst enclosures.

To expose dung beetles to anthelmintic residues, we spiked dung with ivermectin. Following Römbke *et al*. (2009), we prepared dilute solutions of ivermectin (5mg/mL Molemec pour-on, Mole Valley Farmers, South Molton, Devon EX36 3LH, UK) in acetone at three concentrations: 275 ppm solution (high), 68.75 ppm solution (low), and a solution of pure acetone (control). A third party coded the solutions by randomly allocating a unique colour to each, which blinded researchers to treatment levels for the duration of the experiment.

Each pat was formed after homogenising a 1-mL aliquot of the anthelmintic solution into 550 g of dung for 60 seconds. Addition of the high and low concentration solutions to

dung resulted in final ivermectin concentrations of *c.* 500 and 125 ppb (wet dung weight), respectively. Enclosures not subject to treatment with anthelmintic residues in a given week were treated with 1 mL of pure acetone as a control.

We formed dung pats within the enclosures using a 12 cm diameter circular frame. Dung pats were placed on a 2 cm wire grid. Each enclosure was then covered by 2-mm white fine mesh to prevent additional insect colonisation, and a 1-mm blue mesh to provide shade. In all cases, we postponed the addition of beetles to enclosures for three days following dung pat formation, allowing time for acetone to volatise (Römbke *et al.*, 2009), and for dung to reach a more favourable condition for *A. fossor,* which prefers older dung (Holter, 1982).

Dung beetles used in the experiment were collected from two pastures close to the experimental site where organically managed beef cattle were grazing semi-improved pasture at low stocking densities. Beetles were hand collected from dung, stored in mixed-sexed terraria, and fed anthelmintic-free cattle dung before being used in the experiment.

We determined the sex of each beetle, and added two males and two females to each enclosure. The experiment began on June 7th, 2016 (Day 1) by adding beetles to the first enclosures (Figure 4.1, Phase 1). We allowed beetles to feed for 7 days before removing beetles through a combination of hand-sorting and flotation (submerging dung pats in water, and capturing beetles which floated to the surface). The numbers of live and dead beetles were recorded, and all living beetles were immediately transferred into the corresponding enclosure (Figure 4.1, Phase 2). Seven days later, on Day 14, the

beetles were removed as before and added to the final enclosure (Figure 4.1, Phase 3) where functions were measured six weeks later (Day 56).

Removing beetles using flotation during this phase, would have prevented our measurement of ecosystem functioning. Thus to estimate beetle survival from Phase 3, we attached a dung-baited emergence trap to each enclosure on Day 21. Emergence traps consisted of translucent 750-mL plastic containers attached to each of the enclosures with a short length of clear 2 cm plastic tubing (Figure 4.1). Each trap was filled with approximately 1 cm of builders sand and baited with 150 g of previously frozen and thawed cattle dung from the same source as the dung that was used in the experiment. An additional 50 g of dung was added one week later, and baits were refreshed by disturbing the dung's crust with a knife. As beetles move between multiple dung pats as adults, olfactory cues emanating from the fresh dung encouraged movement from the enclosure into the emergence trap. As presence of *A. fossor* individuals in dung has been shown to influence *A. fossor* aggregation (Chapter 7), beetles were removed from the traps every two to three days for two weeks until three consecutive evaluations of emergence yielded no additional beetles.

Response variables

We measured five response variables during the experiment: beetle survival, feeding activity of soil invertebrates, dung removal, soil compaction, and primary productivity. *Beetle survival* was calculated as the total number of adult beetles collected from emergence traps between Days 21—34.

We measured the *feeding activity of soil invertebrates (herein, feeding activity)* using the bait lamina test (Terra Protecta GMbH, Berlin, Germany). Six weeks after introducing

beetles to Phase 3 (day 56) we inserted five bait lamina strips through each dung pat (Chapter 2). Each strip was positioned so that the uppermost hole was located immediately beneath the soil surface. Thirty-six strips were placed alongside the experiment and a subset was checked every two days until between 30-50% feeding activity was observed. In our experiment, this occurred after 12 days of exposure. Bait lamina were removed from the ground on Day 68 (August 13th, 2016) and feeding activity was assessed by scoring each bait as 'consumed' or 'intact' by viewing the strips against a light source.

Immediately after removing bait lamina on Day 68 we began measuring all remaining functions. We measured *dung removal* by lifting dung pats from the enclosures using the wire grid. All dung was immediately placed in an aluminium weigh boat and dried for 48 hours at 80°C. Dung removal was calculated as the mass (g) of dung remaining, subtracted from the mean dry mass (91 g) of 550 g dung pats with all invertebrates excluded (n=3).

We assessed *soil compaction* using a measure of bulk density. Under the initial location of the dung pat, we hammered a segment of 5.5 cm diameter PVC pipe into the soil to a depth of 5 cm. We removed the intact sample within the pipe using a trowel, and placed it into an aluminium weigh boat, drying the sample for 48 hours at 80°C. Soil bulk density ($g \cdot cm^{-3}$) was calculated as the dry mass of soil solids, divided by the core volume.

We measured *primary productivity* by planting perennial ryegrass seeds into soil sampled from the enclosures. Two soil cores were removed from the area beneath each dung pat using the same cores, and methods described above. Soil samples were

homogenised, and a 185 mL subsample was added to a 250 mL plant pot. One mL of tetraploid perennial ryegrass (Cotswold Seeds, Moreton-in-Marsh, Gloucestershire, GL56 0JQ, UK) was scattered on the soil surface, and was covered with 15 mL of soil from the same sample. We placed the pots in an unheated greenhouse and watered all pots regularly and equally. Three weeks after planting, we harvested aboveground biomass by clipping plants at the soil surface and drying at 75°C for 48 hours (Zhao *et al*., 2013). Dried biomass was weighed to the nearest 0.1 g.

Analysis

i) *Does exposure to anthelmintic residues have lethal effects on A. fossor?*

For each 'exposure-delay', we tested how exposure to ivermectin residues influenced dung beetle mortality by modelling emergence (proportion emerged) as a function of ivermectin concentration (high, low, control) using analysis of variance. Model residuals met assumptions of homogeneity and normality and thus raw values were left untransformed.

ii) *Does exposure to anthelmintic residues affect the ability of A. fossor to support individual functions concurrently with exposure?*

We tested whether anthelmintic residues at the site of exposure affected the delivery of individual functions using the subset of our data where exposure occurred concurrently alongside measures of functioning (exposure-delay = 0 weeks, Figure 4.1C). We modelled each individual measure as a function of anthelmintic dose (high, low, control) using analysis of variance (ANOVA). Model residuals met assumptions of homogeneity and normality, and thus raw values were left untransformed. When a significant effect of treatment was found, treatment level means were compared using Tukey's Honestly

Significant Differences (Tukey's HSD), and effect sizes were estimated using Cohen's D.

iii) *Does measuring functioning solely at the source of exposure underestimate or overestimate functional impairment associated with anthelmintic use?*

We tested whether solely considering the immediate effect of anthelmintic residues underestimates or overestimates functional losses associated with exposure, using analysis of covariance. We modelled individual functional measures as a function of anthelmintic concentration (high, low, control), exposure-delay (0 weeks, 1 week, 2 weeks), and the interaction between anthelmintic concentration and exposure-delay.

iv) *Does anthelmintic exposure cause persistent declines in ecosystem multifunctionality beyond the source of initial exposure?*

We tested whether the effect of ivermectin exposure on multifunctionality acted mainly at the source of exposure, or whether continued lethal and sublethal effects resulted in loss of multifunctionality using the 'exceeding thresholds approach' (Byrnes *et al.*, 2014). This method, developed originally to describe the relationship between diversity and multifunctionality, works by estimating a linear relationship between a continuous independent variable (exposure-delay ranging from 0-2 weeks), and the number of ecosystem functions occurring at or above a given threshold (0-4 functions). Estimates were achieved using a linear model with a quasi-binomial error structure.

We described the relationship between exposure-delay and multifunctionality for each of the three exposure levels (high, low, control), to the full threshold limits under which our models would converge (10-87%). To visualise the relationship between exposure-delay

and multifunctionality, all slopes and their 95% confidence intervals were plotted across this full range.

If exposure to anthelmintic residues cause a delayed impact on functioning, we would expect to see a negative relationship between exposure-delay and the number of functions surpassing thresholds in both the low and high residue treatments. We would not expect to see any significant relationship between exposure-delay and multifunctionality under control conditions. When differences are found (i.e. the confidence intervals do not overlap with y=0), a number of metrics can be derived from the curve to facilitate comparisons of multifunctionality amongst treatments (e.g. Perkins *et al.*, 2014).

The statistical package R 3.1.1(R Core Team, 2014) was used for all analyses. The exceeding thresholds approach to assessing multifunctionality was analysed using the multifunc package (Byrnes *et al*., 2014). All figures were created using the ggplot2 package (Wickham, 2009). Methodology and code were pre-registered on the Open Science Framework before beginning the experiment (Manning and Lewis, 2016), and are available online.

Results

i) *Does exposure to anthelmintic residues have lethal effects on A. fossor?*

Beetle survival was not significantly affected by anthelmintic residues two weeks after ($F_{2,21} = 1.07$, $P = 0.36$), one week after ($F_{2,21} = 1.64$, $P = 0.21$), or concurrently alongside ($F_{2,21} = 2.39$, $P = 0.11$) exposure (Figure 4.2).

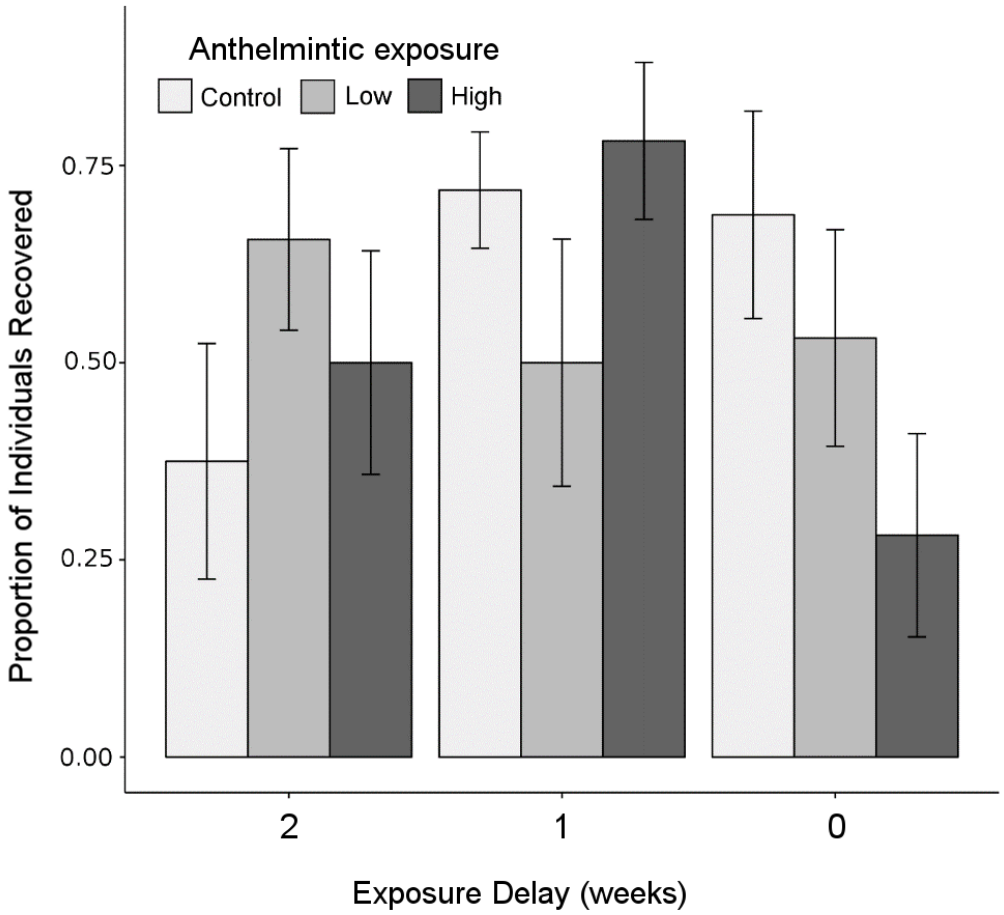

Figure 4.2. Survival as a function of anthelmintic exposure. In no case was survival significantly affected by anthelmintic exposure. Means (bars) are calculated using the number of adult *Aphodius fossor* dung beetles entering emergence traps at the end of the experiment (Phase 3). Means are grouped based on the exposure-delay. Bars represent treatment means with standard errors.

ii) *Does exposure to anthelmintic residues affect the ability of A. fossor to support individual functions concurrently with exposure?*

For concurrent exposure, dung removal was significantly affected by anthelmintic residue level ($F_{2,21} = 6.13$, $P < 0.01$), with approximately twice the level of dung removed from controls relative to dung with high levels of anthelmintic residues (Control: 42.0 ± 4.1 g, High exposure: 21.6 ± 2.95 mean ± se, Cohen's D = -2.00, Figure 4.3A). The

relationship between concurrent anthelmintic exposure and functioning was non-significant in the case of primary productivity ($F_{2,21} = 0.55$, $P = 0.59$), feeding activity ($F_{2,21} = 1.11$, $P = 0.35$), and soil bulk density ($F_{2,21} = 0.10$, $P = 0.91$).

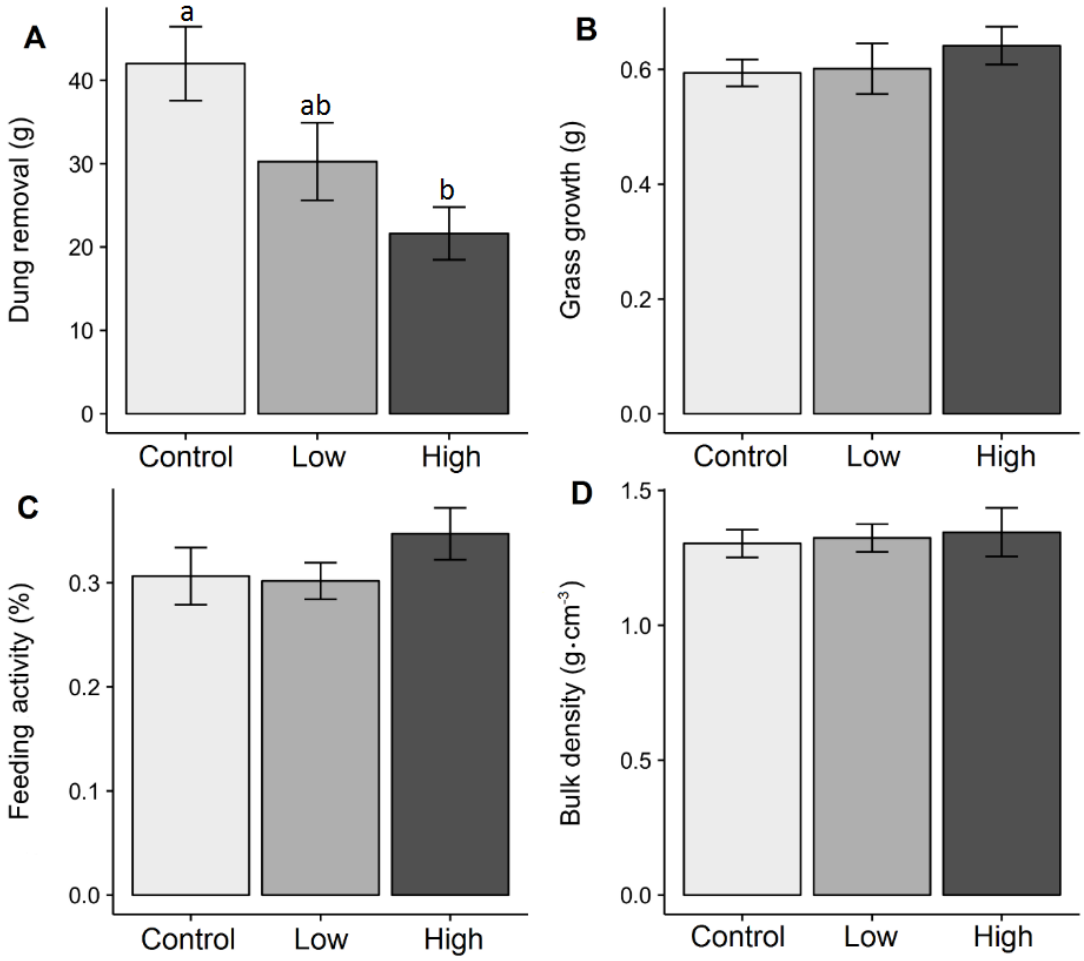

Figure 4.3. The effect of concurrent ivermectin exposure on four ecosystem functions supported by the dung beetle *Aphodius fossor*: dung removal (A), primary productivity (B), feeding activity (C), soil compaction (D). Bars represent treatment means with standard errors. In panel A, sharing of lowercase letter labels indicate that differences between treatment means are non-significant.

iii) *Does measuring functioning solely at the source of exposure underestimate or overestimate functional impairment associated with anthelmintic use?*

For dung removal, we found a significant interaction between exposure-delay and level of anthelmintic residues (Table 4.1). Differences in dung removal for past exposure (Phases 1 and 2) were negligible amongst residue levels, but for concurrent exposure (Phase 3) dung removal was significantly lower at high anthelmintic exposure in comparison to controls (Figure 4.4A). Neither exposure-delay, exposure levels, or their interaction significantly explained any variation in the other three ecosystem functions considered.

Table 4.1. Summary of linear models describing the relationship between exposure-delay (Delay), and the concentration of anthelmintic residues (Residues) in cattle dung.

	F	***P*-value**
Dung removal		
Residues	$F_{2,66} = 0.60$	0.55
Delay	$F_{1,66}=0.65$	0.01
Residues*Delay	$F_{2,66}=0.25$	**0.05**
Primary productivity		
Residues	$F_{2,66} = 0.55$	0.57
Delay	$F_{1,66}=1.02$	0.31
Residues*Delay	$F_{2,66}=0.81$	0.45
Bait Lamina		
Residues	$F_{2,66} = 0.81$	0.45
Delay	$F_{1,66}=0.17$	0.68
Residues*Delay	$F_{2,66}=0.40$	0.67
Bulk Density		
Residues	$F_{2,66} = 0.10$	0.91
Delay	$F_{1,66}= 0.67$	0.42
Residues*Delay	$F_{2,66}= 0.66$	0.52

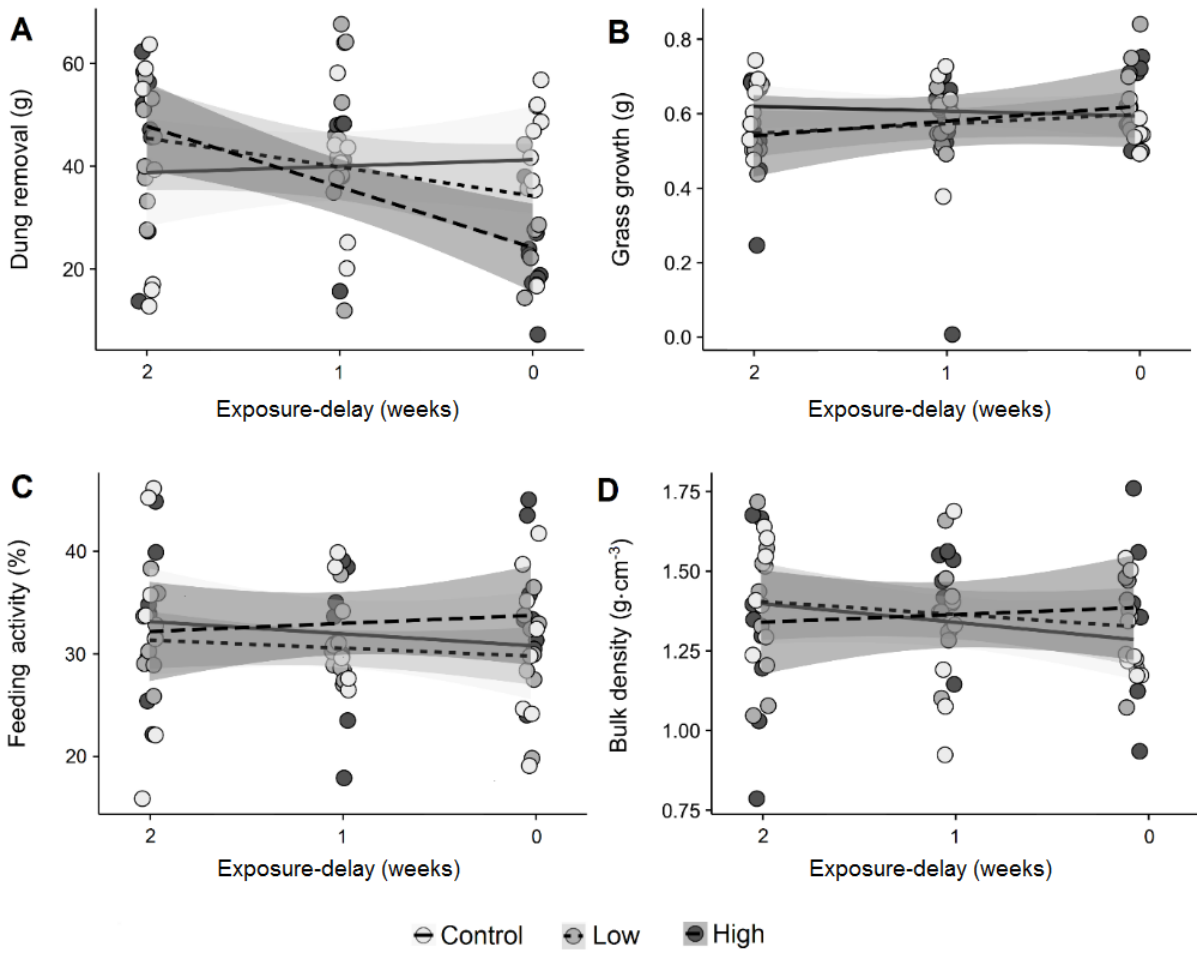

Figure 4.4. The interacting effects of anthelmintic residues and exposure-delay on four ecosystem functions supported by dung beetles. The four functions are: dung removal (A), primary productivity (B), feeding activity (C) and soil compaction (D). Relationships between each function and the timing of exposure are plotted along with 95% confidence intervals at each level of anthelmintic exposure. Light-grey-filled circles with unbroken lines represent anthelmintic-free controls, grey-filled circles with dotted trend lines represent low exposure (125 ppm), dark-grey-filled circles with dashed trend lines represent high exposure (500 ppm).

iv) *Does anthelmintic exposure cause persistent declines in ecosystem multifunctionality beyond the source of initial exposure?*

Using our approach to assessing ecosystem multifunctionality, we found that exposure-delay did not have a marked effect on multifunctionality. In the absence of any

anthelmintic residues, exposure-delay briefly had a positive influence on multifunctionality when thresholds reached 80% of their maxima (Figure 4.5A), although this almost certainly represents a false-positive from random noise. In the case of low exposure levels (Figure 4.5B) and high exposure levels (Figure 4.5C), the number of functions pushed above or below the corresponding thresholds was never significantly different from zero.

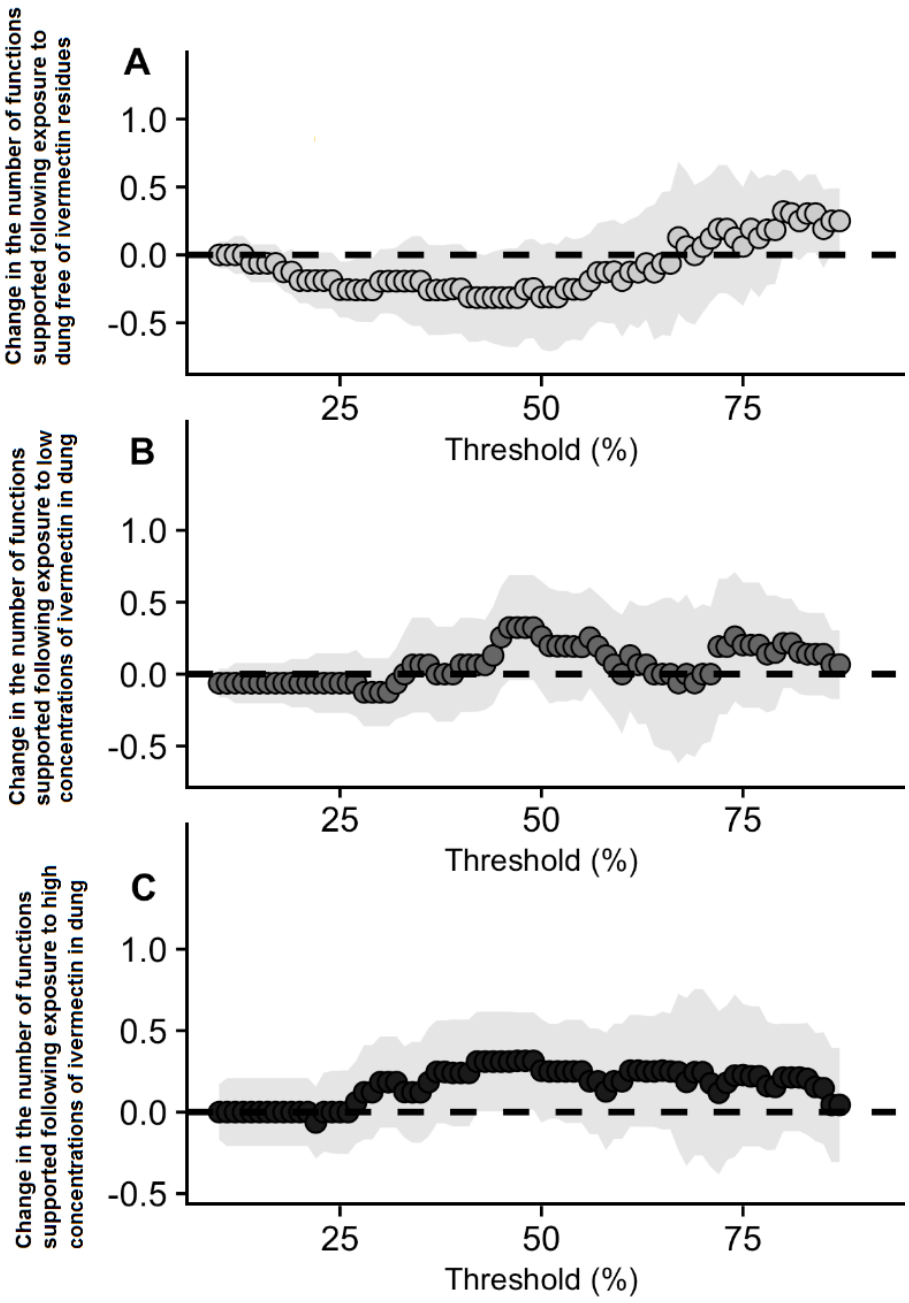

Figure 4.5. Relationships between exposure timing and multifunctionality at control (A), low (B) and high (C) levels of anthelmintic exposure. Thresholds span between 10-87% of the observed maxima. Confidence Intervals (95%) surrounding the slope estimates indicate whether the intervals contain zero. In no case did we find any evidence of a strong loss or gain of multifunctionality associated with anthelmintic exposure.

Discussion

Overall, we found little immediate impact of anthelmintic exposure in directly reducing survival (Figure 4.2), or functioning (with the exception of dung removal) provided by *A. fossor* (Figure 4.3). We found no evidence to suggest that past exposure had any effect on future delivery of individual functions (Figure 4.4, Table 4.1) or for ecosystem multifunctionality (Figure 4.5). This suggests that considering functioning solely at the site of exposure (Figure 4.3) might overestimate the direct losses caused by anthelmintic residues on adult dung beetle functioning, particularly when mortality is low.

Although recent research has demonstrated that low dose ivermectin exposure causes a number of sublethal effects in the dung beetle *Scarabaeus cicatricosus* Lucas (Verdú *et al.*, 2015), exposure was prolonged, as adult beetles were continually supplied with spiked dung. Such prolonged exposure does not reflect typical field conditions, unless anthelmintics are administered through sustained-release boluses (Errouissi *et al.*, 2001), or beetles move amongst groups of livestock treated at different times. Under typical parasite management strategies, not all livestock are simultaneously treated at a farm scale (Boxall *et al.*, 2007). If anthelmintic residues have little impact on dung beetles beyond the site of exposure, ensuring the availability of anthelmintic-free dung through selective or split-treatment of herds could be useful in limiting the non-target impacts of anthelmintic residues on dung dwelling insects. This adds to the benefit of selective treatment in providing genetic refugia which limits the development of anthelmintic resistance in parasite populations (Sissay *et al.*, 2006).

However, for dung beetles to provide any level of functioning they first must disperse to the new patch (dung pat) through flight, and/or walking. Since we moved beetles to their new habitat patch in our experiment, rather than requiring them to disperse we may have underestimated functional consequences of impairment associated with exposure to anthelmintics (Verdú *et al.*, 2015). Research exploring how anthelmintic exposure affects dispersal would be a useful next step in better understanding the risks of anthelmintic use.

In the case of individual functions, we found that dung removal was significantly impaired by anthelmintic residues at high concentrations (Figure 4.4A), consistent with previous studies (Madsen *et al.*, 1990; Beynon *et al.*, 2012a), and is most likely explained by larvae having greater sensitivity to anthelmintic residues than adults (Beynon *et al.*, 2012a). Adult beetles filter out large particles and consume the liquid fraction of dung, whereas bulk-feeding larvae tend to drive dung removal by indiscriminately consuming undigested plant matter and microbial biomass (Holter, 2016). As we estimated dung removal through a measure of dry mass, we likely considered larva feeding only. All other functions considered were not impacted by either high or low levels of anthelmintic residues in dung, which is broadly consistent with previous results (Chapter 3). While dung removal is a useful measure in estimating baseline levels of ecosystem functioning (Kaartinen *et al.*, 2013), it seems to be poorly correlated with other functions of interest, and may not reflect loss of related functions, or ecosystem multifunctionality (Chapter 2).

We found no significant effect of anthelmintic exposure in affecting survival of adult beetles at any point during the experiment (Figure 4.2). This is consistent with Beynon

et al. (2012), but differs from the results reported in Chapter 3, where survival of *A. fossor* was significantly reduced by ivermectin concentrations identical to those used in the 'high' treatment here. Discrepancies between adult survival may have been due to high temperature, which might interact with anthelmintic exposure to cause higher mortality. Considering the fate of developing larvae (e.g. Floate, 1998; Dadour *et al.*, 2000; Errouissi *et al.*, 2001) would reflect more fully the non-target risk anthelmintics pose to dung beetles and other insects.

While the importance of dung beetles in providing dung-associated ecosystem functions within managed and natural systems has attracted significant attention, dung beetles represent only a small portion of the diverse ecological community present in a dung pat (Hanski and Cambefort, 1991; Skidmore, 1991). Coprophagous flies are typically the most abundant taxa in livestock dung (Skidmore, 1991), and have higher sensitivity to chemical residues than dung beetles (Floate, 1998). However, despite their prevalence and known sensitivity to chemical perturbations, with the exception of O'Hea *et al.* (2010) little attention has been given to the ecosystem functioning they support. Furthermore, as our experiments considered only a single species (albeit a functionally important one: Slade and Roslin (2016)) our results, which fail to demonstrate any functional loss, may be different when more sensitive species are considered (Beynon, 2012). *Aphodius fossor* is often common at sites managed intensively (Hutton and Giller, 2003), suggesting that it may have lower sensitivity to perturbations such as anthelmintics, relative to other dung beetle species.

Because we allowed earthworms and other invertebrates to colonise dung from the underlying soil in Phase 3, loss of dung beetle functioning associated with anthelmintic

exposure may have been masked or buffered by soil fauna (Kaartinen *et al.*, 2013). Closed mesocosms, as described by Beynon *et al*. (2012), would allow effects on dung removal by dung beetles to be estimated more precisely, but would not allow other important functions to be measured, as we measured them here. This trade-off between precision and perspective is inevitable, however we believe that our enclosure design allowed for more conservative estimates of functional losses and gives context to the relative importance of dung beetles compared to other taxa in underpinning ecosystem functioning in pastures.

Perhaps the biggest challenge we are faced with in understanding the non-target consequences of livestock parasite control is the difficulty of conducting experiments over multiple generations. Our approach, which considered the functional losses associated with past exposure to anthelmintic residues, goes beyond the typical direct effects but does not consider multi-generational impacts of regular, or occasional use of anthelmintics. Additionally, under intensive systems, dung beetles may be exposed to anthelmintic residues for longer periods, and at higher concentrations than those we considered here. Achieving a better understanding of patterns of anthelmintic use, and chemical sensitivity of common species to anthelmintics (e.g. Beynon, 2012) would be useful in informing models which predict losses of ecosystem functioning caused by the non-target impacts of livestock parasite control.

Chapter 5: Impaired insect activity in cattle dung has no effect on pasture productivity or weed establishment

Paul Manning[1,2], Sarah A. Beynon[1,2], Owen T. Lewis[1]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

Author Contributions: PM, SAB, and OTL designed the experiment. PM and OTL set-up the experiment. PM collected the data, performed all analyses, and wrote the manuscript. SAB and OTL provided multiple rounds of feedback on earlier versions of the manuscript.

Chapter Status: In preparation for submission

Abstract:

Dung produced by grazing livestock represents a pulse of nutrients which can benefit growth of desirable plant species, but may also allow the establishment or proliferation of weeds. As a result, dung may either increase or decrease pasture productivity. A diverse community of coprophagous invertebrates (predominately beetles, flies, and earthworms) consumes, fragments and buries the dung of livestock in a process known collectively as dung removal. This action clears the pasture surface allowing plant growth, while also helping nutrients contained in dung become available to pasture plants. Many invertebrates involved in dung removal are sensitive to perturbations in agricultural landscapes, where a particular concern involves exposure to unmetabolised veterinary anthelmintics (chemicals used to control internal parasites) in livestock dung. Although earthworms are typically unaffected, anthelmintic residues can kill or impair colonising insects which can threaten the delivery of associated ecosystem functions. In a field experiment, we explored how cattle dung affected both pasture productivity and establishment of the perennial pasture weed, broad-leaved dock (*Rumex obtusifolius*) from seed sown under each dung pat. By adding the potent anthelmintic doramectin, we were also able to test whether impairing coprophagous insects affected these functions. Despite high seed viability, we found that broad-leafed dock establishment was negligible (7 out of 1440 seeds), so meaningful comparisons amongst treatments were not possible. Pasture productivity (harvested plant biomass) was significantly higher (by 40%) in treatments with dung than without dung, irrespective of whether coprophagous insects were impaired by anthelmintic residues. An attempt to estimate dung removal through non-destructive image analysis showed no detectable effect of impairing insects on rates of dung removal during the first eight weeks. However, irrespective of insect impairment, dung was fully degraded by the following year. Anthelmintic residues in dung have been shown repeatedly to have lethal and sublethal effects on coprophagous insects, and threaten insect diversity in agricultural ecosystems. However, our data show that consequences for ecosystem functions are not inevitable. In highly improved pasture systems, earthworm activity may buffer against functional losses associated with impairment or death of coprophagous insects.

Introduction

Livestock dung is rich in nutrients (Kirchmann and Witter, 1992) and when incorporated into the soil can improve forage quantity (Bang *et al.*, 2005), and nutritional quality (Fincher, 1981). However, in temperate climates where decomposition is prolonged, this flux of nutrients is paired with fouled pasture which can directly impede forage growth (Anderson *et al.*, 1984), and lower pasture productivity.

Aside from the direct losses associated with pasture fouling, dung on pasture may also limit productivity through indirect pathways. Where dung patches are nutrient rich and generally provide some level of disturbance, livestock dung has been shown to influence the composition of plant communities in pasture. This is achieved through a variety of different mechanisms including direct introduction of propagules (Malo and Suárez, 1995), creating gaps that allow seeds to germinate from the seed bank (Dai, 2000), and allowing rapid vegetative spread of adjacent plants (Welch, 1985). In the context of pasture management, a variety of perennial and annual plants are considered weedy species, causing direct losses of desirable forage growth by competing with the sward for light and nutrients (Seefeldt *et al.*, 2005). Weedy species that are unpalatable can proliferate in landscapes under intensive grazing pressure (Callaway *et al.*, 2000), and some can be toxic when ingested in high quantities (Gorder, 2000).

The speed at which dung decomposes and is ultimately removed from the pasture surface is likely to influence whether the effect of livestock dung on pasture productivity is positive or negative. While decomposition is a microbial process, it is supported by a diverse group of coprophagous invertebrates, particularly flies, worms, and beetles, which use dung for feeding, breeding and shelter (Hanski and Cambefort, 1991). Direct

consumption (O'Hea *et al.*, 2010a; Kaartinen *et al.*, 2013), enhanced microbial decomposition (Yokoyama *et al.*, 1991) and relocation of dung to lower soil horizons (Sowig, 1995) all increase the rate at which dung disappears from the pasture surface (Kaartinen *et al.*, 2013), a function known as dung removal. Alongside the benefits of reducing pasture fouling, dung removal supports a number of ecosystem functions in pastures, including lowering rates of parasite transmission (Gregory *et al.*, 2015), stimulating forage growth (Bang *et al.*, 2005), improving soil structure (Brown *et al.*, 2010), and reducing greenhouse gas emissions (Slade *et al.*, 2016).

Invertebrate groups that support dung removal are sensitive to perturbations associated with agricultural management, and populations may decline or be lost under intensive practices (Hutton and Giller, 2003). For instance, dung beetles with narrow thermal tolerances may become locally extinct when shading features like hedgerows or trees are removed from agricultural landscapes (Horgan, 2002; Hutton and Giller, 2003), and regular soil cultivation associated with short-term ley grazing systems leads to lower earthworm densities (Gerard and Hay, 1979). A perturbation of particular concern for dung invertebrates involves the use of veterinary anthelmintics (Floate, 2007). While highly efficacious against enteric parasites and exhibiting negligible toxicity to mammals (Geary, 2005), unmetabolised anthelmintic residues may enter the environment through dung and have been shown to kill colonising invertebrates or reduce their fitness (Verdú *et al.*, 2015).

The non-target ecological consequences of anthelmintic use for coprophagous insects has received considerable attention (e.g. reviewed by Floate *et al*., 2005). While the majority of work has explored the lethal effects, sublethal effects associated with low

dose exposure are becoming more widely recognised, with exposure linked to lower fecundity (Pérez-Cogollo *et al.*, 2015), impaired chemoreception (Verdú *et al.*, 2015), reductions in egg hatch success (Mann *et al.*, 2015), and shorter lifespan (Verdú *et al.*, 2015). As insects represent a considerable proportion of coprophagous invertebrate communities, these lethal and sublethal effects may also pose a threat for the delivery of related ecosystem functions (Wall and Strong, 1987; Madsen *et al.*, 1990; Beynon *et al.*, 2012a). However, in many temperate systems earthworms also play a key role in promoting dung decomposition (Holter, 1979a; O'Hea *et al.*, 2010a; Kaartinen *et al.*, 2013) and, in contrast to insects, show little or no sensitivity to veterinary anthelmintic residues (Svendsen *et al.*, 2002; Kolar *et al.*, 2008).

To understand how impaired dung decomposition might affect weed emergence and pasture productivity, we conducted a field experiment which involved placing dung pats in an improved pasture on plots manually seeded with a pasture weed. We selected the broad-leaved dock (*Rumex obtusifolius* L.) as a focal weed species. This perennial weed has a widespread distribution on nutrient-rich soils (Holm *et al.*, 1977) and is a noxious and highly competitive weed in many permanent pastures, with high densities leading to declines in pasture productivity and forage quality (Pino *et al*., 1995, Zaller, 2004). We manipulated insect activity by spiking dung with doramectin, a potent veterinary anthelmintic. We investigated (1) whether the addition of cattle dung influenced pasture productivity and weed emergence, and (2) whether pasture productivity and weed emergence were affected by reduced insect activity.

Methods

Experimental Rationale and Design

Our experiment was conducted within an improved sheep-grazed pasture at Dr Beynon's Bug Farm, St. David's, Pembrokeshire, UK (51°53′22″, 5°14′06″). The site is dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.*)*, with other low growing herbaceous plants (primarily *Stellaria media* L., *Ranunculus* spp., *Plantago lanceolata* L.) interspersed through the sward and accounting for less than 5% of the overall cover. The experiment took place within a 14 m × 14 m pasture section surrounded by an electric fence to exclude sheep. The day before we began the experiment (June 3rd 2015), we mowed the sward to a height of *c*. 2 cm, and removed grass clippings by hand.

We divided the experimental area into a 1 × 1 m grid of 160 points. Each point was marked with a steel tent peg, which specified the bottom right hand corner of each plot. Each of these plots measured 15 × 15 cm, which corresponded to the initial size of the dung pats. Plots were randomly assigned one of four treatments (Figure 4.1), with 40 replicates per treatment. On the same day, we collected achenes (dried fruit capsules containing seed) from *Rumex obtusifolius* growing in the margins of a nearby pasture.

We began the experiment on June 4th 2015 by adding achenes to each plot. The location of the achenes was standardised using a plastic reference frame measuring 15 cm × 15 cm. At the innermost 9 cm × 9 cm, we drilled 9 holes spaced equally along a 3 × 3 cm grid. The frame was placed at the reference post, and a single achene was passed through each hole, ensuring each made contact with the underlying soil.

The first two treatments (Figure 4.1a, 1b) involved the adding fresh dung (<6 hours old) collected from Welsh Black cattle grazing an onsite paddock of marshy, and semi-improved dry grassland. The cattle had not been treated within the last 12 months with any anthelmintics known to impact dung invertebrates. All collected dung was combined into a large container, homogenised for several minutes with a mortar mixer, and covered to prevent invertebrate colonisation before beginning the experiment.

Using a factorial design, we tested how the presence of dung (with or without anthelmintic addition) affected the broadleaf dock establishment and pasture productivity relative to control areas without dung (with or without anthelmintic addition). We formed 40 anthelmintic-free pats (control dung, Figure 5.1a) by measuring 600 g batches of dung into a bowl and forming each mass into a dung pat using a 15 x 15 cm square plastic frame. The frame was placed so that it was directly overlaying the dock seeds placed within each plot several hours earlier. Each dung pat was smoothed and edges neatened using a spatula.

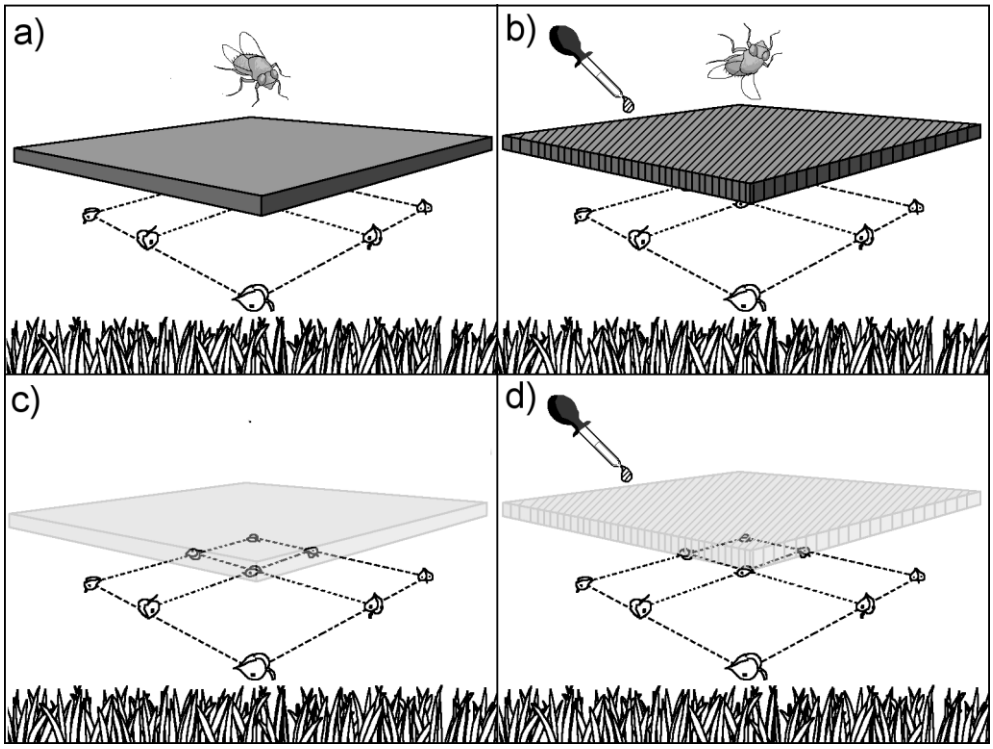

Figure 5.1. Depiction of treatments included in the experiment. a) Untreated dung, b) Treated dung, c) Untreated control, d) Treated control.

To lower insect activity, doramectin (Dectomax Injectable Livestock Wormer – Zoetis) was added to each dung pat in the 'treated dung' (Figure 4.1b). In lowering insect activity, we chose doramectin over more widely used anthelmintics due to its persistence (Suárez *et al.*, 2009), broad-spectrum toxicity to a range of dung insects (Floate *et al.*, 2008), and because it does not exhibit the widespread insect-attractant effects which have been reported for other more widely used anthelmintics (Floate, 2007). We added 0.01 mL of doramectin (Dectomax Injectable Livestock Wormer – Zoetis) to each 600 g dung pat, resulting in a final concentration of *c.* 1,000 ng/g of doramectin in dry dung, a concentration comparable to but below the known peak of c.

1,300 ng/g (Suárez *et al.*, 2009). Doramectin was homogenised within the dung for 60 seconds, before forming 40 dung pats (treated dung) as described above.

To test whether the presence of dung affects dock germination, the remaining 80 plots were used as controls, divided equally into a negative control (untreated control, Figure 5.1c, n=40), and a positive control (treated control, Figure 5.1d, n=40). The untreated control consisted of 500 mL water sprinkled on the plot, which corresponded to the approximate volume of water within the dung. The treated control was treated by sprinkling 500mL of water and 0.01 mL doramectin over the plot.

Pasture Productivity and Weed Germination

Pasture productivity was assessed the following year, on May 26th, 2016. At each sampling point, a 15 × 15 cm quadrat was placed at the location of the initial plot as demarcated by the steel peg. Any germinating dock plants falling within the quadrat were first noted, and all aboveground forage within the quadrat was harvested, separating the sample into broad-leaved dock and non-broad-leaved dock vegetation.

We measured the fresh mass of each sample a day following the sample collection. Due to logistical constraints, we were unable to dry all samples and thus dried a stratified random sample of ten samples per treatment. Samples were oven dried at 60°C for 48 hours. Both fresh mass and dry mass of the forage samples were recorded to the nearest 0.1 g.

Dung Decomposition

To measure the disappearance of dung pats, we used a non-destructive, image based approach. The day after beginning our field experiment (June 5th, 2015), plots

containing dung (untreated dung, treated dung) were photographed from a height of 50 cm, with a measuring tape for scale. Photographs were taken on a biweekly basis for eight weeks, at which point grass obscured the edges of the dung pats beyond a point where edges could be reliably discerned. Thus, each dung pat has five non-independent measures taken over a period of eight weeks.

The area of dung within each photograph was calculated using ImageJ (Schneider *et al.*, 2012). The measurer was blind to the treatment, and calculated the area twice for each dung pat. If two areas calculated for the same photograph differed by more than 2%, a third estimate was taken. In all cases, this was within 2% of one of the initial estimates, and a mean of the two most similar estimates for each photograph was then used as the measure of dung removal rates.

Analysis

We used two-way analysis of variance to test how the presence of dung and doramectin influenced the mass and the moisture content of the forage samples. In both cases, assumptions of normality and homogenous variation of residuals were met, and values were left untransformed. All analyses were completed using R (R Core Team, 2014), and figures were drawn using ggplot (Wickham, 2009).

Preliminary examination of the dung decomposition data revealed that mean dung pat area decreased 38% between week zero and week two (Figure 5.3). This change in area seemed to be caused by a contraction of the dung as it dried, as no external weathering or feeding evidence on the dung pat edges were observed. As observations following the initial two-week period were indicative of feeding (localised weathering on dung pat edges), we used photographs taken between Week 2 to 8 to determine

whether decomposition rates were affected by impaired insect activity. We used linear models to describe changes in dung pat area occurring over this six-week period, and used the slope estimates to test (using a two sample t-test) whether changes in area over time ($cm^2\,week^{-1}$), were influenced by impairing insects.

Results

Only *c.* 0.5% of dock seeds (7 out of the 1440 seeds sown) established as seedlings within the sward, and no meaningful statistical analysis could be used to compare treatments. In all cases, these plants were small (three or four leaves, 0-15 cm h) and were unlikely to cause any immediate direct loss to pasture productivity.

Moisture content did not differ significantly among treatments (Table 5.1), with 70.7 ± 1.2 % (mean ± se) forage moisture content found across all samples.

Table 5.1. Results of analyses testing the effect of dung and doramectin on the quantity and moisture content of harvested biomass.

Term	F	*P*
a) Fresh biomass		
Dung	$F_{1,156} = 28.49$	**< 0.001**
Doramectin	$F_{1,156} = 0.09$	0.76
Dung*Doramectin	$F_{1,156} = 1.96$	0.16
b) Moisture content		
Dung	$F_{1,36}=0.001$	0.98
Doramectin	$F_{1,36}=0.860$	0.32
Dung*Doramectin	$F_{1,36}=0.357$	0.55

Regardless of whether insects were impaired, the addition of dung significantly increased the quantity of forage harvested from the pasture the following year (Table 5.1). Plots with dung had *c.* 40% higher levels of forage growth than plots without dung (Figure 5.2).

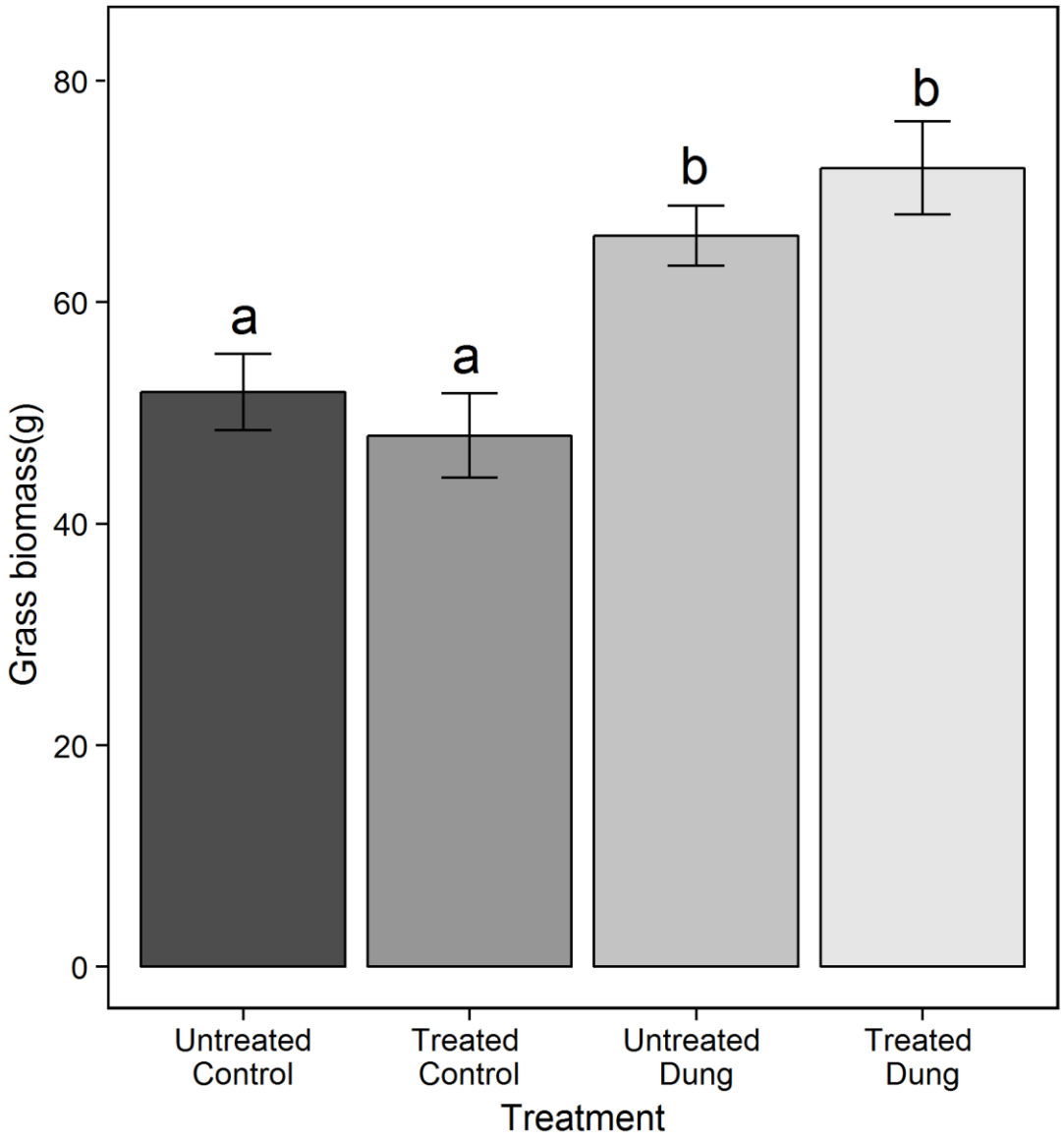

Figure 5.2. Treatments containing dung promote higher forage growth relative to dung free controls. Impairing insect activity does not influence this relationship. Bars are treatment means with standard errors.

There was no significant influence of insect impairment on dung removal rates (t= 0.177, df=78, *P* = 0.86). In both treatments, dung pats lost an average of *c.* 8 cm^2 area per week between weeks 2 and 8 (Figure 5.3).

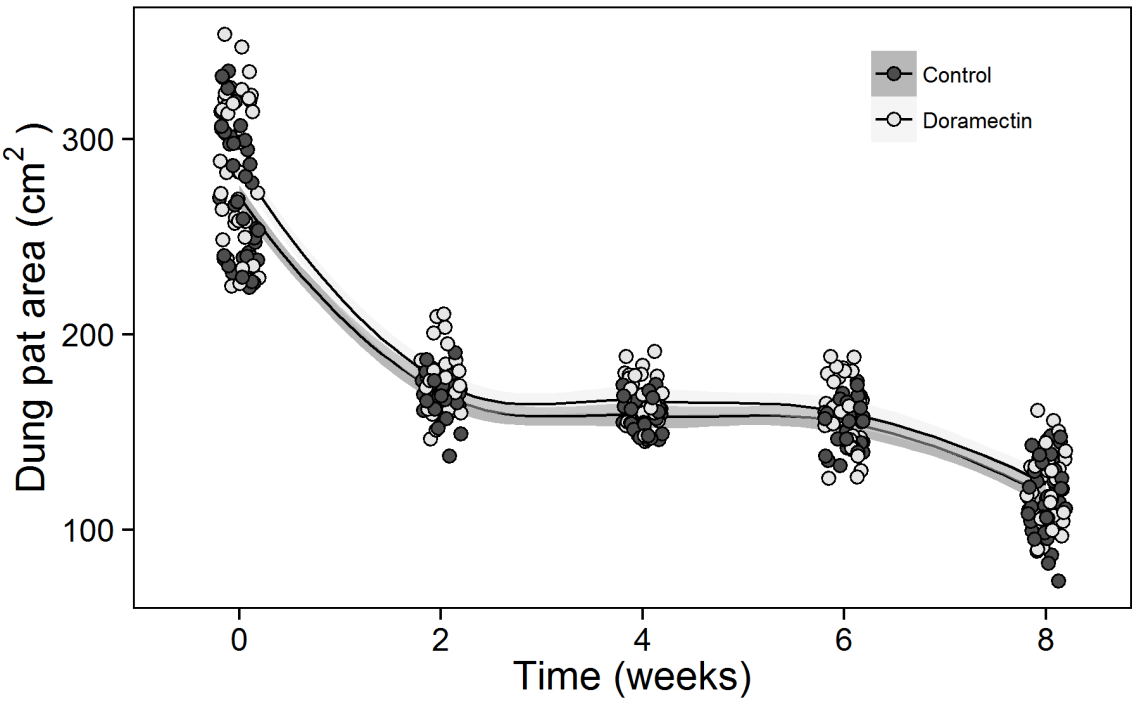

Figure 5.3. Area of dung pats as a function of insect-impairment (addition of doramectin) and time. There was no significant effect of treatment: dung area declined irrespective of the presence of doramectin. Data points have been jittered along the x-axis for clarity.

Discussion

Pasture productivity

We found that harvested plant biomass was higher in the presence of dung regardless of whether the dung was treated with doramectin (Figure 5.2). This suggests that dung deposition promotes pasture productivity, and that this benefit occurs even in the absence of insect-mediated dung decomposition. Research on the effect of coprophagous insects on plant productivity suggests that considerable amount of nutrients leach into the soil even in the absence of dung beetles (Bang *et al.*, 2005; Bertone *et al.*, 2006). This is most likely due to a combination of feeding by earthworms, microbial decomposition, and abiotic weathering of the dung pat.

While a number of studies have shown that dung beetles can support higher levels of forage growth (Bornemissza *et al.*, 1970; Borghesio *et al.*, 1999; Bang *et al.*, 2005), no such effects were evident in our experiment. Most such experiments have taken place over relatively short time periods. The increase in forage mass but consistency in water content observed in our experiment suggest that the improved grassland where this experiment was conducted might be relatively nutrient limited. However, nutrients contained within the dung seemed to become available to the plants the following year, regardless of whether coprophagous insects were impaired.

Although the presence of dung enhanced grass biomass production, our measure does not take into the account the pasture area under dung which would have been unavailable for grazing until sufficient decomposition occurred (Fincher, 1981). This would likely negate the positive growth associated with the presence of dung in the short-term. Conversely, it is possible that nutrients from decomposed dung provide longer-term benefits, if yield increases from dung incorporation continue past the first round of grazing (e.g. Bang *et al.*, 2005). Considering the long-term impacts of livestock-dropped dung on pasture productivity, would merit further investigation.

Weed Establishment

Despite using highly viable seed (a germination test conducted mid-August showed 95% viability), it is likely the environmental conditions required for successful germination and establishment of *R. obtusifolius* were not met during our experiment. While dock is readily observed growing in disturbed, nutrient-rich soil, successful establishment is dependent on a number of environmental factors. Humphreys *et al*. (1997) found just 1.8% seedling establishment under heavy slurry application and 1.7%

establishment under intensive physical disturbance of the soil, even with highly viable seed (85.0%). The same study found *R. obtusifolius* were unable to establish from seed in the absence of a physical disturbance or addition of nutrients, which is consistent with our findings.

As our experiment occurred in the absence of grazing livestock, we may have removed an element of disturbance key to the establishment of broad-leafed dock in pasture systems. While mature broad-leaved docks can quickly spread vegetatively and out-compete the sward, seedlings are poorly competitive in dense vegetation (Cavers and Harper, 1967), and survival is negatively correlated with grass root density (Martinkova *et al.*, 2009). The improved ryegrass-clover sward at our study site may not have provided adequate gaps for establishment. Experiments which consider the interaction between high nutrient availability (dung and urine patches), and frequent soil disturbances (often associated with gates, feeding areas and shelter) could better explain how broad-leafed dock establishes in permanent pastures.

While we found little evidence to support cattle dung (with or without insect impairment) serving as an appreciable microhabitat for broad-leaved docks to establish in pasture, our design considered only a single route to germination and establishment. Broad-leaved docks can establish in grazed pastures through a number of different paths including germination after being passed in livestock dung (Dai, 2000; Harrington *et al.*, 2011), or recruitment from the seed bank following soil disturbance (Van Assche and Vanlerberghe, 1989).

Estimation of dung removal

Our ancillary estimate of dung removal suggested that our attempt to impair coprophagous insects had no influence on dung removal rates, as estimated through changes in area (Figure 5.3). While we did not complement our design with insect emergence data, a large body of scientific literature has demonstrated lethal consequences of doramectin to dung insects at concentrations far lower than we tested (e.g. Dadour *et al.*, 2000; Floate *et al.*, 2001; Suárez *et al.*, 2009).

Assuming that our experimental manipulation did in fact kill or impair colonising insects, it is possible our methodology was not sensitive enough to detect any appreciable effect. The vast majority of decomposition mediated by insect feeding activity occurs within the confines of the dung pat (Owen *et al.*, 2006) and may not be reflected by assessing the exterior. Measuring changes in dung mass (Beynon *et al.*, 2012a; Kaartinen *et al.*, 2013), or organic matter loss (Holter, 1979b) would provide a better understanding of how anthelmintic residues influence invertebrate-mediated dung decomposition and related functions. Our non-destructive method may be more informative when tested under more heavily controlled experimental conditions, such as the mesocosms described by Beynon *et al*. (2012). Under such conditions the underlying heterogeneity in soil conditions across replicates will be greatly reduced, allowing finer treatment effects to be detected.

Our study was conducted in an improved pasture historically under intensive management, where coprophagous insect populations may have been relatively low (Hutton and Giller, 2003), perhaps contributing to the absence of a significant effect of insect impairment. Additionally, large-bodied tunnelling dung beetles (Coleoptera:

Geotrupidae), were largely absent from the study site during the period of our experiment when dung would have been attractive to coprophagous insects. Species from this group have been widely shown to be an important contributor to dung removal in agricultural landscapes (Kaartinen *et al.*, 2013).

Despite low rates of dung removal, no dung remained on the experimental plots in the spring of 2016. This was likely due to the activity of earthworms, with high densities of casts observed within the plots. A soil invertebrate survey conducted every three months at an adjacent improved pasture under similar management found a high annual mean density of 314 ± 58 individuals/m^2 (mean ± se) (P.Manning, unpublished data). Earthworms have been repeatedly shown to play a significant role in dung decomposition (Holter, 1979; Kaartinen *et al*., 2013; O'Hea *et al*., 2010b; Zhao *et al*., 2013), and are not affected by the presence of doramectin or other avermectin-based anthelmintics (Kaneda *et al.*, 2006). Because earthworms tend to colonise dung later during succession (Holter, 1979a), and many studies of dung decomposition are relatively short-term, it is likely the significant contribution of earthworms to dung removal is often underestimated. As earthworm activity is limited by low soil moisture levels (Zaller and Arnone, 1999) and populations are heavily reduced by cultivation (Rothwell *et al.*, 2011), dung removal and related functions mediated by earthworms may be at risk under ley-based grazing systems.

Despite the wealth of non-target consequences associated with use of veterinary anthelmintics for dung invertebrates and for the wider environment (Floate *et al.*, 2005), our data suggest minimal consequences of insect impairment for the functions we considered. However, as we relied on natural colonisation by a community with

relatively low abundance and diversity, we are likely underestimating the functional benefits delivered by a more complex community. Emergence data (e.g. Floate *et al*., 2002) would be a useful addition to future experiments of this sort, allowing assessment of the efficacy of insect-impairment manipulations, and revealing the composition of the dung insect community interacting with the experiment. Given the likelihood that the invertebrate community involved in our study was relatively depauperate (Hutton and Giller, 2003), the absence of any impact of insect-impairment is consistent with previous studies (Borghesio *et al.*, 1999).

Chapter 6: Non-target effects of moxidectin on the survival and functioning of dung beetles

Paul Manning[1,2], Sarah A. Beynon[1,2], Owen T. Lewis[1]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

Author Contributions: PM and SAB designed the experiment. PM conducted the experiment, performed the analyses, and wrote the manuscript. SAB and OTL provided multiple rounds of feedback on earlier versions of the manuscript.

Chapter Status: In preparation for submission

Abstract:

Macrocyclic Lactones (MLs) are a class of chemicals widely administered to livestock to control parasites. While highly effective, ML residues in dung can cause a range of lethal and sublethal effects on colonising insects. One group of insects affected by ML residues are dung beetles, where lethal and sublethal exposure can cause both demographic, and functional declines. Moxidectin has been shown to be less toxic to dung beetles than other MLs, which has led to this compound being marketed as 'dung beetle friendly'. Although evidence from studies of related MLs reveals substantial variation in sensitivity amongst species, and such claims are likely oversimplified. By selecting two species of dung beetle with differing sensitivities to agricultural intensification, we asked whether exposure to moxidectin residues influenced: survival, reproductive output, and functioning (short and long term estimates of dung removal). The species sensitive to agricultural intensification (*Geotrupes spiniger*) experienced a 43% reduction in survival relative to the control. In contrast, the non-sensitive species (*Aphodius rufipes*) was unaffected by moxidectin residues. We were unable to determine how exposure affected reproductive output of either species. A short-term assessment of dung removal found equivalent levels of dung removal were supported by the two species, with mean remaining dung weighing 58% of controls for *G. spiniger*, and 36% in *A. rufipes*. Long-term measures of dung removal were not possible to assess, as dung was fully decomposed regardless of exposure to moxidectin residues, or the addition of dung beetles. While the functional consequences of moxidectin residues seem relatively small, exposure has significant lethal effect on a sensitive species shown to provide high levels of functioning. Fully appreciating the functional losses moxidectin may have for non-target fauna would need to consider any persistent, multi-generational declines caused by repeated exposure.

Introduction

The Macrocyclic Lactones (MLs) are chemicals with broad applications in controlling internal and external parasites of livestock. As MLs are highly efficacious, have negligible toxicity to mammals, and can be conveniently applied thorough a variety of formulations (Boxall *et al.*, 2007), they have become a widely used tool for optimising livestock health.

Despite their popularity and efficacy, there is growing evidence that frequent use of MLs can have non-target impacts in pasture-based production systems (Lumaret *et al.*, 2012). Macrocyclic lactones are poorly metabolised, with active ingredients passed relatively unchanged in dung and urine (González Canga *et al.*, 2009). Invertebrates that colonise the dung for food and shelter come into direct exposure with toxic residues with lethal (Beynon *et al.*, 2012a) and sublethal (Verdú *et al.*, 2015) consequences for some taxa. One sensitive group are the dung beetles (Coleoptera: Scarabaeoidea), which play a key role in mediating dung decomposition (Nichols *et al.*, 2008).

Death or impairment of dung beetles and other insects can slow rates of dung decomposition, which in turn may lead to other detrimental consequences for ecosystem functioning in pastures. For instance, dung remaining unincorporated into the soil acts as a direct impediment to primary productivity (Fincher, 1981) and delayed decomposition is also thought to impair a number of economically important ecosystem functions (Beynon *et al.*, 2015). These negative effects of delayed decomposition include: nitrogen loss through volatilisation (Nichols *et al.*, 2008), increased probability of parasite reinfection (Gregory *et al.*, 2015), and spread of faecal pathogens (Jones *et*

al., 2015). Despite these difficulties, relative to other anthelmintic classes ML parasite-resistance is less widespread (Boxall *et al.*, 2003), and MLs are likely to remain an important tool under current production systems for optimising animal health. Finding ways to limit the non-target impacts of ML use on beneficial fauna, and the functions they provide is an important step in improving the environmental sustainability of livestock production.

Limiting the non-target impacts anthelmintics pose in the environment can be achieved through management decisions which are predominately practiced to slow parasite resistance to anthelmintics. For instance, the selective treatment of livestock with high intestinal burdens (van Wyk *et al.*, 2006) reduces exposure on a population level by providing sources of dung free from anthelmintic residues. Additional action can be taken under conventional management by housing animals during peak faecal excretion (Lumaret *et al.*, 2005). While these management practices show potential in minimising exposure risk, they may be difficult to implement at scale. Development and identification of active ingredients and formulations which exhibit lower toxicity to non-target taxa remains an important goal (Floate *et al.*, 2002; Beynon *et al.*, 2012b).

In this last regard, moxidectin is a ML that has shown particular promise. Moxidectin is effective in controlling a wide variety of livestock parasites (Losson and Lonneux, 1996) but has greatly reduced impacts on non-target species (Lumaret *et al.*, 2012). For instance, Doherty *et al*. (1994) found that moxidectin was required at 64 times greater concentration than abamectin (a related ML) to induce comparable LC_{50} mortalities in the dung beetle *Onthophagus taurus* Schreber. The low mortality of non-target taxa exposed to moxidectin relative to other MLs has been confirmed by numerous studies

(e.g. Blanckenhorn *et al*., 2013; Floate *et al*., 2005; Lumaret *et al*., 2012; Wardhaugh *et al*., 2001).

While moxidectin is a useful alternative to more toxic MLs, the assumption that this compound poses negligible toxicological risk to all dung beetle species is likely to be an oversimplification. Dung beetles exhibit significant interspecific differences in anthelmintic sensitivity (Beynon *et al.*, 2012a), and failing to consider impacts on the more sensitive species could underestimate the non-target effects of anthelmintic treatment on community structure and functioning. In particular, large tunnelling beetles tend to be more sensitive to anthelmintic residues than beetles from other functional groups (Beynon, 2012), potentially due to limited photodegradation of anthelmintics buried in soil (Halley *et al.*, 1989). As large tunnelers play a key role in promoting dung decomposition (Roslin and Koivunen, 2001; Kaartinen *et al.*, 2013), leading to concern that the functions they support may be lost to anthelmintic exposure.

Here, we compare how moxidectin residues affect the functioning, survival, and reproductive output of two representative dung beetle species: a large-bodied tunnelling species (*Geotrupes spiniger* Marsham), and a smaller-bodied species with a variable feeding strategy (*Aphodius rufipes* L.). These two species occur commonly during late summer throughout the Palearctic and have differing sensitivities to agricultural intensification (Hutton and Giller, 2003). However, their demographic and functional sensitivity to moxidectin have not previously been quantified and compared. We used manipulative experiments to investigate the influence of moxidectin residues on survival, reproductive output, and short-term and long-term dung removal.

Methods

The experiment was conducted on an improved pasture at Dr Beynon's Bug Farm, St. David's, Pembrokeshire, UK (51°53′22″, 5°14′06″). The pasture was in long-term rotation, converted from arable (barley) production to pasture in 2006. The vegetation consisted of a mixture of perennial ryegrass (*Lolium perenne L.) and white clover (Trifolium repens L.). A*t the time of the experiment, the field was grazed by a flock of 40 Welsh Mountain ewes and their lambs. Prior to beginning the experiment, we cut a 7×7 m area sward to a height of *c.*1cm. Sheep were excluded from the immediate experimental area with electric fencing.

Beetles were contained in 14 L enclosures (N=36), made from black plastic buckets with the bases removed. Each enclosure was dug into the soil to a depth of 8 cm which allowed colonisation by soil invertebrates, which often play an important role in supporting dung removal (Kaartinen *et al.*, 2013). Enclosures were randomly assigned to one of three beetle treatments (*G. spiniger* added, *A. rufipes* added, or no beetles added). Each beetle treatment was replicated in two dung treatments (dung containing moxidectin residues, or untreated dung), giving a total of six treatments with equal replication (n=6 replicates of each of the six treatments).

Dung was collected from a herd of Simmental cattle from a pasture adjacent to the experimental site (Upper Harglodd Farm, 51°53′23″, 5°14′07″). The cattle had not been treated with any veterinary medications for the past four months. The experiment coincided with a scheduled treatment of a moxidectin product (Cydectin pour-on solution, 0.5% w/v moxidectin) at the recommended dose of 1mL/10kg body weight. Fresh (<10 minutes old) dung was collected prior to anthelmintic treatment (control

dung), and again nine and ten days following treatment (moxidectin dung), a period known to correspond to peak faecal concentration of moxidectin following pour-on application (Steel, 1998). All dung was frozen immediately after collection for a minimum of seven days to kill any insects that might have colonised.

Dung beetles used in the experiment were collected onsite through a combination of hand-searching cattle dung, and dung-baited pitfall trapping. Prior to the experiment, all beetles were stored in mixed-sex terraria containing cattle dung and sterilised compost-based potting soil, and housed in a cool, well-ventilated shed.

The experiment began on August 23rd 2014 when *G. spiniger*, and *A. rufipes* were commonly observed flying at the farm. Dung was thawed and homogenised, with control (Day 0) and treated (a mixture of 50:50 Days 9 and 10) dung kept separated. We measured 600g batches of dung with a kitchen balance accurate to 1g and, using a circular 12cm diameter plastic mould, formed dung pats using the methods of Beynon *et al*. (2012). Dung pats were placed on a 2 cm aperture wire mesh, so that they could be lifted from the pasture surface allowing non-destructive dung removal estimates in the field. To allow more meaningful comparisons among treatments we standardised dung beetle biomass for all replicates using dry biomass (Table 6.1), following Beynon *et al*. (2012). Immediately after forming the dung pats, beetles were added to the enclosures and contained using a fine (2 mm aperture) plastic mesh.

Table 1. Dung beetle assemblages used within enclosures. Dung beetle dry biomass was approximately 1550mg in each treatment

Species	Functional group	Individual dry mass (mg)	Quantity	Overall mass (mg)
Aphodius rufipes (L.)	Soil-ovipositing endocoprid / kleptoparasite / paracoprid	29.9	52	1555
Geotrupes spiniger (Marsham)	Paracoprid	387.0	4	1548

[a]Dry mass values from Gittings and Giller (1997), [b] Dry mass values from Beynon *et al*. (2012)

Enclosures were monitored four to five times per day for beetle activity. When beetle activity was first observed (Day 14), we covered each enclosure with a ceramic tile and fitted an emergence trap: a 500 mL clear plastic bottle attached using a short length of 2 cm diameter clear plastic tubing (Chapter 2), so that beetles would move towards the light to exit the enclosure and enter the trap. Enclosures were checked for emerging beetles three times a day for ten days (Days 14–24), until a four-day period without any additional emergence occurred (Days 20–24). At this point, we assumed no further beetles would emerge, removed the covers from all enclosures and replaced them with mesh coverings.

We estimated dung removal, by measuring the remaining dung mass on Day 42, corresponding to a period when larval *A. rufipes* would have completed development within the dung pat and pupated in the soil (Stevenson and Dindal, 1985). As previous work has shown the benefits of beetles in supporting dung removal may become more apparent during the next grazing season (Beynon *et al.*, 2012a), we took a second measure of dung removal early in the next grazing season (May 14th, 2015). Enclosures were covered, and emergence traps were reattached on August 6th 2015 to estimate

impacts of moxidectin residues on larval development, as assessed by emergence of the next generation of adult beetles. Traps remained attached until September 30th, 2015, at which point we assumed that all adult beetles would have emerged.

Analysis

The proportion of beetles added that exited the enclosure into the emergence traps was used to estimate beetle survival. The effect of moxidectin on adult survival was tested for each species by comparing the proportion emerged between the moxidectin residue treatment and the untreated-dung control using a two-sample *t*-test.

Dung removal was modelled as a function of dung beetle treatment (*G. spiniger*, *A. rufipes*, or beetles absent) and the presence, or absence, of moxidectin using a two-way analysis of variance (ANOVA). Model residuals met assumptions of homogeneity and normality and thus raw values were left untransformed. Post hoc comparisons amongst means were made using Tukey's HSD test. All analyses used R 3.1.1 (R Core Team, 2014), and figures were drawn using the ggplot2 package (Wickham, 2009).

Results

Survival of *A. rufipes* was low in both dung types (control 0.66±0.06, treated 0.54 ± 0.07, mean ± se) and was not significantly affected by the presence of moxidectin residues (t = 1.29, df = 9.87, *P* = 0.23, Figure 6.1). However, survival of *G. spiniger* was significantly affected by the presence of moxidectin residues (t = 4.77, df = 7.71 , *P* = 0.002, Figure 6.1), being reduced by approximately 43% in treated dung (0.54 ±

0.07) relative to the control (0.96 ± 0.04). No beetles from the F1 generation were captured in emergence traps from either control or moxidectin residue dung.

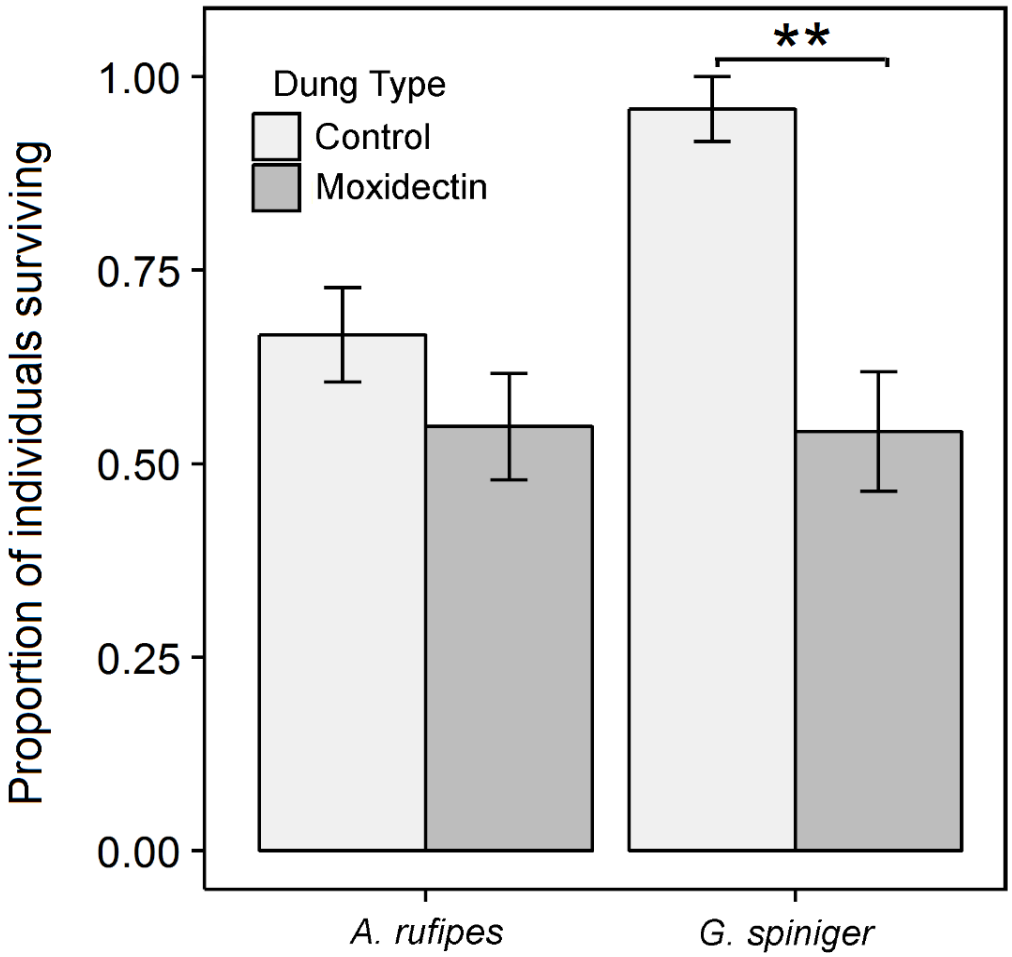

Figure 6.1. The proportion of surviving individuals captured in emergence traps, in response to moxidectin residues. *Aphodius rufipes* was not significantly affected. *Geotrupes spiniger* emergence was reduced by approximately 43% relative to controls. Bars are estimated means with standard errors.

For short-term dung removal, the interaction between dung type and beetle treatment was non-significant ($F_{2,30} = 2.79$, $P=0.078$). There was a significant main effect of the beetle treatment ($F_{2,32} = 10.98$, $P<0.001$) with significantly more dung removed when beetles were present compared to when beetles were absent. The presence of

moxidectin residues did not significantly affect dung removal for either species ($F_{1,32} = 0.37$, $P = 0.55$). *Aphodius rufipes* provided the highest level of dung removal (Figure 6.2), with the quantity of dung remaining in enclosures (56.5 ± 5.8 g) weighing *c.* 36% of dung pats without beetles added (154.2 ± 18.1 g). The addition of *G. spiniger* resulted in intermediate levels of dung removal, with a mean remaining dung mass (89.5 ± 19.3 g) weighing *c.* 58% of dung pats without beetles added, although there were no significant differences in functional efficiency between the two species (Figure 6.2).

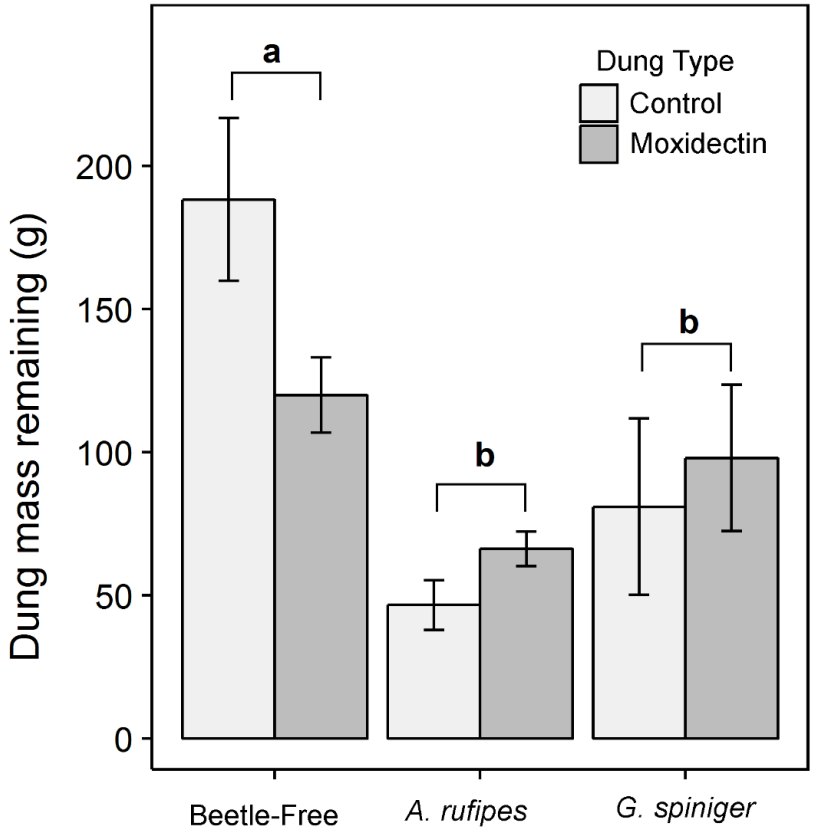

Figure 6.2. Mass of dung remaining (g) after six weeks as a function of the presence of dung beetles and moxidectin residues. Bars are estimated means with standard errors. Groups within beetle treatments sharing the same letter are non-significantly different from one another, as tested using Tukey's HSD.

It was not possible to estimate the effect of that the beetle treatment, and moxidectin residues had in explaining long-term functioning (dung removal at the beginning of the next grazing season). This was due to dung being fully decomposed in all treatments, almost certainly due to earthworms; where we observed high densities of earthworm casts in all enclosures.

Discussion

Overall we found little evidence that moxidectin impaired functioning both in the short- and long-term, but found that survival of one species (*Geotrupes spiniger*) was severely reduced by exposure to residues. In contrast to *G. spiniger*, *Aphodius rufipes* appeared to be unaffected by moxidectin residues (Figure 6.1). This species tends to perform relatively poorly in enclosures (Chapter 3), perhaps because of its susceptibility to heat stress as a nocturnal species (Jessop, 1986).

In dung beetles, larvae are typically more sensitive to anthelmintic residues than adults (Beynon *et al.*, 2012a). We attempted to test how moxidectin residues affected larval development by quantifying emergence in the year following the experiment, however we captured no beetles from the F1 generation. As we observed significant amounts of larval activity by *A. rufipes*, and evidence of brood ball partitioning by *G. spiniger* when estimating functioning in the short-term (Figure 6.2), it is unlikely that no beetles developed. It is more likely that our failure to recover any offspring in our experiment was a consequence of horizontal movement in the soil, with the subsequent generation emerging outside of the enclosures. Deeper enclosures (e.g. Rosenlew and Roslin, 2008), which limit horizontal movement in the soil, or larger enclosures to facilitate such movement, might be more appropriate for future experiments. An additional explanation

for the absence of emerging beetles in the second generation is the failure of beetles to develop within the timeframe of the experiment. While Palaearctic dung beetles are well-studied, detailed ecological and natural history information on the development of species in field conditions is relatively scarce for most species (but see: Floate *et al*., 2014; Vessby, 2001; Vessby and Wiktelius, 2003). Understanding the environmental factors which limit the development of dung beetles would be useful for future work.

While we found no significant effect of moxidectin on short-term measures of dung removal (Figure 6.2), this was likely due to high heterogeneity in the moisture content of dung, combined with a tendency for water content (as indicated by mass) to differ initially between the dung treatments. In the absence of beetles, the mass of dung containing moxidectin residues (120 ± 13 g) was on average 37% lower than control dung (188 ± 28 g) although a comparison using a two sample t-test found no significant difference attributable to the presence of moxidectin (t=-2.18, df =7.04, P = 0.065). A small sample size, and variability in remaining dung mass (likely caused by the colonisation of soil fauna) meant that we could not make any meaningful *post hoc* corrections for differences in dung quality. More precise estimates of dung removal, including use of dry mass (Chapter 3), or loss-on-ignition (Menéndez *et al.*, 2016), might permit better estimates of whether dung decomposition is impaired by anthelmintic residues. Despite variability in initial dung conditions, our long-term estimate of dung removal revealed that these differences may not have appreciable applied relevance. We found that dung was fully degraded the following spring, regardless of the addition of beetles, or the presence of moxidectin residues. This was likely due to the activity of earthworms, which are highly effective in removing dung from pastures (O'Hea *et al.*,

2010a), and have little sensitivity to moxidectin (Svendsen *et al.*, 2002), and other veterinary anthelmintics (Kolar *et al.*, 2008).

In the short-term, we found that *A. rufipes* provided levels of dung removal equivalent to a similar total biomass of *G. spiniger*. Large tunnelling dung beetles like *G. spiniger* are often considered to be the most important guild in supporting ecosystem functioning (Slade *et al.*, 2007; Kaartinen *et al.*, 2013; Nervo *et al.*, 2014). However, our results do show that high densities of a small-bodied dung beetle (with similar total mass) can support equivalent levels of functioning in the short term. As differences in dung removal do not always correspond to differences in other beneficial functions in pasture (Chapter 2), studies that consider other relevant functions while maintaining dung beetle biomass constant, or using models that integrate body size (Nervo *et al.*, 2014) might be useful in understanding how high abundance of a small-bodied species could compensate for absence of a large-bodied species known to contribute disproportionately to functioning.

While dung beetles are important in supporting ecosystem functions that underpin pasture production, they represent only a small proportion of the diverse invertebrate assemblage associated with livestock dung in temperate agroecosystems (Skidmore, 1991). For instance, improved pasture in the UK can support dozens of earthworm (King *et al.*, 2008) and coprophagous fly species (Skidmore, 1991). Both groups can provide considerable benefit in supporting dung removal. The earthworm *Eisenia fetida* Savigny, the fly *Scathophaga stercoraria* L., and two species of *Aphodius* dung beetle larvae were all found to make similar contributions to dung decomposition (O'Hea *et al.*, 2010a). Research focusing on the importance of these alternative groups is limited to a

small handful of published studies (Hendriksen, 1991; O'Hea *et al.*, 2010a; Kaartinen *et al.*, 2013), and their wider importance in supporting associated ecosystem functions have not been explored. While economic models show that conserving dung beetles in agroecosystems may have considerable benefits to farmers (Losey and Vaughan, 2006; Beynon *et al.*, 2015), these estimates do not take into account the role other invertebrates play in supporting dung removal. If earthworm activity fully removes dung even in the presence of anthelmintic residues, high earthworm activity could compensate, in the longer-term, for losses caused by the impairment of more sensitive taxa.

Our findings suggest that while anthelmintic residues pose a threat to dung beetles in agroecosystems, death or impairment of these species may not always have marked functional consequences. For measures of short-term functioning (in the case of dung removal), high densities of a common, non-sensitive species may compensate for the absence of a more functionally important but sensitive species. Additionally, when the same function is considered over the long-term, a combination of earthworm activity and abiotic weathering apparently compensates for the absence of dung beetles entirely. A more comprehensive assessment of functional impacts associated with treating livestock with moxidectin would need to consider multiple ecosystem functions (Chapters 2, 3). In addition, any persistent, multi-generational declines in dung beetles caused by repeated exposure to moxidectin should be investigated to fully appreciate the non-target risks of use of this anthelmintic in the livestock industry.

Chapter 7: Evidence that sex-specific signals may support mate finding and limit aggregation in the dung beetle *Aphodius fossor*

Paul Manning[1,2], Jack P. Ford[3]

1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. David's, Pembrokeshire SA62 6BX, UK

3. School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK

Author Contributions: PM designed the experiment. PM and JPF conducted the experiment. PM performed the analyses, and wrote the manuscript. JPF commented on an earlier version of the manuscript.

Chapter Status: Published as a short communication (Manning and Ford, 2016)

Abstract:

In temperate climates, dung is often colonised by several species of endocoprid (dwelling) dung beetles which use pats for feeding, shelter, and reproduction. Endocoprid beetles aggregate even when offered patches (dung pats) of consistent age, size, and origin, suggesting that beetles themselves might influence the attractiveness of patches to members of their own species. Both pheromones, and physical changes to the structure of dung pats caused by colonising beetles have been suggested as mechanisms facilitating intraspecific aggregation, but neither of these hypotheses have been empirically tested. Using a common European dung beetle (*Aphodius fossor* L.), we conducted a simple choice experiment designed to test whether (i) earlier colonisation by conspecifics could alter dung attractiveness and (ii) whether attraction was influenced by sex-specific signals. We found that female beetles are repelled by dung colonised by conspecific females and are attracted to dung colonised by conspecific males. Male beetles show no evidence of attraction or repellence for dung colonised by either sex. Neither in females nor males was uncolonised dung found to be significantly more or less attractive than predicted by non-preference. Our results suggest that for *A. fossor* male-produced signals may support mate finding in patchy environments, and that female-produced signals may serve to discourage subsequent colonisation by additional females.

Introduction

Dung beetles (Coleoptera: Scaraboidea) are a popular model taxon for testing ecological theory, including biodiversity-ecosystem functioning relationships (Beynon *et al.*, 2012a), metapopulation dynamics (Roslin, 2000; Roslin and Koivunen, 2001) and species-area relationships (Lobo and Martín-Piera, 1999). The spatial ecology of these beetles has been studied extensively, as habitat patches (dung pats) are easily delimited and species distributions can be studied readily over a wide range of scales (e.g. Finn *et al.*, 1998). In contrast to the intense competition experienced in tropical dung beetle communities (Hanski and Cambefort, 1991), in temperate ecosystems competition is relatively weak and dung monopolisation rarely occurs (for review see Finn and Gittings (2003)). Instead, dung beetle communities tend to be characterised by dwelling species, with density and diversity showing an aggregated distribution across habitat patches (Palestrini *et al.*, 1998).

An important element that influences dung beetle aggregation within a localised patch is dung attractiveness. This can be influenced by a variety of factors including time of day at which the dung was produced (Holter, 1979b), exposure of the dung pat (Landin, 1961), weather during dispersal (Finn *et al.*, 1998), age of the dung (Landin, 1961) and the presence of veterinary pharmaceutical residues (Floate, 2007). However, highly variable beetle aggregation is observed even when using dung from a homogenous source with patches having both an identical size and age (Hanski and Cambefort, 1991; Palestrini *et al.*, 1998). This suggests additional unidentified mechanisms may be acting to promote aggregation.

A study exploring the spatial distribution of temperate dung beetles (Palestrini *et al.*, 1998) found both inter- and intraspecific aggregation. They proposed that the activity of early colonising beetles might increase attractiveness and habitability of dung for potential future colonisers. A second hypothesis offered in discussion within the same paper suggested pheromones might be contributing to intraspecific aggregation.

We conducted a preliminary investigation of these hypotheses with a choice experiment using the widely-distributed temperate dung beetle *Aphodius fossor* L. We offered male and female beetles a choice of three dung pats which were either: uncolonised, or manipulated to contain either four conspecific males or four conspecific females. By comparing observed frequencies with expectations of non-preference, we were able to test whether colonisation patterns supported aggregations promoted by (i) enhanced attractiveness as a result of the activity of colonised beetles; or (ii) sex-specific responses.

Methods

Collection of dung beetles

Adult *A. fossor* were hand-collected from cattle dung at Dr Beynon's Bug Farm, St. David’s, Pembrokeshire, U.K. (51°53′20″, 5°14′09″) on 30 May 2015. Adults were separated by sex into well-ventilated 4 L plastic tubs containing damp builders sand and 500 ml of fresh cattle dung. All beetles were stored in a cool, dark shed before beginning the experiment.

Experimental set-up

Dung was collected on site from a herd of Welsh Black cattle immediately following defecation - before any insect colonisation could occur. The dung was homogenised

and formed into nine 250 mL dung pats. Each dung pat was placed into a well-ventilated, 5 L rectangular tub (22 × 15 × 15 cm), on top of 8 cm of moist, washed builders sand. Either four female (*n* = 3) or four male (*n* = 3) beetles were randomly assigned to tubs. The three remaining dung pats were left uncolonised to serve as controls. Tubs were stored for 48 h in a cool, dark shed to allow beetles to acclimatise before beginning the experiment.

Three arenas were constructed using circular, 30 cm diameter, 14 litre black plastic plant pots filled to 10 cm with moist, washed builders sand (Figure 7.1). Three circular holes measuring 2 cm in diameter were drilled at intervals of 120°, with the bottom of the hole sitting flush with the sand surface. A 4 cm length of clear plastic tubing (2 cm diameter) was used to join the larger arena to the tubs containing the dung pats. One end of the tubing was placed flush against the side of the arena with the other end extended into the smaller tubs (Figure 7.1).

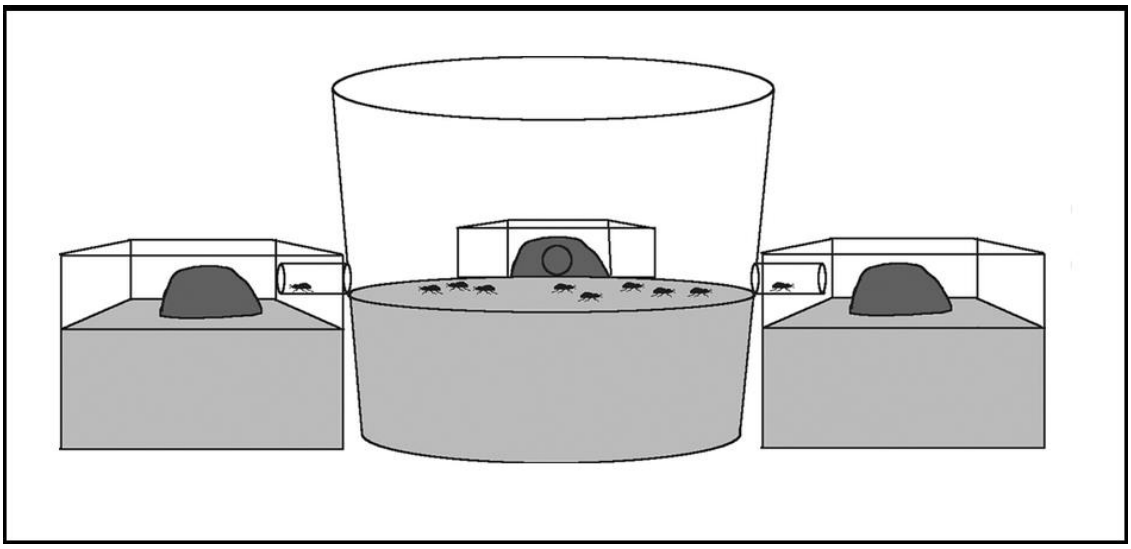

Figure 7.1. Dung choice experiment apparatus. Opaque tubs and arena are shown as transparent for clarity. Beetles are placed in the centre of the central arena, and move into one of the three surrounding tubs.

Dung choice tests

On June 1st at 19.00 hours, 2 h before the experiment began, the tubs containing dung pats were attached to the larger arena. The first round of the experiment began at 21.00 hours when *A. fossor* becomes most active (P. Manning, pers. obs.). A total of 90 males and 90 females were run through the experiment for a total of 18 separate rounds (occurring in six successive periods). Sand was re-moistened with water between rounds, using a spray bottle. The experiment was conducted indoors, under fluorescent light. Arenas and tubs were rotated 120° after each round to account for any differences caused by variation in ambient light. We ran male beetles through the experiment first, replacing the top *c*. 3 cm of sand within the arena before testing female beetles.

Three arenas were run simultaneously. As pilot trials found that beetles frequently took longer than 20 min to select a dung pat, beetles were run in groups of 10. In each round, the 10 beetles of a single sex were added to the middle of each arena and were initially confined to the innermost area of the arena using a circular, steel tea strainer (8 cm diameter). Beetles were kept in place for 120 s before the tea strainer was lifted and beetles were able to move towards a dung pat in one of the surrounding tubs. Each round of the experiment continued until either all beetles had reached a tub (each beetle being promptly captured and removed from the experiment before it could enter the dung) or after 25 min when the round was terminated.

Statistical analysis

Data for male and female beetles were analysed separately using a goodness-of-fit test for discrete multivariate data, comparing observations to null expectations of non-preference. When observed counts were found to be significantly different from null

expectations ($P < 0.05$), a post hoc test was performed, testing each category of observation against its expected frequency (McDonald, 2009) using a Bonferroni corrected significance threshold ($\alpha = 0.0167$). Analyses were carried out using the 'EMT' package (Menzel, 2013) for R 3.1.1 (R Core Team, 2014).

Results and Discussion

Of the 90 females tested, 67 reached the dung within the allotted 25 min. The distribution of observed counts of females attempting to colonise dung differed significantly from expectation ($\chi^2 = 11.32$, df = 2, $P = 0.002$*). Post hoc* testing suggested that female beetles avoided dung colonised by other females ($\chi^2 = 10.024$, df = 1, $P = 0.001$) while preferentially selecting dung colonised by males ($\chi^2 = 6.52$, df = 1, $P = 0.013$) (Figure 7.2a). Of the 90 males tested, 62 reached the dung within the allotted 25 min. The majority of male beetles were attracted to dung colonised by females (Figure 7.2b), but the observed values were not significantly different from expectations of indiscriminate choice ($\chi^2 = 4.004$, df = 2, $P = 0.146$). In neither case did we find uncolonised dung was selected more or less frequently than predicted by non-preference, suggesting there was no significant influence of beetle colonisation on dung attractiveness.

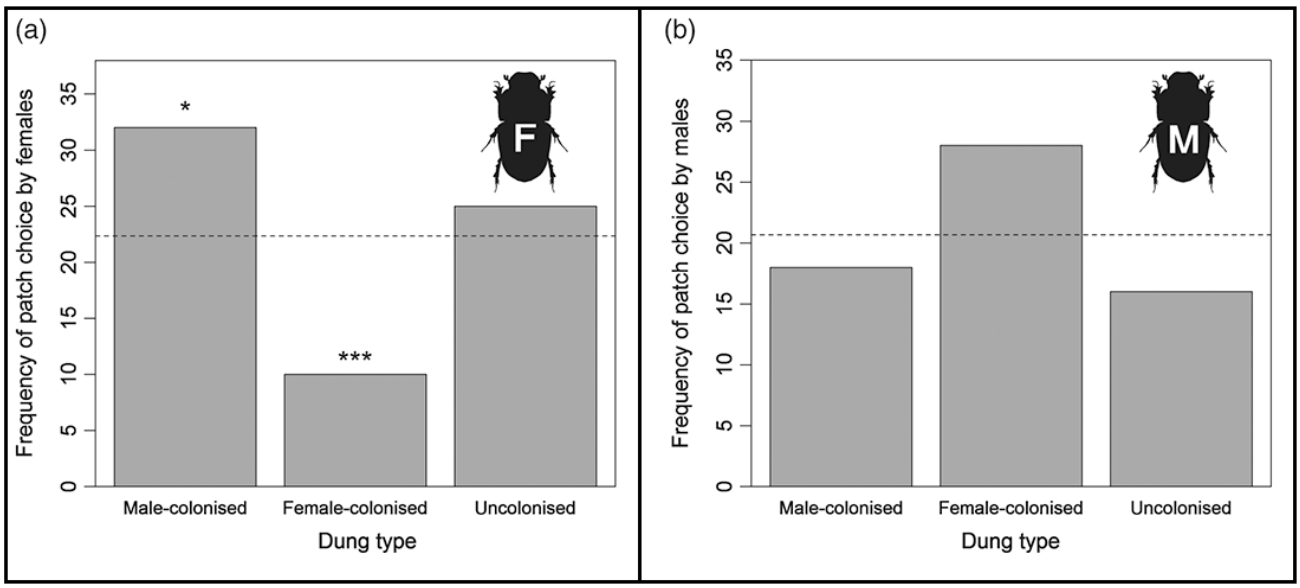

Figure 7.2. Preference of (a) female and (b) male *Aphodius fossor* in selecting dung which is uncolonised, or colonised by either male or female conspecifics. The dashed line marks the expected number of individual selections based on non-preference. Stars indicate frequencies which are significantly different from expectations of non-preference, values (*P < 0.05, ***P < 0.001).

The most striking result of our experiment was the avoidance of female-colonised dung by newly colonising female beetles. We suggest this might be attributable to deterrent pheromones produced by females, which serve to repel other females, many being classified as ‘anti-aggregation’ or ‘oviposition deterring’ pheromones. These signals discourage crowding and egg laying, thus reducing potential competition for offspring. The production of deterrent pheromones is known from many insects (e.g. tephritid flies (Prokopy, 1975), bark beetles (Njihia *et al.*, 2014) and green lacewings (Růžička, 2013)). We suggest that upon receiving a signal indicating the presence of a female beetle, the dispersing female selects an alternative resource where her offspring are less likely to experience density-dependent mortality.

A comprehensive literature review suggests that endocoprid dung beetle larvae can be space-limited within small dung pats (Finn and Gittings, 2003). As *A. fossor* is one of the largest species of endocoprid dung beetle in Europe (Jessop, 1986), low-cost strategies (production and reception of chemical signals) could play an important role in reducing density-dependent mortality.

The preference of male-colonised dung by female beetles could be supported by a male-produced attraction pheromone, a phenomenon documented in other Scarabaeoidea (e.g. Tribe, 1975; Edwards and Aschenborn, 1988; Larsson *et al.*, 2003). The timing of our study occurred approximately 2 weeks after teneral adults began emerging, corresponding to when *A. fossor* females reach reproductive maturity (Gittings and Giller, 1997), and would be receptive to mating. *Aphodius fossor* is found almost exclusively in coupled pairs, although densities may exceed six pairs within a dung pat (P. Manning, pers. obs.). Males of the temperate species *Typhaeus typhoeus* L. (Geotrupidae) have been observed burrowing part-way into dung where they defecate on the dung surface — a behaviour thought to represent simultaneous pheromone release (Brussaard, 1983). During our experiment, we observed both males and females entering part-way into the dung where they remained for several minutes with their abdomen partially exposed: while we did not observe defecation, this stance could have potentially facilitated pheromone release.

Alternatively, sex-specific chemical signals influencing dung beetle aggregation may not be pheromone based. The activity of endocoprid dung beetles alters the composition of gases fluxing from cow dung (Penttilä *et al.*, 2013) which might act to modify its attractiveness to colonising beetles. If male or female beetles behave differently within

the dung pat (e.g. higher activity), this could potentially induce different gas fluxes. As we have no mechanistic evidence for the sex-specific responses, non-pheromone-based chemical cues should not be discounted.

Furthermore, sex-specific signals in dung beetles may not be chemical: acoustic signals have been shown to play a role in aggregation in other *Aphodius* species (Hirschberger, 2001; Kasper and Hirschberger, 2005). Acoustic signals are unlikely to have contributed to dung choice in this experiment as they are effective only at close range, being received mechanically from within the dung pat (Hirschberger, 2001).

As this experiment was conducted only 48 h after dung was first produced, these data are not fully representative of the entirety that beetles remain resident within a dung pat. Adult *A. fossor* often inhabit older dung and it is likely that beetle activity could play a more influential role in altering attractiveness as dung ages and desiccates.

While our experimental design necessitated running beetles in groups of 10 as a consequence of logistical constraints, we recognise that individuals may have been influenced by odours released, and/or signals displayed by other beetles in the group. However, we observed that beetles frequently stopped when reaching the tunnel end, with their head suspended into the airspace above the dung. After a brief (15–30 s) period of antennal movement, beetles would either turn, walking back into the arena, or walk forward – dropping into the tub. This observation suggested that beetles were responding to cues emanating from the dung pat itself, rather than solely following chemical trails laid by other beetles in the group.

Although our data show female patch choice is influenced by the sex of earlier colonisers, it is less clear how this might lead to dung beetle aggregations observed in nature. Female preference for male-colonised patches can explain observations of mate pairing, but female–female avoidance likely acts to limit aggregation size. One explanation could be that when both males and females occupy the same pat, female attraction to males outweighs female–female repellence. A second explanation could be that the female–female repellence observed in our experiment was induced by high densities (four beetles per 250-ml dung pat), and the effect we observed may not persist when beetles occupy larger patches.

Our results with *A. fossor* demonstrate that sex-specific attraction and repellence could have a strong influence in explaining intraspecific dung beetle aggregations. We also suggest that alongside more refined methodologies, simple olfactometer studies could play an important role in achieving a better understanding of the underlying mechanisms that influence the spatial distribution of dung beetles.

Chapter 8: General Discussion

The value of biodiversity for ecosystem functioning in pasture production

Diverse communities generally deliver greater functional benefits than species-poor communities (Cardinale *et al.*, 2012). Intensive agricultural practices often lead to the loss of biodiversity (Tuck *et al.*, 2014), and in some cases these losses can have negative implications for functions which support production (Snyder *et al.*, 2006; Beynon *et al.*, 2012a). For agricultural production to continue in the wake of declining biodiversity, it may become necessary to supplement (Collier and Van Steenwyk, 2004) or replace (Partap and Ya, 2012) freely delivered ecosystem functions with costly inputs.

Despite the inherent appeal of biodiversity conservation directly benefitting agricultural productivity, functional arguments may provide insufficient justification to support these efforts (Norgaard, 2010). For instance, a mere 2% of the bee species found in agricultural ecosystems provide 80% of all crop pollination (Kleijn *et al.*, 2015). In the case of dung beetles in pastures ecosystems, the value of biodiversity in underpinning functioning appears uncertain. While in Chapter 2, we found evidence to suggest that species rich dung beetle assemblages offered considerable promise in supporting multiple functions, these results were not in agreement with our findings in Chapter 3.

An important caveat of our diversity-manipulation experiments (Chapters 2 and 3), was that the highest level of dung beetle species richness considered was four species. While this is representative of a typical assemblage within the study area, the functional benefits of species richness may be stronger when a larger species pool is considered

(e.g. Palestrini *et al.*, 1998). A second important caveat is that we only manipulated the richness of a single taxon (dung beetles). This group represents a relatively small component of the dung-associated invertebrate community (Skidmore, 1991), and its contribution to functioning may be negligible over longer time-scales when and where earthworms are present (Chapters 5 and 6).

Selecting from a larger species pool for diversity manipulations (Rosenlew and Roslin, 2008; Beynon, 2012), or conducting natural colonisation experiments which selectively exclude particular taxa (Slade *et al.*, 2007; Kaartinen *et al.*, 2013) may help to provide a better understanding of the roles invertebrate diversity plays in supporting ecosystem functions in pasture.

Is invertebrate abundance more important than diversity in supporting functioning?

While the functional benefits of maintaining species-rich dung beetle assemblages were not fully clear, we found the addition of dung beetles supported higher levels of functioning than what was supported by soil invertebrates alone (Chapters 2 and 3). Assuming that the relationship between dung beetle biomass and functioning is relatively linear (Yamada *et al.*, 2007), reductions in abundance and biomass associated with agricultural intensification (Hutton and Giller, 2003) may have greater negative implications for ecosystem functioning than loss of diversity. Approaches that manipulate both diversity and abundance of multiple invertebrate groups either through field experiments, or with mathematical models (Sherratt *et al.*, 1998; Vale and Grant, 2002) would be useful in understanding how these factors interact to shape ecosystem functioning in pastures.

Sensitivity to perturbation, and resilience, of dung associated invertebrates

Chemical perturbations associated with macrocyclic lactone (ML) residues in dung were a consistent theme throughout this body of work (Chapters 3–6). While these perturbations represent a significant threat to sensitive invertebrates (Chapters 3 and 6) not all species in pasture communities are similarly affected (Kaneda *et al.*, 2006; Beynon *et al.*, 2012a).

In Chapter 6, we showed that in the short-term a small-bodied species (*Aphodius rufipes* L.) provided equivalent levels of functioning to a large-bodied species (*Geotrupes spiniger* Marsham) with known functional importance (Rosenlew and Roslin, 2008; Kaartinen *et al.*, 2013) and high sensitivity to MLs (Beynon, 2012). We also showed that, despite significant benefits of dung beetles in providing short-term functioning (Chapter 6), their absence may be compensated for by earthworm activity when considered over a longer timescale.

Expanding scope to consider the role of associated taxa

While dung beetles clearly play an important role in promoting a number of important ecosystem functions (Nichols *et al.*, 2008), there is an apparent bias in the literature favouring dung beetles over other dung-associated taxa. Dung beetles constitute only a small part of the diversity found within dung invertebrate communities, and the role of other invertebrate taxa should be more critically evaluated.

Several lines of evidence within this study suggest that earthworms may have a significant role in supporting dung removal in pasture ecosystems (Chapters 5 and 6). Earthworms show little sensitivity to ML residues in dung (Svendsen *et al.*, 2002; Kaneda *et al.*, 2006; Kolar *et al.*, 2008), and are long-established ecosystem engineers

that improve the physical (Blanchart *et al.*, 1999; Shipitalo and Le Bayon, 2004) and chemical (Cortez *et al.*, 2000) qualities of agricultural soils. Earthworm populations tend to show positive responses to applications of organic (Timmerman *et al.*, 2006), and mineral fertilisers (Curry *et al.*, 2008), but populations are severely reduced by cultivation (Rothwell *et al.*, 2011). Given these associations, management practices undertaken to increase earthworm populations could play an important role in ensuring the delivery of ecosystem functions, particularly in the presence of chemical perturbations.

A limitation of our findings is that we did not test how dung removal supported by earthworms affects the delivery of associated functions. Developing a greater understanding of the role which earthworms play in providing related functions associated with dung removal (e.g. regulating greenhouse gas fluxes (Penttilä *et al.*, 2013), limiting parasite transmission (Gregory *et al.*, 2015), or preventing spread of faecal pathogens (Jones *et al.*, 2015)) warrants further study.

Another group whose contribution to dung removal has been largely ignored is the community of flies associated with dung. In many cases, flies are the most abundant (Skidmore, 1991; Floate, 1998; Braga *et al.*, 2012) and diverse (Skidmore, 1991) order of invertebrates associated with livestock dung**.** The majority of work which considers the interaction between dung and coprophagous flies, involves testing the susceptibility of laboratory populations of *Neomyia cornicina* L., and *Scathophaga stercoraria* L. to anthelmintic residues (Lumaret *et al.*, 2005; Römbke *et al.*, 2009). The other notable consideration of coprophagous flies in the literature is how populations of veterinary livestock pests (predominately *Musca autumnalis* De Geer and *Haematobia irritans* L.

might be reduced by dung beetles (Bornemissza, 1970; Hughes *et al.*, 1978; Wallace and Tyndale-Biscoe, 1983). With the exception of O'Hea *et al.*, (2010) contributions of flies in facilitating dung decomposition have largely gone unquantified, and underappreciated.

A final component of the dung invertebrate community which has not been fully explored is the role of invertebrate predators. Rove beetles (Coleoptera: Staphylinidae), water beetles (Coleoptera: Hydrophilidae), and hister beetles (Coleoptera: Histeridae) represent some of the common invertebrate predators found in dung (Skidmore, 1991); however their role in affecting decomposition and related functions is poorly understood. Work by Zhao *et al.* (2013) has shown that the presence of predators can alter earthworm behaviour, causing individuals to move to lower soil horizons which prompts higher levels of forage productivity. Additionally, while the direct role of predatory rove beetles and water beetles in dung pats is well known (Valiela, 1974; Walsh and Posse, 2003), little work has considered the role of these groups in controlling pest fly populations (but see, Fay and Doube, 1987).

Fixating on a sole component of diversity (Chapters 2 and 3), and even categorising entire insect orders, such as Diptera, as pests (Braga *et al.*, 2012), makes it difficult to tease apart the functional importance which different taxa play in underpinning ecosystem functioning. A more comprehensive understanding of how ecosystem functions in pasture are affected by agricultural intensification could be achieved using experiments that consider: a diversity of invertebrate taxa (e.g. O'Hea *et al.*, 2010), their specific sensitivities (e.g. Floate, 1998), and their functional significance (e.g. Beynon, 2012) in supporting multiple functions (e.g. Chapters 2–4).

Sublethal effects of chemical perturbations

A comprehensive body of evidence has documented the lethal effects of anthelmintic exposure on insects developing in livestock dung (reviewed by Lumaret *et al.*, 2012; Wall and Beynon, 2012). However, relatively little work has concentrated on the sublethal impacts of exposure.

Available evidence suggests the impacts of sublethal exposure are varied and significant. Exposure to ML residues can cause impaired chemoreception (Verdú *et al.*, 2015), atypical morphological characteristics (Strong and James, 1992), prolonged larval development (Pérez-Cogollo *et al.*, 2015), reduced muscle strength (Verdú *et al.*, 2015), and lower fecundity (McGarry, 1986). While in Chapter 4, we found that previous exposure to MLs did not affect subsequent functioning, beetles were physically transported between enclosures, removing dispersal from the equation. Given the indispensable importance of successful dispersal in supporting survival, reproduction, and therefore functioning, experiments which explore how sublethal exposure affects dispersal would be invaluable in achieving a more nuanced picture of how ML residues affect the delivery of ecosystem functions.

Wider environmental ramifications of intensive pasture management

Given the dominance of pastures within the global landscape (Steinfeld *et al.*, 2006), intensification of these systems will have ecological ramifications extending beyond the communities they directly support. For example, aerial insectivores are in general decline (Ambrosini *et al.*, 2012) and often forage over pastures where flying insect density may be twice as high as on arable land (Evans *et al.*, 2007). Loss of invertebrate biomass has also been attributed as a factor in the decline of other

insectivorous birds with close associations to agricultural landscapes, including corn bunting *Milaria calandra* L. (Brickle *et al.*, 2000) and yellowhammer *Emberiza citronella* L. (Morris *et al.*, 2005).

The intensive management of pastures can also have costs for ecosystems off-site. Poor nutrient management planning can spread water-borne pathogens (Ferguson *et al.*, 2003) and lead to eutrophication which has numerous adverse outcomes for freshwater (Anderson *et al.*, 2002) and marine (Howarth and Marino, 2006) ecosystems. Additionally, as the production and application of mineral nitrogen fertilisers acts as a significant source of greenhouse gas emissions (Erisman *et al.*, 2008), intensification of pasture production has considerable potential to undermine the role of pastures as net sinks for carbon sequestration (Slade *et al.*, 2016).

An important final consideration is that pasture management is practiced over a wide continuum of intensity (Steinfeld *et al.*, 2006). While intensification may allow for considerable productivity benefits in some areas of the world, other may not be well suited due to geographic, climatic or economic constraints. In many of these cases, management aligned more closely to a land-sharing philosophy may be a more feasible option in producing animal products, offering additional value for biodiversity conservation, ecosystem functions (Silver *et al.*, 2000), and the associated economic benefits.

Conclusion

While addressing only a small component of the vast multidisciplinary challenge of simultaneously feeding the world while conserving biodiversity, our findings offer some insight into how intensive pasture management affects biodiversity and ecosystem

functioning. We show that the functional benefits of maintaining dung beetle diversity are not always consistent (Chapters 2 and 3). We show that resilient taxa may support the delivery of ecosystem functions even when communities come under chemical perturbation (Chapters 5 and 6). While these perturbations may not always result in appreciable functional consequences (Chapters 4–6), we show they remain a significant threat to the conservation of sensitive groups (Chapters 3 and 6).

As the global population continues to expand (United Nations, 2015) and the developing world begins to incorporate more livestock products into their diet (Godfray *et al.*, 2010), it is important that we find sustainable ways to meet this demand while simultaneously conserving biodiversity. As loss of biodiversity does not always translate into loss of ecosystem functioning, it is important that wider justifications for conservation remain integrated into agricultural policy and practice.

Acknowledgements

First and foremost, I offer my sincerest gratitude to Professor Owen Lewis and Dr. Sarah Beynon for their supervision and mentorship over the past three years. Their collective efforts have been instrumental in helping me become a better scientist, entomologist, and communicator. I cannot thank them enough for their time, energy, ideas, or discussions. I am deeply thankful to have had their support and guidance throughout this learning experience.

I thank the wonderful members of my research group - Community Ecology Research Oxford. I am grateful to have landed amongst such bright, supportive, and kind individuals. I thank all members for the spirited ecological discussions, sharing laughter and lunch, inspiring new ideas, and teaching me something new each and every day.

I thank Dr. Eleanor Slade for looking out for me, and reminding me to take it easy. Aside from being a brilliant and reliable collaborator, Ele frequently checked-in, and made sure that everything was ok. Her thoughtfulness was, and is, incredibly appreciated.

I thank Pauline and John Beynon (Penweathers Farm, St. David's), Charlie Gee (Manor Medley Farm, Oxford), Jo Copping (Farm Animal Initiative, Wytham), and Dr. Sarah Beynon (Lower Harglodd Farm, St. David's) for allowing me to collect beetles for experiments. I thank Mark and Emma Evans (Upper Harglodd Farm), Jo Copping, and Dr. Sarah Beynon for allowing me to collect hundreds of kilograms of cow dung to use in these experiments.

I thank Sarah Johnstone and Ben Sharpe for their friendship and kindness throughout the field work seasons. I thank the legendary Andy Holcroft (Grub Kitchen) for the lively conversations, helping move heavy things, catch beetles, and kindly cooking delicious meals at the end of some knackering days in the field. I thank Phil Smith (Wytham Field Station), and Jo Copping for their invaluable contribution in helping with organisation, and logistics during the field seasons in Wytham. Thank-you to Hannah Banks, Sarah Johnstone, Andy Holcroft, Warwick Wainwright, Chris McCloskey, Chris Terry, Sarah Jane Fanning, Megan Daniel, and Olalekan Faniran for their help in the field. Many hands indeed make light work.

I thank my wonderful friends, both new and old – and globally distributed for three excellent years of adventures. Exploring the winding paths of Oxford, pub-crawling, hiking the coast, bird watching, early runs, late breakfasts, punting, learning new languages, indulging in delicious meals, jamming on the guitar or keyboard, cramming in a quick phone call, fishing for mackerel, travelling to new cities, swimming in the Thames, wine-tasting, arguing about science, pancake-flipping, drinking endless cups of tea, and playing bananagrams; these were welcome and necessary distractions from research. You have all made this experience so rich and memorable. Again, thank-you.

I thank my other-half Sarah Jane Fanning for her love and support over the past three years. Simultaneously pursuing independent graduate programs on different continents, paired with a four-hour time difference was not always easy, but was it ever worthwhile. Reading Sarah Jane's carefully chosen, and loving words each morning was always the best way to begin the day; hearing her voice in the evening, was always the best way to end it. Thank-you Janey, for being my person.

I offer heartfelt thanks to my family. I eagerly awaited telephone calls on Sunday evenings, and treasured each moment of jam-packed visits home. It was incredibly difficult to live so far away. Thank-you for always being there, for understanding, and for always making me feel unconditionally and whole-heartedly loved. A special thanks to my father for sending weekly letters in the post; they made me homesick in the best sort of way.

Finally, I'd like to thank my funding bodies for investing in my potential as an early career scientist. Funding for this research was provided by a Rhodes Scholarship, and through a Natural Sciences Engineering Research Council Postgraduate Scholarship (Canada).

References

Alderfer, R.B.B. and Robinson, R.R.R. (1947) Runoff from pastures in relation to grazing intensity and soil compaction. *Journal of the American Society of Agronomy*, **39**, 948–958.

Alexandratos, N. and Bruinsma, J. (2012) *World Agriculture Towards 2030/2050: the 2012 Revision.* (No. 12–3). Food Agriculture Organisation, Rome.

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., *et al.* (2015) Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecology Letters*, 1–10.

Ambrosini, R., Rubolini, D., Trovò, P., Liberini, G., Bandini, M., Romano, A., *et al.* (2012) Maintenance of livestock farming may buffer population decline of the barn swallow *Hirundo rustica*. *Bird Conservation International*, **22**, 411–428.

Anderson, D.M., Glibert, P.M. and Burkholder, J.M. (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, **25**, 704–726.

Anderson, J.R., Merritt, R.W. and Loomis, E.C. (1984) The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *Journal of Economic Entomology*, **77**, 133–141.

Bang, H.S., Lee, J.-H., Kwon, O.S., Na, Y.E., Jang, Y.S. and Kim, W.H. (2005) Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology*, **29**, 165–171.

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., *et al.* (2011) Has the Earth's sixth mass extinction already arrived? *Nature*, **471**, 51–57.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., *et al.* (2014) Package "lme4." *R Foundation for Statistical Computing, Vienna*.

Bengtsson, J., Ahnström, J. and Weibull, A. (2005) The effects of organic agriculture on biodiversity and abundance: a meta-analysis. *Journal of Applied Ecology*, **42**, 261–269.

Bertone, M.A., Green, J.T., Washburn, S.P., Poore, M.H. and Watson, D. (2006) The contribution of tunneling dung beetles to pasture soil nutrition. *Forage and Grazing Lands*, **4**, doi: 10.1094/FG-2006-0711-02-RS.

Beynon, S.A. (2012) *Factors affecting ecosystem service provision by dung-associated invertebrates*. DPhil Thesis. University of Oxford. Oxford, UK.

Beynon, S.A., Mann, D.J., Slade, E.M. and Lewis, O. (2012a) Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. *Journal of Applied Ecology*, **49**, 1365–1372.

Beynon, S.A., Peck, M., Mann, D.J. and Lewis, O.T. (2012b) Consequences of

alternative and conventional endoparasite control in cattle for dung-associated invertebrates and ecosystem functioning. *Agriculture, Ecosystems and Environment*, **162**, 36–44.

Beynon, S.A., Wainwright, W.A. and Christie, M. (2015) The application of an ecosystem services framework to estimate the economic value of dung beetles to the UK cattle industry. *Ecological Entomology*, **40**, 124–135.

Bhagwat, S.A., Willis, K.J., Birks, H.J.B. and Whittaker, R.J. (2008) Agroforestry: a refuge for tropical biodiversity? *Trends in Ecology and Evolution*, **23**, 261–267.

Bistrom, O., Silfverberg, H. and Rutanen, I. (1991) Abundance and distribution of coprophilous *Histerini* (Histeridae) and *Onthophagus* and *Aphodius* (Scarabaeidae) in Finland (Coleoptera). *Entomologica Fennica*, **2**, 53–66.

Blanchart, E., Albrechf, A., Alegre, J., Duboisset, A., Giloe, C., Pashanasf, B., *et al.* (1999) Effects of earthworms on soil structure and physical properties. In *Earthworm Management in Tropical Agroecosystems*. pp. 149–172.

Blanckenhorn, W.U., Puniamoorthy, N., Scheffczyk, A. and Römbke, J. (2013) Evaluation of eco-toxicological effects of the parasiticide moxidectin in comparison to ivermectin in 11 species of dung flies. *Ecotoxicology and Environmental Safety*, **89**, 15–20.

Bloomquist, J.R. (1996) Ion channels as targets for insecticides. *Annual Review of Entomology*, **41**, 163–190.

Borghesio, L., Luzzatto, M. and Palestrini, C. (1999) Interactions between dung, plants and the dung fauna in a heathland in northern Italy. *Pedobiologia*, **43**, 97–109.

Bornemissza, G.F. (1970) Insectary studies on the control of dung breeding flies by the activity of the dung beetle, *Onthophagus gazella* F. (Coleoptera: Scarabaeinae). *Australian Journal of Entomology*, **9**, 31–41.

Bornemissza, G.F., Williams, C.H. and others. (1970) An effect of dung beetle activity on plant yield. *Pedobiologia*, **10**, 1–7.

Boxall, A.B.A., Kolpin, D.W., Halling-Sørensen, B. and Tolls, J. (2003) Are veterinary medicines causing environmental risks? *Environmental Science and Technology*, **37**, 286–294.

Boxall, A.B.A., Sherratt, T.N., Pudner, V. and Pope, L.J. (2007) A screening level index for assessing the impacts of veterinary medicines on dung flies. *Environmental Science and Technology*, **41**, 2630–2635.

Braga, R.F., Korasaki, V., Audino, L.D. and Louzada, J. (2012) Are dung beetles driving dung-fly abundance in traditional agricultural areas in the Amazon? *Ecosystems*, **15**, 1173–1181.

Brickle, N.W., Harper, D.G.C., Aebischer, N.J. and Cockayne, S.H. (2000) Effects of agricultural intensification on the breeding success of corn buntings *Miliaria calandra*. *Journal of Applied Ecology*, **37**, 742–755.

Brown, J., Scholtz, C.H., Janeau, J.-L., Grellier, S. and Podwojewski, P. (2010) Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology*, **46**, 9–16.

Brussaard, L. (1983) Reproductive behaviour and development of the dung beetle *Typhaeus Typhoeus* (Coleoptera, Geotrupidae). *Tijdschrift voor Entomologie*, **126**, 203–231.

Bryan, R.P. (1973) The effects of dung beetle activity on the numbers of parasitic gastrointestinal helminth larvae recovered from pasture samples. *Crop and Pasture Science*, **24**, 161–168.

Butler, S.J., Vickery, J.A. and Norris, K. (2007) Farmland biodiversity and the footprint of agriculture. *Science*, **315**, 381–384.

Byerlee, D., Stevenson, J. and Villoria, N. (2014) Does intensification slow crop land expansion or encourage deforestation? *Global Food Security*, **3**, 92–98.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., *et al.* (2014) Investigating the relationship between biodiversity and ecosystem multifunctionality: Challenges and solutions. *Methods in Ecology and Evolution*, **5**, 111–124.

Callaway, R.M., Kikvidze, Z. and Kikodze, D. (2000) Facilitation by unpalatable weeds may conserve plant diversity in overgrazed meadows in the Caucasus Mountains. *Oikos*, **89**, 275–282.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., *et al.* (2012) Biodiversity loss and its impact on humanity. *Nature*, **486**, 59–67.

Carvalho, F.P. (2006) Agriculture, pesticides, food security and food safety. *Environmental Science and Policy*, **9**, 685–692.

Cavers, P.B. and Harper, J.L. (1967) Studies in the dynamics of plant populations: I. The fate of seed and transplants Introduced into various habitats. *The Journal of Ecology*, **55**, 59–71.

Charlier, J., Höglund, J., Samson-Himmelstjerna, G. von, Dorny, P. and Vercruysse, J. (2009) Gastrointestinal nematode infections in adult dairy cattle: impact on production, diagnosis and control. *Veterinary Parasitology*, **164**, 70–9.

Clayden, B. and Hollis, J.M. (1985) *Criteria for Differentiating Soil Series*. Harpenden, Hertfordshire.

Clough, Y., Barkmann, J., Juhrbandt, J., Kessler, M., Wanger, T.C., Anshary, A., *et al.* (2011) Combining high biodiversity with high yields in tropical agroforests. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 8311–8316.

Collier, T. and Steenwyk, R. Van. (2004) A critical evaluation of augmentative biological control. *Biological Control*, **31**, 245–256.

Cordell, D., Drangert, J.-O. and White, S. (2009) The story of phosphorus: Global food

security and food for thought. *Global Environmental Change*, **19**, 292–305.

Cortez, J., Billes, G. and Bouché, M.B. (2000) Effect of climate, soil type and earthworm activity on nitrogen transfer from a nitrogen-15-labelled decomposing material under field conditions. *Biology and Fertility of Soils*, **30**, 318–327.

Curran Cournane, F., McDowell, R.W., Littlejohn, R.P., Houlbrooke, D.J. and Condron, L.M. (2011) Is mechanical soil aeration a strategy to alleviate soil compaction and decrease phosphorus and suspended sediment losses from irrigated and rain-fed cattle-grazed pastures? *Soil Use and Management*, **27**, 376–384.

Curry, J.P., Byrne, D. and Schmidt, O. (2002) Intensive cultivation can drastically reduce earthworm populations in arable land. *European Journal of Soil Biology*, **38**, 127–130.

Curry, J.P., Doherty, P., Purvis, G. and Schmidt, O. (2008) Relationships between earthworm populations and management intensity in cattle-grazed pastures in Ireland. *Applied Soil Ecology*, **39**, 58–64.

Dadour, I.R., Cook, D.F. and Hennessy, D. (2000) Reproduction and survival of the dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) exposed to abamectin and doramectin residues in cattle dung. *Environmental Entomology*, **29**, 1116–1122.

Dai, X.B. (2000) Impact of cattle dung deposition on the distribution pattern of plant species in an alvar limestone grassland. *Journal of Vegetation Science*, **11**, 715–724.

DEFRA. (2013) *Farming Statistics - Livestock Populations at 1 December, 2012, UK and England*.

Doherty, W.M., Stewart, N.P., Cobb, R.M. and Keiran, P.J. (1994) In-vitro comparison of the larvicidal activity of moxidectin and abamectin against *Onthophagus gazella* (F.) (Coleoptera: Scarabaeidae) and *Haematobia irritans exigua* De Meijere (Diptera: Muscidae). *Australian Journal of Entomology*, **33**, 71–74.

Donald, P.F., Green, R.E. and Heath, M.F. (2001) Agricultural intensification and the collapse of Europe's farmland bird populations. *Proceedings of the Royal Society of London B: Biological Sciences*, **268**, 25–9.

Doube, B. (1990) A functional classification for analysis of the structure of dung beetle assemblages. *Ecological Entomology*, **15**, 371–383.

Douglas, J.T. and Crawford, C.E. (1991) Wheel-induced soil compaction effects on ryegrass production and nitrogen uptake. *Grass and Forage Science*, **46**, 405–416.

Dubroca, J., Ducrot, P.H., Alvinerie, M., Galtier, P., Mougin, C. and Kollmann, A. (2003) Fate of the veterinary medicine ivermectin in soil. *Environmental Chemistry Letters*, **1**, 131–134.

Edwards, P.B. and Aschenborn, H.H. (1988) Male reproductive behaviour of the African ball-rolling dung beetle, *Kheper Nigroaeneus* (Coleoptera: Scarabaeidae). *The Coleopterists Bulletin*, **42**, 17–27.

Egan, J.F. and Mortensen, D.A. (2012) A comparison of land-sharing and land-sparing strategies for plant richness conservation in agricultural landscapes. *Ecological Applications*, **22**, 459–471.

Erisman, J.W., Sutton, M.A., Galloway, J., Klimont, Z. and Winiwarter, W. (2008) How a century of ammonia synthesis changed the world. *Nature Geoscience*, **1**, 636–639.

Errouissi, F., Alvinerie, M., Galtier, P., Kerboeuf, D. and Lumaret, J.-P. (2001) The negative effects of the residues of ivermectin in cattle dung using a sustained-release bolus on *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae). *Veterinary Research*, **32**, 421–427.

Evans, K.L., Wilson, J.D. and Bradbury, R.B. (2007) Effects of crop type and aerial invertebrate abundance on foraging barn swallows *Hirundo rustica*. *Agriculture, Ecosystems and Environment*, **122**, 267–273.

Evenson, R.E. and Gollin, D. (2003) Assessing the impact of the green revolution, 1960 to 2000. *Science*, **300**, 758–762.

Fay, H.A.C. and Doube, B.M. (1987) The effect of some coprophagous and predatory beetles on the survival of immature stages of the African buffalo fly, *Haematobia thirouxi potans*, in bovine dung. *Zeitschrift für Angewandte Entomologie*, **95**, 460–466.

Ferguson, C., Husman, A.M. de R., Altavilla, N., Deere, D. and Ashbolt, N. (2003) Fate and transport of surface water pathogens in watersheds. *Critical Reviews in Environmental Science and Technology*, **33**, 299–361.

Fincher, G.T. (1981) The potential value of dung beetles in pasture ecosystems. *Journal of the Georgia Entomological Society*, **16**, 316–333.

Finn, J.A., Gittings, T. and Giller, P.S. (1998) *Aphodius* dung beetle assemblage stability at different spatial and temporal scales. *Applied Soil Ecology*, **10**, 27–36.

Finn, J. a. and Gittings, T. (2003) A review of competition in north temperate dung beetle communities. *Ecological Entomology*, **28**, 1–13.

Fischer, J., Batáry, P., Bawa, K.S., Brussaard, L., Chappell, M.J., Clough, Y., *et al.* (2011) Conservation: Limits of Land Sparing. *Science*, **334**, 593–593.

Floate, K.D. (1998) Off-target effects of ivermectin on insects and on dung degradation in southern Alberta, Canada. *Bulletin of Entomological Research*, **88**, 25–35.

Floate, K.D. (2007) Endectocide residues affect insect attraction to dung from treated cattle: implications for toxicity tests. *Medical and Veterinary Entomology*, **21**, 312–22.

Floate, K.D. (2011) Arthropods in cattle dung on Canada's grasslands. In *Arthropods of Canadian Grasslands*. Citeseer, pp. 71–88.

Floate, K.D., Bouchard, P., Holroyd, G., Poulin, R. and Wellicome, T.I. (2008) Does doramectin use on cattle indirectly affect the endangered burrowing owl. *Rangeland Ecology and Management*, **61**, 543–553.

Floate, K.D., Colwell, D.D. and Fox, A.S. (2002) Reductions of non-pest insects in dung

of cattle treated with endectocides: a comparison of four products. *Bulletin of Entomological Research*, **92**, 471–481.

Floate, K.D., Spooner, R.W. and Colwell, D.D. (2001) Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Medical and Veterinary Entomology*, **15**, 117–120.

Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A. and Sherratt, T.N. (2005) Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. *Annual Review of Entomology*, **50**, 153–79.

Floate, K.D., Watson, D.W., Coghlin, P. and Olfert, O. (2014) Degree-day models for development of the dung beetles *Onthophagus nuchicornis*, *O. taurus*, and *Digitonthophagus gazella* (Coleoptera: Scarabaeidae), and the likelihood of *O. taurus* establishment in southern Alberta, Canada. *The Canadian Entomologist*, **11**, 1–11.

Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., *et al.* (2005) Global consequences of land use. *Science*, **309**, 570–574.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., *et al.* (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, **320**, 889–892.

Geary, T.G. (2005) Ivermectin 20 years on: maturation of a wonder drug. *Trends in Parasitology*, **21**, 530–532.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., *et al.* (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, **11**, 97–105.

Gerard, B.M. and Hay, R.K.M. (1979) The effect on earthworms of ploughing, tined cultivation, direct drilling and nitrogen in a barley monoculture system. *The Journal of Agricultural Science*, **93**, 147–155.

Gibbs, H.K., Ruesch, A.S., Achard, F., Clayton, M.K., Holmgren, P., Ramankutty, N., *et al.* (2010) Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 16732–16737.

Gillard, P. (1967) Coprophagous beetles in pasture ecosystems. *Journal of Australian Institute of Agricultural Science*, **33**, 30–37.

Gittings, T. and Giller, P.S. (1997) Life history traits and resource utilisation in an assemblage of north temperate *Aphodius* dung beetles (Coleoptera: Scarabaeidae). *Ecography*, **20**, 55–66.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., *et al.* (2010) Food security: the challenge of feeding 9 billion people. *Science*, **327**, 812–818.

González Canga, A., Sahagún Prieto, A.M., José Diez Liébana, M., Martínez, N.F., Vega, M.S. and Vieitez, J.J.G. (2009) The pharmacokinetics and metabolism of

ivermectin in domestic animal species. *The Veterinary Journal*, **179**, 25–37.

Gorder, V. de L.-V. (2000) Tansy ragwort poisoning in a horse in southern Ontario. *The Canadian Veterinary Journal*, **41**, 409–410.

Green, R.E., Cornell, S.J., Scharlemann, J.P.W. and Balmford, A. (2005) Farming and the fate of wild nature. *Science (New York, N.Y.)*, **307**, 550–5.

Gregory, N., Gómez, A., Oliveira, T.M.F. de S. and Nichols, E. (2015) Big dung beetles dig deeper: trait-based consequences for faecal parasite transmission. *International Journal for Parasitology*, **45**, 101–105.

de Groot, R.S. de. (1992) *Functions of nature: Evaluation of nature in environmental planning, management and decision making.* Wolters-Noordhoff BV, Groningen, The Netherlands.

de Groot, R.S. de, Wilson, M.A. and Boumans, R.M. (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological Economics*, **41**, 393–408.

Gross, S.J., Ryan, W.G. and Ploeger, H.W. (1999) Anthelmintic treatment of dairy cows and its effect on milk production. *The Veterinary Record*, **144**, 581–7.

Håkansson, I. and Reeder, R.C. (1994) Subsoil compaction by vehicles with high axle load-extent, persistence and crop response. *Soil and Tillage Research*, **29**, 277–304.

Halley, B.A., Nessel, R.J. and Lu, A.Y.H. (1989) Environmental aspects of ivermectin usage in livestock: general considerations. In *Ivermectin and Abamectin*. Springer New York, New York, NY, pp. 162–172.

Hanski, I. and Cambefort, Y. (1991) The dung insect community. In *Dung Beetle Ecology*. Princeton University Press, Princeton, NJ, pp. 5–22.

Harrington, K.C., Beskow, W.B., Hodgson, J. and others. (2011) Recovery and viability of seeds ingested by goats. *New Zealand Plant Protection*, **64**, 75–80.

Hättenschwiler, S., Tiunov, A. V. and Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 191–218.

Hector, A. and Bagchi, R. (2007) Biodiversity and ecosystem multifunctionality. *Nature*, **448**, 188–190.

Helling, B., Pfeiff, G. and Larink, O. (1998) A comparison of feeding activity of collembolan and enchytraeid in laboratory studies using the bait-lamina test. *Applied Soil Ecology*, **7**, 207–212.

Hendriksen, N. (1991) Consumption and utilization of dung by detritivorous and geophagous earthworms in a Danish pasture. *Pedobiologia*, **35**, 65–70.

Hirschberger, P. (2001) Stridulation in *Aphodius* dung beetles: Behavioral context and intraspecific variability of song patterns in *Aphodius ater* (Scarabaeidae). *Journal of Insect Behavior*, **14**, 69–88.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., *et al.* (2016) Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology*, **53**, 1023–1034.

Holm, L.G., Plucknett, D.L., Pancho, J. V and Herberger, J.P. (1977) *Rumex crispus* and *Rumex obtusifolius*. *The world's worst weeds: Distribution and biology*, 401–408.

Holter, P. (1979a) Effect of dung-beetles (Aphodius spp.) and earthworms on the disappearance of cattle dung. *Oikos*, **32**, 393–402.

Holter, P. (1979b) Abundance and reproductive strategy of the dung beetle *Aphodius rufipes* (L.) (Scarabaeidae). *Ecological Entomology*, **4**, 317–326.

Holter, P. (1982) Resource utilization and local coexistence in a guild of scarabaeid dung beetles (*Aphodius* spp.). *Oikos*, **39**, 213–227.

Holter, P. (2016) Herbivore dung as food for dung beetles: elementary coprology for entomologists. *Ecological Entomology*, **41**, 367–377.

Holter, P. and Spangenberg, A. (1997) Oxygen uptake in coprophilous beetles (*Aphodius, Geotrupes, Sphaeridium*) at low oxygen and high carbon dioxide concentrations. *Physiological Entomology*, **22**, 339–343.

Holzschuh, A., Steffan-Dewenter, I., Kleijn, D. and Tscharntke, T. (2007) Diversity of flower-visiting bees in cereal fields: effects of farming system, landscape composition and regional context. *Journal of Applied Ecology*, **44**, 41–49.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Hooper, D.U. and Vitousek, P.M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs*, **68**, 121–149.

Horgan, F.G. (2002) Shady field boundaries and the colonisation of dung by coprophagous beetles in Central American pastures. *Agriculture, Ecosystems and Environment*, **91**, 25–36.

Howarth, R.W. and Marino, R. (2006) Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnology and Oceanography*, **51**, 364–376.

Hughes, R.D., Tyndale-Biscoe, M., Walker, J., Bornemissza, G.F., Blume, R.R., Matter, J.J., *et al.* (1978) Effects of introduced dung beetles (Coleoptera: Scarabaeidae) on the breeding and abundance of the Australian bushfly, *Musca vetustissima* Walker (Diptera: Muscidae). *Bulletin of Entomological Research*, **68**, 361–367.

Huiting, H.F., Moraal, L.G., Griepink, F.C. and Ester, A. (2006) Biology, control and luring of the cockchafer, *Melolontha melolontha*. *Applied Plant Research*, 1–34.

Hutton, S.A. and Giller, P.S. (2003) The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology*, **40**, 994–

1007.

Jessop, L. (1986) *Dung Beetles and Chafers, Coleoptera: Scarabaeoidea.* Royal Entomological Society of London, London, UK.

Jones, M.S., Tadepalli, S., Bridges, D.F., Wu, V.C.H. and Drummond, F. (2015) Suppression of *Escherichia coli* O157:H7 by dung beetles (Coleoptera: Scarabaeidae) using the lowbush blueberry agroecosystem as a model system. *Plos One*, **10**, e0120904.

Kaartinen, R., Hardwick, B. and Roslin, T. (2013) Using citizen scientists to measure an ecosystem service nationwide. *Ecology*, **94**, 2645–52.

Kaneda, S., Yamashita, N., Uchida, T., Shimano, S., Miyoshi, N., Sasaki, M., *et al.* (2006) Effects of ivermectin in dung pats on earthworm (Megascolecidae) populations and pat degradation in Japanese grassland. *Applied Soil Ecology*, **31**, 280–285.

Kasper, J. and Hirschberger, P. (2005) Stridulation in *Aphodius* dung beetles: Songs and morphology of stridulatory organs in North American *Aphodius* species (Scarabaeidae). *Journal of Natural History*, **39**, 91–99.

Keeler, B.L., Gourevitch, J.D., Polasky, S., Isbell, F., Tessum, C.W., Hill, J.D., *et al.* (2016) The social costs of nitrogen. *Science Advances*, **2**, e1600219.

King, R.A., Tibble, A.L. and Symondson, W. (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology*, **17**, 4684–4698.

Kirchmann, H. and Witter, E. (1992) Composition of fresh, aerobic and anaerobic farm animal dungs. *Bioresource Technology*, **40**, 137–142.

Kleijn, D., Baquero, R.A., Clough, Y., Díaz, M., Esteban, J., Fernández, F., *et al.* (2006) Mixed biodiversity benefits of agri-environment schemes in five European countries. *Ecology Letters*, **9**, 243–254.

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., *et al.* (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications*, **6**, 7414.

Kolar, L., Kožuh Eržen, N., Hogerwerf, L. and Gestel, C.A.M. van. (2008) Toxicity of abamectin and doramectin to soil invertebrates. *Environmental Pollution*, **151**, 182–189.

Kratz, W. (1998) The bait-lamina test: General aspects, applications and perspectives. *Environmental Science and Pollution Research International*, **5**, 94–6.

Kremen, C., Williams, N.M. and Thorp, R.W. (2002) Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 16812–16816.

Krüger, K. and Scholtz, C.H. (1997) Lethal and sublethal effects of ivermectin on the dung-breeding bettles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agriculture, Ecosystems and Environment*, **61**, 123–131.

Laffont, C.M., Alvinerie, M., Bousquet-Mélou, A. and Toutain, P.-L. (2001) Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. *International Journal for Parasitology*, **31**, 1687–1692.

Lähteenmäki, S., Slade, E.M., Hardwick, B., Schiffler, G., Louzada, J., Barlow, J., *et al.* (2015) MESOCLOSURES - increasing realism in mesocosm studies of ecosystem functioning. *Methods in Ecology and Evolution*, **6**, 916–924.

Lambin, E.F. and Meyfroidt, P. (2011) Global land use change, economic globalization, and the looming land scarcity. *Proceedings of the National Academy of Sciences*, **108**, 3465–3472.

Landin, B.-O. (1961) *Ecological studies on dung beetles (Coleoptera: Scarabaeidae)*. Berlingska Boktryckeriet, Lund.

Larsson, M.C., Hedin, J., Svensson, G.P., Tolasch, T. and Francke, W. (2003) Characteristic odor of *Osmoderma eremita* identified as a male-released pheromone. *Journal of Chemical Ecology*, **29**, 575–587.

LeBauer, D.S. and Treseder, K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.

Lefcheck, J.S., Byrnes, J.E.K., Isbell, F., Gamfeldt, L., Griffin, J.N., Eisenhauer, N., *et al.* (2015) Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nature Communications*, **6**, 6936.

Lobo, J.M. (2001) Decline of roller dung beetle (Scarabaeinae) populations in the Iberian peninsula during the 20th century. *Biological Conservation*, **97**, 43–50.

Lobo, J.M. and Martín-Piera, F. (1999) Between-group differences in the Iberian dung beetle species-area relationship (Coleoptera: Scarabaeidae). *Acta Oecologica*, **20**, 587–597.

Losey, J.E. and Vaughan, M. (2006) The economic value of ecological services provided by insects. *Bioscience*, **56**, 311–323.

Losson, B. and Lonneux, J.F. (1996) Field efficacy of moxidectin 0.5% pour-on against *Chorioptes bovis, Damalinia bovis, Linognathus vituli* and *Psoroptes ovis* in naturally infected cattle. *Veterinary Parasitology*, **63**, 119–130.

Lumaret, J.-P., Errouissi, F., Floate, K., Rombke, J. and Wardhaugh, K. (2012) A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Current Pharmaceutical Biotechnology*, **13**, 1004–1060.

Lumaret, J.-P., Errouissi, F., Galtier, P. and Alvinerie, M. (2005) Pour-on formulation of eprinomectin for cattle: fecal elimination profile and effects on the development of the dung-inhabiting diptera *Neomyia cornicina* (L.) (Muscidae). *Environmental Toxicology and Chemistry*, **24**, 797.

MacQueen, A. (1973) Horn fly breeding, nitrogen loss and nutrient immobilization associated with cattle dung in the southern interior of British Columbia. PhD Thesis. Simon Fraser University.

Madsen, A.M., Nielsen, B.O., Holter, P., Pedersen, O.C., Jespersen, J.B., Jensen, K.V., *et al.* (1990) Treating cattle with ivermectin : Effects on the fauna and decompsition of dung pats, **27**, 1–15.

Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., *et al.* (2012) Plant species richness and ecosystem multifunctionality in global drylands. *Science*, **335**, 214–8.

Malo, J. and Suárez, F. (1995) Establishment of pasture species on cattle dung : the role of endozoochorous seeds. *Journal of Vegetation Science*, **6**, 169–174.

Mann, C.M., Barnes, S., Offer, B. and Wall, R. (2015) Lethal and sub-lethal effects of faecal deltamethrin residues on dung-feeding insects. *Medical and Veterinary Entomology*, **29**, 189–95.

Manning, P. and Ford, J.P. (2016) Evidence that sex-specific signals may support mate finding and limit aggregation in the dung beetle *Aphodius fossor*. *Ecological Entomology*, **41**, 500–504.

Manning, P. and Lewis, O.T. (2016) Effect of anthelmintic exposure on dung beetle mediated ecosystem functions across multiple patch movements [WWW Document]. *Open Science Framework*. URL osf.io/6zgq4 [accessed on October 20th 2016].

Manning, P., Slade, E.M., Beynon, S.A. and Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems and Environment*, **218**, 87–94.

Martinkova, Z., Honek, A., Pekar, S. and Strobach, J. (2009) Survival of *Rumex obtusifolius* L. in an unmanaged grassland. *Plant Ecology*, **205**, 105–111.

Martínez, L.. and Zinck, J.. (2004) Temporal variation of soil compaction and deterioration of soil quality in pasture areas of Colombian Amazonia. *Soil and Tillage Research*, **75**, 3–18.

Matson, P.A., Parton, W.J., Power, A.G. and Swift, M.J. (1997) Agricultural intensification and ecosystem properties. *Science*, **277**, 504–509.

McDonald, J.H. (2009) *Handbook of Biological Statistics*. Sparky House Publishing, Balitmore.

McGarry, J.W. (1986) Effects of ivermectin on metamorphosis of *Lucilia sericata* (Meigen 1826) following exposure to 3rd instar larvae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 339–340.

McLintock, J., Depner, K.R., Bruce, W.G., Blakeslee, E.B., Glaser, R.W., Glaser, R.W., *et al.* (1954) A review of the life-history and habits of the horn fly, *Siphona irritans* (L.) (Diptera: Muscidae). *The Canadian Entomologist*, **86**, 20–33.

Meehl, G.A. and Tebaldi, C. (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, **305**, 994–997.

Menalled, F.D., Smith, R.G., Dauer, J.T. and Fox, T.B. (2007) Impact of agricultural

management on carabid communities and weed seed predation. *Agriculture, Ecosystems and Environment*, **118**, 49–54.

Menéndez, R. and Gutiérrez, D. (1996) Altitudinal effects on habitat selection of dung beetles (Scarabaeoidea: Aphodiidae) in the northern Iberian peninsula. *Ecography*, **19**, 313–317.

Menéndez, R., Webb, P. and Orwin, K.H. (2016) Complementarity of dung beetle species with different functional behaviours influence dung–soil carbon cycling. *Soil Biology and Biochemistry*, **92**, 142–148.

Menzel, U. (2013) EMT: Exact Multinomial Test: Goodness-of-fit test for discrete multivariate data.

Morris, A.J., Wilson, J.D., Whittingham, M.J. and Bradbury, R.B. (2005) Indirect effects of pesticides on breeding yellowhammer (Emberiza citrinella). *Agriculture, Ecosystems and Environment*, **106**, 1–16.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C. and Rolando, A. (2014) The effects of body mass on dung removal efficiency in dung beetles. *PloS one*, **9**, e107699.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S. and Favila, M.E. (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation*, **141**, 1461–1474.

Njihia, T.N., Jaramillo, J., Murungi, L., Mwenda, D., Orindi, B., Poehling, H.-M., *et al.* (2014) Spiroacetals in the colonization behaviour of the coffee berry borer: a “push-pull” system. *PloS one*, **9**, e111316.

Norgaard, R.B. (2010) Ecosystem services: From eye-opening metaphor to complexity blinder. *Ecological Economics*, **69**, 1219–1227.

O’Hea, N.M., Kirwan, L. and Finn, J. a. (2010a) Experimental mixtures of dung fauna affect dung decomposition through complex effects of species interactions. *Oikos*, **119**, 1081–1088.

O’Hea, N.M., Kirwan, L., Giller, P.S. and Finn, J.A. (2010b) Lethal and sub-lethal effects of ivermectin on north temperate dung beetles, *Aphodius ater* and *Aphodius rufipes* (Coleoptera: Scarabaeidae). *Insect Conservation and Diversity*, **3**, 24–33.

O’Neil, K.J. and Carrow, R.N. (1983) Perennial ryegrass growth, water use, and soil aeration status under soil compaction. *Agronomy Journal*, **75**, 177–180.

Ollerton, J., Erenler, H., Edwards, M. and Crockett, R. (2014) Pollinator declines. Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science*, **346**, 1360–1362.

Owen, W.K., Lloyd, J.E., Legg, D.E. and Kumar, R. (2006) Endocoprid activity of Aphodius fossor (Coleoptera: Scarabaeidae) related to bovine dung decomposition in a mixed grass prairie. *Journal of Economic Entomology*, **99**, 2210–2215.

Palestrini, C., Barbero, E. and Ronaldo, A. (1998) Intra- and interspecific aggregation

among dung beetles (Coleoptera: Scarabaeoidea) in an Alpine pasture. *Journal of Zoology*, **245**, 101–109.

Partap, U. and Ya, T. (2012) The human pollinators of fruit crops in Maoxian County, Sichuan, China. *Mountain Research and Development*, **32**, 176–186.

Paull, J. (2011) The uptake of organic agriculture: A decade of worldwide development. *Journal of Social and Development Sciences*, **2**, 111–120.

Penttilä, A., Slade, E.M., Simojoki, A., Riutta, T., Minkkinen, K. and Roslin, T. (2013) Quantifying beetle-mediated effects on gas fluxes from dung pats. *PloS one*, **8**, e71454.

Pérez-Cogollo, L.C., Rodríguez-Vivas, R.I., Delfín-González, H., Reyes-Novelo, E. and Ojeda-Chi, M.M. (2015) Lethal and sublethal effects of Iivermectin on *Onthophagus landolti* (Coleoptera: Scarabaeidae). *Environmental Entomology*, **44**, 1634–40.

Perkins, A.J., Maggs, H.E., Watson, A. and Wilson, J.D. (2011) Adaptive management and targeting of agri-environment schemes does benefit biodiversity: a case study of the corn bunting *Emberiza calandra*. *Journal of Applied Ecology*, **48**, 514–522.

Perkins, D.M., Bailey, R. a., Dossena, M., Gamfeldt, L., Reiss, J., Trimmer, M., *et al.* (2014) Higher biodiversity is required to sustain multiple ecosystem processes across temperature regimes. *Global Change Biology*, **21**, 396–406.

Perkins, D.M., McKie, B.G., Malmqvist, B., Gilmour, S.G., Reiss, J. and Woodward, G. (2010) Environmental warming and biodiversity-ecosystem functioning in freshwater microcosms: partitioning the effects of species identity, richness and metabolism. In *Integrative Ecology: From Molecules to Ecosystems*. Elsevier Science, pp. 177–205.

Phalan, B., Onial, M., Balmford, A. and Green, R.E. (2011) Reconciling food production and biodiversity conservation: land sharing and land sparing compared. *Science*, **333**, 1289–1291.

Pickens, L.G. and Miller, R.W. (1980) Biology and control of the face fly, *Musca autumnalis* (Diptera: Muscidae). *Journal of Medical Entomology*, **17**, 195–210.

Pino, J., Haggar, R.J., Sans, F.X., Masalles, R.M. and Hamilton, R.N.S. (1995) Clonal growth and fragment regeneration of *Rumex obtusifolius* L. *Weed Research*, **35**, 141–148.

Prokopy, R.J. (1975) Oviposition-deterring fruit marking pheromone in *Rhagoletis fausta*. *Environmental Entomology*, **4**, 298–300.

Qualls, R.G. and Richardson, C.J. (2000) Phosphorus enrichment affects litter decomposition, immobilization, and soil microbial phosphorus in wetland mesocosms. *Soil Science Society of America Journal*, **64**, 799–808.

R Core Team. (2014) R: A Language and Environment for Statistical Computing.

Rauset, G.R., Andrén, H., Swenson, J.E., Samelius, G., Segerström, P., Zedrosser, A., *et al.* (2016) National parks in northern Sweden as refuges for illegal killing of large carnivores. *Conservation Letters*, **9**, 334–341.

Robinson, R.A. and Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, **39**, 157–176.

Römbke, J., Floate, K.D., Jochmann, R., Schäfer, M.A., Puniamoorthy, N., Knäbe, S., *et al.* (2009) Lethal and sublethal toxic effects of a test chemical (ivermectin) on the yellow dung fly (*Scathophaga stercoraria*) based on a standardized international ring test. *Environmental Toxicology and Chemistry*, **28**, 2117–2124.

Rosenlew, H. and Roslin, T. (2008) Habitat fragmentation and the functional efficiency of temperate dung beetles. *Oikos*, **117**, 1659–1666.

Roslin, T. (2000) Dung beetle movements at two spatial scales. *Oikos*, **91**, 323–335.

Roslin, T., Avomaa, T., Leonard, M., Luoto, M. and Ovaskainen, O. (2009) Some like it hot: microclimatic variation affects the abundance and movements of a critically endangered dung beetle. *Insect Conservation and Diversity*, **2**, 232–241.

Roslin, T. and Koivunen, A. (2001) Distribution and abundance of dung beetles in fragmented landscapes. *Oecologia*, **127**, 69–77.

Rothwell, A., Chaney, K. and Haydock, P. (2011) The impact of cultivation techniques on earthworm populations. In *Biology of Earthworms*. Springer Berlin Heidelberg, pp. 159–172.

Růžička, Z. (2013) Oviposition-deterring pheromone in *Chrysopa oculata* (Neuroptera: Chrysopidae). *European Journal of Entomology*, **91**, 361–370.

Sanchez, J., Dohoo, I., Carrier, J., Carrier, J. and DesCôteaux, L. (2004) A meta-analysis of the milk-production response after anthelmintic treatment in naturally infected adult dairy cows. *Preventive Veterinary Medicine*, **63**, 237–56.

Sands, B. and Wall, R. (2016) Dung beetles reduce livestock gastrointestinal parasite availability on pasture. *Journal of Applied Ecology*, doi: 10.1111/1365-2664.12821.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. and others. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Seefeldt, S.S., Stephens, J.M.C., Verkaaik, M.L. and Rahman, A. (2005) Quantifying the impact of a weed in a perennial ryegrass-white clover pasture, **53**, 113–120.

Sherratt, T.N., Macdougall, A.D., Wratten, S.B. and Forbes, A.D. (1998) Models to assist the evaluation of the impact of avermectins on dung insect populations. *Ecological Modelling*, **110**, 165–173.

Shipitalo, M. and Bayon, R.-C. Le. (2004) Quantifying the effects of earthworms on soil aggregation and porosity. In *Earthworm Ecology*. CRC Press, Boca Raton, pp. 183–200.

Silver, W.L., Ostertag, R. and Lugo, A.E. (2000) The potential for carbon sequestration through reforestation of abandoned tropical agricultural and pasture lands. *Restoration Ecology*, **8**, 394–407.

Simpson, J.E., Slade, E., Riutta, T. and Taylor, M.E. (2012) Factors affecting soil fauna

feeding activity in a fragmented lowland temperate deciduous woodland. *PloS one*, **7**, e29616.

Sissay, M.M., Asefa, A., Uggla, A. and Waller, P.J. (2006) Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: Exploitation of refugia to restore anthelmintic efficacy. *Veterinary Parasitology*, **135**, 337–346.

Skidmore, P. (1991) *Insects of the British cow-dung community.* Field Studies Council, Shrewsbury, UK.

Slade, E.M., Mann, D.J. and Lewis, O.T. (2011) Biodiversity and ecosystem function of tropical forest dung beetles under contrasting logging regimes. *Biological Conservation*, **144**, 166–174.

Slade, E.M., Mann, D.J., Villanueva, J.F. and Lewis, O.T. (2007) Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. *Journal of Animal Ecology*, **76**, 1094–104.

Slade, E.M., Riutta, T., Roslin, T. and Tuomisto, H.L. (2016) The role of dung beetles in reducing greenhouse gas emissions from cattle farming. *Scientific Reports*, **6**, 18140.

Slade, E.M. and Roslin, T. (2016) Dung beetle species interactions and multifunctionality are affected by an experimentally warmed climate. *Oikos*, **125**, 1607-1616.

Snyder, W.E., Snyder, G.B., Finke, D.L. and Straub, C.S. (2006) Predator biodiversity strengthens herbivore suppression. *Ecology Letters*, **9**, 789–796.

Soutello, R.G.V., Seno, M.C.Z. and Amarante, A.F.T. (2007) Anthelmintic resistance in cattle nematodes in northwestern São Paulo State, Brazil. *Veterinary Parasitology*, **148**, 360–364.

Sowig, P. (1995) Habitat selection and offspring survival rate in three paracoprid dung beetles: the influence of soil type and soil moisture. *Ecography*, **18**, 147–154.

Steel, J.W. (1993) Pharmacokinetics and metabolism of avermectins in livestock. *Veterinary Parasitology*, **48**, 45–57.

Steel, J.W. (1998) Assessment of the effects of the macrocyclic lactone class of chemicals on dung beetles and dung degradation in Australia. *NRA Special Review of Macrocyclic Lactones*, **2**, 15–79.

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V. and Haan, C. de. (2006) *Livestock's Long Shadow: Environmental Issues and Options*. Food and Agriculture Org.

Stern, N.H., Peters, S., Bakhshi, V., Bowen, A., Cameron, C., Catovsky, S., *et al.* (2006) *Stern Review: The Economics of Climate Change*. Cambridge University Press Cambridge.

Steudel, B., Hector, A., Friedl, T., Löfke, C., Lorenz, M., Wesche, M., *et al.* (2012) Biodiversity effects on ecosystem functioning change along environmental stress

gradients. *Ecology Letters*, **15**, 1397–1405.

Stevenson, B.. and Dindal, D.. (1985) Growth and development of *Aphodius* beetles (Scarabaeidae) in laboratory microcosms of cow dung. *The Coleopterists Bulletin*, **39**, 215–220.

Strong, L. and James, S. (1992) Some effects of rearing the yellow dung fly *Scatophaga stercoraria* in cattle dung containing ivermectin. *Entomologia Experimentalis et Applicata*, **63**, 39–45.

Suárez, V.H., Lifschitz, A.L., Sallovitz, J.M. and Lanusse, C.E. (2009) Effects of faecal residues of moxidectin and doramectin on the activity of arthropods in cattle dung. *Ecotoxicology and Environmental Safety*, **72**, 1551–1558.

Sutherland, I.A. and Leathwick, D.M. (2011) Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends in Parasitology*, **27**, 176–181.

Svendsen, T.S., Sommer, C., Holter, P. and Grønvold, J. (2002) Survival and growth of *Lumbricus terrestris* (Lumbricidae) fed on dung from cattle given sustained-release boluses of ivermectin or fenbendazole. *European Journal of Soil Biology*, **38**, 319–322.

Swift, M.J., Heal, O.W. and Anderson, J.M. (1979) *Decomposition in Terrestrial Ecosystems, Volume 5*. University of California Press.

Tilman, D. (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 5995–6000.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., *et al.* (2001) Forecasting agriculturally driven global environmental change. *Science*, **292**.

Timmerman, A., Bos, D., Ouwehand, J. and Goede, R.G.M. de. (2006) Long-term effects of fertilisation regime on earthworm abundance in a semi-natural grassland area. *Pedobiologia*, **50**, 427–432.

Tixier, T., Bloor, J.M.G. and Lumaret, J.-P. (2015) Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. *Acta Oecologica*, **69**, 31–34.

Tribe, G.D.D. (1975) Pheromone release by dung beetles (Coleoptera: Scarabaeidae). *South African Journal of Science*, **71**, 277–278.

Tuck, S.L., Winqvist, C., Mota, F., Ahnström, J., Turnbull, L.A. and Bengtsson, J. (2014) Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis. *The Journal of Applied Ecology*, **51**, 746–755.

United Nations. (2015) *World Population Prospects: The 2015 Revision, Highlights and Advance Tables*. New York, NY.

Vale, G.A. and Grant, I.F. (2002) Modelled impact of insecticide-contaminated dung on the abundance and distribution of dung fauna. *Bulletin of Entomological Research*, **92**, 251–263.

Valiela, I. (1974) Composition, food webs and population limitation in dung arthropod communities during invasion and succession. *American Midland Naturalist*, **92**, 370–385.

Van Assche, J.A. and Vanlerberghe, K.A. (1989) The role of temperature on the dormancy cycle of seeds of *Rumex obtusifolius* L. *Functional Ecology*, **3**, 107–115.

Van Grinsven, H.J.M., Spiertz, J.H.J., Westhoek, H.J., Bouman, A.F. and Erisman, J.W. (2013) Nitrogen use and food production in European regions from a global perspective. *The Journal of Agricultural Science*, **152**, 9–19.

Van Wyk, J.A., Hoste, H., Kaplan, R.M. and Besier, R.B. (2006) Targeted selective treatment for worm management--how do we sell rational programs to farmers? *Veterinary Parasitology*, **139**, 336–46.

Vercruysse, J. and Claerebout, E. (2001) Treatment vs non-treatment of helminth infections in cattle: Defining the threshold. *Veterinary Parasitology*, **98**, 195–214.

Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-Pinna, J., Lumaret, J.-P., *et al.* (2015) Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Scientific Reports*, **5**, 13912.

Vessby, K. (2001) Habitat and weather affect reproduction and size of the dung beetle *Aphodius fossor*. *Ecological Entomology*, **26**, 430–435.

Vessby, K. and Wiktelius, S. (2003) The influence of slope aspect and soil type on immigration and emergence of some northern temperate dung beetles. *Pedobiologia*, **47**, 39–51.

Wall, R. and Beynon, S. (2012) Area-wide impact of macrocyclic lactone parasiticides in cattle dung. *Medical and Veterinary Entomology*, **26**, 1–8.

Wall, R. and Strong, L. (1987) Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature*, **327**, 418–421.

Wallace, M.M.H. and Tyndale-Biscoe, M. (1983) Attempts to measure the influence of dung beetles (Coleoptera: Scarabaeidae) on the field mortality of the bush fly *Musca vetustissima* Walker (Diptera: Muscidae) in south-eastern Australia. *Bulletin of Entomological Research*, **73**, 33–44.

Walsh, G.C. and Posse, M.C. (2003) Abundance and seasonal distribution of predatory coprophilous Argentine rove beetles (Coleoptera: Staphylinidae), and their effects on dung breeding flies. *The Coleopterists Bulletin*, **57**, 43–50.

Wardhaugh, K.., Longstaff, B.. and Morton, R. (2001) A comparison of the development and survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. *Veterinary Parasitology*, **99**, 155–168.

Wardle, D.A. (1999) Is “sampling effect” a problem for experiments investigating biodiversity-ecosystem function relationships? *Oikos*, **87**, 403–407.

Weeda, W.C. (1967) The effect of cattle dung patches on pasture growth, botanical composition, and pasture utilisation. *New Zealand Journal of Agricultural Research*, **10**, 150–159.

Welch, D. (1985) Studies in the grazing of heather moorland In North-east Scotland. IV. Seed dispersal and plant establishment in dung. *The Journal of Applied Ecology*, **22**, 461–472.

Wickham, H. (2009) *ggplot2: elegant graphics for data analysis*. Springer New York.

Wilson, A., Vickery, J. and Pendlebury, C. (2007) Agri-environment schemes as a tool for reversing declining populations of grassland waders: Mixed benefits from environmentally sensitive areas in England. *Biological Conservation*, **136**, 128–135.

Wolstenholme, A.J. (2012) Glutamate-gated chloride channels. *The Journal of Biological Chemistry*, **287**, 40232–40238.

Wood, S. and Scheipl, F. (2014) gamm4: Generalized additive mixed models using mgcv and lme4.

Wright, S.J. and Muller-Landau, H.C. (2006) The future of tropical forest species. *Biotropica*, **38**, 287–301.

Wu, X., Duffy, J.E., Reich, P.B. and Sun, S. (2011) A brown-world cascade in the dung decomposer food web of an alpine meadow: effects of predator interactions and warming. *Ecological Monographs*, **81**, 313–328.

Yamada, D., Imura, O., Shi, K. and Shibuya, T. (2007) Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. *Grassland Science*, **53**, 121–129.

Yokoyama, K., Hideaki, K. and Hirofumi, T. (1991) Paracoprid dung beetles and gaseous loss of nitrogen from cow dung. *Soil Biology and Biochemistry*, **23**, 643–647.

Zaller, J., and Arnone, J. (1999) Earthworm and soil moisture effects on the productivity and structure of grassland communities. *Soil Biology and Biochemistry*, **31**, 517–523.

Zaller, J.G. (2004) Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (Polygonaceae): a review. *Weed Research*, **44**, 414–432.

Zhao, C., Griffin, J.N., Wu, X. and Sun, S. (2013) Predatory beetles facilitate plant growth by driving earthworms to lower soil layers. *Journal of Animal Ecology*, **82**, 749–58.