The long-term impacts of upland livestock grazing pressure on the breeding productivity, nestling diet and nutrition of a common insectivorous passerine.

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Abstract

Changes in grazing management of upland habitats during the last decades have contributed to declines of many bird species. In order to determine drivers of population change of upland birds, a mechanistic understanding of how land management affects breeding conditions is needed.

Using a long-term, landscape-scale experiment, this study examined the effect of livestock grazing intensity and type on: a) the breeding productivity of the meadow pipit *Anthus pratensis*, a common insectivorous passerine in the British uplands; b) the abundance of arthropod groups common in upland bird diets; c) nestling diet composition using DNA-metabarcoding; and d) nutrient quality of provisioned prey. The grazing experiment started in 2003 and had four treatments: I) intensive sheep; II) extensive sheep; III) extensive mixed sheep and cattle and IV) ungrazed. Meadow pipit nests were monitored and arthropods were sampled in 2004/5 and 2015/16, to compare differences between early and late stages of the experiment. Faecal sacs of nestlings were used to identify prey DNA and estimate diet nutrient quality.

Egg-stage nest survival was highest in plots with extensive sheep grazing but no statistically significant change in nest survival between grazing treatments was detected over time. Total arthropod mass and abundance was highest in extensively sheep grazed and ungrazed plots. Nestling faecal samples contained a higher concentration of an aggregated measure of micro-nutrients in intensively sheep grazed plots, where the diet analysis also suggested that nestlings were fed prey from a wider range of invertebrate orders.

The higher breeding abundance, diet diversity and concentration of some essential nutrients in intensely grazed plots may be an indication of more favourable foraging conditions. However, this was not reflected in nest survival, which was mainly affected by predation. The applicability and forthcoming obstacles with DNA-based methods for upland bird diet assessments are discussed.

Declaration of work carried out by others

I declare that the work submitted in this thesis has been carried out by myself unless otherwise stated, and for this thesis only. Work carried out by others or assistance by others have been stated below for each chapter. All experiments were planned by myself together with my supervisors: Darren Evans, Newcastle University; Nick Littlewood, The James Hutton Institute; Ali Karley, The James Hutton Institute; and James Pearce-Higgins, British Trust for Ornithology.

Chapter 2: Arthropod sampling and processing in the first sampling year (2005) was carried out by Peter Dennis, who also set up the sampling techniques repeated in 2015. The arthropod sampling in the late sampling year (2015) was assisted by Ewa Karaszewska. Processing of samples was assisted by Robert Jaques.

Chapter 3: Meadow pipit breeding surveys in the first sampling period (2003/4) was carried out by Darren Evans. Breeding surveys were assisted by Ewa Karaszewska in 2015 and by Robert Jaques in 2016. Meadow pipit territory mapping was carried out by Darren Evans during the early sampling period and by Nick Littlewood in the late sampling period. I also got help with nest searching by Darren Evans, Afionn Evans and Charlotte Dufferwiel in 2015 and -16.

Chapter 4: The meadow pipit faecal samples used in this chapter were collected during nest monitoring in 2015 and 2016 and therefore also assisted by Ewa Karaszewska and Robert Jaques. Guidance on sample processing and preparation for sequencing was provided by James Kitson, Newcastle University in addition to guidance provided by my supervisors. Sequencing was done by NU-OMIX, Northumbria University.

Chapter 5: The meadow pipit faecal samples used in this chapter were collected during nest monitoring in 2015 and therefore also assisted by Ewa Karaszewska. The samples were prepared for nutrient analysis by myself (e.g. microwave digestion) and analysed by inductively coupled plasma mass spectrometry by Jaqueline Thompson, The James Hutton Institute, and by elemental analyser by Gill Banks, The James Hutton Institute.

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1.1 Landscape change in the British uplands

1.1.1 Drivers of management change

An increased demand for meat and dairy has caused a recent, worldwide trend towards larger farms with higher livestock densities, which is expected to continue to increase for several decades (Bouwman *et al.* 2005; Erb *et al.* 2012; Eurostat 2016). However, in many mountain and upland areas, including some areas within the Alps and the British uplands, natural grazing has declined or ceased completely, which has led to important changes in ecosystem functions (Cernusca *et al.* 1996; Dirnböck *et al.* 2003; Laiolo *et al.* 2004). Low-intensity farming provides a high biodiversity and unique species composition that is lost under other types of land management (Bignal & Mccracken 1996). The British uplands are mainly managed for grazing or game shooting, and livestock farming in upland regions contributes to meat and wool provisioning while also maintaining a cultural heritage (Chesterton 2009). However, other interests such as afforestation (Read et al. 2009) which can aid in management for combating increased flood risks caused by climate change (Carver 2016) may not be compatible with traditional land use requiring deforested areas. Moreover, increased interest in rewilding, (i.e. increased wilderness through, for example, reintroduction of large predators and natural woodlands) will cause further challenges around traditional natural grazing which today covers large upland areas (Pettorelli et al. 2018). Cattle and mixed livestock grazing has decreased in many areas of UK (Silcock *et al.* 2012) and in Scotland, sheep numbers decreased by 34% from 1991 to 2013, after a longer period of increasing stocking densities since the 1950's (Critchlow-Watton *et al.* 2014).

The recent declines in stocking densities in the UK are partially effects of a generally poor profitability for keeping livestock in upland regions, but also by changes in subsidies systems (Critchlow-Watton *et al.* 2014). Financial support is not necessarily designed in a way that encourages systems that will provide the most ecosystem services (Silcock *et al.* 2012) and due to the dependence on subsidies in many areas, a large part of the responsibility to provide financial support that contributes to land use methods leading to diverse and functioning ecosystems lies within the planning of subsidies. Given that the British uplands cover almost a third of the country, with unique moorland habitats and species

communities, appropriate management regimes are necessary to meet national biodiversity and conservation aims (Fielding & Haworth 1999) in which appropriate grazing management for maintained biodiversity (Fraser *et al.* 2014) is an important part. Britton et al. (2017) suggested that climate change, pollution (nitrogen and sulphur) and grazing (by red deer *Cervus elaphus* and sheep *Ovis aries*) have all contributed to vegetation change in Scottish moorlands, where ubiquitous species have increased, and specialised species have decreased, although factor specific effects depend on habitats and level of disturbance.

Grazing intensity can affect the ecosystem through a range of ways such as nutrient concentrations in soil and plants (Hilder 1964; Haynes & Williams 1993), vegetation structure and plant composition (Noy-Meir *et al.* 1989; Pakeman & Nolan 2009; Britton *et al.* 2017), abundance of arthropods (Gibson *et al.* 1992; Dennis *et al.* 1998), voles and foxes (Wheeler 2008; Villar *et al.* 2013, 2014) and carbon uptake (Allard *et al.* 2007; McSherry & Ritchie 2013). Although the potential ecophysiological effects of grazing are relatively well understood compared to other types of land management (Erb *et al.* 2017), effects of grazing on soil, plants and animals are not necessarily consistent across habitats and under different environmental conditions (McSherry & Ritchie 2013; Speed *et al.* 2013), and observed effects of grazing may also change with time since the grazing commenced (Allombert *et al.* 2005; Allard *et al.* 2007; Jerrentrup *et al.* 2014). Therefore, a habitat-specific and long-term perspective is needed for providing an applicable decision basis for sustainable management policies or land-management changes for conservation purposes.

1.1.2 Potential drivers of avian population change in grazed upland habitats

British uplands are internationally important for holding large proportions of avian species' populations that are of high conservation concern globally (Pearce-Higgins *et al.* 2008). Birds in uplands have shown stronger range distribution contractions than birds in all other habitat types in the UK during the last few decades and many typical upland bird species in the UK are showing widespread population declines, including breeding waders and some passerines such as Ring Ouzel *Turdus torquatus* and Twite *Linaria flavirostris* (Sim *et al.* 2005; Balmer *et al.* 2013). Many of these population declines occurred parallel to increases in stocking densities during the 19th century (Fuller & Gough 1999; Newton 2004). However, livestock densities, which could be a potential driver of bird population declines, ceased to increase before the end of the 20th century, while many species, such as curlew *Numenius*

arquata, lapwing *Vanellus vanellus*, whinchat *Saxicola rubetra* and meadow pipit *Anthus pratensis* are still declining (Hayhow *et al.* 2017). Understanding the potential underlying mechanisms of changes in grazing management to population declines of upland birds is therefore essential for further management improvements.

Vegetation structure is important for meeting habitat requirements of species connected to traditionally grazed habitats, with a mosaic of vegetation composition and structure most likely to support the highest densities of many species and a more diverse assemblage (Báldi *et al.* 2005; Pearce-Higgins & Grant 2006; Pearce-Higgins *et al.* 2008). Habitat requirements also vary within the species connected to traditionally grazed habitats (Báldi *et al.* 2005; Pearce-Higgins & Grant 2006) where some species, such as red grouse, have declined in areas where heather dominated areas have progressively been taken over by grasslands through grazing (Thirgood *et al.* 2000), while numbers of breeding redshank *Tringa totanus* were higher after changes from none or low grazing to higher grazing pressure (Norris *et al.* 1998). Moreover, lower sheep densities have shown positive effects on breeding success in hen harriers through increased prey densities (Amar *et al.* 2011).

Many upland wader, grouse and passerine species of conservation concern feed on an invertebrate diet and heterogeneous habitats are likely to support a wider range of suitable arthropod prey (Buchanan *et al.* 2006) as insectivorous birds and their prey often have different habitat requirements. Therefore, increasingly homogenous landscapes in farming and increased distance between different habitat types has been suggested as a reason for the decline of some farmland bird species (Benton *et al.* 2003), which is probable to be seen in homogenous, semi-natural grazed areas as well. Moreover, abundances of arthropod groups important in bird diets have been shown to be higher in ungrazed enclosures and less abundant in intensively grazed enclosures in upland areas (Dennis *et al.* 2008; Littlewood 2008; Mysterud *et al.* 2010).

Ground nesting birds in UK have shown particular declines when comparing avian groups according to a range of traits (Sullivan *et al.* 2015) which brings focus to predation, and a potentially increased predation pressure. The possibility of increased nest predation is important to consider when estimating effects of altered or diminished breeding grounds (Evans 2004) and effects of food availability, predation risks and the interaction between

them need to be accounted for when estimating habitat effects on avian population declines (McNamara 1987). Baines (1990) showed that predation was the main reason for increased breeding failure of lapwing *Vanellus vanellus* breeding in upland grasslands altered by agricultural intensification compared to lapwings breeding in non-intensified pastures and meadows while food abundance mainly had an effect on breeding density. Moreover, areas with higher nest densities of one or several prey species can attract higher predator abundances (Chamberlain *et al.* 1995; Götmark & Andersson 2005) and form "ecological traps", a habitat where the suitability in terms of survival and breeding success does not match its attractiveness (see Misenhelter & Rotenberry 2000; Bro *et al.* 2004). Nest sites, food availability and predation may therefore all be altered through grazing. For improved implementations of research outputs to management actions, it is important to describe the process of how management change may affect upland bird populations, relative to other potential drivers such as climate change and predator management (Buchanan *et al.* 2017). Knowledge of avian diets is therefore a vital part in understanding species specific habitat requirements (Hildén 1965; Fuller 2012).

1.2 A mechanistic understanding of foraging birds' responses to environmental change

1.2.1 Observing interactions rather than food abundance

Land management change may not only change communities' species composition, but can also alter important ecological interactions and associated ecosystem functions (Chapin *et al.* 2000; Tylianakis *et al.* 2008). Environmental effects mediated through species interactions can even be stronger than direct effects on the abundance of whole trophic levels (Suttle *et al.* 2007). Interactions can be altered without strong changes to species richness (Tylianakis *et al.* 2007) and changes of land use, such as grazing, can alter climatic effects on ecological networks (Câmara *et al.* 2018). This makes interactions of predators and prey under changed management hard to predict. For example, herbivores (of which some are important invertebrate prey to birds), are adapted to specific chemical and physiological traits of plants (Mattson 1980). These traits may change as a response to environmental factors such as increased stress through drought or grazing, which can affect suitability of these plants for foraging, and eventually the abundance of herbivorous arthropods (White 1984). Moreover, ground-foraging insectivorous birds may not respond most positively to management where prey abundance is highest; For example, abundance of arthropods in grazed uplands have

been shown to be higher where grazing intensity is lower (Dennis *et al.* 2008; Littlewood 2008; Mysterud *et al.* 2010). Ungrazed or extensively grazed areas do, however, have more vegetation and plant biomass (Evans *et al.* 2015), and although food abundance is important, many ground foraging birds have been observed to prefer short vegetation or bare patches of soil or peat for foraging (Pearce-Higgins & Yalden 2003, 2004; Douglas *et al.* 2008; Vandenberghe *et al.* 2009; Schaub *et al.* 2010). The types of habitat necessary for successful foraging are not always the areas with the highest food abundance since prey detectability (e.g. through sparser vegetation) can be more crucial for efficient foraging (Atkinson *et al.* 2005; Ontiveros *et al.* 2005).The abundance of food does not therefore equate to availability of food, and food availability and diet composition needs to be confirmed by direct observations of food intake.

1.2.2 Nutrient intake in wild birds

Wild bird populations' diets are rarely investigated from a nutritional composition perspective, most likely due to the complexity of analysing feeding habits in a variable environment where food intake can neither be controlled nor observed in detail. However, Kaspari and Joern (1993) showed that birds select food with higher nutrient contents and Hungerford *et al.* (1993) suggested that a monotonous diet in an insectivorous passerine, eastern bluebirds, *Sialia sialis,* that would consist of only one prey species, grasshoppers (Orthoptera), crickets (Orthoptera) or Lepidoptera larvae, would all result in deficiencies of important nutrients, and that a varied diet is important for meeting nutrient requirements. Important effects of nutrient composition on reproduction and other life history traits have been observed in birds such as passerines (Blount *et al.* 2006) and poultry (Hocking 1987) in laboratory environments when given diets intentionally low in specific nutrient elements. In free living birds, a deficiency of Calcium has been observed to cause egg defects in great tits *Parus major* (Graveland & Gijzen 1994) and a widespread Thiamine (B1) deficiency has been suggested to explain increased mortality of juvenile common eiders *Somateria mollissima* (Mörner *et al.* 2017). As avian diets may differ between breeding habitats (Heiss *et al.* 2009), and therefore change as a consequence of management change (Britschgi *et al.* 2006), land management may have important implications on nutrient provisioning to nestling birds. Standardised measures of nutrient intake could well be used to complement diet assessments to better understanding the importance of foraging habitats and diet composition in wild birds.

1.3 New tools for avian diet analyses

1.3.1 Limitations and advantages of conventional methods for diet assessments

Collecting the samples when digested items are to be avoided for traditional, morphological identification has proven to be challenging; innovative attempts in the early 1900's include hand puppets resembling nestlings in active nest boxes to directly collect prey items, stone throwing at breeding grey herons *Ardea cinerea* to induce defensive regurgitation and cotton balls to obstruct warblers from swallowing their prey (Hartley 1948). Direct observations with the purpose of determining prey items are often difficult for birds feeding on small prey or nesting in dense vegetation, and a range of methods for sampling dietary items or determining diet composition from gut or faecal material has been developed and evaluated (e.g. Kluijver 1933; Moreby & Stoate 2000; Pearce-Higgins & Yalden 2004; Jedlicka *et al.* 2013).

Firstly, material for diet analysis can be collected in a range of ways. Neck ligatures were first used by Kluijver (1933) to determine the prey fed to starling *Sturnus vulgaris vulgaris L.* nestlings. The ligature consists of an aluminium ring that is placed around the throat and obstructs the nestling from swallowing food items, which can then be collected. Since the ligature stops the nestlings from swallowing food, they cannot be used for more than 30-60 minutes at the time. There is also a short time span during the nestling period in which the ligatures can be used. Passerine nestlings within their first days can be strangled and the ligature should never be used on nestlings close to fledging, since they cannot get rid of the ligature themselves and would end up starving (Orians 1966; Jenny 1990; Moreby & Stoate 2000). However, ligatures have provided a useful way of controlling items fed to nestlings during the evaluation of other methods for diet assessment (Moreby & Stoate 2000).

Another method of sample collection is to gather regurgitated material from the gullets. Emetics for induced vomiting have been successfully used on rooks *Corvus frugilegus* and starlings *Sturnus vulgaris (Kadochnikov 1967)*, and several species of passerines with varying dietary ranges using potassium tartrate (Prŷs-Jones *et al.* 1974) and apomorphine (Valera *et al.* 1997) of which apomorphine has been shown to have a lower mortality rate than potassium tartrate (Valera *et al.* 1997). The method works with varying success between species and life stages but gullets are usually not completely empty and the diversity of food

items found is not as high as in dissected birds of the same species (Prŷs-Jones *et al.* 1974; Valera *et al.* 1997). Swallowed items can also be flushed out and Gales (1987) found it to be an effective method on several species of penguins if the individual was flushed just after feeding.

Faecal sacs of birds can be collected without any interaction at all if information about the excreting individual is not necessary (Blair & Tucker 1941) or after observation of marked (e.g. colour ringed) individuals. Where fresh faeces are necessary, they can often easily be collected from passerine nestlings while they are being handled (Moreby & Stoate 2000; Michalski *et al.* 2011). However, caution still needs to be taken since breeding pairs may abandon nests after intrusion, although sensitivity varies between species, and visits may also make nests more easily detected by predators through, for example, changes to the vegetation surrounding a nest (Steenhof & Kochert 1982; Ferguson-Lees *et al.* 2011).

Lastly, prey remains of larger prey around nest sites or in nests may be used for bird species feeding on larger prey that cannot be swallowed whole, although detectability may differ between prey species. For example, fish remains in breeding territories of Skuas *Catharacta skua* was shown to give a high taxonomic accuracy for prey identification, but left out information on prey swallowed whole (Votier *et al.* 2003). Raptors also leave partly eaten remains and regurgitate pellets around nests from relatively large prey (e.g. small mammals or fish) but both pellets and remains have been shown to give results biased towards certain groups of prey and should preferably be compensated by direct observations (Redpath *et al.* 2001).

Samples collected by the above-mentioned or other methods have traditionally been analysed by morphologically identifying prey remains. The success of this method mainly depends on the taxonomic group of prey and type of sample that is studied. Identification of insectivorous birds through faeces can sometimes be done to species or genus (Moreby 1988; Poulsen & Aebischer 1995). Some groups, such as spiders, may be easier to identify than other orders due to clear differences of the genitalia and may even be identified to species (Klink *et al.* 2014). The possibility of getting data on relative abundance may be a benefit of morphological identification compared to molecular methods (described below) that are more reliable in providing presence/ absence data (Elbrecht & Leese 2015). However, when counting individuals of insects in faecal samples, beetles are often

overestimated while soft bodied groups, such as spring-tails, can be underestimated (Moreby & Stoate 2000) and complementary methods such as feeding observations may still be necessary to accurately estimate abundances.

1.3.2 Advances in DNA-based methods for studying trophic interactions

Important developments have been made in the methods available to analyse avian diets, where the largest improvements are in molecular, DNA-based techniques (Symondson 2002; Oehm *et al.* 2017). Briefly, these methods consist of using primers to select specific genetic regions suitable for differentiation between prey species, which are thereafter sequenced and matched with a reference barcode for species, genus or order identification. Such studies of avian diets have shown promising results and remarkable progress since the beginning of the 21st century; it has shown to be possible to target just one group of prey, such as krill (Euphausiacea) species in adelie penguin *Pygoscelis adeliae* (Jarman *et al.* 2002) or the whole range of species, shown by Deagle *et al.* (2007) on macaroni penguins *Eudyptes chrysolophus*. Oehm *et al.* (2011) were able to identify insect larvae in faeces of carrion crow *Corvus corone corone* in up to 5 day old samples stored without ethanol. The substrate the samples were resting on was of significant importance for DNA detectability, where soil was worse than leaves, branches and plastic tubes and rain and sunshine also decreased the amount of amplifiable DNA. However, being able to detect prey DNA in faecal samples from leaves or branches collected up to a few days after defecation makes it possible to analyse a diet without any interaction at all with the birds, which is convenient when studying species sensitive to disturbance. DNA gets degraded during digestion, but identifying small invertebrate prey in digested material and degraded DNA has become possible with the design of primers that select shorter fragments of DNA, which still provide enough variation for species differentiation (Grubwieser *et al.* 2003; Oehm *et al.* 2011; Zeale *et al.* 2011; Joo & Park 2012; Leray *et al.* 2013), as long as a matching reference barcode of the prey is available in a reference library. For example, relatively short DNA segments (up to 600 base-pairs) of the mitochondrial Cytochrome c Oxidase subunit I gene (COI) can provide successful identification of a range of invertebrate and vertebrate prey species (Oehm *et al.* 2011; Jedlicka *et al.* 2013, 2017; King *et al.* 2015; Trevelline *et al.* 2016, 2018). The amount of food items detected (Oehm *et al.* 2017) and taxonomic resolution (e.g. Jedlicka *et al.* 2013; King *et al.* 2015; Trevelline *et al.* 2018) of diet items has increased compared to what has been possible using morphological methods of diet analyses. Moreover, considerable progress has

been made in the convenience of using DNA-based methods for studies of species interactions, as a wide range of species from the same sample can be sequenced simultaneously by using massively parallel "Next Generation Sequencing" (NGS) (Pompanon *et al.* 2012; Leray *et al.* 2013). Each sample can also be marked individually by individual Molecular Identification Tags (MIDs) prior to sequencing, which makes it possible to sequence several hundreds of samples simultaneously and yet allocate all sequnces to its original sample (Binladen *et al.* 2007).

1.3.3 Challenges of DNA-based methods in ornithological research

Although DNA-based methods and metabarcoding suggests great potential in analyses of avian diet composition, there are potential causes of bias that should not be ignored. For example, primers may more easily amplify some species than others (Clarke *et al.* 2014; Elbrecht & Leese 2017) causing a bias in the observed abundance or frequency of some species groups in a diet. The potential primer bias does, at least to this date, limit the possibility of estimating relative abundances of specific species in the same sample (Elbrecht & Leese 2015; Piñol *et al.* 2015). This may be a disadvantage compared to morphological methods, although relative abundances may still be estimated from presence/absence data from a larger set of samples from the same local population (see Trevelline *et al.* 2018). Another, although solvable, limitation may be a lack of reference barcodes from species within the diet, which may limit the identification of prey to genus or order rather than species, depending on the taxonomically closest reference. DNA-based methods may therefore need to be evaluated in a local and species-specific context before applying it to ecological questions.

1.4 Setting up an experiment that can disentangle local land management from surrounding environmental factors on trophic interactions

For a mechanistic understanding of the processes driving population declines and poor breeding success, the effects of management also needs to be explicitly tested and disentangled from other potential environmental effects and factors that may be connected to land management, such as soil quality or topography. Moreover, disturbance from herbivory or other management change may need many years or decades to show its full effects on community composition (Allombert *et al.* 2005). Therefore, replicated

experiments with a long management continuity are needed to describe the ultimate effects of grazing through vegetation structure, arthropod prey abundance and predation pressure on breeding upland birds.

At Glen Finglas, Scotland, a long-term, replicated grazing experiment was set up in 2003 using four consistent grazing types: intensive sheep, extensive sheep, extensive mixed sheep and cattle, and no grazing, which has remained the same since the experiment commenced. Results from the first years of this experiment showed that arthropods common in upland bird diets were most abundant in ungrazed plots and least abundant in intensively grazed plots (Dennis *et al.* 2008; Littlewood 2008) which may be an effect of higher vegetation biomass with decreasing grazing pressure (Evans *et al.* 2015). Similarly, voles (Villar *et al.* 2014) and foxes (Villar *et al.* 2014) were most abundant in ungrazed plots and least abundant in intensively grazed plots. Meadow pipits *Anthus pratensis* had the highest breeding density (Evans *et al.* 2006) and largest egg volume (Evans *et al.* 2005) in extensively, mixed sheep and cattle grazed plots and a higher ratio of male to female offspring in extensively grazed plots compared to intensively grazed or ungrazed plots. These results therefore suggested that, although prey abundance was highest in ungrazed plots, extensively grazed plots (particularly those with mixed sheep and cattle) provided more favourable breeding conditions for meadow pipits. However, these results only described the effects of short-term (2-3 years) of grazing management and the effects may therefore be stronger or completely different after a longer time of continuous management. For example, there were no effects of breeding output of meadow pipits in the first few years of the experiment (Evans *et al.* 2005).

1.5 Thesis aims

Land management change is continuously happening in the British Uplands and Worldwide (Bouwman *et al.* 2005; Pearce-Higgins *et al.* 2008), and may occur faster than many species' ability to adapt to habitat modification, while other species adapt with changed behaviour (Polechová & Barton 2015). Management change may therefore cause important changes to avian species prey availability and predation pressure. As diet assessments allows for increasingly detailed prey determination (e.g. Oehm *et al.* 2017), there is a potential to describe the response of predator-prey interactions under habitat modifications with greater

detail and better understand how management affects breeding upland birds. Many studies have investigated the response of land management such as grazing on birds breeding abundance or breeding success (e.g. Newton 2004; Smart *et al.* 2013; Sternalski *et al.* 2013), but none have described the effects of land management on diets using a DNA-based method and estimates of nutrient intake. Moreover, many correlative studies and experiments have investigated the effects of land management in the short-term, but few have evaluated the effect of time since management change on both insectivorous birds and a range of common prey groups, even though effects of management such as grazing may likely change over time on both arthropods and birds (Calladine *et al.* 2002; Jerrentrup *et al.* 2014).

This thesis aims to study i) the potential mechanisms underlying the response to altered grazing management of breeding meadow pipits *Anthus pratensis*, a common upland passerine, ii) analyse how grazing intensity affects breeding conditions of meadow pipits in the short- and long-term after grazing management change, and iii) assess the utility of new, DNA-based tools and nutrient intake estimates in aiding our understanding of how grazing intensity affects breeding meadow pipits.

These two aspects of grazing management change in uplands and its effects on breeding, insectivorous birds are investigated in the four chapters described in more detail below. The platform for all studies of grazing intensity is the long-term, replicated grazing experiment in Scotland, UK. The meadow pipit is a common bird in upland moorlands and builds concealed nests on the ground (Ferguson-Lees *et al.* 2011). It also forages by walking on the ground, and can therefore be affected by vegetation structure in both nest survival and foraging. Meadow pipits have declined by over 60 % in Europe since the 1980s and in the UK by about 46 % since the 1970s (Balmer *et al.* 2013) and is therefore on the list of species with Amber conservation status, the second most critical category after Red-listed species. This decline may also have effects on other birds of conservation concern, such as hen harriers *Circus cyaneus* and peregrine falcons *Falco peregrinus* (Redpath & Thirgood 1999) due to their importance in the diets, particularly of hen harriers (Picozzi 2008). Due to their foraging and nesting requirements, meadow pipits are a suitable species to study for the effects of habitat change through grazing intensity, as they may respond to breeding conditions altered through both food availability and nest survival.

1.6 Chapter structure

Chapter 2. The effects of short- and long-term grazing management on the abundance of arthropods common in upland bird diets

This chapter aims to increase the understanding of how common arthropod prey groups; Araneae, Hemiptera, Coleoptera, Lepidoptera, Diptera: Tipuloidea and Diptera: Brachycera, respond to different grazing types, and whether these treatment effects have varied with time since the applied management commenced. More specifically, it aims to:

I. Describe how grazing intensity and livestock type affect the total mass and abundance of arthropods common in upland bird diets, at the time meadow pipits start feeding their nestlings;
II. Investigate how the effects of grazing treatments differ under short- and long-term management by comparing abundance data collected two and 12 years after the grazing experiment commenced;
III. Compare the response of different arthropod groups to grazing management.

Chapter 3. Livestock grazing impacts upon the breeding productivity of a common upland insectivorous passerine: results from a long-term experiment

This chapter is a study of the effects of grazing intensity on meadow pipit breeding success after 12 and 13 years of continuous grazing management, and compares these effects to the treatment effects on breeding observed when the experiment started and one year into the experiment, in 2003-4. The aims of this chapter are to:

I. Test how grazing treatment affects breeding density, clutch size, Julian hatch date, number of fledglings per nest, egg- and nestling-stage nest survival and overall nest survival;
II. Test if the treatment effects change during short- and long-term management by comparing treatment effects on the above mentioned variables between the two sampling periods (2003-4 and 2015-6).

Chapter 4. Testing and evaluating DNA-metabarcoding to examine the impacts of livestock grazing on avian predator-prey interactions

This is a study of the meadow pipit nestlings' diets in the four grazing treatments. Samples were collected in 2015 and 2016 and analysed using metabarcoding and high-throughput sequencing of faecal samples. This chapter will also go into details of the molecular technique being used and describe its current shortcomings and applicability to other research around upland bird diets. The aims in this chapter are:

I. To develop and evaluate a nested-tagging DNA-metabarcoding methodology using avian faecal sacs to identify prey DNA provisioned to nestlings of the meadow pipit;
II. To produce a comprehensive list of prey species and further evaluate this method by comparing these results to other studies of meadow pipit diets using morphological methods;
III. To compare diet diversity between meadow pipit nestlings under different grazing management.

Chapter 5. Effects of upland grazing intensity on the nutrient composition of prey provisioned to meadow pipit nestlings: a stoichiometric analysis of faeces

In this chapter, the nutrient availability to meadow pipit nestlings is estimated by measuring the concentration of a range of essential elements (N, P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu) and generally limiting element ratios (C:N and N:P) in food-webs in nestling faecal samples using elemental analysis and inductively coupled plasma mass spectrometry. These estimates were used to compare nutrient availability between grazing treatments during the breeding season. The aims of this study are to:

I. Compare the estimated nutrient availability to nestlings in plots under different grazing management;

II. Test how nutrient availability and nutrient ratios vary as the season progresses and whether this potential variation differs between the four types of grazing management;
III. Evaluate the method of using faecal samples for estimates of nutrient intake and between different types of grazing management.

Chapter 6. General discussion and conclusions

The discussion will first link together all chapters by discussing overall trends in grazing management and how they may be more or less advantageous for breeding meadow pipits. It will also discuss how the effects of grazing management on the breeding variables studied in chapter 3 are reflected in arthropod abundance (short-term and longer-term changes), meadow pipit nestling diet and nutrient concentrations/ratios in nestling faecal samples. Suitable grazing management for meadow pipits, how this may be applicable to other insectivorous upland birds and the need for experiments with a long continuity are also discussed.

The applicability of the methods used for diet and nutrient intake assessment will be evaluated, as well as future improvements that may be necessary before these tools can be standardised across avian species and habitats.

Lastly, the discussion is concluded by highlighting how the results in the previous chapters may strengthen the understanding of how grazing intensity can affect breeding insectivorous birds and how the methods applied here can contribute to a mechanistic understanding of birds' responses to habitat modifications.

1.7 References

Allard, V., Soussana, J.-F., Falcimagne, R., Berbigier, P., Bonnefond, J.M., Ceschia, E., *et al.* (2007). The role of grazing management for the net biome productivity and greenhouse gas budget (CO2, N2O and CH4) of semi-natural grassland. *Agric. Ecosyst. Environ.*, 121, 47–58.

Allombert, S., Stockton, S. & Martin, J.-L. (2005). A Natural Experiment on the Impact of

Overabundant Deer on Forest Invertebrates. *Conserv. Biol.*, 19, 1917–1929.

Amar, A., Davies, J., Meek, E., Williams, J., Knight, A. & Redpath, S. (2011). Long-term impact of changes in sheep Ovis aries densities on the breeding output of the hen harrier Circus cyaneus. *J. Appl. Ecol.*, 48, 220–227.

Atkinson, P.W., Fuller, R.J., Vickery, J.A., Conway, G.J., Tallowin, J.R.B., Smith, R.E.N., *et al.* (2005). Influence of agricultural management, sward structure and food resources on grassland field use by birds in lowland England. *J. Appl. Ecol.*, 42, 932–942.

Baines, D. (1990). The Roles of Predation, Food and Agricultural Practice in Determining the Breeding Success of the Lapwing (Vanellus vanellus) on Upland Grasslands. *J. Anim. Ecol.*, 59, 915–929.

Báldi, A., Batáry, P. & Erdős, S. (2005). Effects of grazing intensity on bird assemblages and populations of Hungarian grasslands. *Agric. Ecosyst. Environ.*, 108, 251–263.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I. & Fuller, R. (2013). *Bird Atlas 2007-11: The Breeding and Wintering Birds of Britain and Ireland*. BTO Books, Thetford.

Benton, T.G., Vickery, J.A. & Wilson, J.D. (2003). Farmland biodiversity: is habitat heterogeneity the key? *Trends Ecol. Evol.*, 18, 182–188.

Bignal, E.M. & Mccracken, D.I. (1996). Low-Intensity Farming Systems in the Conservation of the Countryside. *J. Appl. Ecol.*, 33, 413–424.

Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R., *et al.* (2007). The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS One*, 2, e197.

Blair, R.H. & Tucker, B.W. (1941). Nest sanitation. *Br. Birds*, 34, 206–255.

Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F. & Monaghan, P. (2006). Effects of neonatal nutrition on adult reproduction in a passerine bird. *Ibis* , 148, 509–514.

Bouwman, A.F., Van der Hoek, K.W., Eickhout, B. & Soenario, I. (2005). Exploring changes in world ruminant production systems. *Agric. Syst.*, 84, 121–153.

Britschgi, A., Spaar, R. & Arlettaz, R. (2006). Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the Whinchat Saxicola rubetra: Lessons for overall Alpine meadowland management. *Biol. Conserv.*, 130, 193–205.

Britton, A.J., Hester, A.J., Hewison, R.L., Potts, J.M. & Ross, L.C. (2017). Climate, pollution and grazing drive long-term change in moorland habitats. *Appl. Veg. Sci.*, 20, 194–203.

Bro, E., Mayot, P., Corda, E. & Reitz, F. (2004). Impact of habitat management on grey

partridge populations: assessing wildlife cover using a multisite BACI experiment. *J. Appl. Ecol.*, 41, 846–857.

Buchanan, G.M., Grant, M.C., Sanderson, R.A. & Pearce-Higgins, J.W. (2006). The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis*, 148, 615–628.

Buchanan, G.M., Pearce-Higgins, J.W., Douglas, D.J.T. & Grant, M.C. (2017). Quantifying the importance of multi-scale management and environmental variables on moorland bird abundance. *Ibis*, 159, 744–756.

Calladine, J., Baines, D. & Warren, P. (2002). Effects of reduced grazing on population density and breeding success of black grouse in northern England. *J. Appl. Ecol.*, 39, 772–780.

Câmara, T., Leal, I.R., Blüthgen, N., Oliveira, F.M.P., Queiroz, R.T. de & Arnan, X. (2018). Effects of chronic anthropogenic disturbance and rainfall on the specialization of ant-plant mutualistic networks in the Caatinga, a Brazilian dry forest. *J. Anim. Ecol.*, 87, 1022–1033.

Cernusca, A., Tappeiner, U., Bahn, M., Bayfield, N., Chemini, C., Fillat, F., *et al.* (1996). Ecomont Ecological Effects of Land Use Changes on European Terrestrial Mountain Ecosystems. *Pirineos*, 147-148, 145–172.

Chamberlain, D.E., Hatchwell, B.J. & Perrins, C.M. (1995). Spaced out Nests and Predators: An Experiment to Test the Effects of Habitat Structure. *J. Avian Biol.*, 26, 346–349.

Chapin, F.S., 3rd, Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., *et al.* (2000). Consequences of changing biodiversity. *Nature*, 405, 234–242.

Clarke, L.J., Soubrier, J., Weyrich, L.S. & Cooper, A. (2014). Environmental metabarcodes for insects: in silico PCR reveals potential for taxonomic bias. *Mol. Ecol. Resour.*, 14, 1160–1170.

Critchlow-Watton, N., Dobbie, K.E., Bell, R., Campbell, S.D.G., Hinze, D., Motion, A., *et al.* (2014). *Scotland's State of the Environment Report*. Scotland's Environment Web.

Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., *et al.* (2007). Studying Seabird Diet through Genetic Analysis of Faeces: A Case Study on Macaroni Penguins (Eudyptes chrysolophus). *PLoS One*, 2, e831.

Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., *et al.* (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.*, 45, 279–287.

Dennis, P., Young, M.R. & Gordon, I.J. (1998). Distribution and abundance of small insects

and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. *Ecol. Entomol.*, 23, 253–264.

Dirnböck, T., Dullinger, S. & Grabherr, G. (2003). A regional impact assessment of climate and land-use change on alpine vegetation: Alpine vegetation change. *J. Biogeogr.*, 30, 401–417.

Douglas, D.J.T., Evans, D.M. & Redpath, S.M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study*, 55, 290–296.

Elbrecht, V. & Leese, F. (2015). Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass--Sequence Relationships with an Innovative Metabarcoding Protocol. *PLoS One*, 10, e0130324.

Elbrecht, V. & Leese, F. (2017). Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Front. Environ. Sci. Eng. China*, 5, 11.

Erb, K.-H., Luyssaert, S., Meyfroidt, P., Pongratz, J., Don, A., Kloster, S., *et al.* (2017). Land management: data availability and process understanding for global change studies. *Glob. Chang. Biol.*, 23, 512–533.

Erb, K.-H., Mayer, A., Kastner, T., Sallet, K.-E. & Haberl, H. (2012). The impact of industrial grain fed livestock production on food security: an extended literature review. *Commissioned by compassion in world farming. The Tubney Charitable Trust and World Society for the Protection of Animals, London, UK*.

Eurostat. (2016). *Agriculture, forestry and fishery statistics — 2016 edition*. European Union.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A. & Dennis, P. (2005). Livestock grazing affects the egg size of an insectivorous passerine. *Biol. Lett.*, 1, 322–325.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A., Gardner, C.J., Dennis, P., *et al.* (2006). Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine. *Biol. Lett.*, 2, 636–638.

Evans, D.M., Villar, N., Littlewood, N.A., Pakeman, R.J., Evans, S.A., Dennis, P., *et al.* (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere*, 6, 42.

Evans, K.L. (2004). The potential for interactions between predation and habitat change to cause population declines of farmland birds. *Ibis* , 146, 1–13.

Ferguson-Lees, J., Castell, R. & Leech, D. (2011). *A Field Guide to Monitoring Nests*. British

Trust for Ornithology, Thetford, UK.

Fielding, A.F. & Haworth, P.F. (1999). *Upland Habitats*. Ill edition. Routledge, Abingdon, UK.

Fraser, M.D., Moorby, J.M., Vale, J.E. & Evans, D.M. (2014). Mixed grazing systems benefit both upland biodiversity and livestock production. *PLoS One*, 9, e89054.

Fuller, R.J. (2012). *Birds and Habitat: Relationships in Changing Landscapes*. Cambridge University Press.

Fuller, R.J. & Gough, S.J. (1999). Changes in sheep numbers in Britain: implications for bird populations. *Biol. Conserv.*, 91, 73–89.

Gales, R. p. (1987). Validation of the stomach-flushing technique for obtaining stomach contents of Penguins. *Ibis* , 129, 335–343.

Gibson, C.W.D., Brown, V.K., Losito, L. & McGavin, G.C. (1992). The Response of Invertebrate Assemblies to Grazing. *Ecography* , 15, 166–176.

Götmark, F. & Andersson, M. (2005). Predation by sparrowhawks decreases with increased breeding density in a songbird, the great tit. *Oecologia*, 142, 177–183.

Graveland, J. & Gijzen, T.V. (1994). Arthropods and Seeds are not Sufficient as Calcium Sources for Shell Formation and Skeletal Growth in Passerines. *Ardea*, 82, 299–314.

Grubwieser, P., Mühlmann, R. & Parson, W. (2003). New sensitive amplification primers for the STR locus D2S1338 for degraded casework DNA. *Int. J. Legal Med.*, 117, 185–188.

Hartley, P.H.T. (1948). The Assessment of the Food of Birds*. *Ibis* , 90, 361–381.

Hayhow, D.B., Bond, A.L., Douse, A., Eaton, M.A., Frost, T., Grice, P.V., *et al.* (2017). *The state of the UK's birds 2016*. The RSPB; BTO; WWT; DAERA; JNCC; NE; NRW; SNH.

Haynes, R.J. & Williams, P.H. (1993). Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. In: *Advances in Agronomy* (ed. Sparks, D.L.). Academic Press, pp. 119–199.

Heiss, R.S., Clark, A.B. & McGowan, K.J. (2009). Growth and nutritional state of American Crow nestlings vary between urban and rural habitats. *Ecol. Appl.*, 19, 829–839.

Hildén, O. (1965). Habitat selection in birds: a review. In: *Annales Zoologici Fennici*. JSTOR, pp. 53–75.

Hilder, E.J. (1964). The distribution of plant nutrients by sheep at pasture. In: *Proceedings of the Australian Society of Animal Production*. pp. 241–248.

Hocking, P.M. (1987). Nutritional interactions with reproduction in birds. *Proc. Nutr. Soc.*, 46, 217–225.

Hungerford, B.S., Studier, E.H., Szuch, E.J., Pace, G.L. & Taylor, S. (1993). Aspects of caloric, nitrogen and mineral nutrition during growth in nestling eastern bluebirds, Sialia sialis.

Comp. Biochem. Physiol. A Physiol., 106, 385–389.

Jarman, S.N., Gales, N.J., Tierney, M., Gill, P.C. & Elliott, N.G. (2002). A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. *Mol. Ecol.*, 11, 2679–2690.

Jedlicka, J.A., Sharma, A.M. & Almeida, R.P.P. (2013). Molecular tools reveal diets of insectivorous birds from predator fecal matter. *Conserv. Genet. Resour.*, 5, 879–885.

Jedlicka, J.A., Vo, A.-T.E. & Almeida, R.P.P. (2017). Molecular scatology and high-throughput sequencing reveal predominately herbivorous insects in the diets of adult and nestling Western Bluebirds (Sialia mexicana) in California vineyards. *Auk*, 134, 116–127.

Jenny, M. (1990). Diet ecology of the skylark Alauda arvensis in a intensively cultivated agroecosystem in the Swiss midlands. *Ornithol. Beob.*, 87, 31–53.

Jerrentrup, J.S., Wrage-Mönnig, N., Röver, K.-U. & Isselstein, J. (2014). Grazing intensity affects insect diversity via sward structure and heterogeneity in a long-term experiment. *J. Appl. Ecol.*, 51, 968–977.

Joo, S. & Park, S. (2012). Identification of bird species and their prey using DNA barcode on feces from Korean traditional village groves and forests (maeulsoop). *Animal Cells Syst.* , 16, 488–497.

Kadochnikov, N.P. (1967). A procedure of vital study of feeding habits of adult birds. *Byul Mosk Obshchest Ispyt Prer Otd Biol*, 72, 29–34.

Kaspari, M. & Joern, A. (1993). Prey Choice by Three Insectivorous Grassland Birds: Reevaluating Opportunism. *Oikos*, 68, 414–430.

King, R.A., Symondson, W.O.C. & Thomas, R.J. (2015). Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas. *Bull. Entomol. Res.*, 105, 261–272.

Klink, R. van, Mandema, F.S., Bakker, J.P. & Tinbergen, J.M. (2014). Foraging site choice and diet selection of Meadow Pipits Anthus pratensis breeding on grazed salt marshes. *Bird Study*, 61, 101–110.

Kluijver, H.N. (1933). Contribution to the biology and the ecology of starling (Sturnus vulgaris vulgaris L.) during its reproductive period. University of Wageningen.

Laiolo, P., Dondero, F., Ciliento, E. & Rolando, A. (2004). Consequences of pastoral abandonment for the structure and diversity of the alpine avifauna. *J. Appl. Ecol.*, 41, 294–304.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., *et al.* (2013). A new

versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front. Zool.*, 10, 34.

Littlewood, N.A. (2008). Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.*, 1, 151–160.

Mattson, W.J.J. (1980). Herbivory in Relation to Plant Nitrogen Content. *Annu. Rev. Ecol. Syst.*, 11, 119–161.

McNamara, J.M. (1987). Starvation and Predation as Factors Limiting Population Size. *Ecology*, 68, 1515–1519.

McSherry, M.E. & Ritchie, M.E. (2013). Effects of grazing on grassland soil carbon: a global review. *Glob. Chang. Biol.*, 19, 1347–1357.

Michalski, M., Nadolski, J., Marciniak, B., Loga, B. & Bańbura, J. (2011). Faecal Analysis as a Method of Nestling Diet Determination in Insectivorous Birds: A Case Study in Blue Tits Cyanistes caeruleus and Great Tits Parus major. *Acta Ornithol.* , 46, 164–172.

Misenhelter, M.D. & Rotenberry, J.T. (2000). CHOICES AND CONSEQUENCES OF HABITAT OCCUPANCY AND NEST SITE SELECTION IN SAGE SPARROWS. *Ecology*, 81, 2892–2901.

Moreby, S.J. (1988). An aid to the identification of arthropod fragments in the faeces of gamebird chicks (Galliformes). *Ibis* , 130, 519–526.

Moreby, S.J. & Stoate, C. (2000). A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study*, 47, 320–331.

Mörner, T., Hansson, T., Carlsson, L., Berg, A.-L., Ruiz Muñoz, Y., Gustavsson, H., *et al.* (2017). Thiamine deficiency impairs common eider (Somateria mollissima) reproduction in the field. *Sci. Rep.*, 7, 14451.

Mysterud, A., Aaserud, R., Hansen, L.O., Åkra, K., Olberg, S. & Austrheim, G. (2010). Large herbivore grazing and invertebrates in an alpine ecosystem. *Basic Appl. Ecol.*, 11, 320–328.

Newton, I. (2004). The recent declines of farmland bird populations in Britain: an appraisal of causal factors and conservation actions. *Ibis* , 146, 579–600.

Norris, K., Brindley, E., Cook, T., Babbs, S., Brown, C.F. & Yaxley, R. (1998). Is the density of redshank Tringa totanus nesting on saltmarshes in Great Britain declining due to changes in grazing management? *J. Appl. Ecol.*, 35, 621–634.

Noy-Meir, I., Gutman, M. & Kaplan, Y. (1989). Responses of Mediterranean Grassland Plants to Grazing and Protection. *J. Ecol.*, 77, 290–310.

Oehm, J., Juen, A., Nagiller, K., Neuhauser, S. & Traugott, M. (2011). Molecular scatology: how to improve prey DNA detection success in avian faeces? *Mol. Ecol. Resour.*, 11, 620–628.

Oehm, J., Thalinger, B., Eisenkölbl, S. & Traugott, M. (2017). Diet analysis in piscivorous birds: What can the addition of molecular tools offer? *Ecol. Evol.*, 7, 1984–1995.

Ontiveros, D., Pleguezuelos, J.M. & Caro, J. (2005). Prey density, prey detectability and food habits: the case of Bonelli's eagle and the conservation measures. *Biol. Conserv.*, 123, 19–25.

Orians, G.H. (1966). Food of Nestling Yellow-Headed Blackbirds, Cariboo Parklands, British Columbia. *Condor*, 68, 321–337.

Pakeman, R.J. & Nolan, A.J. (2009). Setting sustainable grazing levels for heather moorland: a multi-site analysis. *J. Appl. Ecol.*, 46, 363–368.

Pearce-Higgins, J.W. & Grant, M.C. (2006). Relationships between bird abundance and the composition and structure of moorland vegetation. *Bird Study*, 53, 112–125.

Pearce-Higgins, J.W., Murray, C., M, G.B.C. & Graeme, M. (2008). International importance and drivers of change of upland bird populations. In: *Drivers of Environmental Change in Uplands* (eds. Bonn, A., Allott, T., Hubacek, K. & Stewart, J.). Routledge, pp. 209–227.

Pearce-Higgins, J.W. & Yalden, D.W. (2003). Variation in the use of pasture by breeding European Golden Plovers Pluvialis apricaria in relation to prey availability: Pasture use by breeding Golden Plovers. *Ibis*, 145, 365–381.

Pearce-Higgins, J.W. & Yalden, D.W. (2004). Habitat selection, diet, arthropod availability and growth of a moorland wader: the ecology of European Golden Plover Pluvialis apricaria chicks: Ecology of Golden Plover chicks. *Ibis*, 146, 335–346.

Picozzi, N. (2008). Dispesion, breeding and prey of the hen harries *Circus cyaneus* in Glen Dye, Kincardineshire. *Ibis* , 120, 498–509.

Piñol, J., Mir, G., Gomez-Polo, P. & Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Mol. Ecol. Resour.*, 15, 819–830.

Polechová, J. & Barton, N.H. (2015). Limits to adaptation along environmental gradients. *Proc. Natl. Acad. Sci. U. S. A.*, 112, 6401–6406.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. (2012). Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.*, 21, 1931–1950.

Poulsen, J.G. & Aebischer, N.J. (1995). Quantitative Comparison of Two Methods of Assessing Diet of Nestling Skylarks (Alauda arvensis). *Auk*, 112, 1070–1073.

Prŷs-Jones, R.P., Schifferli, L. & Macdonald, D.W. (1974). The Use of an Emetic in Obtaining Food Samples from Passerines. *Ibis* , 116, 90–94.

Redpath, S.M., Clarke, R., Madders, M. & Thirgood, S.J. (2001). Assessing raptor diet: comparing pellets, prey remains, and observational data at hen harrier nests. *Condor*, 103, 184–188.

Redpath, S.M. & Thirgood, S.J. (1999). Numerical and functional responses in generalist predators: hen harriers and peregrines on Scottish grouse moors. *J. Anim. Ecol.*, 68, 879–892.

Schaub, M., Martinez, N., Tagmann-Ioset, A., Weisshaupt, N., Maurer, M.L., Reichlin, T.S., *et al.* (2010). Patches of Bare Ground as a Staple Commodity for Declining Ground-Foraging Insectivorous Farmland Birds. *PLoS One*, 5, e13115.

Silcock, P., Brunyee, J. & Pring, J. (2012). *Changing livestock numbers in the UK LFA – an analysis of likely biodiversity implications*. RSPB.

Sim, I.M.W., Gregory, R.D., Hancock, M.H. & Brown, A.F. (2005). Recent changes in the abundance of British upland breeding birds: Capsule Breeding wader populations have more often shown declines than passerine populations during the last 10–20 years. *Bird Study*, 52, 261–275.

Smart, J., Bolton, M., Hunter, F., Quayle, H., Thomas, G. & Gregory, R.D. (2013). Managing uplands for biodiversity: Do agri-environment schemes deliver benefits for breeding lapwing Vanellus vanellus? *J. Appl. Ecol.*, 50, 794–804.

Speed, J.D.M., Austrheim, G. & Mysterud, A. (2013). The response of plant diversity to grazing varies along an elevational gradient. *J. Ecol.*, 101, 1225–1236.

Steenhof, K. & Kochert, M.N. (1982). An Evaluation of Methods Used to Estimate Raptor Nesting Success. *J. Wildl. Manage.*, 46, 885–893.

Sternalski, A., Blanc, J.-F., Augiron, S., Rocheteau, V. & Bretagnolle, V. (2013). Comparative breeding performance of Marsh Harriers Circus aeruginosus along a gradient of land-use intensification and implications for population management. *Ibis* , 155, 55–67.

Sullivan, M.J.P., Newson, S.E. & Pearce-Higgins, J.W. (2015). Using habitat-specific population trends to evaluate the consistency of the effect of species traits on bird population change. *Biol. Conserv.*, 192, 343–352.

Suttle, K.B., Thomsen, M.A. & Power, M.E. (2007). Species interactions reverse grassland

responses to changing climate. *Science*, 315, 640–642.

Symondson, W.O.C. (2002). Molecular identification of prey in predator diets. *Mol. Ecol.*, 11, 627–641.

Thirgood, S.J., Redpath, S.M., Haydon, D.T., Rothery, P., Newton, I. & Hudson, P.J. (2000). Habitat loss and raptor predation: disentangling long- and short-term causes of red grouse declines. *Proc. Biol. Sci.*, 267, 651–656.

Trevelline, B.K., Latta, S.C., Marshall, L.C., Nuttle, T. & Porter, B.A. (2016). Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (Parkesia motacilla). *Auk*, 133, 415–428.

Trevelline, B.K., Nuttle, T., Hoenig, B.D., Brouwer, N.L., Porter, B.A. & Latta, S.C. (2018). DNA metabarcoding of nestling feces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat. *Oecologia*, 187, 85–98.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.*, 11, 1351–1363.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007). Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, 445, 202–205.

Valera, F., Gutiérrez, J.E. & Barrios, R. (1997). Effectiveness, Biases and Mortality in the Use of Apomorphine for Determining the Diet of Granivorous Passerines. *Condor*, 99, 765–772.

Vandenberghe, C., Prior, G., Littlewood, N.A., Brooker, R. & Pakeman, R. (2009). Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic Appl. Ecol.*, 10, 662–670.

Villar, N., Cornulier, T., Evans, D., Pakeman, R., Redpath, S. & Lambin, X. (2014). Experimental evidence that livestock grazing intensity affects cyclic vole population regulation processes. *Popul. Ecol.*, 56, 55–61.

Villar, N., Lambin, X., Evans, D., Pakeman, R. & Redpath, S. (2013). Experimental evidence that livestock grazing intensity affects the activity of a generalist predator. *Acta Oecol.*, 49, 12–16.

Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. & Furness, R.W. (2003). Assessing the diet of great skuas, Catharacta skua, using five different techniques. *Polar Biol.*, 26, 20–26.

Wheeler, P. (2008). Effects of sheep grazing on abundance and predators of field vole

(Microtus agrestis) in upland Britain. *Agric. Ecosyst. Environ.*, 123, 49–55.

White, T.C.R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, 63, 90–105.

Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. & Jones, G. (2011). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Mol. Ecol. Resour.*, 11, 236–244.

Chapter 2. The effects of short- and long-term grazing management on the abundance of arthropods common in upland bird diets

2.1 Abstract

Intensive farming methods can negatively affect the abundance of arthropods, potentially causing declines of insectivorous bird populations. Short-term grazing experiments have demonstrated direct effects of changes on arthropod abundance, but less is known of how arthropods respond to management changes in the long-term. Studies of plant species composition suggest that vegetation changes require several years or decades to become fully apparent. This may result in further changes in foraging conditions and hence abundance of arthropods in addition to those observed immediately after management change.

Here, the impacts of four different grazing types: intensive sheep, extensive sheep, extensive mixed sheep and cattle and no grazing, on six arthropod groups common in upland bird diets were compared after 2 and 12 years of continuous grazing management. The six groups (Araneae, Hemiptera, Coleoptera, Diptera: Brachycera, Diptera: Tipuliodea, and Lepidoptera) were sampled in spring by suction sampling and sweep nets in an upland estate in Scotland, UK.

The total mass of all arthropod groups was, across both sampling years, significantly higher in ungrazed plots compared to intensively grazed plots. Abundances of Araneae and Hemiptera were significantly higher in extensively sheep grazed plots and ungrazed plots compared to intensively grazed plots across both years. However, in the extensively sheep grazed plots, total mass and abundance of Araneae and Hemiptera were as low as intensively grazed plots in the early sampling year, and as high as ungrazed plots only in the later sampling year. Remaining groups showed a trend towards higher abundances in ungrazed plots, although some were sampled in numbers that were too low to test this statistically.

Whilst acknowledging the importance of inter-annual variation on arthropod populations, these results suggest that when studying the effects of grazing after more than a decade,

plots extensively grazed by sheep may have the same abundances of some arthropod groups as ungrazed plots. The ultimate impacts of altered grazing management on the abundance of important arthropod groups in bird diets may therefore not occur or become apparent during the first few years following management change.

2.2 Introduction

Recent decades have seen growing evidence for declining abundances of arthropods worldwide across a range of habitats (Thomas *et al.* 2004; Conrad *et al.* 2006; Brooks *et al.* 2012; Dirzo *et al.* 2014; Hallmann *et al.* 2017). Similarly, although populations have stabilized during the last years (Balmer *et al.* 2013), abundances of many farmland birds in the United Kingdom (UK) have also declined substantially during the last century. These declines are suggested to be a result of intensified farming practises (Burns *et al.* 2013), partially through the lower availability and quality of arthropod prey, in both lowland and upland farming systems (Benton *et al.* 2002; Newton 2004). However, only 4% of invertebrate species in the UK are being monitored for population trends, compared to 58% of all vertebrate species (Burns *et al.* 2013; Hayhow *et al.* 2016), hence while insectivorous birds are well monitored, we know relatively little about how their prey resources have changed over time and according to land management.

The 20th century saw substantial increases in farming intensity and productivity, while forthcoming management improvements will require more focus on sustainable farming for maintaining biodiversity (Dimitri *et al.* 2005; Jain 2012). In the UK, farming intensification has been shown to be a main driver of wildlife loss (Robinson & Sutherland 2002; Fox *et al.* 2014) and many species across different taxa benefit from less intense farming, such as lower grazing pressure where agri-environment schemes have been adopted (Hayhow *et al.* 2016). In the British uplands, farming takes up a large proportion of the land use, with sheep grazing being the most common. Although the majority of land is categorised as Less Favoured Areas (LFA's) (European Union 2013), i.e. areas with poor profitability due to environmental conditions often at risk of human depopulation, these areas have also experienced farming intensification during the last century that in many areas has led to overgrazing (Condliffe 2009). However, due to changes in farming subsidies and low

profitability (Critchlow-Watton *et al.* 2014), grazing pressure in general and grazing by cattle and mixed livestock in particular (Silcock *et al.* 2012) has decreased in many upland areas. Grazing abandonment has been shown to lead to a change in plant community composition and decreased soil fertility (Peco *et al.* 2012) and it is clear that grazing abandonment can have dramatic effects on fauna that depend on grazing animals, such as dung beetles (Tonelli *et al.* 2018). However, indirect effects of large herbivores on arthropod communities through vegetation change can be harder to predict a priori (Suominen *et al.* 2008).

There are a range of ways in which plant traits in grassland and moorland can affect arthropod communities. For example, Carabid species composition in uplands varies with ground wetness, vegetation height and, within heather *Calluna vulgaris*-covered habitats, on plant density. This is probably due to differences in temperature, insolation and humidity according to species' individual preferences which all affect the observed abundances of a group of arthropods (Gardner 1991). Lepidoptera larvae show responses to similar factors, and are both more abundant and more diverse in taller stands of heather (Haysom & Coulson 1998). Moreover, arthropod responses to vegetation change vary with taxonomic groups and requirements (Scohier & Dumont 2012). Gibson et al. (1992) found that abundances of carnivorous Heteroptera and Araneae were more affected by plant structure, while leaf mining insects were more sensitive to the species composition of plants due to specific foraging requirements. Seemingly similar vegetation of moorlands and grasslands can support considerably different arthropod communities and, therefore, a mosaic of plants and different vegetation structures is more likely to hold a wide range of arthropod groups, of which several are important food to birds (Butterfield & Coulson 1983; Haysom & Coulson 1998; Buchanan *et al.* 2006; García *et al.* 2009).

Grazing can change the vegetation structure and increase diversity by holding back otherwise dominant plants (Milchunas *et al.* 1988), and arthropod groups such as Hemipterans have been shown to be more diverse in habitats with a higher plant diversity (Hartley *et al.* 2003). However, plant diversity does not necessarily lead to higher arthropod abundances (Holmquist *et al.* 2014). Grazing can also alter the nutrition available to herbivorous arthropods; for example, grazing by American bison *Bison bison* in prairie grasslands increased arthropod abundance without changing the plant species composition. Instead, a higher nutrition level of the plants, which can be induced by grazing as a stress

response, is likely to explain the increase in arthropods (Moran 2014). Contrary to this, Newbold *et al.* (2014) found no effects of grazing or grazing intensity on the abundance or species richness of the most common arthropod groups in the study area (Coleoptera and Araneae) while the vegetation within the same treatments varied in both species composition and structure. García *et al.* (2010) showed that mixed grazing, goats combined with either sheep or cattle, compared to either sheep or cattle alone, led to higher abundances of most arthropod groups, although total arthropod abundance did not vary between grazing types. This was due to goats grazing in a way that held down otherwise abundant species and maintained a higher biodiversity. Other studies have shown a negative impact of increasing grazing pressure; sheep grazing at a low intensity rather than a higher stocking rate has been shown to result in a higher diversity of several arthropod groups such as Orthoptera, Coleoptera and Lepidoptera (Scohier & Dumont 2012). Lower grazing pressure by sheep can also result in lower mortality of butterflies and other species which hibernate as larvae in the vegetation during autumn and winter, resulting in higher abundances the next spring (Noordwijk *et al.* 2012). Moreover, higher grazing pressure leading to less heather cover and more grass has had negative effects on the abundance of Hemiptera (Hartley *et al.* 2003). At a grazing experiment with different levels of sheep grazing in Norway, a high density of sheep has been shown to negatively affect the abundance of some common beetle species while others remained unaffected (Mysterud *et al.* 2010). However, the majority of examples comparing arthropod abundances in areas under different grazing management study the immediate and short-term effects, which may not necessarily be consistent in the long-term.

Although intense overgrazing often has a negative impact on both plant and animal diversity and is hard to combine with most conservation objectives (Morris 2000), effects of grazing are habitat specific and other factors such as humidity, latitude, altitude, and seasonality of grazing also play an important role for the effects of grazing on vegetation and arthropod abundance (Scohier & Dumont 2012; Koerner *et al.* 2014). The applied grazing management itself may also be driven by environmental conditions, such as soil quality, altitude and climatic factors (Critchlow-Watton *et al.* 2014). Replicated grazing experiments can overcome such drivers and provide information on grazing effects without environmental bias or individual management variation between farms, such as livestock breeds, extent of grazing or other land changes.

Impacts of grazing on arthropod abundance may be stronger after several years of continuous grazing; Allombert et al. (2005) showed that negative impacts of browsing by mule deer *Odocoileus hemionus sitkensis* on arthropod abundance were more prominent after over 50 years of continuous browsing, with variation in responses apparent between arthropod groups. Moreover, Jerrentrup *et al*. (2014) showed that the positive effect on grasshopper abundance of low intensity cattle grazing compared to higher grazing pressure increased over a nine-year period while Lepidopterans showed a similar trend but without a significant change over time. In order to provide guidelines for sustainable management with confidence, it is important to evaluate both short- and longer-term effects of altered grazing management.

In this study, the short- and long-term effects of different grazing pressure were investigated for six arthropod groups of particular importance in upland bird diets (Buchanan *et al.* 2006): Aranea, Hemiptera, Coleoptera, Lepidoptera, Diptera: Tipuloidea and Diptera: Brachycera. A long-term grazing experiment established in 2003 at Glen Finglas, Scotland was used, with intensive sheep, extensive sheep, extensive mixed sheep and cattle and no grazing. Sampling of arthropod abundances was done after two and twelve years into the experiment, to compare the impacts of grazing in the immediate years after the experiment began and after twelve years of continuous grazing management. Earlier work at the same study site by Dennis *et al.* (2008) showed that there was an increasing total arthropod mass and abundance of Hemiptera, Coleoptera and Araneae with decreased grazing pressure after two years. Littlewood (2008) showed that moth abundance was lowest in plots with intensive sheep grazing compared to the three other treatments with extensive or no grazing. Evans *et al.* (2015) showed that arthropod abundance was positively related to vegetation biomass at the same study site, which would explain the positive response of arthropod abundance to decreasing grazing pressure. However, these studies show the short-term effects of different grazing pressure, and further studies are needed to test the effects of long-term management.

The grazing treatment effects on abundance and change of treatment effects between the two sampling years was tested on each arthropod group, including total mass of all groups sampled by suction sampling. Given the previously observed treatment effects, it was

hypothesized that: i) the effects from the early stage of the experiment, with higher arthropod abundances and total arthropod mass in the ungrazed treatment, intermediate abundances in the extensively grazed treatments and lowest abundances in the intensively grazed treatment, would be reflected in the late sampling year although the magnitude of these effects would increase with time; ii) groups strongly dependent on vegetation structure, such as Araneae (Gibson *et al.* 1992), would show a particularly strong positive response to ungrazed plots over time, compared to mainly herbivorous groups such as Hemiptera and Lepidoptera larvae. The latter groups were predicted to benefit from increased nutrient availability induced by grazing in grazed plots: (Boyer *et al.* 2003; Moran 2014), and may therefore show similar abundances in extensively grazed plots as in ungrazed plots.

2.3 Methods

2.3.1 Study area and experimental design

A grazing experiment was set up in 2003 in Glen Finglas, Scotland, UK, (56°16'03"N, 4°25'08"W), where four grazing treatments were established with six replicates, each measuring 3.3 ha. The experiment plots were spread out across three block areas with two replicates in each block and a random allocation of one of each treatment types in each replicate set. The four treatments were: I) commercial stocking density of sheep with 9 ewes per plot (2.73 ewes ha^{-1}); II) one-third of the commercial stocking density with 3 ewes per plot; III) 2 ewes per plot and 2 cows with suckling calves during four weeks each autumn; and IV) ungrazed. Treatments II and III were designed to have the same grazing intensity by using the same number of livestock units each season, in order to compare the effects of sheep only and mixed cattle and sheep grazing with the same biomass offtake. The experiment blocks are placed ca 5 km apart and are located at an altitude of 220 m to 500 m (see Chapter 2, Figure 2.1). The study site consists of acid grassland with a majority of the ground covered by grass fam. *Poaceae,* but heather *Calluna vulgaris*, various small shrubs (e.g. *Salix* spp) and bracken *Pteridium aquilinum* are also frequent.

2.3.2 Sample collection

Sampling was done in 2005 and 2015, following Dennis *et al.* (2008) using two sampling techniques; suction sampling and sweep nets. Suction samples were collected between 24 May and 9 June in both years, using a converted leaf blower from five randomly selected

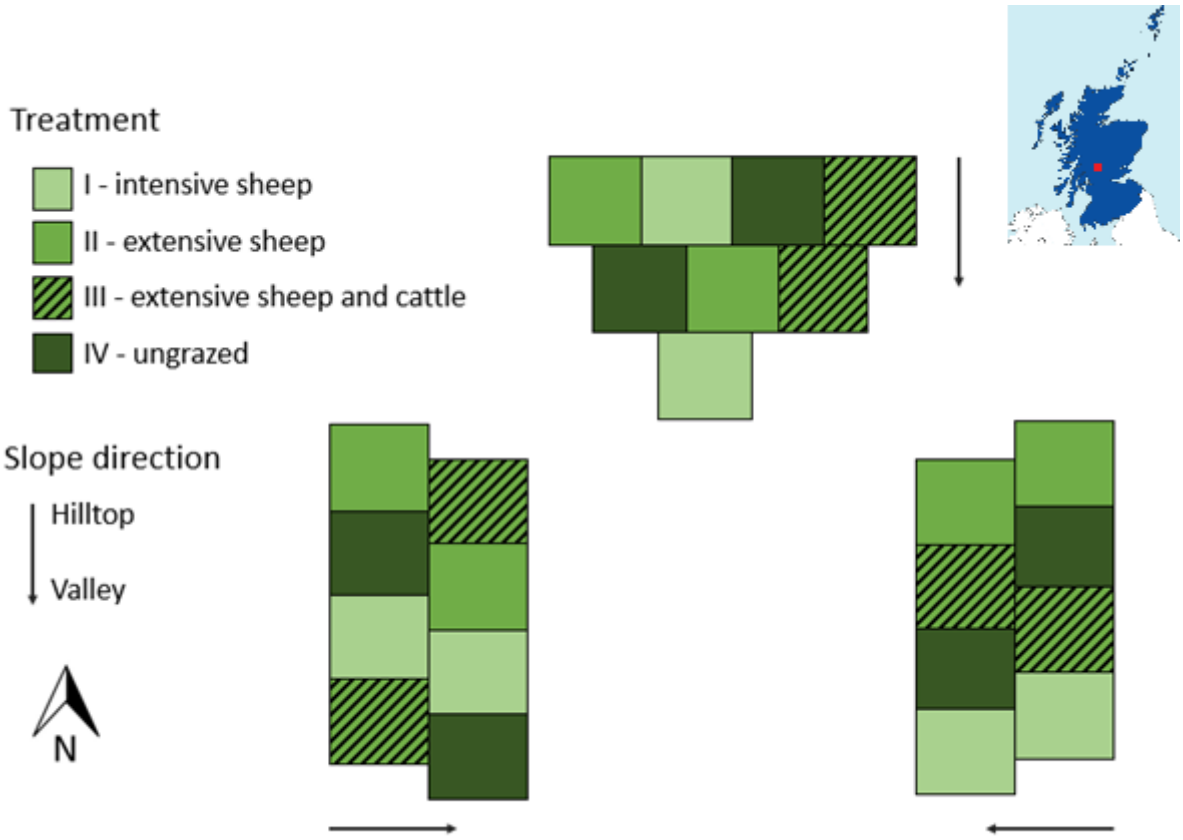


Figure 2.1 Experiment blocks and plots at Glen Finglas, Scotland, UK. The map in the upper right corner shows the location of the Glen Finglas Estate in Scotland. The four grazing treatments were: I) commercial stocking density of sheep with 9 ewes per plot (3 ewes per ha-1), II) one third of the commercial stocking density with 3 ewes per plot, III) 2 ewes per plot and 2 cows with suckling calves during four weeks each autumn and IV) ungrazed. Each plot was 3.3 ha. Shapes of plots are approximate due to limitations such as varying topography and roads.

points within each experiment plot, located by a hand-held global positioning system (GPS) navigator. These points were randomly selected each year from 25 points evenly spread out in each plot. Each sample consisted of five sub-samples, each collected by sampling for 45 sec within a round, deep sampling frame measuring 34.3 cm in diameter, held against the ground to limit the sampling area for each sub-sample. As a complement to suction samples, sweep net samples were collected in both 2005 and 2015 from the same points as the suction sample points used in 2015. Sweep net sampling was done during the same dates as suction sampling in 2015, but during a longer sampling period in 2005, (into mid-July instead of June) since more samples were collected that year. To be able to make a fair comparison and controlling for sample date, the same sample points were used for sweep nets in both years (see data analysis). Sweep net sample collection was done on a transect of 20 m along

the hillside (perpendicular to the uphill direction), with the middle of the transect 2 m above each sampling point to avoid interference with the already sampled area from suction sampling. The vegetation just above the ground was sampled vigorously from left to right while walking along the transect using sweep nets that were approximately 33 cm in diameter. Neither suction nor sweep net sampling was done in wet or very windy conditions. All samples were stored at -20°C in individual zip-lock bags.

2.3.3 Sample processing

Samples from suction sampling were identified to the following groups: Aranea, Hemiptera, Coleoptera, Lepidoptera, Diptera: Tipuloidea and Diptera: Brachycera. All five sub-samples were pooled together to form one sample when counting the number of specimens from each taxonomic group. From sweep net samples, Lepidopteran larvae (caterpillars) and Tipuloidea were counted as these groups were less well sampled by suction sampling. To better estimate the difference in prey abundance overall, total mass of all arthropod groups counted from suction samples were estimated from each pooled sample. In order to do this, specimens from each arthropod group from 10 randomly selected samples collected by suction sampling from a previous year (2004) from the same study site were weighed. The average mass was calculated by taking the wet weight of each specimen after drying off excessive ethanol.

2.3.4 Statistical analysis

The effects of grazing treatment between the two sampling years on the abundance of arthropods in groups sampled by suction sampling, total mass of all arthropods in these groups from suction samples and arthropod groups counted from sweep nets, were all tested using Generalized Linear Mixed effects Models (GLMMs). The fixed effects of all models were “grazing treatment”, which states the four different grazing treatments as a factor, and “sampling year”, which was 2005 or 2015, also as a factor. Both the interaction and separate effects of grazing treatment and sampling year were tested on the arthropod groups’ abundance and mass. A significant effect of the interaction of grazing treatment and sampling year would indicate that the treatment effects have changed markedly and a separate effect of treatment across both years would suggest that treatment effects already in place at the early sampling year persisted through the experiment period until the later sampling year. The models for response variables collected by sweep nets also included

"Date" as a fixed effect, which was the Julian Date of sample collection. All models included the random effects of "Block" (one of three experiment areas), "Replicate" within Block (i.e. lower or higher part of the Block on the hillside) and "Plot" (the enclosure units). Means of counts/mass per sample from each plot were used for suction sample-variables and counts per sample were used for sweep net samples in order to include the sampling date as a covariate for sweep net samples. Models for suction sampled Araneae, Hemiptera, Coleoptera and total mass were created using a normal distribution, although numbers of Araneae and Hemiptera were log-transformed prior to analysis. Tipuloidea and Lepidopteran larvae from sweep net samples were modelled using a negative binomial distribution. As not only the overall difference in treatment effects were of interest here, but also the difference between treatments for each year and how each year contributed to an overall treatment effect across both years, pairwise comparisons were always produced regardless of a significant interaction of grazing treatment and sampling year. Pairwise comparisons of treatment effects were done by comparing means drawn from selected models, and p-values of the comparisons were adjusted with the Holm-Bonferroni method (Holm 1979). Models were validated by checking residuals for normality and non-skewness. All models and graphs were conducted in R version 3.3.2. (R Core Team 2016). GLMMs were carried out using package "lme4" (Bates *et al.* 2015), pairwise comparisons based on model means were carried out using package "lsmeans" (Lenth 2016) and "multcomp" (Hothorn *et al.* 2008) and graphs were drawn using the packages "yarrr" (Phillips 2017) and "ggplot2" (Wickham 2009).

2.4 Results

In total, across all treatments and both years, 31,597 arthropods were collected by suction sampling and 2,945 arthropods by sweep netting of the groups included in this study (Table 2.1, Table 2.2).

a)

Suction sampling:

Year	Araneae	Hemiptera	Coleoptera	Diptera: Brachycera	Diptera: Tipuloidea	Lepidoptera	Total
2005	2692	7817	592	153	2217	6	13477
2015	4063	12476	1148	319	58	56	18120

b)

Sweep nets:

Year	Lepidoptera (Caterpillars)	Diptera: Tipuloidea	Total
2005	2435	325	2760
2015	120	65	185

Table 2.1 Sums of sampled arthropods by group from a) suction sampling and b) sweep nets for each sampling year.

Year	Treatment	Suction sampling	Sweep nets
2005	**I**	6	28
	II	6	31
	III	6	30
	IV	6	24
2015	**I**	6	28
	II	6	31
	III	6	30
	IV	6	25

Table 2.2 Sample size used in statistical analysis for arthropods sampled by suction sampling and sweep nets. *Each suction "sample" used in statistical analyses was an average of the abundance of all samples within each plot, based on 5 samples per plot.

2.4.1 Early and late effects of grazing treatment on arthropod abundance - suction samples

Araneae, Hemiptera, and Coleoptera were collected in sufficient numbers by suction sampling to statistically test their abundance between treatments. Araneae, Hemiptera or Coleoptera were not significantly affected by the interaction of grazing treatment and sampling year ($p > 0.05$), which was the main output of interest to this study. This suggests that there were no significant overall change in treatment effects between the two sampling years. However, pairwise comparisons of treatment effects separately for the two years showed that Araneae were only significantly more abundant in Treatment IV, ungrazed (mean specimens per sample ± SD = 33.1 ± 9.4), than I, intensive sheep grazing (15.3 ± 6.2), in 2005 while more abundant in both Treatment II, extensive sheep grazing (43.3 ± 19), and IV (39.2 ± 11.1) compared to Treatment I (21.1 ± 16.3) in 2015 (Figure 2.2). Hemiptera numbers were not significantly different between any pairs of treatments compared in 2005 but were more abundant in Treatments II (142.3 ± 66.4), III, extensive cattle and sheep grazing (90.4 ± 30.3), and IV (128.5 ± 49.7) compared to I (54.7 ± 52.1) in 2015 (Table 2.4). There were no significant treatment pairwise comparisons for the abundance of Coleoptera, either in 2005 or in 2015 (Figure 2.2).

There was an effect across both years of grazing treatment as a single factor on the abundance of Araneae and Hemiptera but not Coleoptera (Araneae: $\chi^2=20.39$, $p=0.0001$; Hemiptera: $\chi^2=14.55$, $p=0.002$; Coleoptera: $\chi^2=3.53$, $p>0.05$) (Table 2.3). A pairwise comparison showed that numbers of both Araneae and Hemiptera were, overall, more abundant in Treatments II (Araneae: 32.8 ± 17.2, Hemiptera: 100.3 ± 62.3) and IV (Araneae: 36.2 ± 10.3, Hemiptera: 112.9 ± 54.7) compared to Treatment I (Araneae: 18.5 ± 12.2, Hemiptera: 53.1 ± 40.9) (Figure 2.2, Table 2.4). Other pairwise comparisons were not significant. Araneae, Hemiptera and Coleoptera were also significantly more abundant in 2015 than 2005 across all treatments (Table 2.1 and 2.3).

The estimated total mass of all groups counted from suction sampling was not significantly affected by the interaction of grazing treatment and sampling year ($p>0.05$), but pairwise comparisons of the treatment effects for each sampling year showed no significant differences between treatments in 2005, but higher total mass per sample in Treatment II

	χ^2	p-value
Suction sampling: Araneae		
Treatment x Year	2.43	0.487
Treatment	**20.39**	**0.0001**
Year	**8.43**	**0.004**
Suctions sampling: Hemiptera		
Treatment x Year	6.53	0.089
Treatment	**14.55**	**0.002**
Year	**6.39**	**0.012**
Suction sampling: Coleoptera		
Treatment x Year	1.66	0.646
Treatment	3.53	0.317
Year	**16.75**	**4.264e-05**
Suction sampling: Total mass		
Treatment x Year	5.68	0.129
Treatment	**9.07**	**0.028**
Year	**18.77**	**0.00001**
Sweep nets: Lepidoptera (caterpillars)		
Treatment x Year	1.95	0.583
Treatment	**12.67**	**0.005**
Year	0.19	0.659
Date	**11.80**	**0.0006**
Sweep nets: Diptera, Tipuloidea		
Treatment x Year	4.44	0.217
Treatment	1.33	0.723
Year	2.63	0.105
Date	**5.08**	**0.024**

Table 2.3 Outputs of GLMMs of treatment effects on the abundance of arthropods important in bird diets sampled by suction sampling or sweep nets, and estimated total mass of all groups counted from suction samples (Araneae, Hemiptera, Coleoptera, Diptera: Brachycera, Diptera: Tipuloidea and Lepidoptera). Tables are showing χ2 values and p values of all fixed effects in each model. Significant effects are indicated in bold. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Year is a factor testing the effect of sampling year (2005 or 2015).

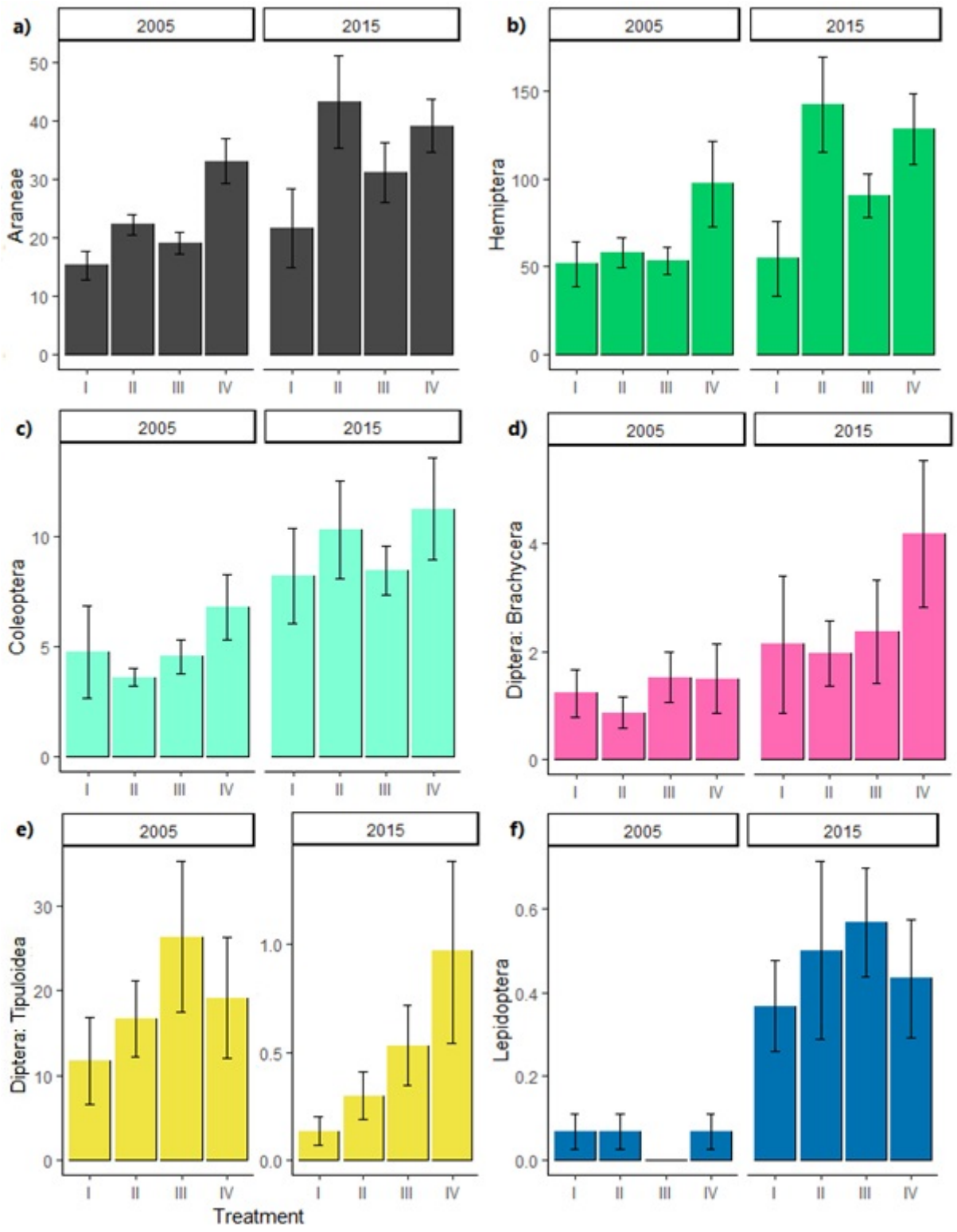


Figure 2.2 Abundance of a) Araneae, b) Hemiptera, c) Coleoptera, d) Diptera:Brachycera, e) Diptera:Tipuloidea and f) Lepidoptera collected by suction sampling. Note that all graphs are on different scales, and graph e, for Diptera:Tipuloidea, show different scales between the two years due to very low abundances in 2015. Bars indicate sample means and error bars indicate standard errors for each treatment and year. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

(262.5 ± 111.7 mg) and IV (245.7 ± 103.4 mg) compared to Treatment I (135.5 ± 95.1 mg) in 2015. Total mass was also affected by the effect of grazing treatment alone (i.e. across both years) (χ^2=9.07, p=0.028). A pairwise comparison of grazing treatments on total arthropod mass showed that there was a significant difference between Treatment IV (203.4 ± 95.1 mg) and I (114.8 ± 80.3 mg) (Figure 2.3, Table 2.4). There was also a significant effect of sampling year (Table 2.3) with higher total mass in 2015 (211.6 ± 100.3) than 2005 (114.0 ± 55.2).

The groups Diptera: Brachycera, Lepidoptera and Diptera: Tipuloidea from suction sampling did not fit any of the standard distributions and did not converge well, hence they did not result in models with reliable results and where therefore not analysed further. The poor fit was likely due to few sampled specimens in general and differences in abundance and variation in data between the two sampling years (Table 2.1, Figure 2.2).

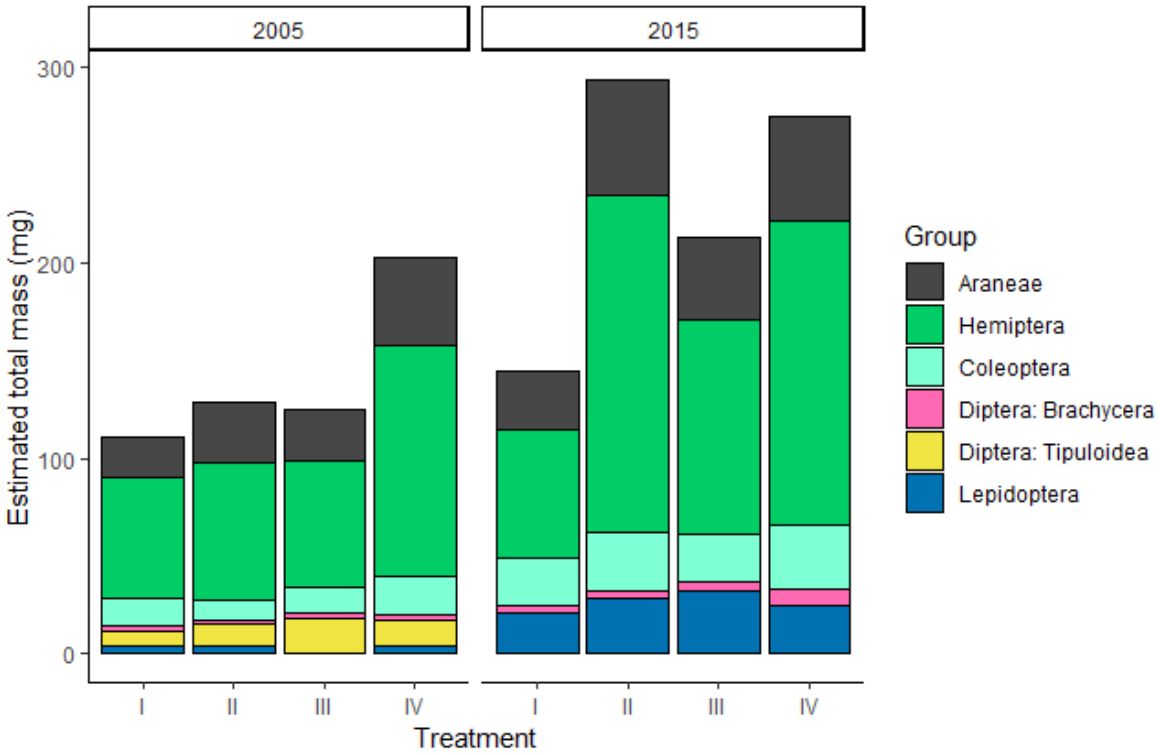


Figure 2.3 Estimated total mass per sample of all arthropod groups counted from suction sampling (Araneae, Hemiptera, Coleoptera, Diptera: Brachycera, Diptera: Tipuloidea and Lepidoptera). Stacked bars indicate the estimated total weight of all groups and each coloured part represents the weight contribution of each arthropod group per treatment and year. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

2.4.2 Early and late effects of grazing treatment on arthropod abundance - Sweep net samples

Lepidoptera larvae sampled by sweep nets were not significantly affected by the interaction of grazing treatment and sampling year, but pairwise comparisons showed that their abundance was significantly lower in Treatment I (mean specimens per sample ± SD = 0.54 ± 0.48) and II (1.97 ± 8.95) compared to Treatment IV (12.58 ± 32.53) in 2005, while no significant differences were found in 2015. There was also a significant effect of grazing treatment across both years on the abundance of Lepidoptera larvae (χ^2=12.67, p=0.005) with abundance being higher in Treatment IV (6.39 ± 23.34), compared to Treatments I (0.36 ± 1.14), II (1.06 ± 6.34) and III (1.87 ± 8.23) (Table 2.3 and 2.4, Figure 2.4). Tipuloidea (Diptera) abundance was not affected by the interaction of grazing treatment and sampling year overall or when looking at pairwise comparisons, or by grazing treatment as a separate effect (p>0.05). Both abundance of Lepidoptera larvae and Tipuloidea increased with sampling date across both sampling years (Lepidoptera: χ^2=12.67, p=0.005; Tipuloidea: χ^2=12.67, p=0.005) (Table 2.3).

2.4.3 Differences in treatment responses between arthropod groups

In the later sampling year, Araneae and Hemiptera were both significantly affected by grazing treatment while Coleoptera was not (Table 2.3), although Araneae and Hemiptera showed similar responses with higher abundances in Treatment II and IV and lowest in Treatment I in the later year. Remaining groups, including Lepidoptera, were sampled in too low numbers in one or both years by both sampling methods to make a fair comparison to the more well-sampled groups (see Figure 2.2 and 2.3).

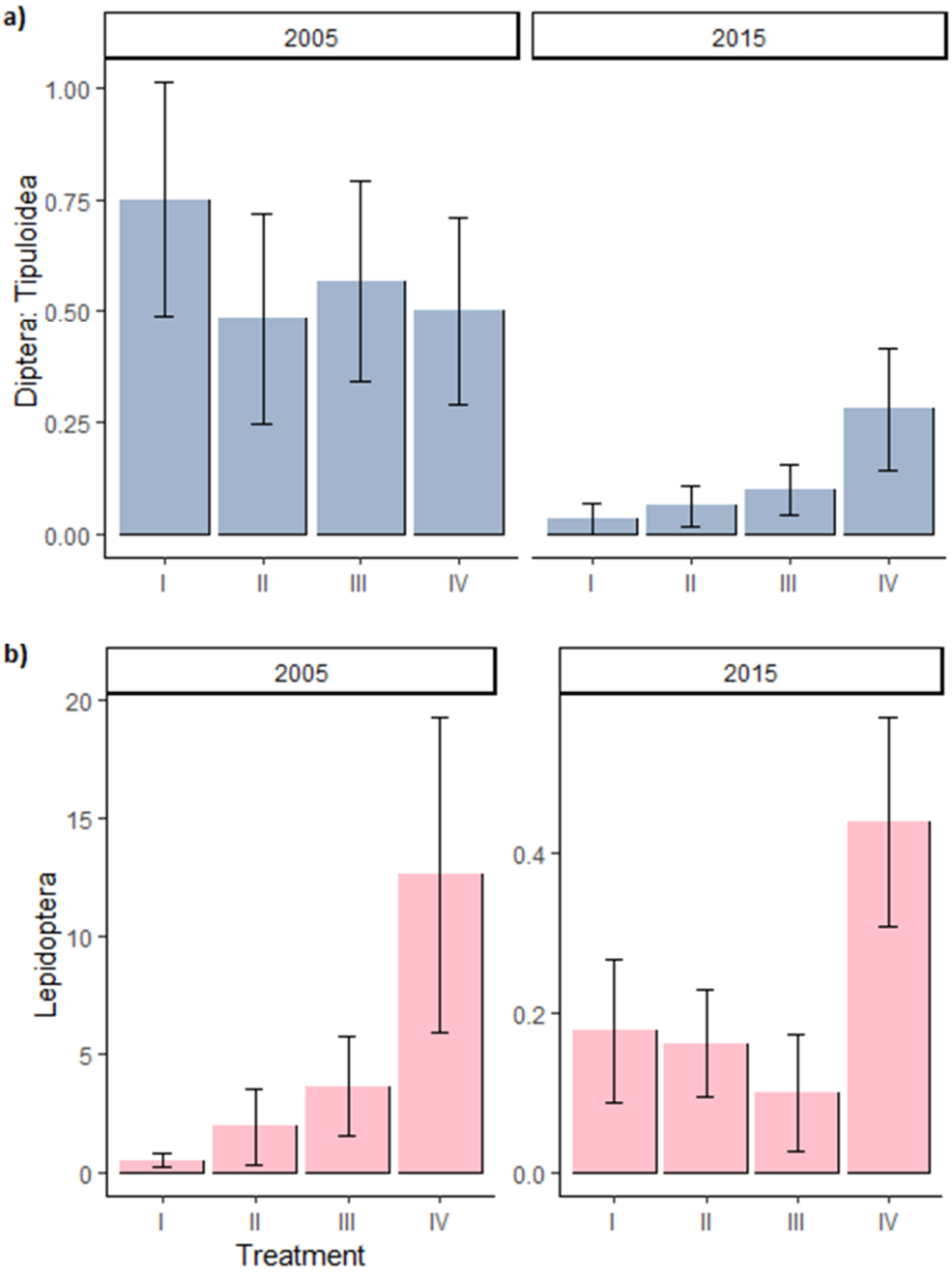


Figure 2.4 Mean numbers of a) Diptera: Tipuloidea and b) Lepidoptera larvae per sample collected by sweep nets. Note that the graph for Lepidoptera is on two different scales due to large differences in abundances between the two sampling years. Bars indicate means and error bars indicate standard errors. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

		2005		2015		Both years	
Suction sampling:		**Est.**	**p**	**Est.**	**p**	**Est.**	**p**
Araneae^{log}	**I-II**	-0.43	0.329	**-0.84**	**0.009**	**-0.63**	**0.001**
	I-III	-0.26	0.713	-0.51	0.182	-0.39	0.084
	I-IV	**-0.79**	**0.015**	**-0.78**	**0.017**	**-0.79**	**<0.001**
	II-III	0.16	0.913	0.33	0.557	0.24	0.315
	II-IV	-0.37	0.457	0.06	0.996	-0.16	0.370
	III-IV	-0.53	0.160	-0.27	0.700	-0.40	0.084
Hemiptera^{log}	**I-II**	-0.25	0.847	**-1.28**	**0.001**	**-0.76**	**0.004**
	I-III	-0.16	0.951	**-0.86**	**0.041**	-0.51	0.092
	I-IV	-0.64	0.187	**-1.18**	**0.003**	**-0.91**	**<0.001**
	II-III	0.09	0.992	0.42	0.543	0.25	0.530
	II-IV	-0.38	0.603	0.09	0.990	-0.15	0.530
	III-IV	-0.47	0.432	-0.32	0.729	-0.40	0.236
Total weight	**I-II**	-7.56	0.997	**-127.03**	**0.011**	-67.29	0.130
	I-III	-4.77	0.999	-67.18	0.309	-35.97	0.703
	I-IV	-67.01	0.311	**-110.18**	**0.032**	**-88.59**	**0.020**
	II-III	2.79	0.999	59.85	0.409	31.32	0.703
	II-IV	-59.44	0.415	16.85	0.971	-21.30	0.703
	III-IV	-62.24	0.375	-43.00	0.675	-52.62	0.327
Sweep nets:							
Lepidoptera	**I-II**	-0.65	0.771	-0.16	0.998	-0.44	0.846
	I-III	-1.14	0.300	0.36	0.983	-0.67	0.643
	I-IV	**-2.35**	**0.003**	-1.28	0.414	**-1.93**	**0.001**
	II-III	-0.49	0.826	0.52	0.948	-0.23	0.846
	II-IV	**-1.70**	**0.019**	-1.13	0.488	**-1.49**	**0.009**
	III-IV	-1.21	0.119	-1.65	0.251	**-1.27**	**0.019**

Table 2.4 Pairwise comparisons of all grazing treatments (I-IV) for all arthropod groups were significant differences between treatments were found. Pairwise comparisons are made for the two sampling years (2005 and 2015) separately and for both years combined. Model estimates show the direction and the strength of the effect in relation to other pairwise comparisons in the same model. "p" indicates p-values and significant pairwise comparisons are marked in bold. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

2.5 Discussion

These results suggest that Araneae and Hemiptera, the two most common groups sampled here, show differential responses to extensive sheep grazing compared to other treatments in the first few years after treatments were initiated and after twelve years with the same grazing pressure. This was seen as significantly different pairwise comparisons, although the overall treatment effect for all treatments was not significantly different between the two sampling years. While a higher abundance of Araneae and Hemiptera in ungrazed plots compared to intensively grazed plots was apparent already in the early stage, it was only in the later stage that these groups were as abundant in extensively sheep grazed plots as in ungrazed plots. These patterns may be confirmed with further years of sampling in the later stage to avoid effects of annual fluctuations, particularly as different total numbers of each group were collected between the two sampling years. However, these results suggest that long-term effects can be different from those observed in the first few years after management change, and that differences between intensive and extensive grazing may take longer to become apparent than differences between grazed and ungrazed areas.

2.5.1 Early and late effects of grazing treatment on arthropod abundance

The first hypothesis suggested that the treatment effects observed in the early stage of the grazing experiment (2005), with higher abundances and total mass in ungrazed plots, intermediate abundances in extensively grazed plots and lowest in intensively grazed plots, would be reflected in the later stage of the experiment (2015), although with stronger treatment effects in the later year. The groups sampled in sufficient numbers to test this statistically (Araneae, Hemiptera and Coleoptera from suction sampling and Lepidoptera larvae and Tipuloidea from sweep net sampling) showed higher abundances in Treatment IV, ungrazed, compared to Treatment I, intensive sheep grazing, across both years, although this was only statistically significant for Araneae, Hemiptera, and Lepidoptera larvae. However, Araneae and Hemiptera also had abundances that were similarly high in Treatment II as Treatment IV when both sampling years were tested together, which was not expected. Moreover, the pairwise comparisons for each year suggest that Araneae was significantly more abundant, about twice the amount, in Treatment II and IV compared to Treatment I in 2015 (on average 21 specimens per sample in Treatment I, 43 in Treatment II and 39 in

Treatment IV), while a significantly higher abundance was only found in Treatment IV compared to I in 2005, where the abundance in Treatment II was in between that in Treatment I and IV (average sampled specimens per sample: I =15, II = 22 and IV = 33). Hemiptera showed higher abundances in Treatment II, III and IV compared to I in 2015, while no significant differences were seen in 2005 (Figures 2.1a and b). The total arthropod mass, likely to be influenced by the most common groups, showed similar results to the abundance of Araneae and Hemiptera, where the total mass was significantly higher in Treatment II compared to Treatment I in 2015 but not in 2005 (Figure 2.3). The hypothesis that arthropod abundance in 2015 would still decrease with increasing grazing pressure, was therefore not confirmed due to the increased abundance in Treatment II, although abundance for several arthropod groups, including total mass, remained significantly higher in Treatment IV than I. The negative effect of the highest grazing pressure (i.e. commercial stocking density) on arthropod abundance aligns with results from other grazing studies. For examples, Mysterud *et al.* (2010) showed that a higher grazing pressure, compared to low or no grazing, in an upland grazing study in Norway resulted in lower abundances of some common Coleoptera and Araneae species even within the first couple of years of the experiment. In the same study site Mysterud *et al.* (2005) found no effect on the abundance of Diptera and Hemiptera, although, the study took place in the first year after the experiment commenced only, which may make it harder to detect effects caused by vegetation change through grazing. For example, Barham and Stewart (2005) did find higher abundances of Auchenorrhyncha (Hemiptera) in ungrazed plots than grazed when sampled after seven years of continuous exclusion of livestock only or both livestock and rabbits.

A clear increase in the magnitude of the effects was expected to be seen as a significant interaction of treatment and sampling year on abundance or total mass, which was not found for any arthropod group or total mass. It is possible that the effective sample size used in statistical analyses as means per plot (N = 48, Table 2.2) and the observed difference was not large enough to detect an overall interaction effect (Heo & Leon 2010). However, pairwise comparisons of treatment effects shed light on how grazing treatments in each year were driving the overall observed treatment differences (i.e. if both years would show the same effect when tested separately, after compensating for multiple comparisons). Although the treatment effects were seemingly different in the two years, they did not appear to be stronger overall in one year than another (Table 2.3, Figure 2.2-4). Abundance of

Lepidopteran larvae showed, surprisingly, stronger effects of grazing in the first sampling year, which may be due to annual variation or a weaker effect after a more extended time period, although low counts of Lepidopterans in the later sampling year probably makes the detection of a treatment effect difficult (Table 2.1b, Figure 2.4b). In a grazing study in Dutch salt marshes, Andresen *et al.* (1990) showed that responses of common spider species to different grazing pressures were stronger in the first three years of the grazing experiment than after eight years, although this may have been due to the disappearance of some species. Long-term studies of a wide range of arthropods with high taxonomic resolution can therefore be useful in providing an understanding of whether some taxa are being replaced by other taxa from the same orders or trophic levels, or if abundances of arthropods, and hence available food to insectivores, are declining.

In this study site, arthropod abundance was also measured by Dennis *et al.* (2008) during the first and second years of the grazing experiment (2003 and 2004) (not statistically compared to these results due to slightly different sampling with longer sampling periods). The abundance in the first year was not yet affected by grazing treatment but the second year showed an effect of the treatments, where abundance of Araneae, Hemiptera and Coleoptera and total mass were higher in Treatment IV, followed by Treatment III. Moreover, Littlewood *et al.* (2012) studied the abundance of Auchenorrhyncha (Hemiptera) in the same grazing experiment five years after the experiment commenced by suction and sweep net samples. Both sampling methods showed that abundances were higher in Treatment IV. The sampling carried out after 12 years of continuous grazing is therefore the first time that abundance of Araneae and Hemiptera and total mass were shown to be as high in Treatment II as Treatment IV, and the difference between Treatment I and II seems to have increased over time. The Glen Finglas estate was grazed before the experiment commenced, approximately at the stocking density of the extensively grazed treatments (II and III), which makes it surprising that the abundance of Araneae and Hemiptera would increase in Treatment II. It is possible that, when sheep grazed over a larger area, these specific plots were more or less intensively grazed which created a less suitable habitat for these groups. These results agree with Jerrentrup *et al.* (2014) who showed that a low grazing pressure led to increased abundances of Orthoptera over a period of nine years, compared to abundances in plots with a higher grazing pressure. Kruess and Tscharntke (2002) showed that abundance of several arthropod groups, Auchenorrhyncha (Hemiptera),

Heteroptera (Hemiptera), Coleoptera and parasitic Hymenoptera, were higher in ungrazed plots than intensively or extensively grazed plots by cattle, and the effect of no grazing increased with time since grazing had ceased. However, although grazed plots had a history of continuous management for at least four years, there was no comparison of short- and long-term effects of intensive or extensive grazing. Long-term, replicated grazing experiments are rare, presumably due to logistic and financial constraints, and long-term studies covering several arthropod groups are even rarer. These results, and long-term perspectives in general, are therefore important to consider before taking actions on management change based on studies presenting short-term (0-5 years) results.

2.5.2 Differences in treatment responses between arthropod groups

The second hypothesis predicted that the advantage of no grazing, which is likely to lead to higher and denser vegetation, would be particularly advantageous to groups connected to a more complex vegetation structure such as Araneae (Gibson *et al.* 1992). The vegetation biomass has been shown to be higher in Treatment IV, ungrazed, compared to the other treatments at the same study site (Evans *et al.* 2015). However, the results presented here did not suggest that ungrazed plots were particularly advantageous for Araneae compared to Hemiptera or Lepidoptera larvae, but Araneae were more affected by grazing than were Coleoptera. It is possible that abundance of Araneae is also linked to the abundance of Hemiptera and other prey such as Collembola (Nentwig 1983, 1987; Harwood *et al.* 2003). Some species of Hemiptera may be important prey of Araneae at the time of sampling as Hemiptera was the most abundant group counted here using suction sampling (Table 2.1), although it is also possible that Araneae and Hemiptera simply require similar vegetation types, or their similar abundance is due to a combination of these two factors. The lack of treatment effect in Coleoptera (Figure 2.2c) may be due to the differences between species requirements within Coleoptera, where herbivorous species may respond differently to grazing compared to carnivorous species and differences are therefore not as strong for the order as a whole, even though there may be differences in species composition between treatments. For example, Mysterud *et al.* (2010) showed that herbivorous Coleoptera species were negatively affected by grazing while carnivorous species were unaffected by grazing.

Nutrients made available through grazing can improve nutrient availability for arthropods requiring fresh leaf tissue or sap through earlier green-up (i.e. earlier appearance of new vegetation) compared to ungrazed plots (Schuman *et al.* 2001). The lower vegetation biomass previously observed in the grazed treatments, including the extensively sheep grazed treatment (II) compared to Treatment IV, ungrazed (Evans *et al.* 2015), may therefore compensate herbivorous arthropods by providing more new plant tissue. Thus, this would result in the same abundance of Hemiptera in extensively grazed as ungrazed plots, driven by the phytophagous species that benefit from fresh leaf tissue early in the season. In a long term grazing experiment in the High Plains Grasslands, United States of America, Schuman *et al.* (1999) found that root uptake of C and N were equally high in enclosures with low and high grazing pressure by cattle (bullocks) and lower in ungrazed enclosures, but the amount of live plant biomass was higher and more similar to ungrazed plots in the low intensity grazed plots, and lower in the intensively grazed plots. This may explain the high abundance of Hemiptera (as the majority of species are phytophagous) in Treatment II and III, but would need to be confirmed by examining plant physiological traits and their direct effect on abundance of sap- and tissue-feeding arthropods.

2.5.3 Implications for avian insectivores' food availability

The sampling here focused on arthropod groups that are important in bird diets according to Buchanan *et al.* (2006), and gives an indication of prey abundance potentially available to insectivorous, ground foraging birds. However, it is important to combine these results with studies of actual prey availability through, for example, feeding observations (Evans *et al.* 2005; Douglas *et al.* 2008) or morphological (Beintema *et al.* 1991; Moreby & Stoate 2000; Pearce-Higgins & Yalden 2004; Michalski *et al.* 2011; Klink *et al.* 2014) and molecular tools (e.g. Deagle *et al.* 2007; Jedlicka *et al.* 2013) to identify arthropod prey in faecal samples or pellets, to get a better picture of how food abundance in combination with vegetation structure affects food availability. Several of the groups sampled here could not be modelled statistically, likely due to low numbers and high variability in the data (Figures 2.1-3). Other sampling techniques, or sampling later in the season, may provide a more complete picture of abundance, depending on the groups of interest.

2.5.4 Conclusions

These results suggest that the intensity, type and duration of grazing may significantly affect the abundance of arthropods important in many insectivorous birds' diets, although both short- and long-term effects suggest a positive effect of no grazing compared to intensive grazing in Araneae. The results also suggest that the most abundant arthropod groups sampled and total arthropod mass are equally high in extensively grazed plots with sheep only and ungrazed plots (and both significantly higher than in Treatment I) in the later stage of the experiment, which was not the case in the earlier sampling year. The overall differences in treatment effects between the two sampling years (i.e. the interaction of Treatment and Year) were, however, not statistically significant and the positive effects of no grazing were already apparing in the early stage for Araneae. The duration for which a certain grazing management has been carried out may be important to consider when studying effects of grazing intensity on nutrient flow and food availability, in which grazing experiments provide invaluable platforms for future controlled experiments.

2.6 References

Allombert, S., Stockton, S. & Martin, J.-L. (2005). A Natural Experiment on the Impact of Overabundant Deer on Forest Invertebrates. *Conserv. Biol.*, 19, 1917–1929.

Andresen, H., Bakker, J.P., Brongers, M., Heydemann, B. & Irmler, U. (1990). Long-term changes of salt marsh communities by cattle grazing. *Vegetatio*, 89, 137–148.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I. & Fuller, R. (2013). *Bird Atlas 2007-11: The Breeding and Wintering Birds of Britain and Ireland*. BTO Books, Thetford.

Barham, D.F. & Stewart, A.J.A. (2005). Differential Indirect Effects of Excluding Livestock and Rabbits from Chalk Heath on the Associated Leafhopper (Hemiptera: Auchenorrhyncha) Fauna. *J. Insect Conserv.*, 9, 351–361.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). *Fitting Linear Mixed-Effects Models Using lme4*. Journal of Statistical Software.

Beintema, A.J., Thissen, J.B., Tensen, D. & Visser, G.H. (1991). Feeding ecology of charadriiform chicks in agricultural grassland, 79, 31–44.

Benton, T.G., Bryant, D.M., Cole, L. & Crick, H.Q.P. (2002). Linking agricultural practice to insect and bird populations: a historical study over three decades. *J. Appl. Ecol.*, 39, 673–687.

Boyer, A.G., Swearingen, R.E., Blaha, M.A., Fortson, C.T., Gremillion, S.K., Osborn, K.A., *et al.*

(2003). Seasonal variation in top-down and bottom-up processes in a grassland arthropod community. *Oecologia*, 136, 309–316.

Brooks, D.R., Bater, J.E., Clark, S.J., Monteith, D.T., Andrews, C., Corbett, S.J., *et al.* (2012). Large carabid beetle declines in a United Kingdom monitoring network increases evidence for a widespread loss in insect biodiversity. *J. Appl. Ecol.*, 49, 1009–1019.

Buchanan, G.M., Grant, M.C., Sanderson, R.A. & Pearce-Higgins, J.W. (2006). The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis*, 148, 615–628.

Burns, F., Eaton, M.A., Gregory, R.D., Al Fulaij, N., August, T.A., Biggs, J., *et al.* (2013). State of Nature report. *The state of nature partnership*.

Butterfield, J. & Coulson, J.C. (1983). The carabid communities on peat and upland grasslands in northern England. *Ecography* , 6, 163–174.

Condliffe, I. (2009). Policy change in the uplands. *Drivers of Environmental Change in Uplands. Routledge, London/New York*, 59–90.

Conrad, K.F., Warren, M.S., Fox, R., Parsons, M.S. & Woiwod, I.P. (2006). Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biol. Conserv.*, 132, 279–291.

Critchlow-Watton, N., Dobbie, K.E., Bell, R., Campbell, S.D.G., Hinze, D., Motion, A., *et al.* (2014). *Scotland's State of the Environment Report*. Scotland's Environment Web.

Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., *et al.* (2007). Studying Seabird Diet through Genetic Analysis of Faeces: A Case Study on Macaroni Penguins (Eudyptes chrysolophus). *PLoS One*, 2, e831.

Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., *et al.* (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.*, 45, 279–287.

Dimitri, C., Effland, A. & Conklin, N. (2005). The 20th Century Transformation of US Agriculture and Farm Policy.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B. & Collen, B. (2014). Defaunation in the Anthropocene. *Science*, 345, 401–406.

Douglas, D.J.T., Evans, D.M. & Redpath, S.M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study*, 55, 290–296.

European Union. (2013). *EU Regulation No 1305. — support for rural development.* Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32013R1305. Last accessed 20 June 2018.

Evans, D.M., Redpath, S.M. & Evans, S.A. (2005). Seasonal patterns in the productivity of Meadow Pipits in the uplands of Scotland. *J. Field Ornithol.*, 76, 245–251.

Evans, D.M., Villar, N., Littlewood, N.A., Pakeman, R.J., Evans, S.A., Dennis, P., *et al.* (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere*, 6, art42.

Ferguson-Lees, J., Castell, R. & Leech, D. (2011). *A Field Guide to Monitoring Nests*. British Trust for Ornithology, Thetford, UK.

Fox, R., Oliver, T.H., Harrower, C., Parsons, M.S., Thomas, C.D. & Roy, D.B. (2014). Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. *J. Appl. Ecol.*, 51, 949–957.

García, R.R., Jáuregui, B.M., García, U., Osoro, K. & Celaya, R. (2009). Effects of livestock breed and grazing pressure on ground-dwelling arthropods in Cantabrian heathlands. *Ecol. Entomol.*, 34, 466–475.

Gardner, S.M. (1991). Ground Beetle (Coleoptera: Carabidae) Communities on Upland Heath and Their Association with Heathland Flora. *J. Biogeogr.*, 18, 281–289.

Gibson, C.W.D., Brown, V.K., Losito, L. & McGavin, G.C. (1992). The Response of Invertebrate Assemblies to Grazing. *Ecography* , 15, 166–176.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., *et al.* (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One*, 12, e0185809.

Hartley, S.E., Gardner, S.M. & Mitchell, R.J. (2003). Indirect Effects of Grazing and Nutrient Addition on the Hemipteran Community of Heather Moorlands. *J. Appl. Ecol.*, 40, 793–803.

Harwood, J.D., Sunderland, K.D. & Symondson, W.O.C. (2003). Web-location by linyphiid spiders: prey-specific aggregation and foraging strategies. *J. Anim. Ecol.*, 72, 745–756.

Hayhow, D.B., Burns, F., Eaton, M.A., Al Fulaij, N., August, T.A., Babey, L., *et al.* (2016). State of Nature 2016. *The state of Nature Partnership*.

Haysom, K.A. & Coulson, J.C. (1998). The Lepidoptera fauna associated with Calluna vulgaris: effects of plant architecture on abundance and diversity. *Ecol. Entomol.*, 23, 377–385.

Heo, M. & Leon, A.C. (2010). Sample sizes required to detect two-way and three-way

interactions involving slope differences in mixed-effects linear models. *J. Biopharm. Stat.*, 20, 787–802.

Holmquist, J.G., Schmidt-Gengenbach, J. & Ballenger, E.A. (2014). Patch-Scale Effects of Equine Disturbance on Arthropod Assemblages and Vegetation Structure in Subalpine Wetlands. *Environ. Manage.*, 53, 1109–1118.

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. *Scand. Stat. Theory Appl.*, 6, 65–70.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.*, 50, 346–363.

Jain, H.K. (2012). Transition to Twenty-First Century Agriculture: Change of Direction. *Agric. Res.*, 1, 12–17.

Jedlicka, J.A., Sharma, A.M. & Almeida, R.P.P. (2013). Molecular tools reveal diets of insectivorous birds from predator fecal matter. *Conserv. Genet. Resour.*, 5, 879–885.

Jerrentrup, J.S., Wrage-Mönnig, N., Röver, K.-U. & Isselstein, J. (2014). Grazing intensity affects insect diversity via sward structure and heterogeneity in a long-term experiment. *J. Appl. Ecol.*, 51, 968–977.

Klink, R. van, Mandema, F.S., Bakker, J.P. & Tinbergen, J.M. (2014). Foraging site choice and diet selection of Meadow Pipits Anthus pratensis breeding on grazed salt marshes. *Bird Study*, 61, 101–110.

Koerner, S.E., Burkepile, D.E., Fynn, R.W.S., Burns, C.E., Eby, S., Govender, N., *et al.* (2014). Plant community response to loss of large herbivores differs between North American and South African savanna grasslands. *Ecology*, 95, 808–816.

Kruess, A. & Tscharntke, T. (2002). Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biol. Conserv.*, 106, 293–302.

Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. *J. Stat. Softw.*, 69, 1–33.

Littlewood, N.A. (2008). Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.*, 1, 151–160.

Littlewood, N.A., Pakeman, R.J. & Pozsgai, G. (2012). Grazing impacts on Auchenorrhyncha diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.*, 5, 67–74.

Michalski, M., Nadolski, J., Marciniak, B., Loga, B. & Bańbura, J. (2011). Faecal Analysis as a Method of Nestling Diet Determination in Insectivorous Birds: A Case Study in Blue Tits Cyanistes caeruleus and Great Tits Parus major. *Acta Ornithol.* , 46, 164–172.

Milchunas, D.G., Sala, O.E. & Lauenroth, W.K. (1988). A Generalized Model of the Effects of

Grazing by Large Herbivores on Grassland Community Structure. *Am. Nat.*, 132, 87–106.

Moran, M.D. (2014). Bison Grazing Increases Arthropod Abundance and Diversity in a Tallgrass Prairie. *Environ. Entomol.*, 43, 1174–1184.

Moreby, S.J. & Stoate, C. (2000). A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study*, 47, 320–331.

Morris, M.G. (2000). The effects of structure and its dynamics on the ecology and conservation of arthropods in British grasslands. *Biol. Conserv.*, 95, 129–142.

Mysterud, A., Aaserud, R., Hansen, L.O., Åkra, K., Olberg, S. & Austrheim, G. (2010). Large herbivore grazing and invertebrates in an alpine ecosystem. *Basic Appl. Ecol.*, 11, 320–328.

Mysterud, A., Ove Hansen, L., Peters, C. & Austrheim, G. (2005). The short-term effect of sheep grazing on selected invertebrates (Diptera and Hemiptera) relative to other environmental factors in an alpine ecosystem. *J. Zool.*, 266, 411–418.

Nentwig, W. (1983). The prey of web-building spiders compared with feeding experiments (Araneae: Araneidae, Linyphiidae, pholcidae, Agelenidae). *Oecologia*, 56, 132–139.

Nentwig, W. (1987). The Prey of Spiders. In: *Ecophysiology of Spiders* (ed. Nentwig, W.). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 249–263.

Newbold, T.A.S., Stapp, P., Levensailor, K.E., Derner, J.D. & Lauenroth, W.K. (2014). Community Responses of Arthropods to a Range of Traditional and Manipulated Grazing in Shortgrass Steppe. *Environ. Entomol.*, 43, 556–568.

Newton, I. (2004). The recent declines of farmland bird populations in Britain: an appraisal of causal factors and conservation actions. *Ibis* , 146, 579–600.

Noordwijk, C.G.E. van, Flierman, D.E., Remke, E., WallisDeVries, M.F. & Berg, M.P. (2012). Impact of grazing management on hibernating caterpillars of the butterfly Melitaea cinxia in calcareous grasslands. *J. Insect Conserv.*, 16, 909–920.

Pearce-Higgins, J.W. & Yalden, D.W. (2004). Habitat selection, diet, arthropod availability and growth of a moorland wader: the ecology of European Golden Plover Pluvialis apricaria chicks: Ecology of Golden Plover chicks. *Ibis*, 146, 335–346.

Peco, B., Carmona, C.P., De Pablos, I. & Azcarate, F.M. (2012). Effects of grazing abandonment on functional and taxonomic diversity of Mediterranean grasslands. *Agric. Ecosyst. Environ.*, 152, 27–32.

Phillips, N. (2017). *yarrr: A Companion to the e-Book "YaRrr!: The Pirate's Guide to R."*

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation

for Statistical Computing, Vienna, Austria.

Robinson, R.A. & Sutherland, W.J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *J. Appl. Ecol.*, 39, 157–176.

Rosa García, R., Ocharan, F.J., García, U., Osoro, K. & Celaya, R. (2010). Arthropod fauna on grassland–heathland associations under different grazing managements with domestic ruminants. *C. R. Biol.*, 333, 226–234.

Schuman, G.E., LeCain, D.R., Reeder, J.D. & Morgan, J.A. (2001). Carbon dynamics and sequestration of a mixed-grass prairie as influenced by grazing. In: *Soil Carbon Sequestration and the Greenhouse Effect*, SSSA SPECIAL PUBLICATIONS (ed. Lal, R.). Presented at the Symposium on Soil Carbon Sequestration and the Greenhouse Effect held at the 90th Annual Meeting of the Soil-Science-Society-of-America, Soil Science Soc Amer, 677 S Segoe Rd, Madison, Wi 53711 USA, pp. 67–75.

Schuman, G.E., Reeder, J.D., Manley, J.T., Hart, R.H. & Manley, W.A. (1999). Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. *Ecol. Appl.*, 9, 65–71.

Scohier, A. & Dumont, B. (2012). How do sheep affect plant communities and arthropod populations in temperate grasslands? *Animal*, 6, 1129–1138.

Silcock, P., Brunyee, J. & Pring, J. (2012). *Changing livestock numbers in the UK LFA – an analysis of likely biodiversity implications*. RSPB.

Suominen, O., Persson, I.-L., Danell, K., Bergström, R. & Pastor, J. (2008). Impact of simulated moose densities on abundance and richness of vegetation, herbivorous and predatory arthropods along a productivity gradient. *Ecography* , 31, 636–645.

Thomas, J.A., Telfer, M.G., Roy, D.B., Preston, C.D., Greenwood, J.J.D., Asher, J., *et al.* (2004). Comparative Losses of British Butterflies, Birds, and Plants and the Global Extinction Crisis. *Science*, 303, 1879–1881.

Tonelli, M., Verdú, J.R. & Zunino, M. (2018). Effects of the progressive abandonment of grazing on dung beetle biodiversity: body size matters. *Biodivers. Conserv.*, 27, 189–204.

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis.

Chapter 3. Livestock grazing impacts upon the breeding productivity of a common upland insectivorous passerine: results from a long-term experiment

3.1 Abstract

The intensity of pastoral management in areas of High Nature Value farming is sensitive to economic and social pressures and, as a result, is declining in some regions of Europe but increasing in others. This affects open habitats of conservation concern, such as the British uplands, where bird species that benefit from low-intensity grazing may be most sensitive to such polarisation. While experimental manipulations of livestock grazing intensities have improved our understanding of upland breeding bird responses in the short-term, none have examined the longer-term impacts of altered management on reproductive success.

Using a replicated landscape-scale experiment that started in 2003, the effects of four grazing treatments (intensive sheep; extensive sheep; extensive mixed sheep and cattle; and no grazing) on the breeding productivity of a common insectivorous passerine: the meadow pipit *Anthus pratensis,* were investigated. Territory mapping and nest monitoring were carried out systematically during early (2003 & 2004) and later (2015 & 2016) sampling periods of the experiment to examine the short and longer-term effects of grazing treatment on abundance and productivity of pipits.

Breeding abundance was lowest in the extensive sheep treatment, where eggs hatched significantly later. Grazing treatment significantly affected egg-stage nest survival, with highest mortality in both the ungrazed and intensive sheep treatments, while no statistically significant treatment effects were detected on overall nest survival or fledgling productivity. There were no significant differences in the treatment effects between the two sampling periods on any breeding variable, but overall nest survival was lower in the later sampling period across all treatments.

Livestock grazing pressure affects several aspects of meadow pipit breeding productivity. However, the lack of interaction between grazing treatment and sampling period on breeding parameters suggests that there are (as yet) no substantial differences in early and late treatment effects on reproductive success after more than a decade of experimental

grazing management. Consistent with recent studies, it was shown that other processes at the wider landscape-scale appear to be important, such as increased rates of nest predation.

3.2 Introduction

One third of the farmland in the European Union (EU) consists of permanent grasslands. However, the proportion of livestock fed through natural grazing is decreasing in the majority of European countries, and in many countries outside Europe (van den Pol-van Dasselaar *et al.* 2015). As a result, more polarised management (i.e. intensification or abandonment) will be applied on traditionally pastoral landscapes, of which many are of High Nature Value (Meiner & Bas 2017).

The British uplands are internationally important for significant proportions of a number of the World's bird species of conservation concern (Pearce-Higgins *et al.* 2009), and several habitat types are designated as Special Areas of Conservation under the European Union's Natura 2000 Habitats Directive: Annex I (EU 1992). Upland areas have historically been grazed for livestock production but predominantly have "Less Favoured Areas" (LFAs) status; areas with a disadvantage, such as poor agricultural profitability. After decades of concerns about unsustainably high levels of grazing (Fuller & Gough 1999), new concerns regarding undergrazing have recently become prevalent (DEFRA 2004), particularly as many non-intensified, High Nature Value grasslands of conservation concern are in areas where grazing management is experiencing the greatest declines in livestock densities (Holland *et al.* 2008).

Balmer *et al.* (2013) found that bird species in upland habitats have shown the strongest range contractions during a 40 year period in Britain and Ireland compared to birds in other habitat types. Some population declines occurred alongside increases in livestock densities since the middle of the 20th century (Fuller & Gough 1999; Newton 2004). However, many species are still declining in abundance, despite lower sheep densities in the British uplands in recent decades (Hayhow *et al.* 2017), particularly those that nest on the ground (Sullivan *et al.* 2015). Given ongoing changes in the intensity of livestock management in marginal upland areas, and large declines in the abundance of many upland bird species, there is an urgent need to test in detail the extent to which grazing pressure is functionally linked to

changes in upland bird populations, relative to other pressures such as changes in climate, land-cover and predator management (Buchanan *et al.* 2017).

Livestock grazing intensity can affect bird populations through a number of mechanisms. Firstly, livestock may have a direct impact upon demographic parameters, for example by trampling or predating nests and chicks (Jarrett *et al.* 2017). Secondly, they may alter vegetation structure by their effect on sward height and density. This can not only alter the suitability of the habitat for different species, thus affecting bird settlement patterns (Loe *et al.* 2007; Bloom *et al.* 2013), but may secondarily alter the abundance and/or availability of their prey (Buchanan *et al.* 2006; Dennis *et al.* 2008; Kőrösi *et al.* 2012; Littlewood *et al.* 2012; Douglas & Pearce-Higgins 2014). Food availability is a result of both food abundance and food access (e.g. through vegetation structure); for example, ground foraging, insectivorous birds have been shown to prefer bare patches between vegetation (Schaub *et al.* 2010) or shorter vegetation with high arthropod abundance and accessibility, rather than simply high arthropod abundance (Pearce-Higgins & Yalden 2003, 2004; Douglas *et al.* 2008; Vandenberghe *et al.* 2009). Thirdly, changes in vegetation structure may also affect the vulnerability of nests to predation (Tapper *et al.* 1996) and predator density may increase as a result of increased habitat suitability, and hence population densities, of other prey such as voles (Evans *et al.* 2015). In the longer-term, changes in grazing pressure, or complete removal of livestock, may alter the composition of vegetation, particularly the ratio of shrubs to sedges and grasses (Fuller & Gough 1999), with further impacts on the abundance of bird species (Pearce-Higgins & Grant 2006).

Experimentally manipulated grazing studies are necessary for identifying the effect of grazing pressure alone, in comparison to confounding effects linked to grazing intensity, such as soil type, topography and climate. Experimentally managed sheep grazing in Norway has shown a higher total abundance of birds with increasing sheep density (Loe *et al.* 2007), while Johnson *et al.* (2012) found that breeding success of two ground nesting passerines did not vary with grazing pressure in a cattle grazing experiment in the United States of America. However, these studies both investigated bird responses in the first few years after experiments had commenced. Land management change can take several decades, or longer, to reach their full effects on plant composition (Hermy & Verheyen 2007) and hence to fully influence surrounding taxa. So far, no experimentally managed grazing experiment

has looked at the long-term effects of grazing management on avian breeding success and population change, largely due to the logistical and financial constraints of maintaining long-term ecological experiments.

Here, a replicated landscape-scale grazing experiment with different levels of grazing intensity and type (i.e. sheep only at two different stocking densities, mixed cattle and sheep grazing and no grazing) was used after 13 years of continuous manipulation to study the impacts of grazing treatment upon meadow pipits *Anthus pratensis*, a common upland passerine. Previous work using this experiment has documented short-term effects upon pipit breeding density (Evans *et al.* 2006, 2015) and egg size (Evans *et al.* 2005b), which were both enhanced under extensive, mixed cattle and sheep grazing. Furthermore, the pipit offspring sex ratio was biased towards more male nestlings in plots with low intensity sheep or mixed livestock grazing (Prior *et al.* 2011), while arthropod abundance and plant biomass increased with decreasing livestock densities (Dennis *et al.* 2008; Evans *et al.* 2015). There was no significant effect of grazing pressure on nest survival during the first years of the experiment (Evans *et al.* 2005b). However, the positive effects of low intensity mixed cattle and sheep grazing on breeding meadow pipits was expected to eventually be reflected in breeding output through high vegetation structure heterogeneity with enhanced prey access (Douglas *et al.* 2008). Here, after more than a decade of continuous grazing, it was predicted that long-term effects of grazing management has significant effects on nest survival and breeding output compared to early results in the experiment. In particular, if meadow pipit nest survival is a function of vegetation structure and arthropod availability, it was hypothesised that the effect of grazing pressure becomes enhanced through time, with lowest productivity in both the intensively grazed and ungrazed treatments. Here, interactions between grazing type and management duration was examined to investigate how long-term grazing pressure affects the following measures of breeding success: i) pipit breeding density, ii) clutch size, iii) Julian hatch date, iv) number of fledglings per nest, v) estimated fledgling output per experiment plot and vi) egg- and nestling-stage nest survival and overall nest survival of meadow pipits.

3.3 Methods

3.3.1 Study area and experimental design

A replicated, randomised block experiment, consisting of six replicates of four treatments was initiated in 2003 at Glen Finglas, in central Scotland, United Kingdom (UK) (56°16'03"N, 4°25'08"W). The study site consists of largely wet and dry acid grassland with smaller areas of dwarf shrubs, bracken and willow scrub. Meadow pipits are the most common breeding birds in the area and other bird species were uncommon in experiment plots when the treatments commenced. Each of the 24 experimental plots measured 3.3 ha and were established over 3 spatial blocks, with random treatment allocation within 6 replicates (Chapter 2, Figure 2.1). The plot size was established to provide an anticipated sample size of meadow pipit pairs large enough for statistical analyses (i.e. ca. 5 pairs/plot). The blocks are situated approximately 5 km apart at an altitude of 200–500 m. The grazing treatments were: I) commercial stocking density of sheep with 9 ewes per plot (2.73 ewes ha^{-1}); II) one-third of the commercial stocking density with 3 ewes per plot; III) 2 ewes per plot and, during four weeks each autumn, 2 cows with suckling calves; and IV) ungrazed. Treatments II and III were set up to both have extensive grazing pressures with the same annual vegetation biomass offtake and to maintain stocking at similar rates to those pre-experiment.

3.3.2 Bird surveys and nest monitoring

Meadow pipits breed in a range of grasslands types and build concealed nests on the ground. Incubation and nestling development each take approximately 13 days before chicks are ready to leave the nest. Following Evans *et al.* (2005a), breeding bird surveys were carried out in 2003–04 and 2015–16 to study immediate and long-term effects of livestock grazing treatments on meadow pipit breeding abundance and success. Breeding territories were assessed by mapping all meadow pipit activity during six surveys of each area in the early part of the breeding season. A territory was defined as an area with several independent observations of which those with displayed breeding behaviour (i.e. singing, alarming or encounters of active nests) were regarded as more indicative of territories. Approximate territories could be confirmed by observing simultaneous singing by two or more meadow pipit males. Each year, territories were assigned by the same method and same person (DME) on maps of accumulated observations from all six surveys. Territories were assigned to the plot/treatment where most or all observations were done, and the plot

surrounding most of the territory was considered to be the main foraging area. Vandenberghe *et al.* (2009) showed in a previous study at the same experiment site that meadow pipits forage at a distance from the nest of 29.3 m ± 2.89 (mean ± 1SE) at intensively, sheep grazed plots and 15.1 m ± 1.85 in extensively, sheep and cattle grazed plots. Assuming a circle shaped foraging area around the nest, each foraging area would then be up to 2642m^2, which makes out 8 % of the surface of a plot measuring 3.3ha, and plots could therefore hold several territories without foraging areas necessarily stretching across several experiment plots. Inevitably, some estimated breeding territories and hence foraging areas would be placed on the border to adjacent experiment plots or the surrounding land, particularly as meadow pipits like to perch and are often observed on fence posts between plots. However, the distribution of breeding territories was fairly even within plots and across each experiment block (Appendix A, Figure S.1), hence, the breeding conditions would be more affected by the plot/treatment in which the nest was found compared to surrounding areas when the treatment effects are averaged across all nests during analyses. Moreover, due to the randomised design of the grazing experiment, any potential effects from adjacent plots or surrounding land would, overall, be even across all treatments and therefore not cause direct confounding effects across all the plots of a whole treatment. Nests were located with standardised sampling effort by searching through plots every 2-5 days, depending on weather conditions, through the whole breeding season. Nests were found by flushing incubating females while walking or rope dragging, and occasionally by observing birds arriving at the nests.

Once found, each nest was checked every 3 days (weather permitting) while active through each stage of the nest period (i.e. egg-laying through to hatching and hatching to fledging, hereafter referred to as the egg- and nestling-stage). Meadow pipits lay one egg per day until a clutch of 2-5 eggs is completed. Partial predation was very unusual, and the clutch size recorded when no additional eggs were found on following visits was considered to be a sufficient representation of clutch size. Partial mortality did occur (in 29% of nests), but unhatched eggs were then found in the nest and dead nestlings were found in or just outside the nest. A nestling was considered as successfully fledged when recorded alive just before fledging, unless it was found dead on the post-fledge visit on day 15-17 after hatching. Most likely, not all nests were found, but the numbers of territories per plot were used as a measure for breeding density. In order to estimate the total output of fledged meadow

pipits per land unit (i.e. experiment plot), the breeding density of each plot was multiplied by the average number of fledglings produced for each plot. The possibility of not finding all nests also made the number of territories the best proxy for breeding density or the number of nests within a plot when estimating the total output of fledged meadow pipits per land unit (i.e. experiment plot). However, the possibility of a second breeding attempt of some pairs may add further to the differences observed within a treatment, hence, this variable is an estimate of expected combined effects of abundance and productivity only, and is, compared to other breeding variables, not a direct measure.

3.3.3 Statistical analyses

All breeding parameters (see below) were analysed using generalised linear mixed effects models (GLMMs). Nests found after hatching were not used in proportional survival analyses for the incubation stage or total numbers of fledglings per nest. Within the models, Treatment (the four grazing treatments) and Sampling Period (early/late) were the primary factors of interest, with the latter indicating either early (2003-04) or late stage (2015-16) effects of the treatments. A significant interaction between Treatment and Sampling Period would indicate that grazing effects have changed between the two Sampling Periods, for example as a result of varying impacts of grazing treatment on vegetation between short- and long-term management. A significant effect of Treatment across both Sampling Periods would suggest that any effect of different grazing pressure was already apparent at the early stage of the experiment. An effect of Sampling Period across all Treatments would indicate that changes occurred between the two sampling periods affecting the whole study area, and therefore were unlikely to be related to grazing intensity. In models where a significant effect of sampling period was found, 2 variables for regional weather during each breeding season were included in the models. These variables were average temperature and average monthly precipitation during the three calendar months of May, June and July (during which the meadow pipits have eggs or nestlings). This was done to test whether weather variables could explain the changes observed across the region, or if the observed differences were more likely to be caused by other regional changes such as increased predation pressure. Weather data for the region (Western Scotland) was available from Met Office, UK (Met Office 2019). All tests for breeding parameters had the same random effects, i.e. Block (one of three experiment areas), Replicate within Block, Plot and Calendar Year. Random effects

were removed sequentially if making the model worse, which was tested with Likelihood Ratio Tests (LRTs).

The GLMM's for Clutch size, Julian hatch date, Number of fledglings, Egg- and Nestling-stage nest survival, and Overall survival all had the same fixed effects; Treatment, Sampling Period, the interaction of Treatment and Sampling Period, Julian Hatch date, Julian Hatch date^2, and number of Territories per plot. The Estimated number of Fledglings/Plot also had the same variables excluding number of territories, as it is directly linked to the estimate of Fledglings/Plot. Julian Hatch date and Julian Hatch date^2 were included in the models (averaged per plot in Fledglings/Plot) to account for a linear and quadratic effect, respectively, of seasonal variation (Perrins 1970), while the number of Territories was included to control for potential density dependence (Coulson *et al.* 1982; Arcese & Smith 1988). Nest ID was included in nest survival models as an observation level random effect (OLRE) to control for over-dispersion (Harrison 2014). Fixed effects in all models were tested with LRTs and removed sequentially if making the model worse in terms of model convergence and AIC score. Details on selected models and probability distributions applied can be found in Appendix A, Table S.1.

Since traditional r2 values are not applicable to GLMM's we calculated marginal and conditional r2's which provide estimates of variance explained by fixed effects only and variance explained by both fixed and mixed effects, respectively (Nakagawa & Schielzeth 2013). All models and graphs were analysed/produced in R version 3.3.2 (R Core Team 2016). GLMMs were done in package "lme4" (Bates *et al.* 2015), post hoc tests for pairwise comparisons were done with package "lsmeans" (Lenth 2016) and p-values of the comparisons were adjusted with the Holm-Bonferroni method (Holm 1979). Graphs were produced using packages "yarrr" (Phillips 2017) and "ggplot2" (Wickham 2009).

3.4 Results

Across the four breeding seasons, 295 nests were found of which 268 were followed from egg or nestling stage until breeding outcome was confirmed (post-fledging) and 240 nests were followed from egg stage until breeding outcome was confirmed (see Table 3.1 for nests monitored per year and treatment).

Year	Treatment	Breeding density	Monitored nests
2003	**I**	6	20
	II	6	11
	III	6	13
	IV	6	16
2004	**I**	6	22
	II	6	20
	III	6	27
	IV	6	19
2015	**I**	6	12
	II	6	11
	III	6	13
	IV	6	14
2016	**I**	6	9
	II	6	11
	III	6	10
	IV	6	12

Table 3.1 Sample size for breeding density and nests monitored from egg laying until fledging for breeding productivity variables. Breeding density was estimated by counting the number of breeding territories per experiment plot, which results in a constant sample size of 6 replicates per treatment and year. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

Parameter		χ^2	p-value	Model Cond. R^2
Breeding Density	Treatment * Sampling period	1.88	0.597	0.414
	Treatment	**20.15**	**< 0.001**	
	Sampling period	1.67	0.196	
Clutch Size	Treatment * Sampling period	0.90	0.825	0.092
	Treatment	0.36	0.940	
	Sampling period	**6.68**	**0.010**	
Hatch Date	Treatment * Sampling period	2.06	0.559	0.150
	Treatment	**13.01**	**0.005**	
	Sampling period	1.51	0.220	
No. of Fledglings	Treatment * Sampling period	5.71	0.127	0.3
	Treatment	2.63	0.452	
	Sampling period	3.62	0.057	
Est. Fledglings /Plot	Treatment * Sampling period	3.89	0.273	0.264
	Treatment	5.87	0.118	
	Sampling period	**4.25**	**0.039**	
Proportional Nest Survival:				
- Eggs to Fledging	Treatment * Sampling period	4.65	0.199	0.853
	Treatment	2.54	0.469	
	Sampling period	**8.99**	**0.003**	
- Egg-stage	Treatment * Sampling period	6.70	0.082	0.194
	Treatment	**10.07**	**0.018**	
	Sampling period	**16.66**	**< 0.001**	
- Nestling-stage	Treatment * Sampling period	0.85	0.838	0.002
	Treatment	0.37	0.946	
	Sampling period	0.13	0.721	

Table 3.2 Results of generalised linear mixed models shown as χ^2 - and p-values and the explained variance by the model as Conditional R^2. The interaction and independent effects of Treatment and Sampling Period were tested in two separate models with the same final model structure. Treatment: factor with 4 grazing treatments: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Sampling period: factor as either early = immediate and one year after the grazing treatments commenced; or late = 12 and 13 years after the grazing treatments commenced with continuous management in each plot. Significant effects are marked in bold.

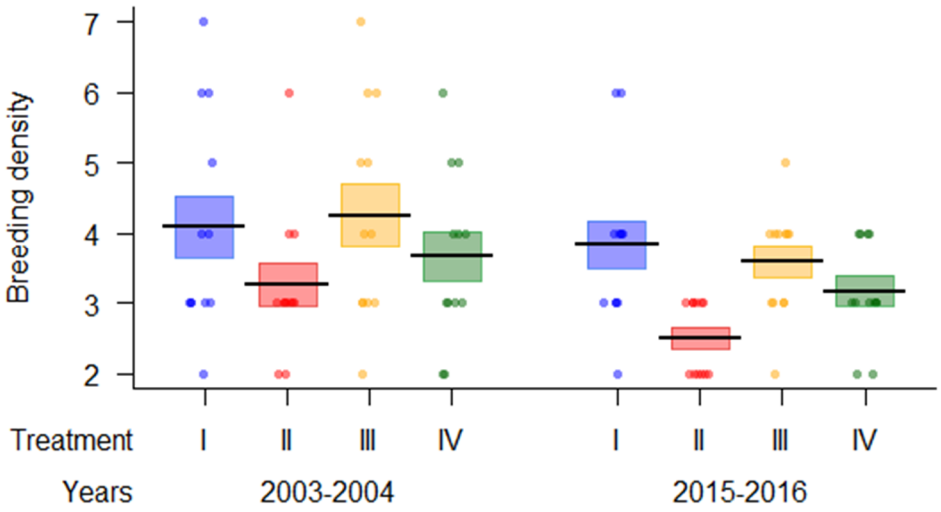


Figure 3.1 Breeding density of meadow pipits at Glen Finglas, Scotland, shown as territories per plot. Each plot measures 3.3 ha. The grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Bars are showing means and standard errors. Raw data points are shown for each treatment and sampling period.

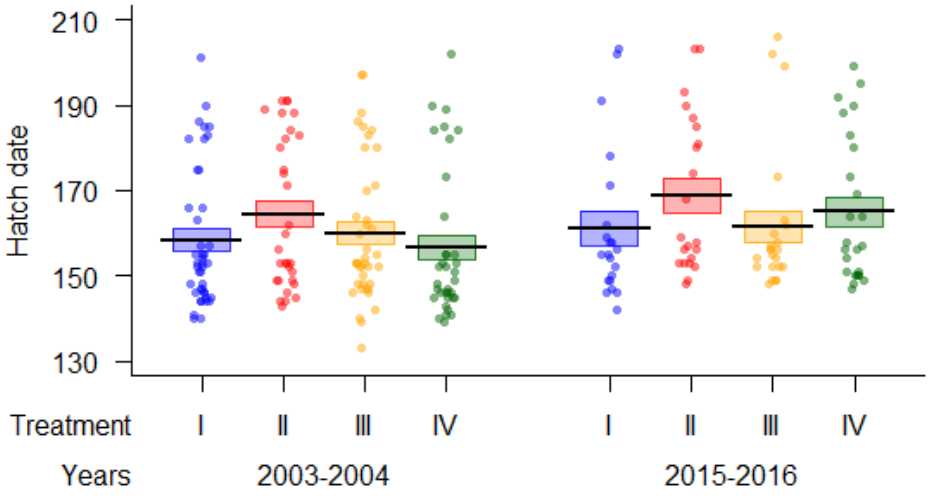


Figure 3.2 Julian hatch date for meadow pipit nests at Glen Finglas, Scotland, under four grazing treatments: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Bars are showing means and standard errors. Raw data points are shown for each treatment and sampling period.

3.4.1 Breeding density

The breeding density of meadow pipits was significantly affected by grazing treatment across all years ($\chi^2 = 20.15$, $p < 0.001$) with lowest numbers of territories per plot in treatment II (mean ± SD = 2.88 ± 0.9) and highest numbers in treatments I (3.96 ± 1.37) and III (3.92 ± 1.25) but there was no interaction between grazing treatment and sampling period (Figure 3.1, Table 3.2).

3.4.2 Clutch size and Hatch date

There was no significant effect of grazing treatment or the interaction of grazing treatment and sampling period on clutch size (Table 3.2), but there were significantly fewer eggs laid per nest in the later sampling period (3.89 ± 0.6) than the early period (4.1 ± 0.62), ($\chi^2 = 6.68$, $p = 0.010$). Hatch date was affected by grazing treatment ($\chi^2 = 13.01$, $p = 0.005$) with later hatching dates in treatment II than the other treatments (Julian date: Treatment I = 159.29 ± 16.72, II = 166.21 ± 17.85, III = 160.49 ± 16.71, IV = 160.07 ± 17.73) although there was no significant change in treatment effects between sampling periods (Figure 3.2, Table 3.2).

3.4.3 Fledgling output and nest survival

The number of fledglings per nest was highest in the extensively grazed treatments (II and III) but there was no statistically significant effects of grazing treatment across all years nor by treatment-sampling period interaction (Figure 3.3, Table 3.2). The estimated total output of nestlings per plot was not significantly affected by the interaction of treatment and sampling period or treatment as a single factor (Figure 3.4, Table 3.2). However, there were significantly fewer fledglings produced per plot in the later sampling period (5.97 ± 5.04) than the early period (9.49 ± 5.06).

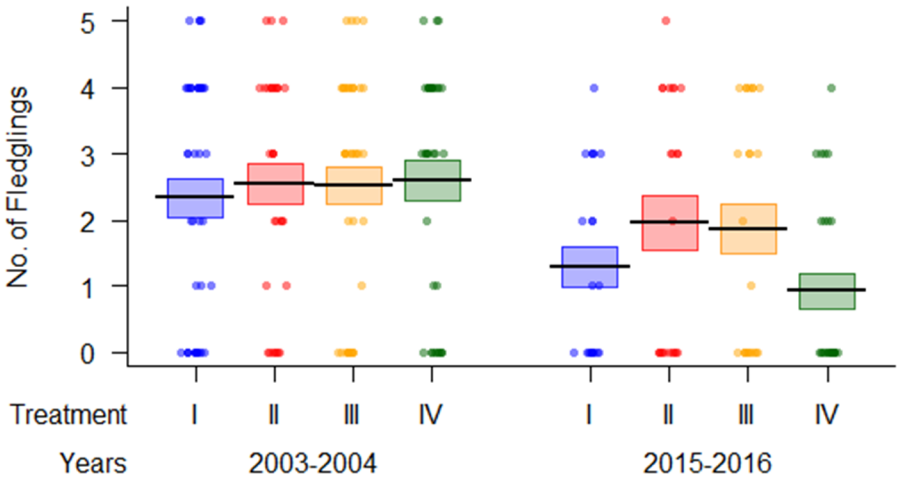


Figure 3.3 Number of meadow pipit fledglings per nest at Glen Finglas, Scotland, under four grazing treatments: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Bars are showing means and standard errors. Raw data points are shown for each treatment and sampling period.

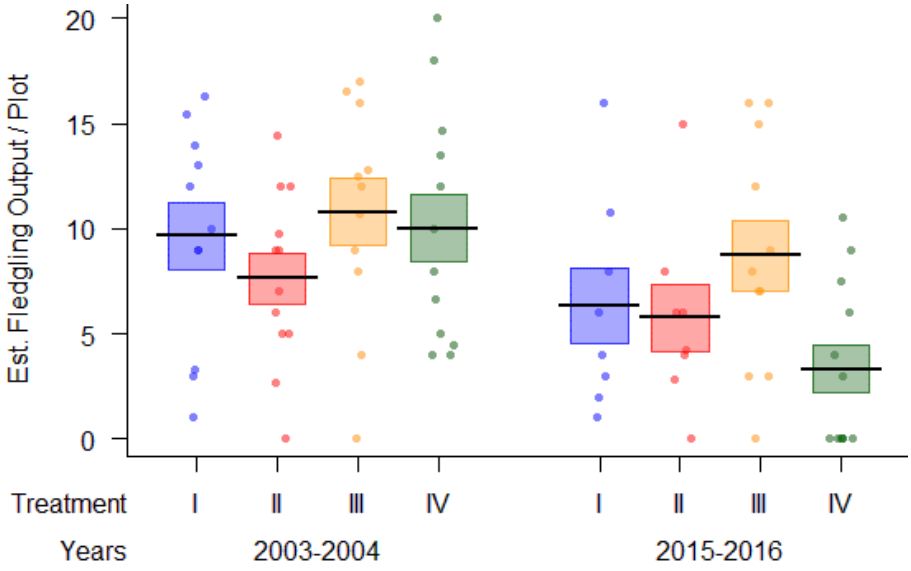


Figure 3.4 Estimated Fledgling output/plot. Breeding density measured as meadow pipit territories per experiment plot multiplied by the average number of fledglings per nest in each plot by treatment and sampling period. Each plot measures 3.3 ha. The grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Bars are showing means and standard errors. Raw data points are shown for each treatment and sampling period.

Nest survival was highest in treatment II and III (Figure 3.5) but there was no significant difference in overall nest survival between grazing treatments or in the interaction of grazing treatment and sampling period. The proportion of eggs surviving until fledging was significantly higher in the early sampling period (early = 0.61 ± 0.42, late = 0.38 ± 0.42, χ^2 = 8.99, p = 0.003), (Figure3.5, Table 3.2). Egg-stage nest survival was significantly affected by grazing treatment (χ^2 = 10.07, p = 0.018) and sampling period (χ^2 = 16.66, p < 0.001) with highest proportional egg-survival in treatment II (I = 0.64 ± 0.43, II = 0.84 ± 0.30, III = 0.71 ± 0.40, IV = 0.72 ± 0.40) and in the early sampling period (early = 0.80 ± 0.34, late = 0.60 ± 0.43); but there was no significant interaction of treatment and sampling period (Figure 3.5, Table 3.2). The nestling-stage nest survival was neither significantly affected by grazing treatment nor the interaction of grazing treatment and sampling period (Figure 3.5, Table 3.2). The significant difference in clutch size, egg-stage nest survival and overall survival still remained after controlling for inter-annual temperature and precipitation differences, but the significant effect of sampling period on estimated total number of fledglings/plot did not remain after including these variables (Appendix A, Table S.2). Out of the nests considered for overall nest survival, 70 nests had partial survival during the incubation or nestling-stage, while 87 nests failed completely and 83 had complete survival. Trampling was very rare (<1 nest per year) and completely failed nests were mainly assumed to be predated as, most often, no eggs or nestlings would be left in the nest.

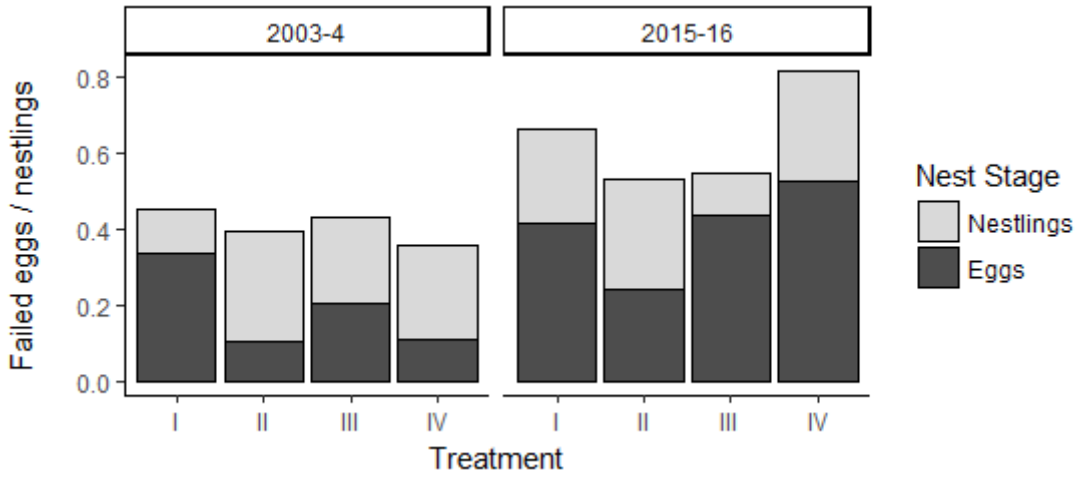


Figure 3.5 Proportional nest mortality during the period between egg laying and hatching (black bars) and hatching to fledging (grey bars) at Glen Finglas, Scotland. Full bars show average mortality proportions per nest for the whole period from egg laying until fledging. The grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. The two time periods represent early (first two years) and late (12-13 years into the experiment) sampling periods under continuous grazing treatments.

		Est.	p
Breeding density^{log}	**I-II**	0.30	**<0.001**
	I-III	0.00	0.966
	I-IV	0.13	0.220
	II-III	-0.30	**<0.001**
	II-IV	-0.17	0.094
	III-IV	0.13	0.220
Hatch date	**I-II**	-0.05	**0.005**
	I-III	-0.01	1.000
	I-IV	-0.01	1.000
	II-III	**0.04**	**0.022**
	II-IV	**0.04**	**0.019**
	III-IV	0.00	1.000
Egg-stage nest survival	**I-II**	**-3.42**	**0.013**
	I-III	-1.22	0.587
	I-IV	-1.57	0.373
	II-III	2.20	0.195
	II-IV	1.84	0.293
	III-IV	-0.35	0.983

Table 3.3 Pairwise comparisons of all grazing treatments (I-IV) for all meadow pipit breeding variables were a significant effect of treatment was found. Model estimates show the direction and the strength of the effect in relation to other pairwise comparisons in the same model. "p" indicates p-values and significant pairwise comparisons are marked in bold. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

3.5 Discussion

This study provides the first long-term experimental results of the effects of livestock grazing intensity on the breeding performance of a common upland insectivorous passerine. After a 13-14 year period, meadow pipit breeding density was significantly lower in plots with extensive, sheep only grazing (where hatch date was also significantly later). Conversely, the highest rates of egg-stage failure when both sampling periods were tested together occurred in intensively sheep grazed and ungrazed plots, where overall nest-survival also tended to be lower, although not significantly so. There was no significant change in grazing treatment effects over time but, across the experiment, both the egg-stage and overall nest survival declined with time, as did clutch size. Over the course of the experiment, these results suggest that other processes at the wider landscape-scale, such as increased predation rates,

may be important for breeding meadow pipits and that these may be more apparent than long-term effects of variation in grazing treatment.

3.5.1 Long-term treatment changes

Given their association with habitat mosaics (Pearce-Higgins & Grant 2006; Douglas *et al.* 2008), it was hypothesised that extensively grazed treatments would maximise meadow pipit reproductive success through changes in the vegetation structure and arthropod availability and that this effect was likely to be more pronounced in the later period of the experiment. Despite some intuitive trends, we found no statistically significant changes over time of grazing treatment effects on breeding parameters.

A clear, causal relationship of grazing through changes in vegetation structure and composition, on both food availability and predation pressure, would be expected to result in a stronger increase of treatment effects over time. Some of our models had a high proportion of unexplained variance, even when including random effects accounting for annual and spatial differences (Table 3.2 and Appendix A, Table S.1) hence, other factors such as weather and predation pressure unrelated to grazing treatments may contribute to a larger proportion of the variation in breeding success. The majority (72%) of all completely failed nests was a result of predation, which was higher in the later sampling period with a predation rate across all monitored nests of 33%, compared to 17% in the early sampling period. This suggests that, at Glen Finglas at least, predation is a more significant driver of breeding failures than lack of food for nestlings. However, increased begging, potentially due to a food shortage, could result in increased predation rates (Leech & Leonard 1997). These findings support previous studies that suggest that regional, environmental factors can be more important than local land-management for bird populations (Buchanan *et al.* 2017) and that precise land use practice becomes less important for ground-nesting birds when breeding success is mainly limited by predation (Smart *et al.* 2013).

3.5.2 Treatment effects

The highest breeding density was found in plots with intensive sheep grazing (Treatment I) followed by extensive, mixed sheep and cattle grazing (Treatment III) which both had significantly higher breeding densities than extensively, sheep grazed plots (Treatment II) when both sampling periods were tested together (i.e. without the interaction of Treatment

and Sampling period). This supports a landscape-scale study by Loe *et al.* (2007) in Norway who found both meadow pipit and total bird abundances to be highest in intensively grazed plots compared to extensively or ungrazed plots in a sheep grazing experiment after short-term management only, although the stocking densities and vegetation structure prior experimental treatments will then be an important factor. However, a grazing study by Báldi *et al.* (2005) showed that, for cattle grazing, extensive grazing had highest abundances of grassland birds, while intensive grazing had a higher diversity of bird species. Previous results from the early period of this experiment suggest that meadow pipit breeding density is mainly driven by a high arthropod abundance, where vegetation heterogeneity is high (Evans *et al.* 2015). The same study showed that vegetation height heterogeneity was greater in intensively grazed plots while vegetation biomass and arthropod abundance was highest in ungrazed plots, which suggests a trade-off between food abundance and food access in selecting breeding habitats. But this does not explain why breeding densities were lowest in Treatment II. Using the same experimental plots as in this study, Pakeman *et al.* (2019) showed that extensive grazing by cattle and sheep and intensive sheep grazing produced a marginally, though significantly higher plant species richness and Shannon diversity index than plots with extensive grazing by sheep only by 2017. This could mean that vegetation heterogeneity has also increased in plots grazed by both cattle and sheep, while extensive grazing by sheep only has a more homogenous vegetation structure and provides poorer prey availability.

The significantly later hatching date observed in the extensive, sheep grazing treatment (Treatment II) compared to all other treatments across both sampling periods, although surprising, may be a result of a less preferred habitat (seen as lower breeding density) and hence being left to individuals arriving late or perhaps being in poorer condition. The lack of a significant treatment effect on number of fledglings and overall nest-survival suggests, as previously mentioned, that other factors, such as parent experience and condition, predation and temporary weather conditions, may have stronger effects on breeding output. However, Treatment II also had the highest egg-stage nest survival (although only significantly higher than Treatment I) which could either suggest a mismatch in site preference versus suitability, or be a consequence of density-dependence. Predation was higher in the later compared to earlier stage of this study and it can be harder for arriving birds to predict predation risks compared to food availability when selecting breeding

territory (Misenhelter & Rotenberry 2000). At Glen Finglas, previous studies found indices that red foxes *Vulpes vulpes* were most abundant in ungrazed plots with abundance declining with increasing grazing pressure (Villar *et al.* 2013). The higher nest failure rate in intensively grazed plots could instead be explained by an increased exposure to predators through lower vegetation biomass (Baines 1990) and/or by predation by sheep (Jarrett *et al.* 2017).

3.5.3 Long-term regional changes

Several breeding parameters were affected by sampling period across all grazing treatments with smaller clutch sizes, lower overall nest survival and lower egg-stage nest survival in the later sampling period (Table 3.2). Considering that most nests fail due to predation, the change in nest survival is likely caused by a regional change in predation pressure. Predators such as red fox *Vulpes vulpes* and carrion crow *Corvus corone* have increased in UK during the last decades, and may limit bird populations within groups such as waders and gamebirds (Roos *et al.* 2018). The abundance of Meadow pipits in the Scottish uplands have declined by 10% between 1994 and 2017 (Scottish Natural Heritage 2018) and meadow pipit breeding success (but not local breeding density) has been observed to increase under experimental predator removal (Fletcher *et al.* 2010). The area of native woodland on the estate on which this study is located has increased during the course of the experiment, which could contribute to the higher nest predation in the later period by providing increased cover for predators (Söderström *et al.* 1998) while predator populations are no longer controlled. This afforestation is reflected in other areas of the British uplands and Europe as planted forests or land use abandonment (Meiner & Bas 2017) and the growing interest in natural woodlands and rewilding will drive the need of finding ways for such management to work effectively in parallel with traditional land use such as grazing (Pettorelli *et al.* 2018). Long-term, landscape-scale experiments are therefore vital in disentangling effects of local land use from ongoing large-scale changes and how local land use is affected by surrounding habitat changes.

3.5.4 Conclusions

These results show that grazing intensity can affect the breeding abundance and egg-stage nest survival of meadow pipits, with lowest survival in intensively sheep grazed plots, but that this has little effect on overall nest survival or number of fledglings produced.

Treatment effects were not significantly stronger in the later sampling period, which would have been expected if grazing gradually changes the vegetation structure and species composition in a way that then primarily affects the breeding outcome of insectivorous, ground-nesting birds. Instead, there was a significant decrease in nest survival between the sampling periods studied, mainly caused by predation, across all grazing treatments. This is consistent with other British studies linking large-scale changes in upland bird populations to increasing predation rates. However, predation may be partially linked to grazing treatments, as indicated by the effect of treatment on egg-stage nest survival. Further studies disentangling effects of regional predator abundances and local management on both predator numbers and predator behaviour would be needed to identify causes of observed predation pressure on breeding birds. More research is also needed to better understand the role of other drivers of change (e.g. atmospheric N deposition, climate change, agricultural practices) in predicting the cascading impacts on upland food-webs in general, especially plant-animal and predator-prey interactions.

3.6 References

Arcese, P., & Smith, J. N. M. (1988). Effects of Population Density and Supplemental Food on Reproduction in Song Sparrows. *The Journal of Animal Ecology*, *57*(1), 119–136.

Baines, D. (1990). The Roles of Predation, Food and Agricultural Practice in Determining the Breeding Success of the Lapwing (Vanellus vanellus) on Upland Grasslands. *The Journal of Animal Ecology*, *59*(3), 915–929.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I., & Fuller, R. (2013). *Bird Atlas 2007-11: The Breeding and Wintering Birds of Britain and Ireland*. Thetford: BTO Books.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4 (Version 64). Journal of Statistical Software. doi:10.18637/jss.v067.i01.

Beintema, A. J., & Muskens, G. J. D. M. (1987). Nesting Success of Birds Breeding in Dutch Agricultural Grasslands. *The Journal of Applied Ecology*, *24*(3), 743–758.

Bloom, P. M., Howerter, D. W., Emery, R. B., & Armstrong, L. M. (2013). Relationships between grazing and waterfowl production in the Canadian prairies. *The Journal of Wildlife Management*, *77*(3), 534–544.

Buchanan, G. M., Grant, M. C., Sanderson, R. A., & Pearce-Higgins, J. W. (2006). The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *The Ibis*, *148*(4), 615–628.

Buchanan, G. M., Pearce-Higgins, J. W., Douglas, D. J. T., & Grant, M. C. (2017). Quantifying the importance of multi-scale management and environmental variables on moorland bird abundance. *The Ibis*, *159*(4), 744–756.

Bunce, R. G. H., Wood, C. M., Smart, S. M., Oakley, R., Browning, G., Daniels, M. J., … Holl, K. (2014). The Landscape Ecological Impact of Afforestation on the British Uplands and Some Initiatives to Restore Native Woodland Cover. *Journal of Landscape Ecology*, *7*(2). doi:10.2478/jlecol-2014-0013

Coulson, J. C., Duncan, N., & Thomas, C. (1982). Changes in the Breeding Biology of the Herring Gull (Larus argentatus) Induced by Reduction in the Size and Density of the Colony. *The Journal of Animal Ecology*, *51*(3), 739–756.

Critchlow-Watton, N., Dobbie, K. E., Bell, R., Campbell, S. D. G., Hinze, D., Motion, A., … Towers, W. (2014). Scotland's State of the Environment Report. Scotland's Environment Web. Retrieved from http://www.environment.scotland.gov.uk/media/92572/state-of-environment-report-2014.pdf

DEFRA. (2004). An assessment of the impacts of hill farming in England on the economic, environmental and social sustainability of the uplands and more widely. DEFRA. Retrieved from http://webarchive.nationalarchives.gov.uk/20110318142221/http://www.defra.gov.uk/evidence/economics/foodfarm/reports/documents/Hillfarm.pdf

Dennis, P., Skartveit, J., McCracken, D. I., Pakeman, R. J., Beaton, K., Kunaver, A., & Evans, D. M. (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *The Journal of Applied Ecology*, *45*(1), 279–287.

Dennis, P., Young, M. R., & Gordon, I. J. (1998). Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. *Ecological Entomology*, *23*(3), 253–264.

Douglas, D. J. T., Evans, D. M., & Redpath, S. M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study: The Journal of the British Trust for Ornithology*, *55*(3), 290–296.

Douglas, D. J. T., & Pearce-Higgins, J. W. (2014). Relative importance of prey abundance and habitat structure as drivers of shorebird breeding success and abundance. *Animal Conservation*, *17*(6), 535–543.

EU. (1992). Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31992L0043

Evans, D. M., Redpath, S. M., & Evans, S. A. (2005). Seasonal patterns in the productivity of Meadow Pipits in the uplands of Scotland. *Journal of Field Ornithology*, *76*(3), 245–251.

Evans, D. M., Redpath, S. M., Evans, S. A., Elston, D. A., & Dennis, P. (2005). Livestock grazing affects the egg size of an insectivorous passerine. *Biology Letters*, *1*(3), 322–325.

Evans, D. M., Redpath, S. M., Evans, S. A., Elston, D. A., Gardner, C. J., Dennis, P., & Pakeman, R. J. (2006). Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine. *Biology Letters*, *2*(4), 636–638.

Evans, D. M., Villar, N., Littlewood, N. A., Pakeman, R. J., Evans, S. A., Dennis, P., … Redpath, S. M. (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere* , *6*(3), art42.

Fletcher, K., Aebischer, N. J., Baines, D., Foster, R., & Hoodless, A. N. (2010). Changes in breeding success and abundance of ground-nesting moorland birds in relation to the experimental deployment of legal predator control. *The Journal of Applied Ecology*, *47*(2), 263–272.

Franks, S. E., Douglas, D. J. T., Gillings, S., & Pearce-Higgins, J. W. (2017). Environmental correlates of breeding abundance and population change of Eurasian Curlew Numenius arquata in Britain. *Bird Study: The Journal of the British Trust for Ornithology*, *64*(3), 393–409.

Fuller, R. J., & Gough, S. J. (1999). Changes in sheep numbers in Britain: implications for bird populations. *Biological Conservation*, *91*(1), 73–89.

Furness, R. W. (1988). Predation on ground-nesting seabirds by island populations of red deer Cervus elaphus and sheep Ovis. *Journal of Zoology*, *216*(3), 565–573.

Haines-Young, R. H., Barr, C. J., Black, H., Briggs, D. J., Bunce, R., Clarke, R. T., … Others. (2000). Accounting for nature: assessing habitats in the UK countryside. DETR. Retrieved from http://nora.nerc.ac.uk/id/eprint/4298/1/exec.pdf

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, *2*, e616.

Hayhow, D. B., Bond, A. L., Douse, A., Eaton, M. A., Frost, T., Grice, P. V., … Wotton, S. (2017). *The state of the UK's birds 2016*. The RSPB; BTO; WWT; DAERA; JNCC; NE; NRW; SNH.

Retrieved from https://www.bto.org/sites/default/files/publications/state-of-uk-birds-2016.pdf

Hermy, M., & Verheyen, K. (2007). Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. *Ecological Research*, *22*(3), 361–371.

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics, Theory and Applications*, *6*(2), 65–70.

Johnson, T. N., Kennedy, P. L., & Etterson, M. A. (2012). Nest success and cause-specific nest failure of grassland passerines breeding in prairie grazed by livestock. *The Journal of Wildlife Management*, *76*(8), 1607–1616.

Kőrösi, Á., Batáry, P., Orosz, A., Rédei, D., & Báldi, A. (2012). Effects of grazing, vegetation structure and landscape complexity on grassland leafhoppers (Hemiptera: Auchenorrhyncha) and true bugs (Hemiptera: Heteroptera) in Hungary. *Insect Conservation and Diversity / Royal Entomological Society of London*, *5*(1), 57–66.

Kosicki, J. Z., & Chylarecki, P. (2013). Predictive mapping of Meadow Pipit density using integrated remote sensing data and an atlas of vascular plants dataset. *Bird Study: The Journal of the British Trust for Ornithology*, *60*(4), 500–508.

Kumstátová, T., Brinke, T., Tomková, S., Fuchs, R., & Petrusek, A. (2004). Habitat preferences of tree pipit (Anthus trivialis) and meadow pipit (A. pratensis) at sympatric and allopatric localities. *Journal of Ornithology / DO-G*, *145*(4), 334–342.

Lenth, R. V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33.

Loe, L. E., Mysterud, A., Stien, A., Steen, H., Evans, D. M., & Austrheim, G. (2007). Positive short-term effects of sheep grazing on the alpine avifauna. *Biology Letters*, *3*(1), 109–111.

Low, M., Arlt, D., Eggers, S., & Pärt, T. (2010). Habitat-specific differences in adult survival rates and its links to parental workload and on-nest predation. *The Journal of Animal Ecology*, *79*(1), 214–224.

Meiner, A., & Bas, P. (2017). *Landscapes in transition* (No. 10). European Environment Agency.

Misenhelter, M. D., & Rotenberry, J. T. (2000). Choices and consequences of habitat occupancy and nest site selection in sage sparrows. *Ecology*, *81*(10), 2892–2901.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution / British Ecological Society*, *4*(2), 133–142.

Newton, I. (2004). The recent declines of farmland bird populations in Britain: an appraisal of causal factors and conservation actions. *The Ibis*, *146*(4), 579–600.

Papanastasis, V. P., & Peter, D. (1998). *Ecological Basis of Livestock Grazing in Mediterranean Ecosystems: Proceedings of the International Workshop Held in Thessaloniki (Greece) on October 23-25, 1997*. Office for Official Publications of the European Communities.

Pearce-Higgins, J. W., & Grant, M. C. (2006). Relationships between bird abundance and the composition and structure of moorland vegetation. *Bird Study: The Journal of the British Trust for Ornithology*, *53*(2), 112–125.

Pearce-Higgins, J. W., Murray, C., M, G. B. C., & Graeme, M. (2008). International importance and drivers of change of upland bird populations. In A. Bonn, T. Allott, K. Hubacek, & J. Stewart (Eds.), *Drivers of Environmental Change in Uplands* (pp. 209–227). Routledge.

Pearce-Higgins, J. W., & Yalden, D. W. (2003). Variation in the use of pasture by breeding European Golden Plovers Pluvialis apricaria in relation to prey availability: Pasture use by breeding Golden Plovers. *The Ibis*, *145*(3), 365–381.

Pearce-Higgins, J. W., & Yalden, D. W. (2004). Habitat selection, diet, arthropod availability and growth of a moorland wader: the ecology of European Golden Plover Pluvialis apricaria chicks: Ecology of Golden Plover chicks. *The Ibis*, *146*(2), 335–346.

Perrins, C. M. (1970). The timing of birds' breeding seasons. *The Ibis*, *112*(2), 242–255.

Pettorelli, N., Barlow, J., Stephens, P. A., Durant, S. M., Connor, B., Bühne, H. S. to, … Toit, J. T. du. (2018). Making rewilding fit for policy. *The Journal of Applied Ecology*. doi:10.1111/1365-2664.13082

Phillips, N. (2017). yarrr: A Companion to the e-Book "YaRrr!: The Pirate's Guide to R" (Version R package version 0.1.4). Retrieved from https://CRAN.R-project.org/package=yarrr

Prior, G. L., Evans, D. M., Redpath, S., Thirgood, S. J., & Monaghan, P. (2011). Birds bias offspring sex ratio in response to livestock grazing. *Biology Letters*, *7*(6), 958–960.

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/

Roos, S., Smart, J., Gibbons, D. W., & Wilson, J. D. (2018). A review of predation as a limiting factor for bird populations in mesopredator-rich landscapes: a case study of the UK: Predation on UK birds. *Biological Reviews*, *63*, 10.

Schaub, M., Martinez, N., Tagmann-Ioset, A., Weisshaupt, N., Maurer, M. L., Reichlin, T. S., … Arlettaz, R. (2010). Patches of Bare Ground as a Staple Commodity for Declining Ground-Foraging Insectivorous Farmland Birds. *PloS One*, *5*(10), e13115.

Sim, I. M. W., Burfield, I. J., Grant, M. C., Pearce-Higgins, J. W., & Brooke, M. de L. (2007). The role of habitat composition in determining breeding site occupancy in a declining Ring Ouzel Turdus torquatus population. *The Ibis*, *149*(2), 374–385.

Smart, J., Bolton, M., Hunter, F., Quayle, H., Thomas, G., & Gregory, R. D. (2013). Managing uplands for biodiversity: Do agri-environment schemes deliver benefits for breeding lapwing Vanellus vanellus? *The Journal of Applied Ecology*, *50*(3), 794–804.

Söderström, B., Pärt, T., & Rydén, J. (1998). Different nest predator faunas and nest predation risk on ground and shrub nests at forest ecotones: an experiment and a review. *Oecologia*, *117*(1-2), 108–118.

Sullivan, M. J. P., Newson, S. E., & Pearce-Higgins, J. W. (2015). Using habitat-specific population trends to evaluate the consistency of the effect of species traits on bird population change. *Biological Conservation*, *192*, 343–352.

Tapper, S. C., Potts, G. R., & Brockless, M. H. (1996). The Effect of an Experimental Reduction in Predation Pressure on the Breeding Success and Population Density of Grey Partridges Perdix perdix. *The Journal of Applied Ecology*, *33*(5), 965–978.

Vandenberghe, C., Prior, G., Littlewood, N. A., Brooker, R., & Pakeman, R. (2009). Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic and Applied Ecology*, *10*(7), 662–670.

van den Pol-van Dasselaar, A., de Vliegher, D., Hennessy, J., Isselstein, J.-L., & Peyraud, A. (2015). The future of grazing. In European Grassland Federation EGF (Ed.), *Grassland Science in Europe*. Aberystwyth, UK: Livestock research, Wageningen. Retrieved from http://www.europeangrassland.org/fileadmin/media/pdf/Grazing/906_The_future_of_grazing_-_Van_den_Pol-van_Dasselaar_et_al.pdf

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. Retrieved from http://ggplot2.org

Chapter 4: Testing and evaluating DNA-metabarcoding to examine the impacts of livestock grazing on avian predator-prey interactions

4.1 Abstract

Changes in large-scale processes, including land management, have been implicated in the significant declines of upland birds during the last decades. At the same time, some upland habitats in the UK that are important for moorland bird populations have seen large changes in livestock grazing intensity and are likely to face further changes in management. It is therefore important to gain a more mechanistic understanding of how land management affect variables such as bird foraging conditions, which in turn can affect population trends. To date, diet analyses of insectivorous birds are often limited to taxonomic identification at the order or family in some invertebrate groups. Recent molecular techniques that use DNA within faecal samples to identify prey species offer unprecedented opportunities to study avian trophic interactions, but no studies have used this method to examine potential changes in diet as a result of habitat modification. In this study the aim was to i) analyse the meadow pipit diet using metabarcoding of faecal samples and evaluate this method, ii) compare these results to previous studies on meadow pipit diets using morphological methods and iii) compare the diets in four different grazing treatments.

The diet of meadow pipit *Anthus pratensis* nestlings, a common upland insectivore, was analysed and compared under four different grazing treatments in a replicated, large-scale grazing experiment. The four different treatments were intensive sheep, extensive sheep, extensive mixed sheep and cattle and no grazing. Faecal samples from nestlings across the experimental treatments were collected and extracted DNA was used in massively parallel "next generation" sequencing of the Cytochrome c Oxidase subunit I gene using a nested tagging approach.

The diet identified using the DNA-metabarcoding approach had several similarities with other studies of meadow pipit nestling diets based on conventional light microscopy approaches and/or direct observations of prey provisioning at the nest, especially those from the same study site. The most frequently occuring prey were Tipulidae spp. (Diptera), but most prey identified to species level were from the orders Lepidoptera and Coleoptera. There were more prey orders detected in intensively grazed plots than the other treatments and fewest in ungrazed plots.

The outcomes of this study suggest that this method has great potential in giving more detailed information on foraging preferences in insectivorous species, and that DNA-based methods can be successfully used to detect dietary differences between habitats. However, a larger number of barcode records of identified species is needed to describe the whole diet to species level, especially within Diptera. The higher diversity of prey orders in intensively grazed plots may be a result of higher food access and therefore more availability and choice of prey types but may also be an effect of lower availability of preferred prey making other orders occurring in the diet. Other technological challenges are identified and discussed.

4.2 Introduction

British upland areas used for natural grazing have been subject to significant management changes, mainly due to shifting subsidies systems for grazing management. Therefore, sheep numbers have decreased by over 30% in Scotland between 2000-2013 (Critchlow-Watton *et al.* 2014) while grazing with mixed sheep and cattle have decreased across all upland areas in the United Kingdom (UK) (Silcock *et al.* 2012). Meanwhile, some upland bird populations have shown important declines during the last decades and are the group of birds with the strongest declines in the UK (Balmer *et al.* 2013). These declines have been linked to management changes (Douglas *et al.* 2017), although large-scale, undetermined factors have been suggested to be of more importance to some populations (Buchanan *et al.* 2017). Moreover, an attempt to increase moorland breeding bird densities within an area where management practices thought to favour upland bird populations were applied failed to increase abundances of many breeding bird species (Calladine *et al.* 2014).

Upland habitats are likely to undergo further habitat changes (Pearce-Higgins *et al.* 2008) as a result of either climate or management changes. Therefore, it is important to disentangle management and other environmental effects on variables related to avian population declines in upland areas such as foraging opportunities, but also to understand how habitat changes through altered management affect links between species, such as access to prey by insectivorous birds (Britschgi *et al.* 2006; Schaub *et al.* 2010; Douglas & Pearce-Higgins 2014). Lower arthropod abundances due to agricultural intensification have been suggested to be a reason for declines of some bird populations (Benton *et al.* 2002). The link between cessation of farming (e.g. grazing) and avian food availability is less investigated, and in both intensified and abandoned farming systems a better understanding of avian trophic interactions, and how these might be affected by environmental change, is needed.

Food availability to birds can be limited by both actual food abundance (Hart *et al.* 2006) but also by food access (Gawlik 2002). Limited availability through, for example, dense vegetation, is suggested to be the reason why several insectivorous, ground foraging birds prefer to forage in shorter vegetation (Douglas *et al.* 2008; Vandenberghe *et al.* 2009; Schaub *et al.* 2010; Douglas & Pearce-Higgins 2014) or higher vegetation heterogeneity (Klink *et al.* 2014). These factors can be significantly affected by large herbivores, especially livestock such as sheep and cattle (Evans *et al.* 2015). Therefore, foraging conditions are better described by actual intake or provisioning to nestlings than estimates of food abundance. Moreover, some species or orders of prey may be actively selected and overrepresented in the diet in comparison to their abundance (Klink *et al.* 2014), presumably to meet nutrient requirements (Kaspari & Joern 1993) and detailed information on diet composition and how it changes in anthropogenically modified habitats can be a useful tool in understanding food limitations.

Diet analyses of insectivorous birds have been conducted using several morphological methods such as analysis of remains in faecal samples (Moreby & Stoate 2000; Michalski *et al.* 2011), manual or video observations (Goodbred & Holmes 1996; Oosten 2016), neck-collars (ligatures) obstructing food from being swallowed (Kluijver 1933; Bureš & Weidinger 2000; Moreby & Stoate 2000; Mennechez & Clergeau 2001), and analysis of gut content by stomach flushing in live birds (Gales 1987) or dissection of dead birds (Walton 1979; Kaspari & Joern 1993). No method of diet identification is completely free from implications or biases. For example, morphologically identified prey can be biased towards large recognisable prey in feeding observations or prey that is hard to digest in analyses of faecal or gut contents; Moreby and Stoate (2000) found that some groups such as Diptera, Lepidopteran Larvae and Araneae are often well recorded from morphological identification of faecal samples in relation to their actual abundance in the diet (observed by neck ligatures), while Coleoptera tend to be overrepresented and soft bodied arthropods such as Collembola underrepresented in relation to actual frequency. Moreover, neck ligatures can only be used under a limited period at the time and not while nestlings are too young as it may harm (strangle) them or too near fledging as it would then be hard to recapture the nestlings and remove the ligature (Orians 1966; Jenny 1990; Moreby & Stoate 2000). Identification of insectivorous birds' prey through faeces can sometimes be done to species

or genus (Moreby 1988; Poulsen & Aebischer 1995; Klink *et al.* 2014), but often only to family (Moreby & Stoate 2000) or order (Michalski *et al.* 2011).

Molecular methods to identify avian prey, most often through faecal samples has received increasing attention since the beginning of the last decade (Symondson 2002; King *et al.* 2008) and several successful studies have identified avian species' prey from a wide taxonomic range (Jarman *et al.* 2002; Deagle *et al.* 2007; McInnes *et al.* 2017). This includes diets of insectivorous birds, where relatively short DNA segments (up to 600 base-pairs) of the mitochondrial Cytochrome c Oxidase subunit I gene (COI) can provide successful prey identification of arthropod prey species, given that a matching reference of the prey is available in a reference library (Oehm *et al.* 2011; Jedlicka *et al.* 2013, 2017; King *et al.* 2015; Trevelline *et al.* 2016, 2018). Knowledge of prey species rather than orders can have several benefits to conservationists: Firstly, it can provide a better understanding of foraging habitats, for example, different types of larvae may be linked to different host plants (Sheck & Gould 1996; Janz & Nylin 1997; Tang *et al.* 2006). Secondly, identification of prey on a species level can give a better understanding of the range between prey types and diversity (number of species of prey) that is being taken, which is useful, for example, as diet specialisation can be an indicator of sensitivity to extinction in both birds (Sekercioglu 2007) and bats (Boyles & Storm 2007). Feeding observations (or by other methods recording measurable amounts of prey) can provide a good estimate of biomass of each prey type in any one sample (i.e. prey delivered per feeding event, proportions of prey remains of a specific order in one faecal sample) (e.g. Douglas *et al.* 2008; Klink *et al.* 2014), which is more difficult to do using DNA-based techniques reporting presence/absence only. However, with a sufficient sample size, frequency of occurrence can, to some extent, be used to estimate the proportion of different prey types in a diet (Trevelline *et al.* 2016).

DNA-based identification of prey has also been more materially costly than morphological methods in terms of consumables and requires a range of task-specific lab equipment. However, there has been considerable progress in the convenience of using DNA and PCR based methods for studies of species interaction in general. This is primarily due to the use of massively parallel "Next Generation Sequencing" (NGS), where a wide range of species can be sequenced simultaneously (Pompanon *et al.* 2012; Leray *et al.* 2013). Within this method, each sample can be individually marked prior to sequencing by individual

combinations of Molecular Identification Tags (MIDs). This makes sequencing of several hundred samples or more possible simultaneously, where all output sequences can be allocated to its original sample (Binladen *et al.* 2007). This method has also been successfully applied to passerines (Vo & Jedlicka 2014) and has great potential to provide more detail than previously achievable on insectivorous birds' diets in uplands that have undergone management change or changes in management intensity. However, to use this method when answering ecological and management questions, it is necessary to test that outputs can provide enough resolution and information, for example by having barcode references available for local prey groups.

Aims

The study had three objectives: i) to develop and evaluate a nested-tagging DNA-metabarcoding methodology using avian faecal sacs to identify DNA of prey provisioned to nestlings of the meadow pipit *Anthus pratensis*, a common insectivorous ground foraging insectivore, ii) produce a comprehensive list of prey species and further evaluate this method by comparing these results to other studies of meadow pipit diets using morphological methods and iii) identify key differences in diets between meadow pipit nestlings under different levels of grazing pressure using a long term, replicated grazing experiment. On the same study site, it has previously been shown that meadow pipits breed in higher abundances in plots with extensive grazing by a mix of sheep and cattle in comparison to sheep only grazing with the same or higher grazing pressure or no grazing (Evans *et al.* 2006). It was also shown that arthropods important in upland bird diets were more abundant with decreasing grazing pressure (Dennis *et al.* 2008) but that meadow pipits preferred to search for food in patches with particularly short vegetation with higher arthropod abundance (Douglas *et al.* 2008; Vandenberghe *et al.* 2009) and that feeding meadow pipits had to fly further away for foraging in intensively grazed plots than in plots with extensive, mixed cattle and sheep grazed plots (Vandenberghe *et al.* 2009). Diet comparisons between treatments are focused on the diversity of prey as it has been suggested to be beneficial to insectivorous birds (Hungerford *et al.* 1993) and higher prey diversity has been linked to more favourable breeding habitats with higher fledgling success in another insectivorous passerine, Whinchat *Saxicola rubetra* (Britschgi *et al.* 2006). As increasing grazing pressure led to shorter vegetation at the same study site (Evans *et al.* 2015) it is hypothesised that extensive (intermediate) grazing pressure would provide a

higher diversity of available prey as it could be more likely to have both shorter vegetation and a higher abundance of arthropod prey, which in turn would provide more available options for foraging meadow pipits.

4.3 Methods

4.3.1 Method development and evaluation – sample collection

All meadow pipit faecal samples were collected during 2015 and 2016 in Glen Finglas, an upland estate in Scotland, UK. Nests of meadow pipits were found by walking the monitored area systematically every 3 days, weather permitting. Nests were usually detected by flushing out the incubating females or occasionally observing feeding parents arriving at the nest. Nestling birds of many species, such as meadow pipits, often produce faecal sacs when handled which makes it possible to collect complete faecal samples separated from faecal sacs of other nestlings in the same nest. Faecal samples were collected during nest visits with a period of 3 days between each visit until the nestlings were 11-12 days old. However, no nests were visited during periods of rainfall and not all chicks and visits produced a collected sample. Samples were collected using disposable gloves and directly placed in individual 5 mL tubes and stored in 99.8% ethanol and at -20°C until processing.

4.3.2 Method development and evaluation - sample processing

Due to practical reasons, two slightly different extraction protocols were used for the samples collected in 2015 and 2016. Both extraction methods were developed for soil samples rather than stool/faecal samples as avian excretions contains a mix of urine and faeces and are high in uric acid, which may inhibit Polymerase Chain Reaction (PCR), but these components can be removed by extracting the DNA using extraction kits developed to handle humic acids in soils (Jedlicka *et al.* 2013). Ethanol was removed from the samples collected in 2015 by pouring and pipetting out excessive ethanol and drying in an oven at 50°C for 15 min or longer (if necessary) until no excessive ethanol remained, although samples were still wet. The samples were homogenised in a TissueLyser II for 2 minutes at 30Hz in 10 ml stainless steel grinding jars (Qiagen) together with 2 mL of nuclease-free water. 250 mL of the homogenised sample mix was then extracted using the PowerSoil Kit (at the time of purchase MoBio, now Qiagen) following provided instructions. Samples from 2016 were first emptied of excessive ethanol by pouring and pipetting out excessive ethanol and then dried in an oven at 50°C until dry. Samples were thereafter homogenised manually

to a powder using a spatula and splitting the sample in half. One half of each sample was then used for extraction following the protocol by Sellers *et al.* (2018) adapted for soil with the following changes: 1460 µL of Lysis solution 1 and 530 µL of Lysis solution 2 was used instead of indicated volumes as this was most optimal for successful grinding. Among the extractions of samples both from 2015 and 2016, a total of 18 blank (nuclease-free water) extractions were also made to check for occurrence of any contamination between samples during the extraction process. The primer pair mICOIintF and jgHCO2198, amplifying a 313 bp fragment in the mitochondrial Cytochrome c Oxidase subunit I gene modified by Kitson *et al.* (2018) originally designed by Leray *et al.* (2013) for metazoan animals, was used. Following the adaptation from (Kitson *et al.* 2018) there were 12 forward primers and 8 reverse primers with an additional, unique 8-nucleotide combination which allowed 96 unique combinations in the first PCR (PCR1) and another set of 12 x 8 unique tags attached to the primers for the second PCR (PCR2) that also enable Illumina sequencing. This "nested tagging" approach using doubble pairs of primers allows for a large number of samples to be sequenced simultaneously while still allowing all sequences to be traced back to their original sample when analysing the sequence data. Unique primer tags were also used for positive and negative PCR controls, for both PCR1 and PCR2. In this study, 48 out of 96 possible combinations were used for PCR1 (including 3 blank extractions, 1 positive and 1 negative [i.e. nuclease-free water]). The primer tag combinations were then replicated on 9 PCR plates. PCR1 was set up in 25 µL reactions using high fidelity hot-start Taq mix (MyTaq HS Red mix, BioLine) which is developed to avoid amplification starting before the reaction is heated up to 95-98°C. 12.5 µL of Taq mix, 4 µL of DNA template and a final concentration of 0.4 µM of each primer was used in each PCR reaction. Each reaction was sealed with a drop of mineral oil inside the reaction tube and a plastic film covering each well to avoid contamination between reaction tubes. The PCRs consisted of an initial step of 98°C for 3 min, followed by 45 cycles containing a first step at 95°C for 15 sec, a second step using a gradient from 63-53°C where the temperature decreased by 1-2°C per cycle during the first 8 cycles and then staying at 53°C during the remaining cycles, and a third step at 72°C for 1 min. The 45 three-step cycles were then followed by a final step at 72°C for 5 min and 5°C for 5 min to cool down all reactions. PCR success (including the one from the positive control) and contamination was examined by running all PCR products on an agarose gel. All samples from one PCR plate with 48 primer combinations were pooled into one pre-library.

To increase the equimolarity of DNA used between the samples from one PCR plate, success of each sample (PCR reaction) was scored as one of 3 levels of success: no band, weak band or strong band, and according to the score: 12, 8 or 4 µL respectively of each sample was used. Although no bands were detected for blank and negative reactions, they were still included in the pooling as a small amount of amplified DNA may still be present. 4 µL of positive controls and 8 µL of the blank extractions and negative controls were added from each PCR plate. The pre-libraries were cleaned up from unused primers using magnetic beads (MAG-bind, Omega Bio-Tek) that bind to PCR products of a selected base-pair length depending on the bead concentration. Each pre-library was run in a separate PCR reaction for PCR2, where each reaction gets a unique primer pair. These primers allows for Illumina Sequencing and bind to the edge of the primers used in PCR1. Each PCR2 reaction was 21.1 µL and consisted of 10.5 µL of Taq mix, 5 µL of cleaned, pre-library DNA template and a final concentration of each primer at 0.52 µM. The PCR2 reactions were also covered with mineral oil and sealed with a plastic film. 1/49th of the ng of DNA of PCR positive control was also added to each pre-library at a volume of 1/49th of the ng of DNA in each pre-library. PCR2 consisted of the following steps: an initial 95^{o}C for 3 min, 11 cycles containing two steps; 98^{o}C for 20 s and 72^{o}C for 1 min and finally 72^{o}C for 5 min and 4^{o}C for 10 min. The products of the second PCR were then tested for tagging-success on an agarose gel. The PCR2 products were then pooled equimolarly by measuring DNA concentrations, cleaned with magnetic beads and pooled into two libraries. Hence, all cleaned reactions from 5 pre-libraries (PCR1-plates) were pooled into one final library and 4 in another final library. This was necessary as two Sequencing runs would be needed to get enough reading depth for this number of samples and amplicon lengths. The two libraries were further diluted to 4 ± 0.2 nM of DNA and sequenced in an Illumina MiSeq using a V2 sequencing kit (2x250bp) with 10% PhiX of the same molarity added as sequencing control.

4.3.3 Method development and evaluation - bioinformatics of sequencing data

A pipeline developed for reproducible analysis of metabarcoding data (i.e. data from universal primers amplifying a large range of taxa): metaBEAT (github.com/HullUni-bioinformatics/metaBEAT), was used to assign sequences to the correct original sample (demultiplexing) and taxonomic ID following Kitson *et al.* (2018): After allocating all reads to each sample ID, reads were end-trimmed to a remaining phred score higher than Q30 which

means that the parts of reads that had more than 1 in 1000 faulty base calls (i.e. assigned nucleotide base pairs) were removed. The reads were also trimmed from primers before merging paired end-sequences. Only reads over 100 base pairs before merging, and 313bp ± 10% after merging, were kept for further analysis. Within each sample ID, reads were assigned to clusters of reads that were 100% similar and had a least 50 reads for each cluster (cluster coverage) and remaining reads were filtered out. Clusters were assigned to a taxonomic ID (species, genus or higher) if it matched an available entry by no less than a given percentage. Four different criteria of cluster similarity to matching sequences in the Basic Local Allignment Search Tool (BLAST) were tested: 0.92, 0.94, 0.96 and 0.98 to test if the number of unassigned reads could be decreased by using a lower BLAST criteria percentage. Although species cannot be reliably identified with a BLAST similarity of 0.92, the output could provide useful information on the orders of prey and the interpretation of diet distribution between orders is more reliable with a larger proportion of reads and clusters. Therefore, the dataset based on 92% was used for frequency of order detection to make use of a read-proportion as large as possible, and the data set based on 98% similarity was used to list the species detected in the diet, as 98% OTUs less similar to a reference than 98% may be assigned to the wrong species (Clare *et al.* 2014; Alberdi *et al.* 2018).

4.3.4 Method development and evaluation - data analysis

Clusters within samples that had been assigned to a taxonomic ID are hereby referred to as Operational Taxonomic Units (OTUs) which may be anything from a species to kingdom, (although most often species to order). Non prey species were filtered out by going through each identified OTU and removing all OTUs that were host DNA, fungi, endoparasites or more likely to be coming from contamination during sample processing. When only prey OTUs remained, the number of unassigned reads and the distribution of identified species, genera, families or orders were compared between the different BLAST similarity criteria.

4.3.5 Diet comparison to morphological studies of meadow pipit diets

In order to compare the diet described in this study to some previous studies of meadow pipit nestling diets using other methods, a table including identification and sampling methods, measure unit, habitat and prey groups was compiled. A list of studies of meadow pipit nestling diets was produced by searching for the words “meadow pipit AND diet” in Web of Science from which all studies available online describing meadow pipit nestling

diets to any extent were included in the table with an addition of one study that was found as a reference in another study but not detected in the first search as no abstract was available. As the studies found by this search provided a range of sampling methods and study locations, no further searches were done. The same prey categories were used as found in the majority of other studies to demonstrate the overall patterns in the diet observed in the present study. This was, apart from dividing prey by orders, also specified to Tipulidae (Diptera) and other Diptera. The measure used for diet distribution differed between studies and here, the number of OTUs detected from each prey group out of all OTU's detected was used.

4.3.6 Effects of grazing pressure on nestling diet - experimental design

A long-term grazing experiment set up in 2003 in Glen Finglas, Scotland, UK was used, from which all faecal samples were systematically collected in this study. The experiment consisted of four grazing treatments with six replicates measuring 3.3 ha of each treatment. The four treatments were I) commercial stocking density of sheep with nine ewes per plot, II) a third of the commercial stocking density with three ewes per plot, III) two ewes per plot and two cows with suckling calves during four weeks in autumn and IV) ungrazed. Each experiment plot was 3.3 ha and the treatments had remained the same in each plot since 2003. The treatments were evenly distributed across three blocks (i.e. two replicates per block) with a random distribution within each replicate. All faecal samples were collected after 12 (2015) and 13 (2016) years of continuous experimentally manipulated grazing management. Based on observations in Vandenberghe *et al.* (2009), the foraging areas around a nest were estimated to only cover 8 % or less of the area of an experiment plot. Therefore, across all nests, most foraging would take place inside the plot/treatment in which the nest was located, and plots could be considered to be large enough to show any potential effect of treatment on diet (see chapter 3 for more details).

4.3.7 Effects of grazing pressure on nestling diet - data analysis

In order to control for a varying number of samples and possible sampling incompleteness, rarefaction curves were created for all OTUs detected and all orders detected in each of the four grazing treatments. This provided both an understanding of how well sampled each

treatment was and showed how many OTUs or orders were detected on average for a given sample size in each treatment.

As it was not possible to control for all factors (i.e. spatial differences in sampling or extraction protocols) when comparing rarefaction curves, the number of orders detected between grazing treatments was also tested statistically. The number of prey orders detected per experiment plot was modelled with a linear mixed effects model (GLMM) using a Gaussian distribution. The number of samples was included both as a linear and quadratic effect to allow the number of orders to be controlled by both a linear or quadratic (i.e. declining increase as sample sizes get larger) effect. The proportion of samples collected and extracted during the first year for each plot out of all samples collected in both sampling years, and the average sample date for all samples collected in each plot were included as fixed effects. Grazing experiment replicate and block were included as random effects. The proportion of samples collected and extracted in the first year were included to control for the effect of slightly different DNA extraction techniques following sampling collection between the two years and average date was included to test and minimise an effect of sampling date on observed treatment effects. It was not possible to extract rarefaction values adjusted to sample size per plot since the sample size often exceeded the number of observed orders (see Oksanen *et al.* 2015 for more details). The variables in the model were removed sequentially if they did not improve the model in terms of AIC scores. Analyses were done in R (R Core Team 2016) version 3.3.3 using package "vegan" (Oksanen *et al.* 2015), "lme4" (Bates *et al.* 2015) and "lsmeans" (Lenth 2016). Graphs were done in Microsoft Excel and in R using package "vegan" (Oksanen *et al.* 2015) or package "ggplot2" (Wickham 2009).

4.4 Results

4.4.1 Method development and evaluation

310 faecal samples were collected from 71 different meadow pipit nests during the two sampling years. PCR1 reactions showed a success rate of 57-75% of all faecal samples on one PCR plate (i.e. as a visible PCR product on agarose gels). All PCR2 reactions were successfully tagged (i.e. addition of the second pair of primers could be observed by an increased PCR product length), and there was no evidence of contamination (i.e. no PCR products from negative or blank samples).

After trimming, a total of 16,259,734 reads remained, of which 2,653,372 reads were from prey DNA. DNA of possible prey species was detected in 127 out of 309 faecal samples and DNA of any kind was detected in 295 samples. Many samples had high numbers of reads of meadow pipit (host) DNA which may have limited the amount of reads that could be produced from prey DNA due to template competition during amplification. Host DNA was on average 20,987 reads per sample (with levels up to 232,939 reads per sample) compared to prey DNA, which was on average 8,559 reads per sample/well. The highest number of reads (546,062 reads) in a single PCR well was in the positive control which was extracted from tissue DNA from killer shrimp *Dicerogammarus villousus.* Although no contamination was detected when visually examining PCR negatives and blank extractions, some contamination was, surprisingly, found in the blank samples after sequencing (average = 4,152 reads per well), mainly from host DNA. Some sequenced DNA was also found in two PCR negative controls, with 405 and 715 reads from human DNA and an unidentified source, respectively. There were more samples that resulted in sequenced prey DNA in samples collected and extracted in the first year (2015) with 136 prey OTUs detected in 122 samples in 2015 and 51 prey OTUs detected in 188 samples in 2016.

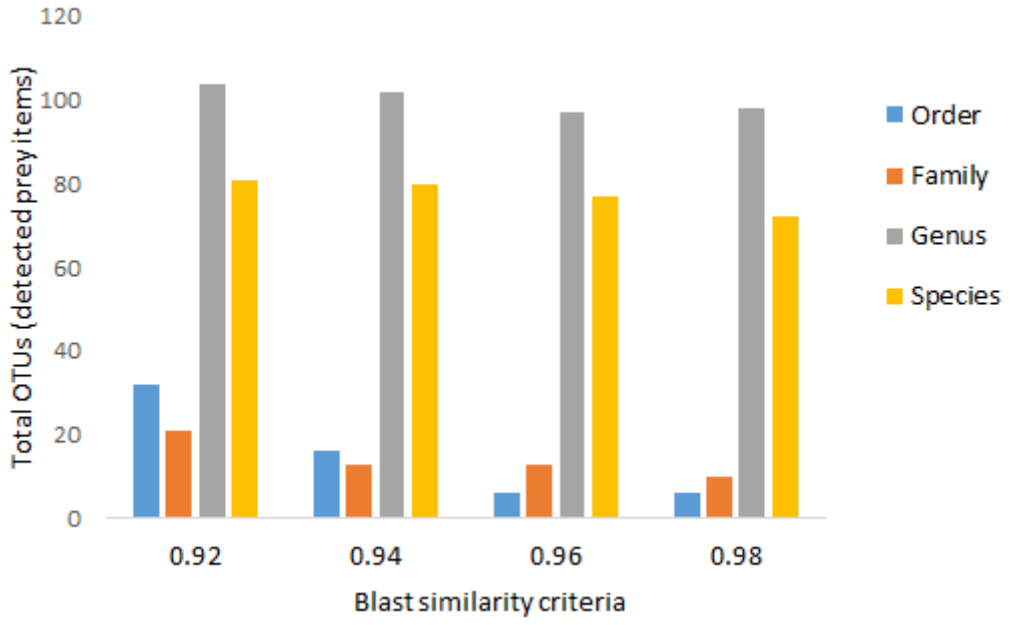


Figure 4.1 Operational Taxonomic Units (OTUs) detected in 309 meadow pipit nestling faecal sacs by level of taxonomic rank under four different BLAST critera. Each OTU consists of a cluster of at least 50 identical sequences different to other sequences in the same sample. All OTUs were tested against entries in BLAST of known taxonomic origin and assigned to the best matching taxonomic ID (species, genus or higher) if the similarity to the known entry was over the set similarity criteria (i.e. 0.92, 0.94, 0.96 or 0.98).

The average number of unassigned reads per well containing a faecal sample under the four different BLAST criteria were: 0.92 = 3,198 reads, 0.94 = 3,742 reads, 0.96 = 3,772 reads and 0.98 = 4,022 reads. There was a slight decrease in detected orders, families, genera and species with increasing BLAST similarity criteria (Figure 4.1) although some were assigned to non-prey taxa such as host (bird) species and fungi. When filtering out non-prey species the only difference in detected prey genera was observed when going from 0.96 to 0.98 BLAST similarity where five genera were lost (i.e. clusters being less than 0.98 similar to nearest entered BLAST reference for those genera) (Table 4.1) but no difference in detected genera was observed between 0.92 - 0.94 or 0.94 - 0.96 BLAST similarity. Under the highest BLAST similarity criterion (0.98) 26 OTUs were determined to species level (Table 4.2).

0.96		0.98	
Genus	**Order**	**Genus**	**Order**
Agriopis	Lepidoptera	*Agriopis*	Lepidoptera
Alopecosa	Araneae	*Alopecosa*	Araneae
Aphantopus	Lepidoptera	*Aphantopus*	Lepidoptera
Aphelia	Lepidoptera	*Aphelia*	Lepidoptera
Aphodius	**Coleoptera**		
Aphrodes	Hemiptera	*Aphrodes*	Hemiptera
Aplotarsus	Coleoptera	*Aplotarsus*	Coleoptera
Athous	Coleoptera	*Athous*	Coleoptera
Bactra	Lepidoptera	*Bactra*	Lepidoptera
Baetis	Ephemeroptera	*Baetis*	Ephemeroptera
Cantharis	Coleoptera	*Cantharis*	Coleoptera
Cerapteryx	Lepidoptera	*Cerapteryx*	Lepidoptera
Chloroperla	**Plecoptera**		
Chlorops	Diptera	*Chlorops*	Diptera
Clostera	Lepidoptera	*Clostera*	Lepidoptera
Ctenicera	Coleoptera	*Ctenicera*	Coleoptera
Culicoides	Diptera	*Culicoides*	Diptera
Dalopius	Coleoptera	*Dalopius*	Coleoptera
Dendrobaena	Haplotaxida	*Dendrobaena*	Haplotaxida
Dendrodrilus	Haplotaxida	*Dendrodrilus*	Haplotaxida
Drassodes	**Araneae**		
Eiseniella	Crassiclitellata	*Eiseniella*	Crassiclitellata
Euclidia	Lepidoptera	*Euclidia*	Lepidoptera
Isoperla	Plecoptera	*Isoperla*	Plecoptera
Javesella	Hemiptera	*Javesella*	Hemiptera
Macrothylacia	Lepidoptera	*Macrothylacia*	Lepidoptera
Mythimna	Lepidoptera	*Mythimna*	Lepidoptera
Nemurella	**Plecoptera**		
Ochsenheimeria	Lepidoptera	*Ochsenheimeria*	Lepidoptera
Oligia	Lepidoptera	*Oligia*	Lepidoptera
Operophtera	Lepidoptera	*Operophtera*	Lepidoptera
Perizoma	Lepidoptera	*Perizoma*	Lepidoptera
Phyllopertha	Coleoptera	*Phyllopertha*	Coleoptera
Plateumaris	Coleoptera	*Plateumaris*	Coleoptera
Polymerus	**Hemiptera**		
Rhagonycha	Coleoptera	*Rhagonycha*	Coleoptera
Rhopobota	Lepidoptera	*Rhopobota*	Lepidoptera
Scoparia	Lepidoptera	*Scoparia*	Lepidoptera
Tenthredopsis	Hymenoptera	*Tenthredopsis*	Hymenoptera
Tetragnatha	Araneae	*Tetragnatha*	Araneae
Tipula	Diptera	*Tipula*	Diptera
Trioza	Hemiptera	*Trioza*	Hemiptera

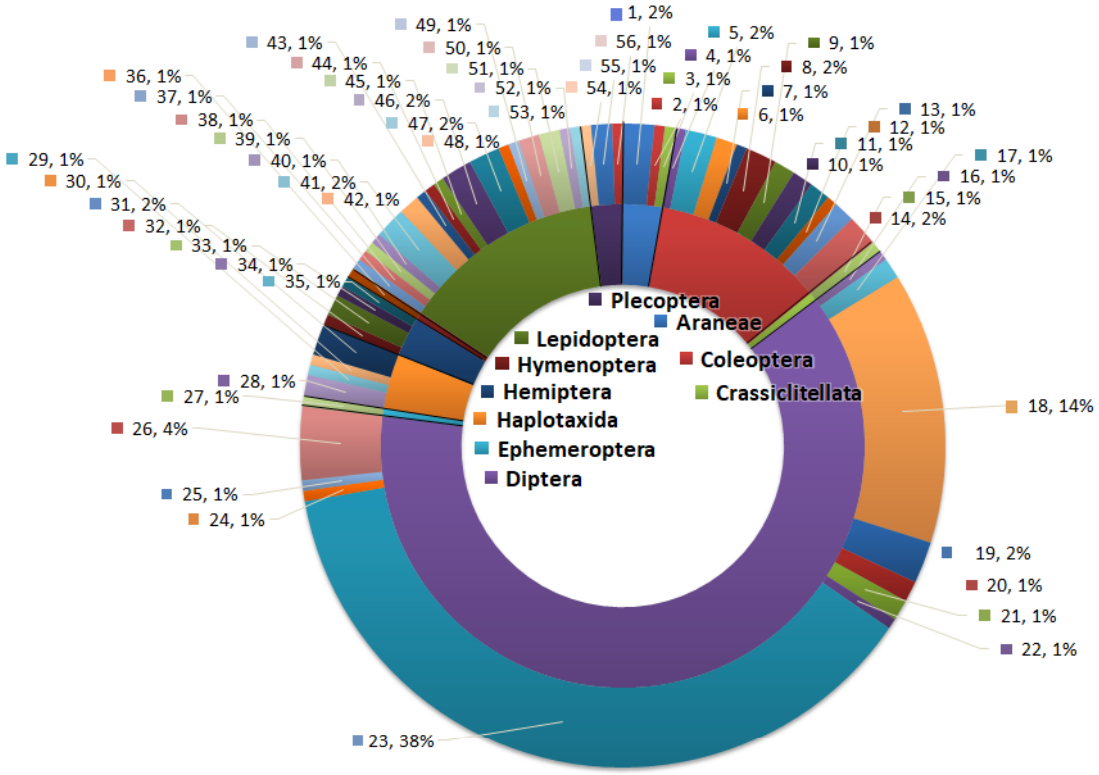
Table 4.1 Prey genera DNA detected in meadow pipit faecal sacs for 0.96 and 0.98 BLAST similarity criteria (i.e. genera of Operational Taxonomic Units (OTUs) that were more than 96 or 98 % similar to existing entries in BLAST). No difference in the number of detected prey genera was observed between 0.92, 0.94 and 0.96 BLAST similarity. Genera only assigned to OTU clusters under 0.96 or lower BLAST similarity are highlighted in bold.

Species			**Treatment Presence**			
OTU ID	Order	English name	I	II	III	IV
Tetragnatha extensa	Araneae		1	0	0	0
Aplotarsus incanus	Coleoptera	Click beetle spp.	0	1	1	1
Cantharis figurata	Coleoptera	Soldier beetle spp.	0	1	0	0
Ctenicera cuprea	Coleoptera	Coppery Click beetle	1	0	1	0
Ctenicera pectinicornis	Coleoptera	Click beetle spp.	1	1	0	0
Dalopius marginatus	Coleoptera	Click beetle spp.	1	1	0	0
Phyllopertha horticola	Coleoptera	Garden Chafer	1	0	0	0
Plateumaris sericea	Coleoptera		0	2	0	0
Rhagonycha nigriventris	Coleoptera		0	3	0	0
Eiseniella tetraedra	Crassiclitellata		1	0	0	0
Culicoides impunctatus	Diptera	Midge sp	1	0	1	0
Diptera sp. L63	Diptera		2	0	0	0
Tipula oleracea	Diptera	Cranefly spp.	1	0	0	0
Tipula varipennis	Diptera	Cranefly spp.	0	1	0	0
Dendrodrilus rubidus	Haplotaxida	Earthworm spp.	1	0	0	0
Aphrodes diminutus	Hemiptera		1	0	0	0
Stenodema holsata	Hemiptera		0	0	1	0
Agriopis marginaria	Lepidoptera	Dotted border	0	1	0	0
Cerapteryx graminis	Lepidoptera	Antler moth	2	1	0	0
Euclidia mi	Lepidoptera	Mother shipton	0	0	1	0
Macrothylacia rubi	Lepidoptera	Fox moth	0	2	0	1
Mythimna impura	Lepidoptera	Smoky wainscot	0	0	1	2
Ochsenheimeria urella	Lepidoptera	Variable Stem-moth	1	0	0	0
Operophtera brumata	Lepidoptera	Winter moth	1	1	0	0
Perizoma didymatum	Lepidoptera	Twin-spot Carpet	1	1	0	0
Rhopobota naevana	Lepidoptera	Holly Tortrix Moth	1	0	0	0
Isoperla grammatica	Plecoptera		1	0	1	0
Sum			19	16	7	4
N, 2015			28	42	33	18
N, 2016			54	46	51	37
N, Total			82	88	84	55

Table 4.2 Operation Taxonomic Unit (OUT) ID according to BLAST assignment, order and English name for all prey items determined to species level from DNA in meadow pipit faecal samples under 0.98 BLAST similarity. Treatment presence indicates the number of samples containing a species in each grazing treatment. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. Total counts of items detected in each Treatment and total sample size for each Treatment are provided in the two last rows.

4.4.2 Diet comparison to morphological studies of meadow pipit diets

For the remaining analysis of general patterns in meadow pipit diets, a BLAST similarity criterion of 0.92 was used since this output had the lowest number of unassigned reads and therefore gave the best picture of the whole diet. A total of 191 prey OTUs belonging to 56 different OTU IDs (i.e. 56 different arthropod taxa) from nine different orders were detected. These OTUs were identified to order, family, genus or species. The most abundant order was Diptera (62% of all OTUs), followed by Lepidoptera (14% of OTUs) and Coleoptera (12% of OTUs). The most abundant taxon (OTU ID) was the genus Tipula with unidentified species, that, when combined with all Tipulidae (family) OTUs made out 42% of all OTUs detected. Second most common OTU ID was undetermined Diptera (14% of all OTUs) (Figure 4.2).



1, Alopecosa
2, Drassodes
3, Tetragnatha extensa
4, Aphodius depressus
5, Aplotarsus incanus
6, Athous
7, Cantharis figurata
8, Chrysomelidae
9, Ctenicera cuprea
10, Ctenicera pectinicornis
11, Dalopius marginatus
12, Phyllopertha horticola
13, Plateumaris sericea
14, Rhagonycha nigriventris
15, Eiseniella tetraedra
16, Chlorops
17, Culicoides impunctatus
18, Diptera
19, Diptera sp. L60
20, Diptera sp. L63
21, Dolichopodidae
22, Syrphidae
23, Tipula
24, Tipula oleracea
25, Tipula varipennis
26, Tipulidae
27, Baetis
28, Aporrectodea
29, Dendrobaena
30, Dendrodrilus rubidus
31, Lumbricidae
32, Aphrodes diminutus
33, Javesella
34, Polymerus
35, Stenodema holsata
36, Tenthredopsis
37, Agriopis marginaria
38, Aphantopus
39, Aphelia
40, Bactra
41, Cerapteryx graminis
42, Clostera
43, Euclidia mi
44, Geometridae
45, Lepidoptera
46, Macrothylacia rubi
47, Mythimna impura
48, Ochsenheimeria urella
49, Oligia
50, Operophtera brumata
51, Perizoma didymatum
52, Rhopobota naevana
53, Scoparia
54, Chloroperla tripunctata
55, Isoperla grammatica
56, Nemurella pictetii

Figure 4.2 Relative abundance of prey items detected from DNA in meadow pipit faecal samples by order (inner circle) and the proportion (%) of all Operational Taxonomic Units (OTUs) in their respective orders (outer circle). The relative abundance was calculated as frequency occurrence across all faecal samples. Orders are indicated inside the circle and OTUs in the legend starting at OTUs within Araneae (12 o'clock in the circle) and moving on clockwise. Dashed lines between OTU's in the legend indicates a new order. Samples = 310, total OTUs = 191, unique OTUs = 56.

Six other studies aiming to describe the diet provisioned to meadow pipit nestlings using a range of morphological methods were found. Different units of measure were applied in these studies, which complicates comparisons, but some patterns were clear. For example, in this study Tipulidae (Diptera) DNA was found in 57% of the faecal samples containing detectable prey DNA and made up 42% of the total prey OTUs identified across all samples (Figure 4.2, Table 4.3). Tipulidae was also the most common group in Coulson *et al.* (1956) and in a previous study at this experimental site by Evans *et al.* (2005a) where a frequency occurrence measure of prey groups (i.e. not biomass estimates) was used. Moreover, two arthropod groups occur in this and all other studies (Diptera and Lepidoptera), and a further two groups (Araneae/Arachnidae and Coleoptera) were detected in all studies except Coulson (1956); in these studies, Diptera (including Tipulida) and Lepidoptera made up larger proportions of the total diet, while Araneae and Coleoptera often comprised a smaller proportion of the diet (Table 4.3), although see Klink *et al.* (2014). There were also important differences between all studies, for example, Bureš & Weidinger (2000) and Weidinger (2000) found a large proportion (20%) of Aphids (Order: Hemiptera) in the diet, Klink *et al.* (2014) found a larger proportion (38%) of Araneae than the other studies and van Oosten (2016) found an unusually large proportion of Orthoptera (19%). The two other studies from this study site (Evans *et al.* 2005a; Douglas *et al.* 2008) showed similar prey types to this study in terms of the most common prey, but there were a few groups present here that were not listed as prey in those two studies: Hemiptera, Hymenoptera, Plecoptera and Ephemeroptera, (Haplotaxida and Crassiclitellata are both often described as "Earthworms", reported by Douglas *et al.* (2008). However, Plecoptera and Ephemeroptera were both detected as prey in Coulson (1956).

Author	Method	Unit	Diet	Habitat	Country
This study	DNA (COI), faecal samples	Frequency detection from samples	Tipulidae (57%) Other Diptera (26%) Lepidoptera (14%) Coleoptera (4%) Haplotaxidae (4%) Araneae (3%) Hemiptera (3%) Plecoptera (2%) Hymenoptera (1%) Ephemeroptera (1%) Crassiclitellata (1%)	Upland grassland (Glen Finglas)	UK
Evans *et al.* 2005	Observation (binoculars)	Frequency in feeding events	Tilpulidae (ca 70%) Other Diptera (ca 15%) Lepidoptera (ca10%) Unknown (ca 7%) Arachnidae (ca 5%) Coleoptera (ca 3%)	Upland grassland (Glen Finglas)	UK
Douglas *et al.* 2008	Observation (telescope)	% diet biomass	Lepidoptera (ca 40%) Tipulidae (ca 25%) Arachnidae (Ca 4%) Other Diptera (Ca 3%) Coleoptera "Earthworms" Mollusca	Upland grassland (Glen Finglas)	UK
Coulson 1956	Observation (binoculars and telescope)	% of prey items observed	*Tipula* spp. (62%) Ephemeroptera (20%) Other Diptera (10%) Lepidoptera (5%) Plecoptera (2%)	Moorland	UK
Bures and Weidinger 2000	Neck ligatures	% of prey items collected	Diptera (46%) Hemiptera: Aphid (20%) Lepidoptera (14%) Hymenoptera (8%) Araneae (3.5%) Coleoptera (2%) Gastropoda (2%) Opilionidae Other Auchenoryncha Diplopoda	Acidified alpine grasslands	Czech Republic
van Klink *et al.* 2014	Morphology, faecal samples	% of prey items detected	Araneae (ca 38%) Lepidoptera (ca 35%) Other larvae (ca 12%) Diptera and Hymenoptera (ca 10%) Mollusca Coleoptera	Grazed salt marshes	The Netherlands
van Oosten 2016	Video recording	% of prey items detected	Unidentified (37%) Diptera (ca 19 %) Orthoptera (ca 19%) Lepidoptera (ca 15 %) Araneae (ca 7%) Others	Dune grasslands	The Netherlands

Table 4.3 Distribution of prey groups in this and other studies of meadow pipit nestling diets. In papers where larvae and adults were recorded as separate groups, these groups have been combined to provide estimates comparable to this study. Percentages were added for the largest groups where the proportion was large enough to make a good estimate as most data was available as count data only.

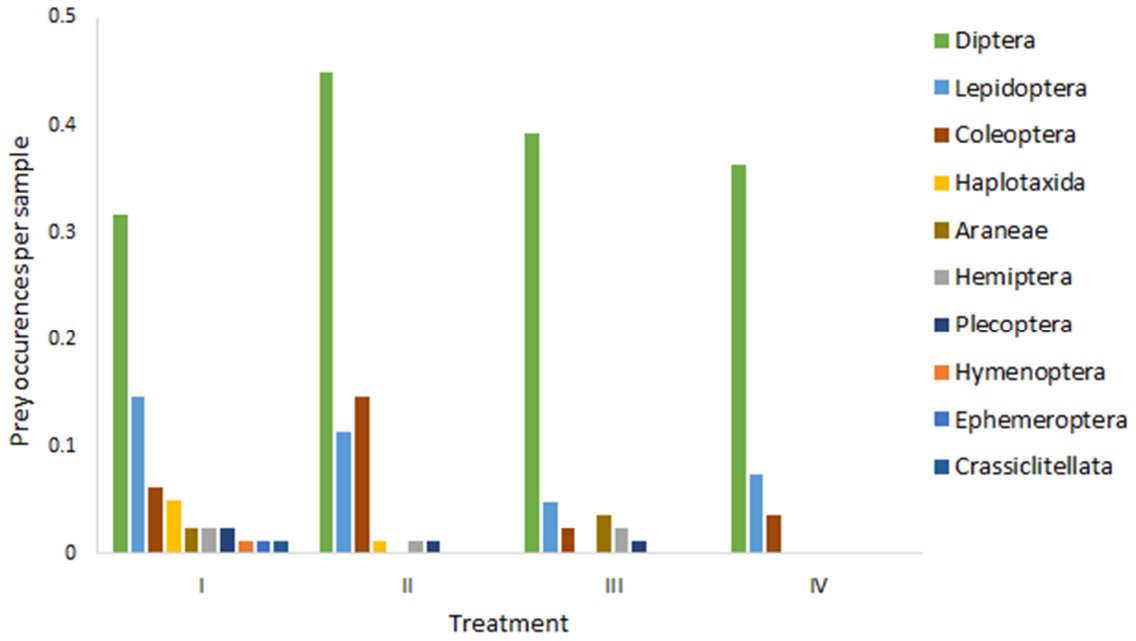
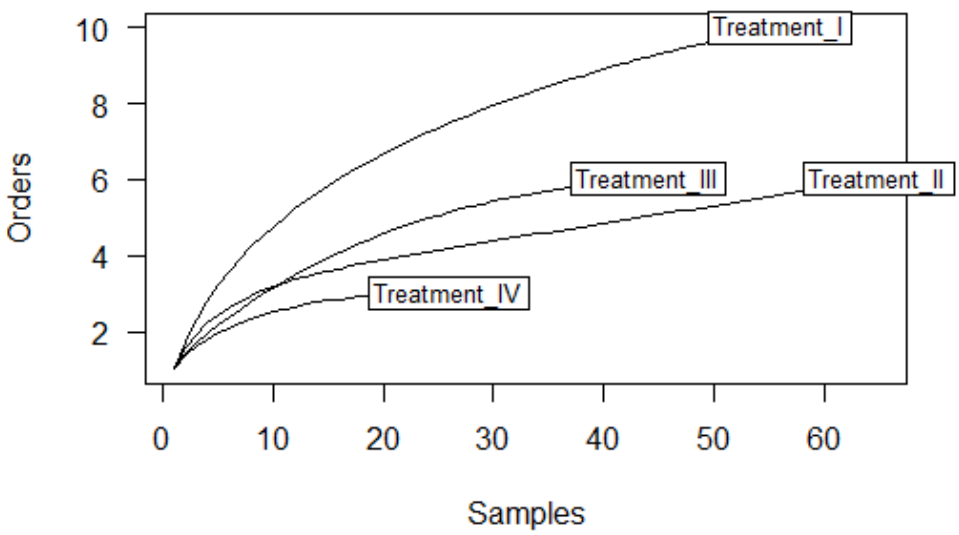


Figure 4.3 Prey DNA occurrence per meadow pipit faecal sample by taxonomic order at a minimum of 0.92 Blast Similarity (i.e. at least 92 % similarity of the assigned Taxonomic ID). Sample size for the four treatments were (I) n = 82, (II) n = 88, (III) n = 84 and (IV) n = 55. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

4.4.3 Effects of grazing pressure on nestling diet

The number of faecal sacs collected in each treatment were: I = 82, II = 88, III = 84, IV = 55. Since the sample size was smaller in Treatment IV, rarefaction curves and rarefaction values were plotted for orders and OTUs detected for the different Treatments based on a pre-set sample size of 25 randomly selected samples from each treatments (Figure 4). 25 was the highest possible sample size for an estimate since it had to be lower than the lowest number of observations in one Treatment, which was 26. The rarefaction curves indicated that there were more OTU IDs and Orders to be detected but that the Treatment with the lowest number of samples (IV) approached saturation in detected orders (Figure 4.a), suggesting that more orders were unlikely to be found in the meadow pipit diets in this treatment. Based on the rarefaction plots (figure 4) it was most logical to test the difference in diet diversity statistically by the number of orders detected as prey since i) order diversity can be

a)



b)

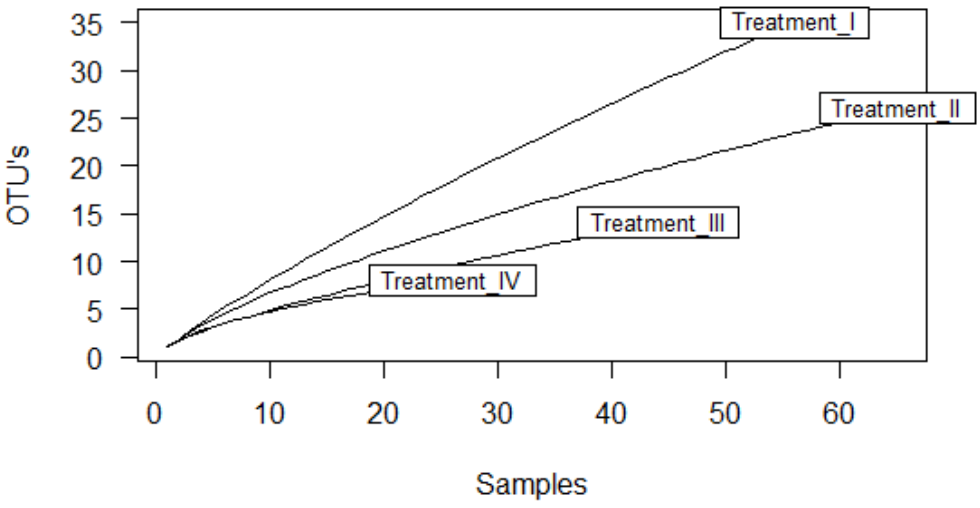


Figure 4.4 Rarefaction curves for a) orders and b) all OTUs from meadow pipit faecal samples for the four different grazing treatments. Rarefaction curves reaching saturation (a diagonal plateau) suggests that sampling for that treatment and taxonomic resolution has been saturated, where no new records are likely to be detected with further sampling. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

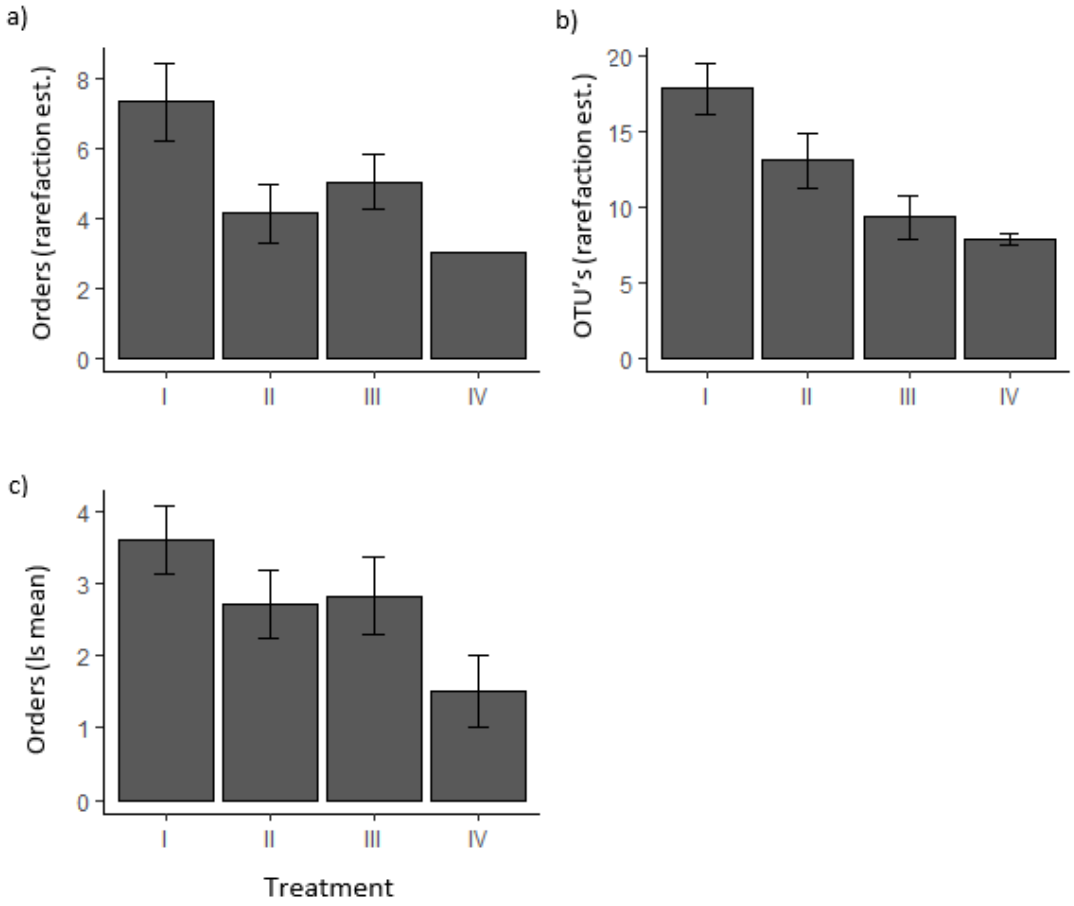


Figure 4.5 Estimated prey a) order and b) OTU ID diversity per Treatment from rarefaction estimates and c) least square means per plot drawn from a general linear mixed effects models (GLMMs) for invertebrate DNA detected in meadow pipit faecal samples. Rarefaction estimates were based on a sub-sample of 25 random samples per treatment. Least square means from GLMMs were adjusted for sample size and proportion of samples extracted during the first year for each treatment. Error bars display standard errors. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed.

considered well sampled since the treatment with fewest samples (and therefore most important group) approached saturation, in terms of new samples detected, with only a small sub-sample size (compared to OTUs) and ii) a measure of order presence is less biased by the greater range of species level-entries in reference libraries for more well studies orders, whose presence seems to vary between treatments. The rarefaction curves showed that there were more orders and OTUs detected in Treatment I than the other Treatments and fewest in Treatment IV (Figure 4.3), even after controlling for sample size (Figures 4.4a and b).

The GLMM for order numbers detected in diets per experiment plot also suggested a difference in diet diversity between treatments where the strongest difference was seen between Treatment I and Treatment IV (Table 4.4, Figure 4.5), with greatest diet diversity in the former (mean ± SD: Treatment I = 3.50 ± 1.87, II = 2.67 ± 1.03, III = 2.80 ± 1.30, IV = 1.00 ± 1.54). The least square means (i.e. means adjusted by model covariates) of order diversity per plot for each treatment were plotted to compare the treatment differences in the model to the rarefaction values to assess the accuracy of model estimates, and similar trends were observed between the two sets of values (Figures 4.5a and c). There was also a positive linear but not quadratic effect of sample size per plot on the number of orders detected per plot and an effect of sampling/extraction year with more orders detected per plot in faecal samples collected and extracted in 2015 (Table 4.4).

Variable	**χ^2**	**p**
Grazing treatment	**13.30**	**0.004**
DNA extraction year	**5.21**	**0.022**
Samples	**11.75**	**<0.001**
Samples^2	0.02	0.896
Sampling date	0.70	0.400

Table 4.4 General linear mixed model of no. of orders detected in meadow pipit faecal sacks per experiment plot. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. DNA extraction year shows the effect of the fraction of samples collected and extracted during the first season (2015) (remaining samples were collected and extracted during the second sampling year (2016)). Samples and Samples^2 indicates the number of faecal sacs collected per plot. Sampling date is the average sampling date for all faecal samples collected in each plot. Significant effects highlighted in bold, which here also demonstrated the variables kept in the final (selected) model.

4.5 Discussion

4.5.1 Method development and evaluation

A total of 56 different prey OTU IDs were detected, of which 27 could be identified to species. Although the number of reads obtained was not low, only 41% of samples gave detectable prey OTUs. It is possible that the amount of host (meadow pipit) DNA was too high for successful amplification due to template competition, while sequencing might have been compromised by more degraded prey DNA. However, bird DNA and prey DNA was

amplified and sequenced successfully in some samples, suggesting that with further optimisation the technique has good potential for prey identification. Other, more prey specific primers are an option (e.g. Zeale *et al.* 2011) although this may lead to higher primer bias between detected prey groups (Elbrecht & Leese 2017) and as invertebrates other than arthropods may be in the diet, arthropod specific primers may leave out information on such prey. There is also further work to be done to successfully extract inhibitor-free DNA from faecal samples of this kind, as many samples (up to 43% per PCR plate) did not provide any visible bands on an agarose gel. Trevelline *et al.* (2016) successfully amplified DNA from 100% of faecal samples from nestlings of another insectivorous passerine, Waterthrush *Parkesia motacilla*, which is promising, but extraction requirements may also vary between avian species depending on the chemical composition of faecal sacs. Crisol-Martínez *et al.* (2016) detected a wide diversity of prey OTUs determined to species level per faecal sample (>100 OTUs per sample) from a range of insectivorous birds in macadamia orchards in Australia, where OTU accumulation curves started to get saturated after ca. 20 samples. This suggests that with optimised methods, a huge amount of samples may not be needed to analyse the complete range of an insectivorous diet, but comparisons between individual birds or broods would still be difficult as normally, only one sample per bird is collected on each encounter/nest visit. Moreover, comparison of published results tend to be biased towards successful studies, and a certain level failure needs to be taken into account depending on previous success with similar samples.

Samples collected and extracted in the first year (2015) had a higher success rate in terms of the proportion of samples where prey DNA was detected compared to the second year (2016). This is likely to be due to the extraction protocols being used. This could be caused by to the longer drying in samples from 2016 where DNA may degenerate even further, which was due to samples required to be dried off before mixing and splitting them. It could also be due to other aspects of the extraction protocol such as small differences in amounts of reagents casuing better DNA purification in one protocol than another, even though they were considered equally successful when compared for PCR amplification success and DNA concentration in extracted DNA on spare samples prior to extraction of the actual samples.

Many studies do not sequence blank (water) extractions or negative PCR controls and rely on the detection (or the lack of) visible bands on an agarose gel to show the absence of DNA contamination. This study shows that this assumption is unsafe: although no bands were

detected on agarose gels for negative and blank controls, it was clear from the sequencing data that contaminant DNA was present. There was a significant amount of host DNA in the blank extractions, which shows that most of this contamination happen during the extraction phase, possibly when samples were dried or while transferring liquids between tubes. It is therefore important to sequence all blank and negative controls before assuming that no contamination has occurred. If a pilot study is being carried out, it can also be useful to do blank extractions starting at different stages in the extraction protocol and find the source of contamination depending on the stage when contamination is detected. In this case, contamination appeared to occur at random and as samples were extracted in a random order, there was no reason to assume that samples from one grazing treatment would be more likely to be more contaminated than from another and was unlikely to affect the comparison of diet diversity between treatments.

It is apparent from the list of prey OTUs identified to species (Figure 4.2) that the diet groups that are more studied in general, i.e. Lepidoptera and Coleoptera (Noyes & Sadka 2003), are also the ones most frequently detected at species level amongst prey OTUs. By contrast, the most frequently detected prey order was Diptera, and a large proportion of prey was not identified beyond arthropod order (Figure 4.2), which is most likely due to fewer available barcodes within Diptera to match these sequences (see Virgilio *et al.* 2010). With more references for Dipteran species, this could be a strong tool in determining diet composition of meadow pipits and other insectivorous birds. The list of prey items determined to species is promising in terms of identifying small and soft prey such as Diptera, and prey often fed on in the larval stage, such as Lepidoptera, where arthropod identification through visual observation can be difficult.

The COI gene is the most widely used DNA region for studies of metabarcoding of invertebrate communities, but it could be prone to bias, as some groups may be more easily detected than others (Clarke *et al.* 2014; Deagle *et al.* 2014; Piñol *et al.* 2015). However, it is probably the best region to use currently for samples containing a wide taxonomic distribution (Trevelline *et al.* 2016; Clarke *et al.* 2017). If more DNA markers (genetic locations) are characterised in a barcode library with sufficient reference entries (i.e. sequences of different species), study-specific markers could be selected to provide more accurate outputs of OTU identification (Deagle *et al.* 2014). The nested tagging method applied here is very useful in building larger datasets on species interactions and networks,

as it allows a large amount of individual samples to be sequenced simultaneously, without losing the information about each sample's origin.

4.5.2 Diet comparison to morphological studies of meadow pipit diets

The diet distribution found here were not remarkably different from diet composition patterns identified in other studies, and were particularly similar to previous studies conducted at the same study site. It is clear from this study and other studies in the UK that Tipulidae and Diptera in general are important prey groups (62 % of recorded OTUs in this study were from Diptera). Evans *et al.* (2005a) also observed Tipulidae to be the most common prey of Meadow Pipits, and although Douglas *et al.* (2008) estimated that Lepidoptera make up a larger mass of provisioned prey to nestlings, the total biomass intake of Tipulidae adults suggested that they were disproportionately selected by birds relative to the actual abundance of Tipulids locally. This also becomes evident here, as Chapter 2 shows very low numbers of sampled Tipulidae in 2015 compared to other arthropod groups this year, which may be due to a high abundance of Tipulidae larvae in the diets as these will not be sampled above ground by suction sampling or sweep nets. Despite some differences in their relative contribution to the meadow pipit diet, Diptera and Lepidoptera were the most common prey types in the two previous studies from this study site (Evans *et al.* 2005a; Douglas *et al.* 2008); their estimated contribution to the bird diet is likely to vary with different methods and units of measurement, between years and according to the time that has passed since the experiment treatments commenced. Moreover, the nestling diets previously observed on this study site differ more than diets observed in other countries than from the results of this study. For example, Bureš & Weidinger (2000) found that about 20% of all prey provided to nestlings in a study in the Czeck Republic were from Hemiptera which was not the case in any other study, and van Klink *et al.* (2014) found mostly Araneae specimens in the faecal samples of nestlings in a study site in the Netherlands. In addition, the differences in diet diversity observed here between grazing treatments also suggested that diet composition differs significantly between habitats, especially when the birds are located in different countries. The similarity between the three studies from this study site, Glen Finglas, suggests that the method applied here is comparable to other non-molecular methods when describing meadow pipit diets (particularly regarding the most common prey groups). However, analysis of faecal samples from feeding trials, where intake of a range of prey are compared to their detectability in faecal samples in captive birds, would be

necessary to confidently make precise estimates of invertebrate presence and composition in bird diets.

4.5.3 Effects of grazing pressure on nestling diet

Due to previous indications that extensively grazed plots with sheep only or mixed cattle and sheep grazing was a better breeding environment for meadow pipits (Evans *et al.* 2005b, 2006), extensively grazed plots were assumed to have a higher diet diversity. Instead, intensive sheep grazing, treatment I, had the highest number of observed orders and the difference between treatment I and IV was surprisingly different with ten and three orders detected, respectively (Figure 4.3). This may be due to the shorter vegetation previously observed in this treatment (Evans *et al.* 2015) which has been shown to be preferred by several ground foraging birds (Low *et al.* 2010; Schaub *et al.* 2010) including meadow pipits (Douglas *et al.* 2008; Vandenberghe *et al.* 2009). Although a varied diet has been suggested to be necessary for insectivorous birds (Hungerford *et al.* 1993) and linked to areas with higher fledgling output of Whinchat (Britschgi *et al.* 2006) it is difficult to confirm whether the increased diet diversity is an effect of more prey and therefore greater choice of available prey, or a lower abundance of preferred prey and therefore a higher intake of less preferred prey. Douglas *et al.* (2008) suggested that earthworms (which could be from both the orders Haplotaxida and Crassiclitellata) were not preferred due to their low presence in the diet in relation to abundance on foraging patches. Haplotaxida showed the highest occurrence in Treatment I in this study and it could be assumed that this was a response to less favourable conditions. On the other hand, the prey type that Douglas *et al.* (2008) showed to make up the largest biomass proportion in this study, Lepidoptera, was one of the prey types that was provided to nestlings disproportionately more than their abundance, and also showed the highest frequency in Treatment I. Although the reason for the observed pattern is not obvious, these results signal clear differences in foraging conditions of breeding insectivorous birds in different grazing treatments. These differences may be investigated further with a dataset containing more samples, or a higher proportion of samples where one or several prey types were detected.

By using orders detected per plot as a sampling unit for the GLMMs, there was a linear and not quadratic effect of the number of samples. A linear increase suggests that the number of detectable orders had not saturated with sampling effort, even with the highest numbers of samples per plot. A quadratic relationship would have indicated that the number of orders

detected would have approached a maximum value with within the number of samples collected and used in this model. To produce models of this kind, it would therefore be better to have a larger sample size for the unit on which diversity is tested, to either reach a saturation of detected orders with increasing sample size, or collect enough data to enable rarefaction curves to be extracted for each sample unit and thereby take potentially missing orders into consideration. Although this would require fairly large numbers of samples per treatment, improvements in DNA extraction and template amplification protocols might also help in this aim by providing a higher number of prey detected per sample.

There were more prey determined to species in treatment I and fewer species were detected with declining grazing pressure (Table 4.2). However, as few detected prey OTUs were identified to species level within Diptera, which were the most common prey type (Figure 4.2), this dataset would not be adequate to compare diet diversity on a species level between treatments.

4.5.4 Conclusions

This study shows that there is good potential to apply this method to study the diet composition of other British upland birds where a more detailed list of prey is needed, for example in order to assess specific habitat requirements. For example, frequent evertebrate prey such as Lepidoptera could more easily be connected to host plants if prey species instead of order was known. Although no diet assessment method comes without sampling bias or logistic problems, a few things are needed to improve the use of DNA-based methods for diet assessments: i) As Diptera and especially Tipulidae are an important part of many upland bird diets (Buchanan *et al.* 2006), more barcode references (COI or other markers) are needed for these taxa, and possibly other groups depending on the diet of the study species; ii) Controlled experiments are needed on the detection of different invertebrate orders after gut digestive processes to avoid biases in assessing diet composition, parallel with comparisons of morphological analyses of faecal samples; and iii) available extraction kits or protocols that can successfully deal with a range of avian faecal samples to allow comparison between species or studies covering a range of bird species. In order to make valid comparisons of diet diversity on a species level between habitats, it is important to make sure that possible prey items in the two habitats are equally well referenced. However, on a higher taxonomic level such as family or order, this should be a smaller problem. The method applied here suggests that habitat differences can be detected by the applied

method and that intensive grazing may provide a habitat that results in a wider diversity of meadow pipit prey compared to extensively or ungrazed plots, possibly due to easier access to some groups when vegetation is shorter.

4.6 References

Alberdi, A., Aizpurua, O., Gilbert, M.T.P. & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods Ecol. Evol.*, 9, 134–147.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I. & Fuller, R. (2013). *Bird Atlas 2007-11: The Breeding and Wintering Birds of Britain and Ireland*. BTO Books, Thetford.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). *Fitting Linear Mixed-Effects Models Using lme4*. Journal of Statistical Software.

Benton, T.G., Bryant, D.M., Cole, L. & Crick, H.Q.P. (2002). Linking agricultural practice to insect and bird populations: a historical study over three decades. *J. Appl. Ecol.*, 39, 673–687.

Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R., *et al.* (2007). The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS One*, 2, e197.

Boyles, J.G. & Storm, J.J. (2007). The perils of picky eating: dietary breadth is related to extinction risk in insectivorous bats. *PLoS One*, 2, e672.

Britschgi, A., Spaar, R. & Arlettaz, R. (2006). Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the Whinchat Saxicola rubetra: Lessons for overall Alpine meadowland management. *Biol. Conserv.*, 130, 193–205.

Buchanan, G.M., Grant, M.C., Sanderson, R.A. & Pearce-Higgins, J.W. (2006). The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis*, 148, 615–628.

Buchanan, G.M., Pearce-Higgins, J.W., Douglas, D.J.T. & Grant, M.C. (2017). Quantifying the importance of multi-scale management and environmental variables on moorland bird abundance. *Ibis*, 159, 744–756.

Bureš, S. & Weidinger, K. (2000). Estimation of calcium intake by Meadow Pipit nestlings in an acidified area. *J. Avian Biol.*, 31, 426–429.

Calladine, J., Critchley, C.N.R., Baker, D., Towers, J. & Thiel, A. (2014). Conservation management of moorland: a case study of the effectiveness of a combined suite of

management prescriptions which aim to enhance breeding bird populations. *Bird Study*, 61, 56–72.

Clare, E.L., Symondson, W.O.C., Broders, H., Fabianek, F., Fraser, E.E., MacKenzie, A., *et al.* (2014). The diet of Myotis lucifugus across Canada: assessing foraging quality and diet variability. *Mol. Ecol.*, 23, 3618–3632.

Clarke, L.J., Beard, J.M., Swadling, K.M. & Deagle, B.E. (2017). Effect of marker choice and thermal cycling protocol on zooplankton DNA metabarcoding studies. *Ecol. Evol.*, 7, 873–883.

Clarke, L.J., Soubrier, J., Weyrich, L.S. & Cooper, A. (2014). Environmental metabarcodes for insects: in silico PCR reveals potential for taxonomic bias. *Mol. Ecol. Resour.*, 14, 1160–1170.

Coulson, J.C. (1956). Biological studies on the meadow pipit (anthus pratensis) and moorland tipulidae; members of a food chain. Doctoral. Durham University.

Crisol-Martínez, E., Moreno-Moyano, L.T., Wormington, K.R., Brown, P.H. & Stanley, D. (2016). Using Next-Generation Sequencing to Contrast the Diet and Explore Pest-Reduction Services of Sympatric Bird Species in Macadamia Orchards in Australia. *PLoS One*, 11, e0150159.

Critchlow-Watton, N., Dobbie, K.E., Bell, R., Campbell, S.D.G., Hinze, D., Motion, A., *et al.* (2014). *Scotland's State of the Environment Report*. Scotland's Environment Web.

Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., *et al.* (2007). Studying Seabird Diet through Genetic Analysis of Faeces: A Case Study on Macaroni Penguins (Eudyptes chrysolophus). *PLoS One*, 2, e831.

Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F. & Taberlet, P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biol. Lett.*, 10.

Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., *et al.* (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.*, 45, 279–287.

Douglas, D.J.T., Beresford, A., Selvidge, J., Garnett, S., Buchanan, G.M., Gullett, P., *et al.* (2017). Changes in upland bird abundances show associations with moorland management. *Bird Study*, 64, 242–254.

Douglas, D.J.T., Evans, D.M. & Redpath, S.M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed

moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study*, 55, 290–296.

Douglas, D.J.T. & Pearce-Higgins, J.W. (2014). Relative importance of prey abundance and habitat structure as drivers of shorebird breeding success and abundance. *Anim. Conserv.*, 17, 535–543.

Elbrecht, V. & Leese, F. (2017). Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Front. Environ. Sci. Eng. China*, 5, 11.

Evans, D.M., Redpath, S.M. & Evans, S.A. (2005a). Seasonal patterns in the productivity of Meadow Pipits in the uplands of Scotland. *J. Field Ornithol.*, 76, 245–251.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A. & Dennis, P. (2005b). Livestock grazing affects the egg size of an insectivorous passerine. *Biol. Lett.*, 1, 322–325.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A., Gardner, C.J., Dennis, P., *et al.* (2006). Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine. *Biol. Lett.*, 2, 636–638.

Evans, D.M., Villar, N., Littlewood, N.A., Pakeman, R.J., Evans, S.A., Dennis, P., *et al.* (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere*, 6, art42.

Gales, R. p. (1987). Validation of the stomach-flushing technique for obtaining stomach contents of Penguins. *Ibis* , 129, 335–343.

Gawlik, D.E. (2002). THE EFFECTS OF PREY AVAILABILITY ON THE NUMERICAL RESPONSE OF WADING BIRDS. *Ecol. Monogr.*, 72, 329–346.

Goodbred, C.O. & Holmes, R.T. (1996). Factors Affecting Food Provisioning of Nestling Black-Throated Blue Warblers. *Wilson Bull.*, 108, 467–479.

Hart, J.D., Milsom, T.P., Fisher, G., Wilkins, V., Moreby, S.J., Murray, A.W.A., *et al.* (2006). The relationship between yellowhammer breeding performance, arthropod abundance and insecticide applications on arable farmland: Insecticides, arthropods and yellowhammer productivity. *J. Appl. Ecol.*, 43, 81–91.

Hungerford, B.S., Studier, E.H., Szuch, E.J., Pace, G.L. & Taylor, S. (1993). Aspects of caloric, nitrogen and mineral nutrition during growth in nestling eastern bluebirds, Sialia sialis. *Comp. Biochem. Physiol. A Physiol.*, 106, 385–389.

Janz, N. & Nylin, S. (1997). The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information processing hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 264, 701–707.

Jarman, S.N., Gales, N.J., Tierney, M., Gill, P.C. & Elliott, N.G. (2002). A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. *Mol. Ecol.*, 11, 2679–2690.

Jedlicka, J.A., Sharma, A.M. & Almeida, R.P.P. (2013). Molecular tools reveal diets of insectivorous birds from predator fecal matter. *Conserv. Genet. Resour.*, 5, 879–885.

Jedlicka, J.A., Vo, A.-T.E. & Almeida, R.P.P. (2017). Molecular scatology and high-throughput sequencing reveal predominately herbivorous insects in the diets of adult and nestling Western Bluebirds (Sialia mexicana) in California vineyards. *Auk*, 134, 116–127.

Jenny, M. (1990). Diet ecology of the skylark Alauda arvensis in a intensively cultivated agroecosystem in the Swiss midlands. *Ornithol. Beob.*, 87, 31–53.

Kaspari, M. & Joern, A. (1993). Prey Choice by Three Insectivorous Grassland Birds: Reevaluating Opportunism. *Oikos*, 68, 414–430.

King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C. (2008). Invited Review: Molecular analysis of predation: a review of best practice for DNA-based approaches. *Mol. Ecol.*, 17, 947–963.

King, R.A., Symondson, W.O.C. & Thomas, R.J. (2015). Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas. *Bull. Entomol. Res.*, 105, 261–272.

Kitson, J.J.N., Hahn, C., Sands, R.J., Straw, N.A., Evans, D.M. & Lunt, D.H. (2018). Detecting host-parasitoid interactions in an invasive Lepidopteran using nested tagging DNA metabarcoding. *Mol. Ecol.*

Klink, R. van, Mandema, F.S., Bakker, J.P. & Tinbergen, J.M. (2014). Foraging site choice and diet selection of Meadow Pipits Anthus pratensis breeding on grazed salt marshes. *Bird Study*, 61, 101–110.

Kluijver, H.N. (1933). Contribution to the biology and the ecology of starling (Sturnus vulgaris vulgaris L.) during its reproductive period. University of Wageningen.

Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. *J. Stat. Softw.*, 69, 1–33.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., *et al.* (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front. Zool.*, 10, 34.

Low, M., Arlt, D., Eggers, S. & Pärt, T. (2010). Habitat-specific differences in adult survival rates and its links to parental workload and on-nest predation. *J. Anim. Ecol.*, 79, 214–

224.

McInnes, J.C., Alderman, R., Lea, M.-A., Raymond, B., Deagle, B.E., Phillips, R.A., *et al.* (2017). High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Mol. Ecol.*

Mennechez, G. & Clergeau, P. (2001). Settlement of breeding European Starlings in urban areas: Importance of lawns vs. anthropogenic wastes. In: *Avian Ecology and Conservation in an Urbanizing World* (eds. Marzluff, J.M., Bowman, R. & Donnelly, R.). Springer US, pp. 275–287.

Michalski, M., Nadolski, J., Marciniak, B., Loga, B. & Bańbura, J. (2011). Faecal Analysis as a Method of Nestling Diet Determination in Insectivorous Birds: A Case Study in Blue Tits Cyanistes caeruleus and Great Tits Parus major. *Acta Ornithol.* , 46, 164–172.

Moreby, S.J. (1988). An aid to the identification of arthropod fragments in the faeces of gamebird chicks (Galliformes). *Ibis* , 130, 519–526.

Moreby, S.J. & Stoate, C. (2000). A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study*, 47, 320–331.

Noyes, J.S. & Sadka, M. (2003). Universal chalcidoidea database.

Oehm, J., Juen, A., Nagiller, K., Neuhauser, S. & Traugott, M. (2011). Molecular scatology: how to improve prey DNA detection success in avian faeces? *Mol. Ecol. Resour.*, 11, 620–628.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B., *et al.* (2015). Package "vegan" - Community Ecology Package, 8, 48–50.

van Oosten, H.H. (2016). A pilot study into nest predators of ground nesting Meadow Pipits Anthus pratensis and European Stonechats Saxicola torquatus in Dutch dune grasslands (in Dutch, with English summary). *Limosa*, 89, 179–184.

Oosten, H.H. van. (2016). Comparative Breeding Biology of Three Insectivorous Songbirds in Dutch Dune Grasslands. *Ardea*, 104, 199–212.

Orians, G.H. (1966). Food of Nestling Yellow-Headed Blackbirds, Cariboo Parklands, British Columbia. *Condor*, 68, 321–337.

Pearce-Higgins, J.W., Murray, C., M, G.B.C. & Graeme, M. (2008). International importance and drivers of change of upland bird populations. In: *Drivers of Environmental Change in Uplands* (eds. Bonn, A., Allott, T., Hubacek, K. & Stewart, J.). Routledge, pp. 209–227.

Piñol, J., Mir, G., Gomez-Polo, P. & Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative

metabarcoding of arthropods. *Mol. Ecol. Resour.*, 15, 819–830.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. (2012). Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.*, 21, 1931–1950.

Poulsen, J.G. & Aebischer, N.J. (1995). Quantitative Comparison of Two Methods of Assessing Diet of Nestling Skylarks (Alauda arvensis). *Auk*, 112, 1070–1073.

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Schaub, M., Martinez, N., Tagmann-Ioset, A., Weisshaupt, N., Maurer, M.L., Reichlin, T.S., *et al.* (2010). Patches of Bare Ground as a Staple Commodity for Declining Ground-Foraging Insectivorous Farmland Birds. *PLoS One*, 5, e13115.

Sekercioglu, C.H. (2007). Conservation ecology: area trumps mobility in fragment bird extinctions. *Curr. Biol.*, 17, R283–6.

Sheck, A.L. & Gould, F. (1996). The genetic basis of differences in growth and behaviour of specialist and generalist herbivore species: Selection of hybrids of *Heliothis virescens* and *Heliothis subflexa* (Lepidoptera). *Evolution*, 50, 831–841.

Silcock, P., Brunyee, J. & Pring, J. (2012). *Changing livestock numbers in the UK LFA – an analysis of likely biodiversity implications*. RSPB.

Symondson, W.O.C. (2002). Molecular identification of prey in predator diets. *Mol. Ecol.*, 11, 627–641.

Tang, Q.-B., Jiang, J.-W., Yan, Y.-H., Loon, J.J.A. & Wang, C.-Z. (2006). Genetic analysis of larval host-plant preference in two sibling species of Helicoverpa. *Entomol. Exp. Appl.*, 118, 221–228.

Trevelline, B.K., Latta, S.C., Marshall, L.C., Nuttle, T. & Porter, B.A. (2016). Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (Parkesia motacilla). *Auk*, 133, 415–428.

Trevelline, B.K., Nuttle, T., Hoenig, B.D., Brouwer, N.L., Porter, B.A. & Latta, S.C. (2018). DNA metabarcoding of nestling feces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat. *Oecologia*, 187, 85–98.

Vandenberghe, C., Prior, G., Littlewood, N.A., Brooker, R. & Pakeman, R. (2009). Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic Appl. Ecol.*, 10, 662–670.

Virgilio, M., Backeljau, T., Nevado, B. & De Meyer, M. (2010). Comparative performances of DNA barcoding across insect orders. *BMC Bioinformatics*, 11, 206.

Vo, A.-T.E. & Jedlicka, J.A. (2014). Protocols for metagenomic DNA extraction and Illumina amplicon library preparation for faecal and swab samples. *Mol. Ecol. Resour.*, 14, 1183–1197.

Walton, K.C. (1979). Diet of Meadow Pipits Anthus Pratensis on Mountain Grassland in Snowdonia. *Ibis* , 121, 325–329.

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis.

Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. & Jones, G. (2011). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Mol. Ecol. Resour.*, 11, 236–244.

Chapter 5: Effects of upland grazing intensity on the nutrient composition of prey provisioned to meadow pipit nestlings: a stoichiometric analysis of faeces

5.1 Abstract

There is growing interest in the study of bird prey composition, in particularly new molecular tools to assess diets. These methods allows for a better understanding of trophic interactions, but not nutrient composition, which is an important but overlooked aspect in avian biology. Nutrient concentrations in diets may be reflected in faecal samples, but studies have rarely been undertaken to test nutrient intake or how this changes as a result of environmental change. Habitat modification, such as altered grazing pressure, can lead to changes in foraging conditions for insectivorous birds through availability and abundance of food. These factors may affect essential nutrients provisioned to nestling birds by parents through changes in insect prey. The aim of this study was to test how grazing type and intensity affected nutrient insect food provisioning by meadow pipit *Anthus pratensis* parents to nestlings by examining nutrient concentrations in faecal sacs as a relatively non-invasive indicator of nutrient intake and assimilation.

Fieldwork was carried out within a replicated grazing experiment located in Glen Finglas, an upland estate in Scotland, UK. The four grazing treatments were intensive sheep, extensive sheep, extensive mixed sheep and cattle, and no grazing. Within each experimental plot, meadow pipit nests were systematically searched for and monitored during the complete nestling stage. Faecal samples were collected from nestlings across all treatments and analysed using elemental analysis and inductively coupled plasma mass spectrometry for a range of essential elements (N, P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu) and element ratios previously identified as limiting in food-webs: C:N and N:P.

An accumulated measure of essential elements in faecal material showed that all elements, except N, were at significantly higher concentrations in intensively grazed sheep plots. The ratios of C:N and N:P were not affected by grazing treatment but C:N decreased and N:P increased as the breeding season progressed and C:N was positively affected by meadow pipit breeding density.

These results suggest that, for several essential elements, grazing intensity may affect nutrient availability to, and assimilation by, insectivorous passerine nestlings and that the methods applied here can be a useful tool in detecting differences in nutrient availability to birds between habitats. However, further knowledge of nutrient egestion levels in relation to nutrient intake and nutrient deficiencies is needed to fully interpret the implications of nutrient element concentrations in avian faecal sacs.

5.2 Introduction

New DNA-based methods have offered an increase in available information on avian insectivorous diets (Jedlicka *et al.* 2013; Vo & Jedlicka 2014; Trevelline *et al.* 2016) with successful identification of hundreds of prey species in a single bird species (Crisol-Martínez *et al.* 2016). These results may give information on prey with a higher taxonomic resolution compared to previous morphological identification of prey remains in guts or faeces (Oehm *et al.* 2017), and will most likely continue to be an increasingly useful tool as the number of available barcodes, required to match a sequence with a species, is increasing. However, these methods do not provide any information on the quality of these prey: their nutritional content and concentration.

Avian diets that do not contain sufficiently available nutrients, such as minerals and vitamins, can cause nutrient deficiencies and limitations in wild, as well as captive, birds (Klasing 1998). For example, a partially theoretical study on eastern bluebirds *Sialia sialis* by Hungerford *et al.* (1993) showed that a diet of only grasshoppers, crickets or lepidopteran larvae would cause a deficiency in Sodium (Na), Calcium (Ca) or Iron (Fe) respectively. Hence access to a wide range of prey types seems necessary for insectivorous birds with similar nutrient requirements. Information about the importance of different nutrients in free living birds is hard to assess, since their intake is difficult to monitor. However, studies on wild populations have shown important effects of nutrient imbalances; lack of Ca availability in prey from vegetation on acidified soils has led to egg defects in great tits *Parus major* (Graveland & Gijzen 1994) and low Ca levels can also cause an increase in body accumulation of heavy metals in birds (Scheuhammer 1996), and thereby an increased sensitivity to

pollution. Thiamine (B1) deficiency has shown strong negative effects on the survival of young common eiders *Somateria mollissima*, which is a possible explanation for recent population declines (Mörner *et al.* 2017). Moreover, Ca supplementation provided to free living, breeding great tits increased both clutch sizes and the condition of the nestlings (Tilgar *et al.* 2002). There is also strong evidence for the importance of several other nutrient elements from studies of captive birds; N (i.e. protein) is necessary for weight retention (Brice & Grau 1991), Zinc (Zn) for nestling growth (Fox & Harrison 1964) and Fe for haemoglobin production (Davis *et al.* 1962).

N can be locally and temporarily limited (Mattson 1980) and is, after Carbon (C), often the element with highest concentration in avian faecal samples. The ratio of C:N can be used to assess nutrient flow and accumulation through food chains (Elser *et al.* 2000) and can also be used to assess protein availability to birds (Ingold & Craycraft 1983), as a higher ratio value implies a higher proportion of C relative to N, suggesting that the food source is less nutrient-dense. This could, for example, be a result of foraging on prey from a lower trophic level, as tissue N concentrations have been shown to increase with higher trophic levels in arthropods (Fagan *et al.* 2002). N:Phosphorus (P) ratios have been used to identify nutrient limitation and the effects on ecological processes at different trophic levels in food webs (Reiners 1986; Elser *et al.* 1996). The proportion of dietary P has been shown to limit primary production and community productivity in aquatic (Fourqurean *et al.* 1992; Bulgakov & Levich 1999) as well as in terrestrial environments (Koerselman & Meuleman 1996; Cleveland & Liptzin 2007). However, we do not know how these nutrient ratios might vary in bird diets in response to environmental, seasonal and breeding density factors.

The contents of avian diets and, therefore, nutrient intake, can be hard to monitor accurately. Feeding observations of small insectivorous prey to nestlings can be hard to record with accuracy (Rosenberg & Cooper 1990), and element concentrations in prey may vary with soil type and management (Janssen & Hogervorst 1993). Therefore, nutrient concentrations in nestling faecal samples may provide a useful source of information for estimating available nutrients in the diet, as a result of both diet composition and nutrient concentration in prey. Although the literature is scarce on this topic for passerine nestling birds, intake levels of nutrient elements as well as variation in element concentration in soil have previously been shown to be reflected in avian faecal samples (Summers 1993; Yin *et*

al. 2008; Ruiz *et al.* 2017). The nutrients egested in faecal material are likely to reflect elements that were not assimilated, either because they were present in excess supply in the bird diet or because they were in an unavailable form. Due to large variation during the course of a day in the diets of insectivorous birds, these samples provide a snapshot in time with large variation between samples (Orłowski *et al.* 2015), meaning that several samples from the same nest/area are necessary to detect compositional changes in response to environmental factors. However, faecal samples of nestlings can be collected when handled and as the nestlings are fed from nearby areas, nestling faecal samples can provide information on available nutrients within the area adult birds use for finding prey provided to nestlings.

Not much is known when it comes to the impact of environmental change on nutrient intake of wild birds. Changes in landscape management can have strong effects on ecological processes and functions through changes in species community structures (Folke *et al.* 2004) and nutrient flow through trophic levels (Holland *et al.* 1992). Such environmental change can also affect avian diets through variation in food availability (Mennechez & Clergeau 2001) with effects on nestling survival (Reynolds *et al.* 2003) and breeding output (Batchelor & Ross 1984; Weiser & Powell 2010). It has been shown that the composition of available prey species may change with altered management (Hollifield & Dimmick 1995; Dennis *et al.* 2008) but less is known about how this affects overall nutrient availability to free living birds. The ability to adapt to management change is partly dependent on the range of suitable prey species consumed by a bird species (Barboza *et al.* 2009). However, arthropod species vary in nutrient composition (Fagan *et al.* 2002) and not only caloric content is selected for when birds make choices among prey species (Tinbergen 1981). The composition of prey in a diet needs to contain sufficient concentrations of a range of nutrients required to maintain vital body functions (Klasing 1998) and some prey types have been shown to be more preferred than others (Kaspari & Joern 1993). Therefore, an involuntary change of diet can lead to a change in nutrient intake. Knowledge of arthropod abundances and prey preferences of birds may not provide a complete picture of available food, as food intake for insectivorous birds can also vary with limitations in prey accessibility through, for example, structural complexity of vegetation (Milsom *et al.* 1998; Douglas & Pearce-Higgins 2014) and competition with other insectivores. Moreover, availability of nutrients obtained through feeding on arthropods can vary with changes in the availability of soil and plant nutrient

status and the ability of different herbivorous and predatory arthropod species to accumulate different elements from their own diet (Janssen & Hogervorst 1993).

Grazing management is declining in many countries and, in Europe, this is mainly a result of higher intensity farming with fewer livestock fed through natural grazing (van den Pol-van Dasselaar *et al.* 2015, EUROSTAT 2016), leading to a loss of grazed habitats and potential loss of biodiversity associated with extensive grazing (Meiner and Bas 2017). Grazing has been shown to increase productivity of some plant species (McNaughton 1979) and can affect arthropod communities through changes in vegetation structure (Gardner *et al.* 1997; Hartley *et al.* 2003). Land management, such as grazing (Hilder 1964; Moran 2014) or the use of fertilizers (Butler *et al.* 2012), can also increase the nutrient content and composition of plant tissues and alter the outcome of plant-arthropod interactions (Mattson 1980). However, herbivory can both accelerate and slow down nutrient cycling (Schoonhoven *et al.* 2005). Some plants respond to stress, including stress caused by nutrient deficiencies, by increasing the mobilization of nitrogen (N) in stressed tissue and thereby availability of N to herbivores feeding on the tissue, which can lead to higher abundances of a range of plant-feeding arthropods that are limited by N availability (White 1984). Moreover, grazing has been shown to increase availability of N to plants (Holland & Detling 1990) and further affect arthropod communities, such as by increase abundances of sap-feeding arthropods, most likely through increased N availability (Moran 2014). Available N can be a limiting factor for both plant and arthropod populations, and herbivorous arthropods have evolved a range of strategies to overcome this N limitation, which varies with plant tissue type and herbivore feeding mode (Mattson 1980), suggesting that variability in vegetation composition and N status could be reflected in arthropod community composition.

Nutritional differences in avian diets have previously been observed between habitat types. For example, diets in rural vs urban areas are thought to explain poorer condition of American Crows *Corvus brachyrhynchos* in urban areas (Heiss *et al.* 2009). However, little is known about the effects of different types of livestock management on grassland birds on this aspect. Grazing management can potentially both affect prey abundance through changed nutrient availability and prey accessibility through changes in vegetation structure. The combined effect of these two processes, including their interactive effects on food

availability to birds were therefore described by Vickery *et al.* (2001) as one of the most important research areas for an increased understanding of ongoing declines in farmland bird populations. Benton *et al.* (2002) showed clear links between farming intensity, arthropod abundances and insectivorous bird populations, and Britschgi *et al.* (2006) showed that food provisioning to nestlings and nestling survival decreased with increasing farming intensity of grasslands. Altered land management is therefore a plausible effector of nutrient availability to breeding insectivorous birds.

Here, the impact of grazing intensity on nutrient availability was tested by using a replicated landscape-scale experiment with four grazing treatments: intensive sheep, extensive sheep, extensive sheep and cattle, and no grazing. Previous results from this study site have shown that the abundance of arthropods that are important in bird diets (Dennis *et al.* 2008) and moth (Lepidoptera) diversity (Littlewood 2008) decreases with increasing grazing pressure, but that meadow pipits prefer shorter vegetation with higher arthropod abundance for foraging rather than high arthropod abundance alone (Douglas *et al.* 2008; Vandenberghe *et al.* 2009). Meadow pipits have also shown higher breeding densities in sites with extensive grazing with mixed sheep and cattle (Evans *et al.* 2006) and higher egg volumes in extensive grazing with sheep only or mixed sheep and cattle (Evans *et al.* 2005) which may indicate better foraging conditions. There was also an effect of grazing intensity on meadow pipit offspring sex ratio where broods in extensive grazing with sheep only or mixed sheep and cattle had a higher proportion of males, which may, again, suggest more favourable breeding conditions in these treatments compared to no grazing or intensive sheep grazing (Prior *et al.* 2011).

This study aims to compare the effects of grazing treatment on nestling provision of a range of essential elements (N, P, Ca, Manganese (Mn), Fe, Zn, Potassium (K), Magnesium (Mg), Na, and Copper (Cu) (Macwhirter 1998) and the ratio of known limiting elements in food chains (C:N and N:P) by measuring the element concentrations in faecal samples of meadow pipit *Anthus pratensis* nestlings. The analysis of element concentrations in faecal samples is a less explored complement to studies of habitat preference and prey availability though it enables relatively non-invasive measurements (e.g. compared to gut content analysis) of the accumulated effects of prey quality and accessibility.

As management and nutrient concentrations often vary with soil type and topography, the aim here is to isolate the effects of grazing type and management from other environmental causes of variation, more specifically, the aims were to i) compare the estimated nutrient availability to nestlings in plots under different grazing management, ii) test how nutrient availability and nutrient ratios vary as the season progresses and whether this potential variation differs between the four types of grazing management and iii) evaluate the usefulness of this method for comparisons of different habitat types. It was hypothesised that grazing management affects nutrient availability to nestling birds. Due to the higher breeding densities and larger egg volumes in extensively grazed plots (Evans *et al.* 2005, 2006), it was predicted that these would provide meadow pipits with higher dietary nutrient availability with lower C:N and N:P ratios than would ungrazed or intensively grazed plots. This would be seen as higher nutrient concentrations and lower nutrient ratios in faecal sacs from extensively grazed plots compared to other treatments. Another possible outcome is that either the benefits of ungrazed plots (high arthropod abundance) or intensive grazing (e.g. high prey accessibility and plant nutrient-mediated effects of grazing) have dominant effects on prey availability and quality that lead to higher faecal concentrations of nutrients and lower nutrient ratios in these plots. It was also predicted that nutrient content in the diet would increase as the season progressed and that the observed increase would vary between grazing treatments. A seasonal increase in plant biomass may be promoted under decreased grazing pressure, which in turn could lead to high arthropod abundances. Possible outcomes could be that i) more vigorous plant growth with less grazing leads to more drastic seasonal increases in diet nutrient concentration, or that ii) grazing increases nutrient concentration in plants for herbivorous arthropod prey (Holland *et al.* 1992; Moran 2014) while also increasing accessibility to prey, leading to a stronger increase in diet nutrient concentration in intensively grazed plots compared to treatments with reduced grazing pressure or no grazing.

5.3 Methods

5.3.1 Experimental design

A grazing experiment was set up in 2003 in the Glen Finglas estate, Scotland (568160 N 48240 W) with four grazing treatments and 6 x 3.3 ha replicates of each treatment. The four treatments were I) commercial stocking density of sheep with nine ewes per plot (2.73 ewes ha^{-1}), II) a third of the commercial stocking density with three ewes per plot, III) two ewes

per plot and two cows with suckling calves during four weeks in autumn and IV) ungrazed. The study site consists of acid grassland with a majority of the ground covered by grass fam. *Poaceae,* but heather *Calluna vulgaris*, various small shrubs (e.g. *Salix* spp) and bracken *Pteridium aquilinum* are also found.

5.3.2 Sample collection

All faecal samples were collected between 29 May and 26 July, 2015, after 12 years of continuous experimentally manipulated grazing management. Nests of meadow pipits were found by walking through all plots systematically every 3 days, weather permitting. Nests were usually detected by flushing out the incubating females or occasionally observing feeding parents arriving at the nest. Nestling birds of many species, such as meadow pipits, often produce faecal sacs when handled (Ibáñez-Álamo *et al.* 2017), which makes it possible to collect complete faecal samples separately from other faecal sacs from nestlings in the same nest. In order to collect a representable amount of samples across all treatments and the whole breeding season, faecal samples were collected during nest visits with a period of 3 days between each visit, until the nestlings were 11-12 days old. However, no nests were visited during periods of rainfall to avoid that the nestlings get wet and cold while they need cover from parent birds or the nest, and not all chicks and visits produced a collected sample. Samples were collected using disposable gloves to avoid contamination and addition of nutrient elements and directly placed in individual 5 mL tubes and were stored in 99.8% ethanol at -20°C until processing. Based on observations in Vandenberghe *et al*. (2009), the foraging areas around a nest were estimated to only cover 8 % or less of the area of an experiment plot. Therefore, across all nests, most foraging would take place inside the plot/treatment in which the nest was located, and plots could be considered to be large enough to show any potential effect of treatment on diet and nutrient availability (see chapter 3 for more details).

5.3.3 Sample processing

Samples were initially analysed for the concentration of all elements measured during elemental analysis (EA) and inductively coupled plasma mass spectrometry (ICP-MS). These were N, C and H (EA) and N, P, Ca, Mn, Fe, Zn, K, Mg, Na, Caesium (Cs), Chloride (Cl), Sulfur (S), C and Cu (ICP-MS), which, together, provided measures of the nutrient ratios (C:N and N:P) and the nutrient elements listed in Macwhirter (1998) that are known to cause

deficiencies in birds and are therefore of interest in this study (P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu). The wide range of nutrients was used as a deficiency of one nutrient element may impact on the function of another (Nishizawa *et al.* 2007), hence a wide estimate provides a better overview of nutritional differences between treatments. The ethanol and water was removed from stored samples by evaporation at 50°C. The dried samples were homogenised in a TissueLyser II in stainless steel grinding jars (Qiagen, Hilden, Germany), and subsequently dried further at 70°C until there was no further weight loss. Samples were then stored in a freezer at -20°C until processing. Just before analysis, the samples were placed in liquid nitrogen and then freeze-dried overnight to ensure complete removal of water. Approximately 1 mg of each sample was weighed out and placed in a tin capsule for EA. For ICP-MS, samples were subjected to acid digestion prior to analysis. For the acid digestion procedure, samples were weighed out, placed in microwave tolerant plastic tubes, then 3 ml of concentrated nitric acid (HNO_3) (approx. 15 M) was added to each sample and left to digest at room temperature for 15 minutes. The tubes were then placed in a microwave digester with the following settings: 3 min ramp time up to 100°C followed by 2 min at 100°C, 1 min ramp time up to 120°C followed by 1 min at 120°C, 3 min ramp time up to 160°C followed by 2 min at 160°C and 2 min ramp time up to 180°C followed by 20 min at 180°C. After cooling, the tubes were vented in a fume hood to release nitrous oxide (N_20), then 1 ml of 30% hydrogen peroxide (H_2O_2) was added to each sample, and samples were left to digest for 15 min. The same microwave digestion program was then repeated. After cooling down, each sample was diluted in water to a final volume of 50 ml.

Each batch of samples processed by microwave acid digestion for analysis by ICP-MS included control blanks (i.e. without addition of biological material) and a standard reference material comprising dried tomato leaves of known nutrient element composition (National Institute of Standards and Technology, Gaithersburg, USA). Extracts of this reference material were included at the beginning, middle and end of each batch of samples analysed on the ICP-MS to standardise samples for digestion efficiency and to control for any drift in measured values during the run. Data processing involved subtraction of blank control values from each sample and inspection of tomato standard values to control for drift during the ICP-MS analysis, although drift was not detected. A subset of samples was run in duplicate with two different sample sizes (weights), and so the weight used in each sample was recorded for use as a covariate in the statistical analysis.

5.3.4 Statistical analyses

Nutrient concentrations (mg or µg per g dry mass or % dry mass) were first subject to Principal Components Analysis (PCA) using a correlation matrix; the sample scores were plotted on the first 2 Principal Components to get an overview of how different elements covaried. Pearson correlation coefficients were extracted for all element combinations. A second PCA was carried out focussing on the elements measured that are known to be limiting, or to cause deficiencies, in bird diets (N, P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu) (Macwhirter 1998). The Principal Components (PCs) from the second PCA with a standard deviation above 1 were analysed in general linear mixed effects models (GLMMs) to detect factors underlying a significant proportion of variation in the selected nutrient elements. GLMMs on the Ca concentrations and the ratio of C:N and N:P were also performed as these variables have previously been shown to be limiting, to wild insectivorous birds, specifically (Ca) (Graveland & Gijzen 1994; Tilgar *et al.* 2002), or more generally in food chains (C:N and N:P) (Ingold & Craycraft 1983; Reiners 1986; Elser *et al.* 1996).

All models included the random effects of "Block" (one of three experiment areas), "Replicate" within Block (i.e. lower or higher part of the Block on the hillside), "Plot" (the enclosure units) and "Nest". For variables where C or N was a part of the analysis, the "Plate" of samples (one of four plates analysed on the EA) was included as an additional random effect. The fixed effects were: "Grazing Treatment", which states the four different grazing treatments as factors, Julian "Date" of sample collection and "Date^2" for a quadratic effect of the Julian Date, "Number of Nestlings" (per nest), "Age of Nestlings" (at sample collection), "Breeding density" (Meadow pipit Territories per plot), "Altitude" (of the nests), "Time of Day" and "Weight x" in mg of samples processed with an elemental analyser and "Weight y" for samples processed by ICP-MS. Date and Date^2 was included to explain seasonal variance with, respectively, a linear and quadratic seasonal increase or decrease. Breeding density, Number of Nestlings and Age of Nestlings would explain variation due to competition of space and recourses from other active nests and number and size of nestlings being fed from the same parents. Altitude would explain variation due to the height on the hillside. Time of Day controls for variation during the day. The weights of the samples being processed were included to control for a possible effect of the sample size on the measure of nutrient concentrations. Fixed effects in all models were tested with Likelihood Ratio Tests (LRTs) and removed sequentially. In order to produce an unbiased result of Treatment

effects, fixed effects were only removed if they made the model worse in terms of model convergence and AIC score, but only a maximum of four variables were kept in the final model due to the relatively small number of observations. The effect of an interaction of Grazing Treatment and Date was also tested on selected models, to evaluate the hypothesis that the effect of date on prey availability and nutrition was different in different grazing treatments. Pairwise comparisons of treatment effects were done by comparing means drawn from selected models, and p-values of the comparisons were adjusted with the Holm-Bonferroni method (Holm 1979). Models were validated by checking residuals for normality and non-skewness. All models and graphs were constructed in R version 3.3.2. (R Core Team 2016). GLMMs were carried out using the package "lme4" (Bates *et al.* 2015), post hoc tests for pairwise comparisons were carried out using the package "multcomp" (Hothorn *et al.* 2008) and graphs were drawn using the packages "yarrr" (Phillips 2017) and "ggplot2" (Wickham 2009).

5.4 Results

58 meadow pipit nests were found during the breeding season, of which 24 survived long enough to have nestlings that could provide faecal samples, as most visits to nests within five days of hatching would not produce any faecal samples. From these 24 nests, 65 faecal samples were collected and processed for elemental concentrations (Table 5.1). The first two PCs explained 51.2% of variation in the data: PC1 separated carbon from a number of other nutrients, particularly Mn, Ca and Zn; PC2 separated N, P, Cl and K from Ni, Fe and Cu (Figure 1). Sample scores on the first two PC values showed a large overlap for the four grazing treatments, although Treatment IV had generally higher values on both PC axes, while Treatment I had the highest variation in PC values and the samples with lowest PC values. The correlation tests confirmed the negative correlation between concentrations of C and those of N, P and K and the high covariance between many of the other elements. The highest correlation coefficients were found between S:Cl, P:K, N:P, and N:K (Table 5.2).

Treatment	**I**	**II**	**III**	**IV**
Nests	4	8	7	5
Samples	14	21	17	13

Table 5.1 Number of nests that provided faecal samples and the number of samples processed by inductively coupled plasma mass spectrometry (ICP-MS) and elemental analysis (EA) for nutrient concentration. All samples were collected in 2015.

5.4.1 Grazing treatment effects on PCs of essential nutrients

The second PCA, based on only essential and limiting nutrient elements (N, P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu), had three PCs with a standard deviation above 1 that together explained 70% (PC1=33%, PC2=27% and PC3=10%) of the variation in nutrient element concentration. The scores on PC1 showed a negative relation with all nutrient elements tested, while PC2 and PC3 showed both negative and positive correlation coefficients with these elements (Table 5.3). The GLMM analyses showed that the scores on PC 1 and PC 3, but not PC 2, did vary with grazing treatment (Table 5.4a; Figure 5.3). A pairwise comparison from the GLMM estimates of Treatment effects showed that samples from Treatment IV (ungrazed) had significantly higher scores (PC score ± SD = 1.17 ± 1.37) on PC 1 (i.e. lower nutrient concentrations) than samples from Treatment I (intensive sheep grazing) (-1.04 ± 2.41). On PC3, Treatment II (extensive sheep grazing) samples had significantly lower scores (-0.10 ± 1.01) than those in Treatment I (0.05 ± 0.80). Remaining pairwise interactions were not significant ($p > 0.05$). PC 1 was also affected by the interaction of Grazing Treatment and Date, suggesting that nutrient concentrations varied differently over time in different grazing treatments (Table 5.4a, Figure 5.2). However, samples from different grazing treatments could not be collected evenly over the season and a larger number of samples would be needed to fully interpret effects of interactions of variables. One data point from Treatment I from late in the season seemed to contribute disproportionately to the difference in date effects between treatments to the PC1 value (Figure 5.2). Therefore, the same model was repeated without this datapoint and in that case, there was no significant interactive effect of the treatment and date ($\chi^2 = 5.521$, $p = 0.137$). Other important factors explaining the variation in scores on the PCs were Date^2, Age of Nestlings and Breeding Density (Table 5.4a), hence these variables remained in the final models.

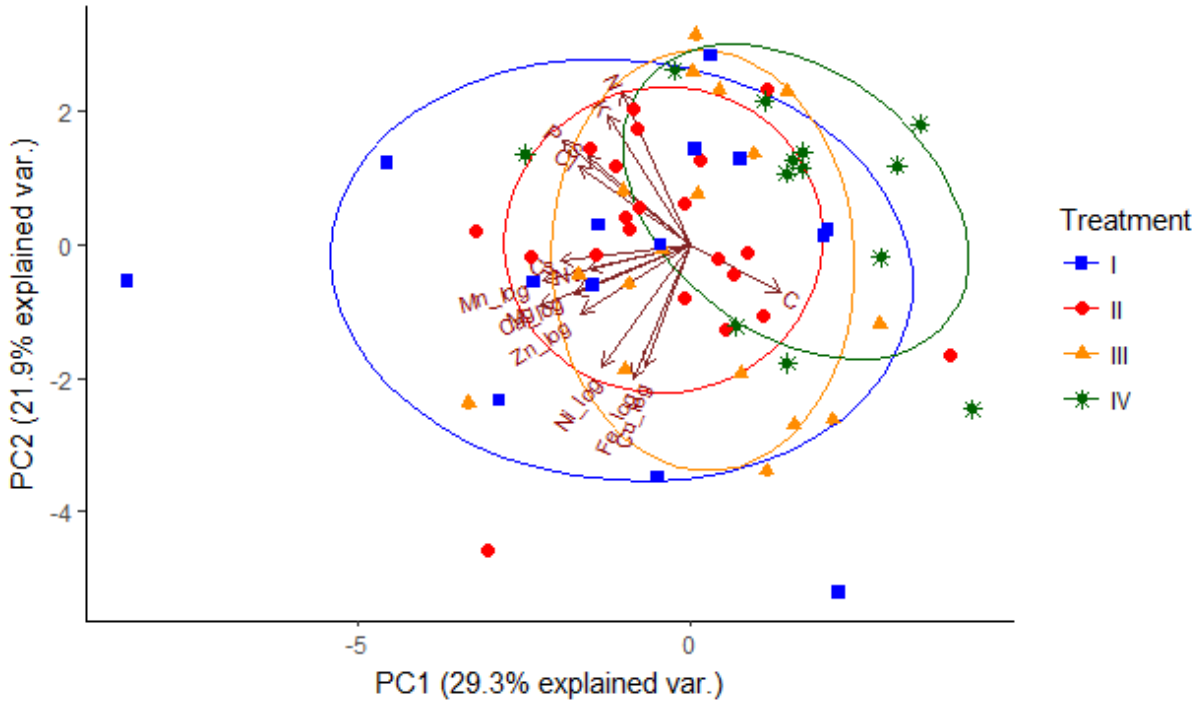


Figure 5.1 Principal Components Analysis (PCA) of concentrations of all nutrients measured (see table 5.2) in meadow pipit nestling faecal sacs. Symbols and colours indicate the four grazing treatments. Circles indicate the main distribution of each treatment according to colour. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. N = 65.

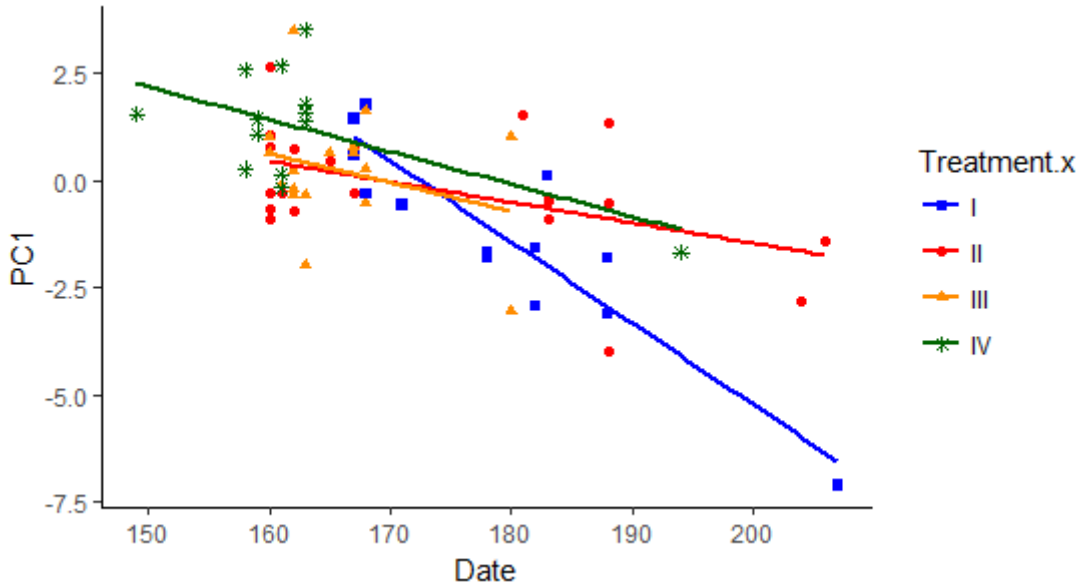


Figure 5.2 The relationship between Julian sampling date of faecal samples and the scores on Principal Component 1 from a PCA of all nutrients measured known to cause deficiencies in bird diets in the four grazing treatments. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. N = 65. Note that a higher PC score indicates a lower nutrient concentration (see table 5.3).

5.4.2 Grazing treatment effect on Ca concentrations

Ca concentrations in faecal sacs were not explained by grazing treatment nor the interaction of grazing treatment and date. Instead there was a positive, linear effect of date, suggesting more available Ca later in the season, and a negative effect of the number of nestlings per nest, indicating that nestlings in larger broods received less Ca per individual nestling (Table 5.4b, Figure 5.3).

5.4.3 C:N and N:P ratios

The ratios of C:N and N:P were not significantly affected by grazing treatment or the interaction of grazing treatment and date. C:N ratio was negatively affected by date, showing that there was more N relative to C later in the season (Figure 5.4). C:N ratio increased with increasing number of nestlings per nest, age of nestlings and breeding density. The N:P ratio was negatively affected by the non-linear, quadratic effect of date (Date^2) suggesting a non-linear increase of P to N over time (Figure 5.4). Altitude and Time of Day did not have a significant effect on any of the measured variables (Table 5.4b).

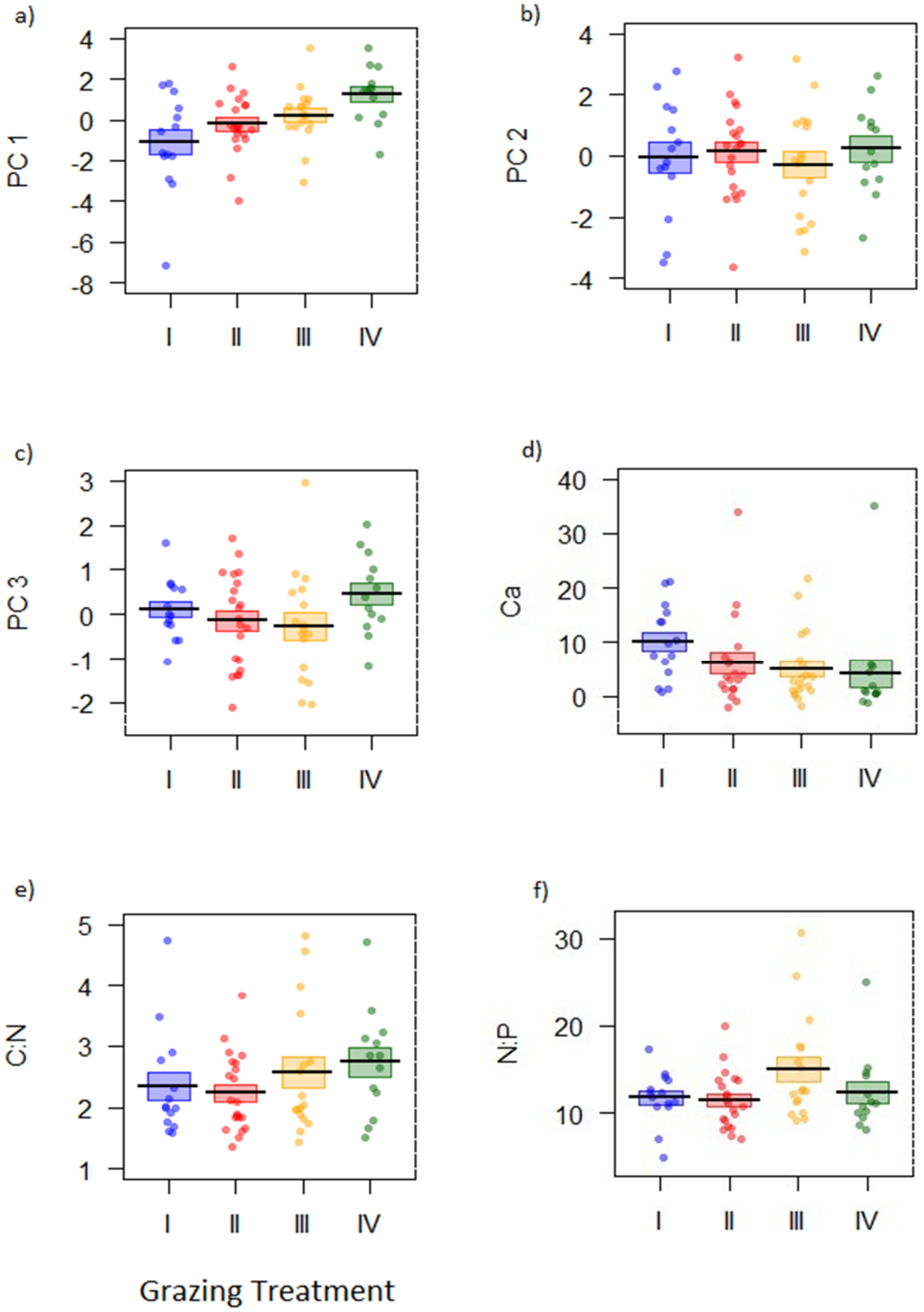


Figure 5.3 Scores on (a-c) Principal Components 1-3, (d) concentration of Ca in ppm and ratios of (e) C:N and (f) N:P in meadow pipit nestling faecal samples by treatment. The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. N = 65. The relationship between nutrient concentrations in faecal samples and the Principal Components is found in Table 5.3. Bars indicate means and surrounding boxes show standard errors. Scattered points show all data points.

a)	N	C	S	Cl	Cs	Ni^{log}	P	Ca^{log}	Mn^{log}	Fe^{log}	Zn^{log}	K	Mg	Na
C	**-0.50**													
S	**0.36**	0.02												
Cl	**0.29**	-0.08	**0.90**											
Cs	0.12	**-0.39**	**0.25**	**0.36**										
Ni^{log}	-0.21	-0.20	-0.11	-0.05	**0.28**									
P	**0.66**	**-0.44**	**0.41**	**0.35**	**0.35**	0.05								
Ca^{log}	-0.04	-0.14	**0.32**	**0.38**	**0.33**	0.18	0.10							
Mn^{log}	0.17	**-0.36**	**0.29**	**0.40**	**0.33**	**0.43**	**0.25**	**0.55**						
Fe^{log}	**-0.30**	-0.23	-0.24	-0.18	**0.31**	**0.85**	-0.13	0.18	**0.36**					
Zn^{log}	-0.05	0.08	**0.32**	**0.25**	**0.28**	0.22	0.14	**0.52**	**0.45**	0.17				
K	**0.66**	**-0.44**	**0.28**	**0.27**	0.19	-0.10	**0.73**	-0.07	0.11	-0.23	-0.10			
Mg	-0.02	-0.20	0.13	0.18	**0.53**	**0.57**	**0.47**	**0.39**	**0.54**	**0.40**	**0.42**	**0.25**		
Na	0.11	-0.04	0.10	0.10	0.23	0.22	**0.38**	0.23	**0.35**	0.02	**0.35**	0.12	**0.51**	
Cu^{log}	**-0.45**	0.13	-0.11	-0.15	0.19	**0.30**	-0.12	**0.29**	0.14	0.20	**0.52**	-0.18	**0.36**	**0.25**

Table 5.2 Correlation coefficients (Pearson's r) between concentrations of all elements measured from meadow pipit nestling faecal samples. N = 65. Significant relationships ($p < 0.05$) are indicated in bold. Variables for which the natural logarithm was used are indicated by "log".

	PC1		PC2		PC3	
	r	**p**	***r***	**p**	***r***	**p**
N	-0.14	0.268	**0.87**	**<0.001**	-0.10	0.423
P	**-0.51**	**<0.001**	**0.76**	**<0.001**	0.06	0.622
Ca^{log}	**-0.65**	**<0.001**	-0.24	0.059	-0.09	0.470
Mn^{log}	**-0.76**	**<0.001**	-0.02	0.862	**-0.40**	**0.001**
Fe^{log}	**-0.35**	**0.005**	**-0.46**	**<0.001**	**-0.65**	**<0.001**
Zn^{log}	**-0.70**	**<0.001**	**-0.29**	**0.020**	**0.27**	**0.027**
K	**-0.25**	**0.048**	**0.83**	**<0.001**	0.02	0.858
Mg	**-0.83**	**<0.001**	0.00	0.996	-0.09	0.494
Na	**-0.64**	**<0.001**	0.10	0.428	**0.36**	**<0.001**
Cu^{log}	**-0.45**	**<0.001**	**-0.55**	**<0.001**	**0.48**	**<0.001**

Table 5.3 Pearson's correlation coefficients and p values of the scores on the first three principal components (PC1-3) and measured nutrient concentrations in meadow pipit nestling faecal samples. PCs are based on all nutrients measured in this study that are known to be limiting in bird diets. Variables for which the natural logarithm has been used are marked by "log". Significant correlations ($p < 0.05$) are marked in bold.

a)

	PC 1			PC 2			PC 3		
	χ^2	p	dir.	χ^2	p	dir.	χ^2	p	dir.
Grazing Treatment	8.02	**0.046**	**NA**	0.49	0.921		8.24	**0.041**	**NA**
Date	7.81	**0.005**	**+**	0.04	0.846		7.46	**0.006**	**-**
Date^2	9.24	**0.002**	**-**	1.28	0.257		6.70	**0.010**	**+**
No of Nestlings	1.03	0.310		2.47	0.116		0.16	0.690	
Age of Nestlings	0.001	0.972		4.28	**0.039**	-	5.41	**0.020**	**+**
Breeding density	6.73	**0.009**	**-**	6.12	**0.013**	-	1.39	0.238	
Altitude	1.05	0.305		3.50	0.061		0.53	0.466	
Time of Day	0.93	0.335		0.02	0.903		0.15	0.705	
Weight x	3.32	0.068		0.000	0.995		0.81	0.369	
Weight y	2.81	0.094		0.26	0.614		1.35	0.245	
Grazing*Date	10.04	**0.018**	**NA**	1.47	0.690		4.43	0.219	

b)

	Calcium			C:N			N:P		
	χ^2	p	dir.	χ^2	p	dir.	χ^2	p	dir.
Grazing Treatment	3.78	0.286		0.47	0.922		6.14	0.105	
Date	**8.29**	**0.004**	**+**	**5.81**	**0.016**	-	**6.25**	**0.012**	**+**
Date^2	0.002	0.966		0.33	0.565		**6.54**	**0.011**	**-**
No of Nestlings	**3.95**	**0.047**	**-**	**6.51**	**0.011**	+	0.05	0.822	
Age of Nestlings	0.001	0.976		**5.54**	**0.019**	+	0.17	0.684	
Breeding density	1.12	0.289		**8.07**	**0.005**	+	3.59	0.058	
Altitude	0.07	0.788		0.04	0.837		0.02	0.888	
Time of Day	0.003	0.954		0.30	0.585		1.33	0.249	
Weight x	-	-		0.33	0.564		0.85	0.356	
Weight y	0.11	0.739		-	-		1.45	0.228	
Grazing Treatment*Date	0.62	0.892		0.61	0.893		4.43	0.218	

Table 5.4 χ^2 value, p-value and direction of significant effects (dir.) from general linear mixed models for a) scores on principal components of all nutrient elements measured that can be limiting in bird diets (N, P, Ca, Mn, Fe, Zn, K, Mg, Na and Cu) and b) the concentration of Ca and the ratio of C:N and N:P in meadow pipit nestling faecal samples. The direction indicates whether a significant relationship was positive or negative, or, when a variable consisted of a categorical factor, not applicable (NA). The four grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. The two weight factors (x and y) are weights for ICP-MS and EA analyses, respectively. Dashed lines instead of estimates are for factors that had little influence on responce variables and had to be excluded before reliable estimates could be made.

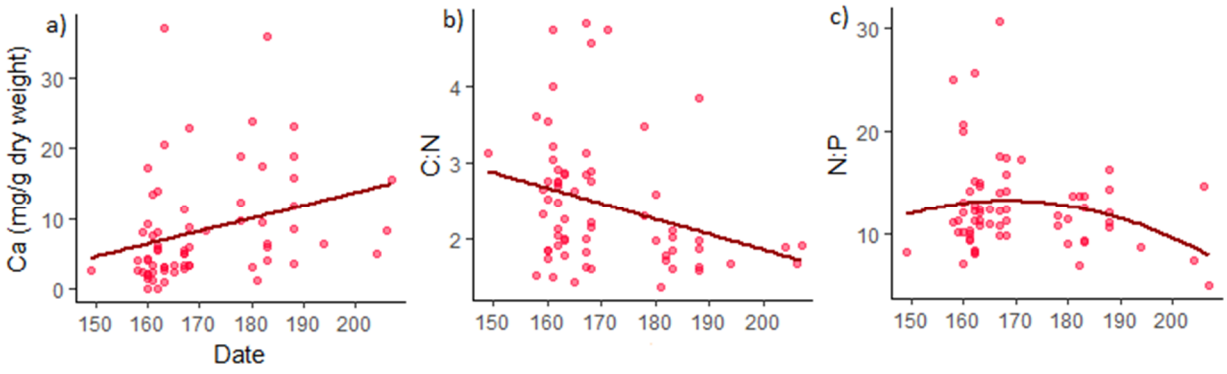


Figure 5.4 a) Ca concentration, b) C:N and c) N:P ratios in meadow pipit nestling faecal samples and Julian dates of sample collection. Trend lines indicate linear relationships between Date and Ca concentration and C:N ratio and a quadratic negative relationship between Date and N:P.

5.5 Discussion

These results suggest that nutritional differences in nestling diets caused by habitat modification can be seen through nutrient concentrations in faecal samples and show that faeces collected from nestlings in intensive sheep grazing treatments tend to have the highest nutrient concentrations in a compiled estimate of all nutrients. However, no significant differences between treatments were found for Ca concentration, C:N and N:P ratios. The observed concentrations and variation between samples and treatments is likely to be caused by variation in nutrient intake (Summers 1993; Hungerford *et al.* 1993; Yin *et al.* 2008; Ruiz *et al.* 2017) although it is important to keep in mind that egested nutrients may be caused by both saturated levels of nutrients in the diet and indigestible nutrients that have not been taken up by the nestling gut. These results also suggest that the combined estimate of nutrient concentrations in faecal samples increases non-linearly as the season progresses and is higher where breeding density is high. C:N ratio was higher with a higher number of nestlings, older nestlings and a higher breeding density, which may suggest that N to C was limited by other nestlings in the same brood and plot. Further studies that could provide tools to better implement results such as these on conservation strategies are discussed.

5.5.1 Treatment differences in nutrient availability

Based on previous results from the same study site showing that meadow pipits breed in higher densities in extensive mixed cattle and sheep grazing, Treatment III (Evans *et al.* 2006,

2015), it was hypothesised that extensive grazing would be more advantageous in terms of availability of prey, with higher nutrient concentrations that would be reflected in nutrient concentrations in nestling faecal sacs. The accumulated measure of nutrient element concentrations (i.e. the Principal Components in this analysis), suggests that the elements related to PC1 and PC3 (Table 5.3) were affected by grazing treatment (Table 5.4a). In PC1, all elements included (except N) were negatively correlated with the PC score. Therefore, as the PC score declined with increasing grazing intensity this indicates that faecal samples collected in intensively grazed plots, Treatment I, had higher concentrations of nutrients than those in other treatments, but only significantly more than in ungrazed plots, Treatment IV (Figure 5.3a). PC2 scores showed the highest correlation with N, K and P, hence the lack of Treatment effect on PC2 suggests that these elements do not vary markedly between Treatments and these nutrient levels seem to instead be negatively affected by competition of other nestlings and breeding density in the same plot (Table 5.4a). PC3 showed the strongest correlation to Fe, which was negative, and highest PC scores in Treatment II and lowest in Treatment I, suggesting that concentrations of Fe and other nutrients with a negative correlation to PC3 were lowest in Treatment II and highest in Treatment I. None of the GLMMs on PC scores provide support for the expected pattern of higher nutrients in Treatment II or III. Although food quality and accessibility may be important, breeding density may also be affected by other factors, such as vegetation that offers concealment of nest sites (Davis 2005), hence, breeding density and nutrient concentration in prey does not necessarily need to be highly correlated. The results presented here do instead indicate that Treatment I, high intensity grazing, had nestlings with the highest, and nestlings in Treatment IV the lowest, faecal nutrient concentrations in the merged estimate of all essential nutrients from the PCA (table 5.4a). This suggests that higher nutrient concentrations were provided to nestlings in intensively grazed plots and that nutrient concentration in the diet may be declining with declining grazing pressure. As the highest nutrient concentrations in faecal samples can reflect those of ingested nutrients, this could indicate a positive effect on access to preferred prey of the shorter vegetation in intensely grazed plots (Evans *et al.* 2015). This would support other studies suggesting that food accessibility through more open vegetation is as important as food abundance in the choice of foraging sites (Douglas *et al.* 2008; Vandenberghe *et al.* 2009). Alternatively, there could be a positive effect of grazing (directly on plants or indirectly through fertilisation from sheep and cattle dung) on nutrient concentrations in prey. However, the effect of Treatment

on the Principal components were just below the significance level of 0.05 and it would be desirable to see if this effect remains on a larger dataset covering several years. Insect populations can show large annual variations in abundance which can have important impacts on food availability and diet composition to insectivorous birds (Denlinger 1980; Marciniak *et al.* 2007). For example, the detected variation in faecal nutrient concentrations may be lower or higher in years when weather conditions are more or less favourable to arthropod groups common in meadow pipit diets, due to a different intake of preferred, and more nutrient rich, prey. The separate analysis of Ca, C:N and N:P ratios in faecal samples suggested that time of year and factors related to the presence of other nestlings and breeding density were more important than grazing treatment (Table 5.4b). Razeng and Watson (2015) showed that micronutrient (K, Ca, Mg, P and Zn) concentrations in prey were more linked to preferred arthropod prey than protein content, which is typically reflected by N concentration (measured in this study). Therefore, it may be more likely that effects are apparent of prey selection on faecal micronutrient concentrations than on C:N ratios if the preferred prey is more abundant in one treatment than another. As no significant treatment effects were found on Ca concentrations and N:P ratios, the nutrient elements driving the difference in observed PC scores between treatments may be other nutrients (highest correlation coefficient to PC1 was seen in Mg followed by Mn and Zn).

5.5.2 Changes in nutrient element levels during the breeding season

Prior to analysis, it was hypothesised that nutrient concentrations in faecal samples would increase and nutrient ratios of C:N and N:P would decrease as the breeding season progressed. This was true for Ca and C:N but not N:P, possibly because N and P both increased during the season (Table 5.4b). There was also a negative quadratic effect of date on the score for PC1 (Table 5.4a) although the graph of PC1 against date (Figure 5.2) suggests that overall, there was a decline in PC score as the season progressed, suggesting that the peak of PC score was very early in the season and then declining. This means that nutrient concentrations which were negatively correlated to PC1 (all but N) (Table 5.3), were increasing in faecal samples during the season. The increasing nutrient element concentrations in faecal samples during the season suggests that there is an increase in nutrient availability and supports the findings of Boyer *et al.* (2003) that suggested a bottom-up limitation of nutrients in grasslands early in the season. This indicates the importance of timing of breeding for birds in temperate climates where summers are short. The increase in

faecal nutrient levels by date could result from increasing availability of prey in general as the season progresses, and therefore greater prey choice, or from an increase in more nutrient-dense arthropods. For example, Coulson (1956) showed that meadow pipits provided a more varied diet to nestlings in the second brood of the season with more prey from Ephemeroptera and Lepidoptera, compared to the first brood which mainly consisted of Tipuloidea (Diptera). Hintz and Dyer (1970) showed that adult red-winged blackbirds *Agelaius phoeniceus* both decreased their intake of arthropods within their diets compared to plant material, and changed the composition of arthropod prey during the time from the end of breeding until early autumn. A similar difference is possible before the breeding season; Holmes (1966) showed that both food supply and diet of red-backed sandpipers *Calidris alpina* in Alaska, United States of America, who mainly feed on larvae from the Dipteran families Tipuloidea and Chironomidae, was more diverse in July compared to earlier and later in the season. Moreover, Beintema *et al.* (1991) showed that the peak in abundance of arthropods that could be suitable prey for Charadriiform waders in grassland habitats in the Netherlands was in late May to early June.

5.5.3 Differences in seasonal increase of nutrient availability between treatments

The significant interaction between Grazing Treatment and Date of PC1 scores suggests a variation in seasonal effects of nutrient concentration between the different grazing Treatments (Table 5.4a) as expected prior to analysis. However, this was only found in the GLMM of PC1 and this relationship was weak (a likely effect of a small data set) and no longer significant when removing one data point, so the results should be interpreted with caution. It is possible that some habitat types (e.g. areas with high vs low vegetation heterogeneity) or a landscape with a mix of the four treatments could provide suitable prey for breeding birds earlier and later in the season compared to a homogeneous landscape, due to peaks in arthropod abundance varying with plant species and vegetation structure (see Cole *et al.* 2015). If this result was consistent in a larger sample size it may be worthwhile considering grazing management options when studying climate effects on peaks in food and nutrient availability to insectivorous birds. Operative management actions that promotes high arthropod abundances and availability both early and late in the season could diminish potential mismatches in nutrient availability and breeding initiation. For example, Burger *et al.* (2012) showed that the seemingly preferred prey of pied flycatchers

Ficedula hypoleuca i.e. caterpillars (Lepidopteran larvae), decreased in abundance during the breeding season in oak habitats but not in other habitats and suggested that flycatchers breeding in oak habitats may be more sensitive to climate change.

5.5.4 Other variables affecting nutrient element levels

C:N ratio increased with increasing number of nestlings per nest, age of nestlings and breeding density, indicating that the N to C in the diet delivered to nestlings was affected by other nestlings in the same nest, particularly as they get bigger, and that N delivery to nestlings is also affected by other nests in the same Plot. The Principal Component of essential elements that explained most of the variation, PC1, was significantly negatively correlated with all included elements except N and was also negatively related to breeding density. This suggests that the elements correlated with PC1 were positively related to breeding density. A possible reason for this could be that meadow pipits selecting territories can predict areas where nutrient provisioning will be high, and breeding density is therefore not affecting nutrient provisioning but varies in response to available nutrients. Studies on insectivorous bird breeding densities and arthropod prey abundance have previously not shown a strong relationship (Yom-Tov 1974; Turner & McCarty 1997; Forsman *et al.* 1998; Vögeli *et al.* 2010; Douglas & Pearce-Higgins 2014). However, Eberhard and Ewald (1994) showed that Anna's Hummingbirds had smaller territories (which would result in higher breeding densities if the population is dense) when food abundance was high, but only if the territory was exposed to several intrusions and the territory had to be defended by conspecifics. Meadow pipits, being the most common bird, breed densely in this study site, which makes it possible that territories are smaller and denser where nutrient availability is high.

5.5.5 Method evaluation

It has previously been shown that insectivorous birds can actively choose the most nutrient rich prey by selecting prey types with high macro- (fat and protein) and micro- (minerals and other essential elements) nutrients (Razeng & Watson 2015) and by avoiding chitin rich prey species (Kaspari & Joern 1993) rather than feeding opportunistically on all available prey. Meadow pipits have also been shown to compensate for low Ca intake from their arthropod diet by feeding on snails shells of *Arianta arbustorum* (Bureš & Weidinger 2000). It is therefore not likely that the observed variations (e.g. by date) in faecal nutrient

concentration are passive effects of an overall increase in nutrient availability through increased prey abundance. Instead, faecal nutrient concentrations might be lower when optimal prey types are unavailable, and increased concentrations may result from diet supplementation with alternative prey, suggesting that elements that vary in relation to the number of nestlings per nest or breeding density may indeed be limited in the arthropod-based diet. Nutrient concentrations observed in faecal sacs are most likely linked to the unabsorbed nutrients in the nestling diet, reflecting dietary nutrient composition but also efficiency of absorbing nutrients as an effect of prey digestibility and nutrient demand by the growing chicks. Unfortunately, literature in this area is scarce. Knowledge of what nutrient concentrations in faecal samples are likely to indicate deficiencies for a range of insectivorous birds would therefore be needed to further interpret the impact of these results and potentially apply this method to inform habitat management for conservation measures. The sample size used here was relatively small (N = 65) and further sampling may reveal clearer effects of, for example, grazing treatments. However, this study can demonstrate that variation in nutrient availability is detectable using faecal samples with a relatively small sample size by this method and that it has has clear potential to complement studies of prey composition. Moreover, biomonitoring of polluting elements has been shown to be possible by using faecal samples from birds as a source of information (Furness & Camphuysen 1997; Yin *et al.* 2008; Clapp 2011). Therefore, faecal samples could potentially also function as a measure of how essential elements move through the food chain due to land management changes such as grazing pressure.

The C:N ratio measured here (approx. 2.5:1, Figure 5.3) were different to previously reported ratios. For example, the ratio of C:N in lesser snow geese *Chen caerulescens caerulescens* faecal samples was much higher (around 23:1) (Ruess *et al.* 1989) but the ratio of N:P (approx. 13:1, Figure 5.3) more similar to the ones of lesser snow geese (around 8:1) (Post, D. M. *et al.* 1998). The difference in C:N is probably caused by the high N concentrations in meadow pipit faecal samples (mean N in dry samples = 15.8%, mean P in dry samples = 0.0014%) compared to lesser snow geeses' lower N concentrations (N in dry samples ~ 1.8%) (Ruess *et al.* 1989). This difference might be caused by the difference in diet as geese in these studies fed on a herbivorous diet, although no other measures of C:N in insectivorous passerines were found for comparison. N:P in meadow pipit nestling samples was instead different to the faecal concentrations measured for great cormorant *Phalacrocorax carbo*

sinensis where the N:P ratios were 1.29:1 (Gwiazda *et al.* 2010). These differences are likely to be caused by differences in diets between species, but the studies mentioned above also used samples from adult birds which may feed on less nutrient-rich diets or have different nutrient requirements than growing nestlings.

5.5.6 Conclusion

This study suggests there may be variation in avian access to important nutrients caused by grazing management, which can be detected in nestling faecal samples, although no significant effect of grazing treatment was found for the concentration of Ca, C:N and N:P. In order to fully understand the impacts of environmental factors on avian conditions through nutrient limitation, it would be necessary to develop standardised measures that can signal nutrient deficiencies and their effects on birds' health based on concentrations in faecal samples. Links between nutrient intake and concentration in faecal samples have been established, but better links between arthropod diversity and nutrient intake are needed. Nutrient requirements also vary between bird species and individual circumstances, such as climate and life stage, which requires more field-based studies. By building a wider understanding of critical nutrient levels in faecal or blood samples, predictions of nutrient shortage as environmental stressors could be facilitated, and ultimately provide an aid in the monitoring of how altered land management and food webs affect avian insectivores.

5.6 References

Barboza, P.S., Parker, K.L. & Hume, I.D. (Eds.). (2009). Integrating Nutrient Supply and Demand in Variable Environments. In: *Integrative Wildlife Nutrition*. Springer Berlin Heidelberg, pp. 257–284.

Batchelor, A.L. & Ross, G.J.B. (1984). The diet and implications of dietary change of cape gannets on Bird Island, Algoa Bay. *Ostrich*, 55, 45–63.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). *Fitting Linear Mixed-Effects Models Using lme4*. Journal of Statistical Software.

Beintema, A.J., Thissen, J.B., Tensen, D. & Visser, G.H. (1991). Feeding ecology of charadriiform chicks in agricultural grassland, 79, 31–44.

Benton, T.G., Bryant, D.M., Cole, L. & Crick, H.Q.P. (2002). Linking agricultural practice to insect and bird populations: a historical study over three decades. *J. Appl. Ecol.*, 39, 673–

687.

Boyer, A.G., Swearingen, R.E., Blaha, M.A., Fortson, C.T., Gremillion, S.K., Osborn, K.A., *et al.* (2003). Seasonal variation in top-down and bottom-up processes in a grassland arthropod community. *Oecologia*, 136, 309–316.

Brice, A.T. & Grau, C.R. (1991). Protein Requirements of Costa's Hummingbirds Calypte costae. *Physiol. Zool.*, 64, 611–626.

Britschgi, A., Spaar, R. & Arlettaz, R. (2006). Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the Whinchat Saxicola rubetra: Lessons for overall Alpine meadowland management. *Biol. Conserv.*, 130, 193–205.

Bulgakov, N.G. & Levich, A.P. (1999). The nitrogen: phosphorus ratio as a factor regulating phytoplankton community structure. *Archiv für hydrobiologie*.

Bureš, S. & Weidinger, K. (2000). Estimation of calcium intake by Meadow Pipit nestlings in an acidified area. *J. Avian Biol.*, 31, 426–429.

Burger, C., Belskii, E., Eeva, T., Laaksonen, T., Mägi, M., Mänd, R., *et al.* (2012). Climate change, breeding date and nestling diet: how temperature differentially affects seasonal changes in pied flycatcher diet depending on habitat variation: Climate change, breeding date and nestling diet. *J. Anim. Ecol.*, 81, 926–936.

Butler, J., Garratt, M.P.D. & Leather, S.R. (2012). Fertilisers and insect herbivores: a meta-analysis. *Ann. Appl. Biol.*, 161, 223–233.

Clapp, J.B. (2011). Avian urate spheres: a non-invasive method to biomonitor environmental pollution and stress in birds.

Cleveland, C.C. & Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, 85, 235–252.

Cole, E.F., Long, P.R., Zelazowski, P., Szulkin, M. & Sheldon, B.C. (2015). Predicting bird phenology from space: satellite-derived vegetation green-up signal uncovers spatial variation in phenological synchrony between birds and their environment. *Ecol. Evol.*, 5, 5057–5074.

Coulson, J.C. (1956). Biological studies on the meadow pipit (Anthus pratensis) and moorland tipulidae; members of a food chain. Doctoral. Durham University.

Crisol-Martínez, E., Moreno-Moyano, L.T., Wormington, K.R., Brown, P.H. & Stanley, D. (2016). Using Next-Generation Sequencing to Contrast the Diet and Explore Pest-Reduction Services of Sympatric Bird Species in Macadamia Orchards in Australia. *PLoS*

One, 11, e0150159.

Davis, P.N., Norris, L.C. & Kratzer, F.H. (1962). Iron deficiency studies in chicks using treated isolated soybean protein diets. *J. Nutr.*, 78, 445–453.

Davis, S.K. (2005). Nest-Site Selection Patterns and the Influence of Vegetation on Nest Survival of Mixed-Grass Prairie Passerines. *Condor*, 107, 605–616.

Denlinger, D.L. (1980). Seasonal and Annual Variation of Insect Abundance in the Nairobi National Park, Kenya. *Biotropica*, 12, 100–106.

Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., *et al.* (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.*, 45, 279–287.

Douglas, D.J.T., Evans, D.M. & Redpath, S.M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study*, 55, 290–296.

Douglas, D.J.T. & Pearce-Higgins, J.W. (2014). Relative importance of prey abundance and habitat structure as drivers of shorebird breeding success and abundance. *Anim. Conserv.*, 17, 535–543.

Eberhard, J.R. & Ewald, P.W. (1994). Food availability, intrusion pressure and territory size: and experimental study of Anna's Hummingbirds (Calypte Anna). *Behav. Ecol. Sociobiol.*, 34, 11–18.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996). Organism Size, Life History, and N:P Stoichiometry. *Bioscience*, 46, 674–684.

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., *et al.* (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408, 578–580.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A. & Dennis, P. (2005). Livestock grazing affects the egg size of an insectivorous passerine. *Biol. Lett.*, 1, 322–325.

Evans, D.M., Redpath, S.M., Evans, S.A., Elston, D.A., Gardner, C.J., Dennis, P., *et al.* (2006). Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine. *Biol. Lett.*, 2, 636–638.

Evans, D.M., Villar, N., Littlewood, N.A., Pakeman, R.J., Evans, S.A., Dennis, P., *et al.* (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere*, 6, 42.

Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A., *et al.* (2002).

Nitrogen in Insects: Implications for Trophic Complexity and Species Diversification. *Am. Nat.*, 160, 784–802.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., *et al.* (2004). Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. *Annu. Rev. Ecol. Evol. Syst.*, 35, 557–581.

Forsman, J.T., Mönkkönen, M., Helle, P. & Inkeröinen, J. (1998). Heterospecific attraction and food resources in migrants' breeding patch selection in northern boreal forest. *Oecologia*, 115, 278–286.

Fourqurean, J.W., Zieman, J.C. & Powell, G.V.N. (1992). Phosphorus limitation of primary production in Florida Bay: Evidence from C:N:P ratios of the dominant seagrass Thalassia testudinum. *Limnol. Oceanogr.*, 37, 162–171.

Fox, M.R. & Harrison, B.N. (1964). Use of Japanese quail for the study of zinc deficiency. *Proc. Soc. Exp. Biol. Med.*, 116, 256–259.

Furness, R.W. & Camphuysen, K. (C J.). (1997). Seabirds as monitors of the marine environment. *ICES J. Mar. Sci.*, 54, 726–737.

Gardner, S.M., Hartley, S.E., Davies, A. & Palmer, S.C.F. (1997). Carabid communities on heather moorlands in northeast Scotland: The consequences of grazing pressure for community diversity. *Biol. Conserv.*, 81, 275–286.

Graveland, J. & Gijzen, T.V. (1994). Arthropods and Seeds are not Sufficient as Calcium Sources for Shell Formation and Skeletal Growth in Passerines. *Ardea*, 82, 299–314.

Gwiazda, R., Jarocha, K. & Szarek-Gwiazda, E. (2010). Impact of a small cormorant (Phalacrocorax carbo sinensis) roost on nutrients and phytoplankton assemblages in the littoral regions of a submontane reservoir. *Biologia*, 65, 828.

Hartley, S.E., Gardner, S.M. & Mitchell, R.J. (2003). Indirect Effects of Grazing and Nutrient Addition on the Hemipteran Community of Heather Moorlands. *J. Appl. Ecol.*, 40, 793–803.

Heiss, R.S., Clark, A.B. & McGowan, K.J. (2009). Growth and nutritional state of American Crow nestlings vary between urban and rural habitats. *Ecol. Appl.*, 19, 829–839.

Hilder, E.J. (1964). The distribution of plant nutrients by sheep at pasture. In: *Proceedings of the Australian Society of Animal Production*. pp. 241–248.

Hintz, J.V. & Dyer, M.I. (1970). Daily Rhythm and Seasonal Change in the Summer Diet of Adult Red-Winged Blackbirds. *J. Wildl. Manage.*, 34, 789–799.

Holland, E.A. & Detling, J.K. (1990). Plant Response to Herbivory and Belowground Nitrogen

Cycling. *Ecology*, 71, 1040–1049.

Holland, E.A., Parton, W.J., Detling, J.K. & Coppock, D.L. (1992). Physiological responses of plant populations to herbivory and their consequences for ecosystem nutrient flow. *Am. Nat.*, 140, 685–706.

Hollifield, B.K. & Dimmick, R.W. (1995). Arthropod Abundance Relative to Forest Management Practices Benefiting Ruffed Grouse in the Southern Appalachians. *Wildl. Soc. Bull.*, 23, 756–764.

Holmes, R.T. (1966). Feeding Ecology of the Red-Backed Sandpiper (Calidris Alpina) in Arctic Alaska. *Ecology*, 47, 32–45.

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. *Scand. Stat. Theory Appl.*, 6, 65–70.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.*, 50, 346–363.

Hungerford, B.S., Studier, E.H., Szuch, E.J., Pace, G.L. & Taylor, S. (1993). Aspects of caloric, nitrogen and mineral nutrition during growth in nestling eastern bluebirds, Sialia sialis. *Comp. Biochem. Physiol. A Physiol.*, 106, 385–389.

Ibáñez-Álamo, J.D., Rubio, E. & Soler, J.J. (2017). Evolution of nestling faeces removal in avian phylogeny. *Anim. Behav.*, 124, 1–5.

Ingold, J.L. & Craycraft, M.J. (1983). Avian frugivory on honeysuckle (Lonicera) in soutwestern Ohio in fall.

Janssen, M.P. & Hogervorst, R. (1993). Metal accumulation in soil arthropods in relation to micro-nutrients. *Environ. Pollut.*, 79, 181–189.

Jedlicka, J.A., Sharma, A.M. & Almeida, R.P.P. (2013). Molecular tools reveal diets of insectivorous birds from predator fecal matter. *Conserv. Genet. Resour.*, 5, 879–885.

Kaspari, M. & Joern, A. (1993). Prey Choice by Three Insectivorous Grassland Birds: Reevaluating Opportunism. *Oikos*, 68, 414–430.

Klasing, K.C. (1998). *Comparative avian nutrition*. Cab International.

Koerselman, W. & Meuleman, A.F.M. (1996). The Vegetation N:P Ratio: a New Tool to Detect the Nature of Nutrient Limitation. *J. Appl. Ecol.*, 33, 1441–1450.

Littlewood, N.A. (2008). Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.*, 1, 151–160.

Macwhirter, P. (1998). 31: Malnutrition. In: *Avian Medicine: Princilpes and Application* (eds. Ritchie, B.W., Harrison, G.J. & Harrison, L.R.). Wingers Publishing, Inc., pp. 842–861.

Marciniak, B., Nadolski, J., Nowakowska, M., Loga, B. & Bańbura, J. (2007). Habitat and Annual Variation in Arthropod Abundance Affects Blue Tit Cyanistes caeruleus Reproduction. *Acta Ornithol.* , 42, 53–62.

Mattson, W.J.J. (1980). Herbivory in Relation to Plant Nitrogen Content. *Annu. Rev. Ecol. Syst.*, 11, 119–161.

McNaughton, S.J. (1979). Grazing as an Optimization Process: Grass-Ungulate Relationships in the Serengeti. *Am. Nat.*, 113, 691–703.

Mennechez, G. & Clergeau, P. (2001). Settlement of breeding European Starlings in urban areas: Importance of lawns vs. anthropogenic wastes. In: *Avian Ecology and Conservation in an Urbanizing World* (eds. Marzluff, J.M., Bowman, R. & Donnelly, R.). Springer US, pp. 275–287.

Milsom, T.P., Ennis, D.C., Haskell, D.J., Langton, S.D. & McKay, H.V. (1998). Design of grassland feeding areas for waders during winter: The relative importance of sward, landscape factors and human disturbance. *Biol. Conserv.*, 84, 119–129.

Moran, M.D. (2014). Bison Grazing Increases Arthropod Abundance and Diversity in a Tallgrass Prairie. *Environ. Entomol.*, 43, 1174–1184.

Mörner, T., Hansson, T., Carlsson, L., Berg, A.-L., Ruiz Muñoz, Y., Gustavsson, H., *et al.* (2017). Thiamine deficiency impairs common eider (Somateria mollissima) reproduction in the field. *Sci. Rep.*, 7, 14451.

Nishizawa, Y., Morii, H. & Durlach, J. (2007). *New Perspectives in Magnesium Research: Nutrition and Health*. Springer.

Oehm, J., Thalinger, B., Eisenkölbl, S. & Traugott, M. (2017). Diet analysis in piscivorous birds: What can the addition of molecular tools offer? *Ecol. Evol.*, 7, 1984–1995.

Orłowski, G., Kamiński, P., Karg, J., Baszyński, J., Szady-Grad, M., Koim-Puchowska, B., *et al.* (2015). Variable contribution of functional prey groups in diets reveals inter- and intraspecific differences in faecal concentrations of essential and non-essential elements in three sympatric avian aerial insectivores: A re-assessment of usefulness of bird faeces in metal biomonitoring. *Sci. Total Environ.*, 518–519, 407–416.

Phillips, N. (2017). *yarrr: A Companion to the e-Book "YaRrr!: The Pirate's Guide to R."*

Post, D. M., Taylor, J.P., Kitchell, J.F., Olson, M.H., Schindler, D.E. & Herwig, B.R. (1998). The Role of Migratory Waterfowl as Nutrient Vectors in a Managed Wetland. *Conserv. Biol.*, 12, 910–920.

Prior, G.L., Evans, D.M., Redpath, S., Thirgood, S.J. & Monaghan, P. (2011). Birds bias

offspring sex ratio in response to livestock grazing. *Biol. Lett.*, 7, 958–960.

Razeng, E. & Watson, D.M. (2015). Nutritional composition of the preferred prey of insectivorous birds: popularity reflects quality. *J. Avian Biol.*, 46, 89–96.

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reiners, W.A. (1986). Complementary Models for Ecosystems. *Am. Nat.*, 127, 59–73.

Reynolds, S.J., Schoech, S.J. & Bowman, R. (2003). Diet quality during pre-laying and nestling periods influences growth and survival of Florida scrub-jay (Aphelocoma coerulescens) chicks. *J. Zool.*, 261, 217–226.

Rosenberg, K.V. & Cooper, R.J. (1990). Approaches to avian diet analysis. *Studies in avian biology*.

Ruess, R.W., Hik, D.S. & Jefferies, R.L. (1989). The role of lesser snow geese as nitrogen processors in a sub-arctic salt marsh. *Oecologia*, 79, 23–29.

Ruiz, S.R., Espín, S., Sánchez-Virosta, P., Salminen, J.-P., Lilley, T.M. & Eeva, T. (2017). Vitamin profiles in two free-living passerine birds under a metal pollution gradient – A calcium supplementation experiment. *Ecotoxicol. Environ. Saf.*, 138, 242–252.

Scheuhammer, A.M. (1996). Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. *Environ. Pollut.*, 94, 337–343.

Schoonhoven, L., van Loon, J. & Dicke, M. (2005). *Insect-Plant Biology*.

Summers, J.D. (1993). Reducing Nitrogen Excretion of the Laying Hen by Feeding Lower Crude Protein Diets. *Poult. Sci.*, 72, 1473–1478.

Tilgar, V., Mänd, R. & Mägi, M. (2002). Calcium shortage as a constraint on reproduction in great tits Parus major: a field experiment. *J. Avian Biol.*, 33, 407–413.

Tinbergen, J.M. (1981). Foraging Decisions in Starlings (Sturnus vulgaris L.). *Ardea*, 69, 1–67.

Trevelline, B.K., Latta, S.C., Marshall, L.C., Nuttle, T. & Porter, B.A. (2016). Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (Parkesia motacilla). *Auk*, 133, 415–428.

Turner, A.M. & McCarty, J.P. (1997). Resource availability, breeding site selection, and reproductive success of red-winged blackbirds. *Oecologia*, 113, 140–146.

Vandenberghe, C., Prior, G., Littlewood, N.A., Brooker, R. & Pakeman, R. (2009). Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic Appl. Ecol.*, 10, 662–670.

Vickery, J.A., Tallowin, J.R., Feber, R.E., Asteraki, E.J., Atkinson, P.W., Fuller, R.J., *et al.* (2001).

The Management of Lowland Neutral Grasslands in Britain: Effects of Agricultural Practices on Birds and Their Food Resources. *J. Appl. Ecol.*, 38, 647–664.

Vo, A.-T.E. & Jedlicka, J.A. (2014). Protocols for metagenomic DNA extraction and Illumina amplicon library preparation for faecal and swab samples. *Mol. Ecol. Resour.*, 14, 1183–1197.

Vögeli, M., Serrano, D., Pacios, F. & Tella, J.L. (2010). The relative importance of patch habitat quality and landscape attributes on a declining steppe-bird metapopulation. *Biol. Conserv.*, 143, 1057–1067.

Weiser, E.L. & Powell, A.N. (2010). Does Garbage in the Diet Improve Reproductive Output of Glaucous Gulls? *Condor*, 112, 530–538.

White, T.C.R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, 63, 90–105.

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis.

Yin, X., Xia, L., Sun, L., Luo, H. & Wang, Y. (2008). Animal excrement: a potential biomonitor of heavy metal contamination in the marine environment. *Sci. Total Environ.*, 399, 179–185.

Yom-Tov, Y. (1974). The Effect of Food and Predation on Breeding Density and Success, Clutch Size and Laying Date of the Crow (Corvus corone L.). *J. Anim. Ecol.*, 43, 479–498.

Chapter 6. General discussion

This thesis aimed to describe the long-term impact of grazing intensity on meadow pipit breeding conditions and explain the mechanisms behind such potential effects. In order to better understand the processes that may limit breeding success in upland birds, two methods previously not applied to avian studies in areas under environmental change were used: DNA metabarcoding of prey in faecal samples and estimates of nutrient concentration intake from faecal samples. Together with the arthropod and meadow pipit breeding surveys (gathered during the early and late stages of a unique, fully-replicated livestock grazing experiment), these studies suggested several effects of treatment on meadow pipit breeding conditions. For example, both diversity of prey orders and estimated nutrient concentration intake were higher in intensively grazed plots, while arthropod mass was highest in extensively sheep grazed plots and ungrazed plots. The methods applied to describe prey composition and nutrient intake were hence able to underline important differences between the grazing treatments and appear to have high potential for further development and applications in avian ecology studies. It was also apparent that the time since management change may have influenced the grazing treatment effects on the abundance of common arthropod prey groups, particularly in extensively, sheep grazed plots compared to other treatments. There was a higher nest survival and fledgling output in extensively grazed plots compared to intensive or no grazing and the estimate of fledglings produced per plot was highest in extensively cattle and sheep grazed plots in the later sampling period, although this trend was non-significant. However, external landscape factors, mainly increased predation pressure between the two sampling periods, had a more pronounced effect on fledgling output and nest survival than grazing treatment. As breeding density was lowest in plots where egg-stage nest survival was highest, this suggests that predation pressure may be difficult to predict prior to breeding and that breeding site selection is rather based on foraging conditions. Large-scale, long-term management experiments are therefore an invaluable resource for disentangling short- and long-term effects of land management from other factors such as surrounding management or different levels of predation pressure.

Chapter	Variable	χ^2	p	I	II	III	IV
2. Arthropods	Araneae	**20.39**	**< 0.001**	**-**	**+**	.	**+**
	Hemiptera	**14.55**	**0.002**	**-**	**+**	.	**+**
	Coleoptera	3.53	0.317	.	.	.	.
	Total mass	**9.07**	**0.028**	**-**	.	.	**+**
	Lepidoptera	**12.67**	**0.005**	**-**	**+**	**+**	**+**
	Tipuloidea	1.33	0.723	.	.	.	.
3. Breeding meadow pipits	Breeding Density	**20.15**	**< 0.001**	**+**	**-**	**+**	.
	Clutch Size	0.36	0.940	.	.	.	.
	Hatch Date	**13.01**	**0.005**	**-**	**+**	**-**	**-**
	No. of Fledglings	2.63	0.452	.	.	.	.
	Est. Fledglings /Plot	5.87	0.118	.	.	.	.
	Eggs to Fledging Survival	2.54	0.469	.	.	.	.
	Egg-stage Survivial	**10.07**	**0.018**	**-**	**+**	.	.
	Nestling-stage Survival	0.37	0.946	.	.	.	.
4. Diets from prey DNA	Diet Diversity by Orders	**13.30**	**0.004**	**+**	.	.	**-**
5. Nutrients in diets	PC1 (all nutrients)*	8.02	**0.046**	**+**	.	.	**-**
	PC2 (all nutrients)	0.49	0.921	.	.	.	.
	PC3 (all nutrients)**	8.24	**0.041**	**a**	**b**	.	.
	Calcium	3.78	0.286	.	.	.	.
	C:N	0.47	0.922	.	.	.	.
	N:P	6.14	0.105	.	.	.	.

Table 6.1 Treatment effects of all variables tested for an effect of grazing treatment. When samples were collected during more than one year (chapters 2,3 and 4) the table is showing the overall effect across both/all years. Significant differences between treatments are shown under the treatment columns where a positive sign indicates a significantly higher response value than in the treatment with a negative sign. No significantly different intermediate response values were observed. The grazing treatments were: I) intensive sheep, II) extensive sheep, III) extensive mixed sheep and cattle and IV) ungrazed. *Significant differences for Principal Component 1 (PC1) shows the relationship between measured nutrient concentrations in faecal samples (i.e. higher values in Treatment I than IV) for easier interpretation, which is the opposite of the relationship of PC1 values (see Table 5.3). **Nutrients significantly correlated to PC3 were both potitvely and negatively correlated to PC3 scores (Table 5.3), although the PC scores were significantly higher in treatment I than II, indicated by different letters insead of "+" or "-".

6.1 Compiled grazing effects on breeding conditions

6.1.1 Arthropod abundance and occurrence in diet

Chapter 2 investigated the effects of grazing on the abundance of common arthropods in insectivorous birds' diets. Few similarities were observed between the meadow pipit nestlings' diet (shown in Chapter 4), and the abundance of specific arthropod groups or total mass from sweep net samples. For example, the most common arthropod groups (Araneae and Hemiptera, Chapter 2, Table 2.1, Figure 2.2) were not the most frequently occurring prey groups. However, this is not surprising as feeding meadow pipits in this study site (Evans *et al.* 2005; Douglas *et al.* 2008) and other insectivorous birds are known to forage selectively in order to maximise nutrient intake (Kaspari & Joern 1993). Moreover, Lepidoptera, a preferred prey type (Douglas *et al.* 2008), occurred most often in the diet of nestlings in intensively grazed plots and least in ungrazed plots (Chapter 4, Figure 4.3). The abundance of Lepidoptera sampled by sweep nets was instead most abundant in ungrazed plots in this (Chapter 2, Figure 2.2f and 2.4b) and other studies from this field site (Dennis *et al.* 2008; Littlewood 2008). Differences in the diet was only statistically tested for the number of prey orders, which was highest in intensively grazed and lowest in ungrazed plots (Chapter 4, Table 4.4, Figure 4.5), and therefore did not show any similarity in treatment effects with the abundance or total mass of arthropods (Chapter 2, Figure 4.2). Abundance of the most common arthropod groups and total arthropod mass was highest in plots extensively grazed by sheep only and ungrazed plots in the later sampling period, when faecal samples for diet assessment were collected. Breeding density was also lowest in the treatment where arthropod mass was highest, extensively sheep grazed plots, which suggests that arthropod mass or abundance alone are insufficient predictors of breeding conditions if food accessibility is not considered. This goes in line with the result from Evans *et al.* (2015) suggesting that prey availability is more important than prey abundance in ungrazed or extensively grazed plots, where vegetation biomass is higher.

6.1.2 Foraging conditions and predation pressure

Chapters 3-5 suggested a common trend in which intensively grazed plots may provide the best foraging conditions: intensively grazed plots had the highest, and extensively sheep grazed plots the lowest, breeding density (especially in the later stage) (Chapter 3, Figure 3.1); Diet range was widest in terms of detected arthropod orders in intensively grazed plots and lowest in ungrazed plots (Chapter 4, Figure 4.3). The estimated nutrient concentration

intake based on the compiled measure of nutrient concentration in nestling faecal samples was also highest in intensively grazed plots and lowest in ungrazed plots (Chapter 5, Figure 5.3). More research on the benefits (or disadvantages) of a varied nestling diet in insectivorous nestling birds would be needed to fully interpret the potential impact of these results. In the meantime, other measures may indicate whether trends observed in diets are reflected by positive effects on breeding variables such as foraging preferences or nestling survival.

Previous results from the Glen Finglas experiment by Vandenberghe *et al.* (2009) suggested that breeding meadow pipits flew longer distances in order to provide food to nestlings in intensively sheep grazed plots compared to extensively grazed plots by both sheep and cattle, which would contradict the results found here that intensively grazed plots would provide better foraging conditions. However, the effects of grazing on vegetation may have changed during the time since the study by Vandenberghe *et al.* (2009) was carried out (sampling took place in 2007). More importantly, seemingly better foraging conditions (based on nutrient concentrations in faecal samples, Chapter 5, and the range of diet prey orders, Chapter 4) are not reflected in the breeding output observed in Chapter 3. The number of fledglings per nest was not significantly affected by grazing treatment, but showed a trend towards higher fledgling output in the two extensively grazed treatments (Chapter 3, Figure 3.3). Hence, if foraging conditions were better in intensively grazed plots, there are no indications of this outweighing other effects on fledgling output or nest survival, such as predation. Predation was the highest cause of nest failure (72% of failed nests) which may be even higher if the proportion of nests that failed due to predation of parents and subsequent starvation of nestlings were known. The rates of nest failure and predation were higher in intensively grazed and ungrazed plots (Chapter 3, Figure 3.4). Although parameters such as higher fledgling weights (Magrath 1991; Naef-Daenzer *et al.* 2001) and early laying dates (Sanz 2002) can be linked to increased juvenile survival through better foraging conditions, nest survival is an important factor for population demography in short lived passerines (Clark & Martin 2007), such as meadow pipits. Therefore, habitats that produce well-fed fledglings while being less exposed to predation are important.

Newton (2004) suggested that increased grazing pressure by sheep has caused declines of many upland birds. The results found here do also show a trend for meadow pipits, the most

common upland passerine, although not significant for fledgling output but significant for egg survival, of higher nest failure in intensively grazed and ungrazed plots. The advantages of intense grazing for Meadow Pipit feeding conditions may have resulted from better prey availability through lower vegetation height (Douglas *et al.* 2008; Vandenberghe *et al.* 2009; Low *et al.* 2010) as the intensively grazed treatments have been shown to have shorter vegetation (Evans *et al.* 2015). The lower mortality during the egg stage in extensively sheep grazed plots may instead be a response to better nest concealment than intensively grazed plots and lower predation pressure than ungrazed plots (Low *et al.* 2010; Villar *et al.* 2013). A heterogeneous landscape with both short and tall vegetation may therefore improve food availability (Dennis *et al.* 1998; Buchanan *et al.* 2006) and provide a better habitat for single species' breeding abundance and/or nest survival (Homberger *et al.* 2017; Whittingham & Evans 2004). A heterogeneous landscape is also favourable for avian diversity as it will meet the habitat requirements of a wider range of bird species (Benton *et al.* 2003; Fuhlendorf *et al.* 2006; Pearce-Higgins & Grant 2006; Coppedge *et al.* 2008).

6.1.3 Management for heterogeneous landscapes suitable for breeding inseVtivorous upland birds

In order to create heterogeneous habitats for birds and other species in upland areas, a range of managements applications at different times and intensities may be needed (Bonari *et al.* 2017; Buchanan *et al.* 2017). In general, Agri-Environment Schemes, which compensate farmers for financial loss as a result of measures taken to increase biodiversity, are more likely to improve ecosystem services, as a consequence of increased biodiversity, in heterogeneous than homogenous landscapes (Whittingham 2011). Increased heterogeneity may be achieved by a mix of, for example, grazing and burning at different times and intensities and thereby creating a mosaic of vegetation at different levels (Hovick *et al.* 2015). However, labour intensive management may be complicated and unprofitable in remote upland areas where soils have low productivity (Critchlow-Watton *et al.* 2014). Moreover, local land management only explains a small proportion of the variation in abundance of many upland bird populations, and management for improved habitats may therefore be more cost-efficient if limited to sites that already meet other environmental criteria (Buchanan *et al.* 2017). One management type may therefore not fit all conservation purposes and environments. For example, this study site at Glen Finglas showed some variation between experimental areas (e.g. proximity to woodland and shrubs, ground

humidity and altitude) which may have resulted in differential effects within the same treatment. This may be a reason for the large variation seen in enclosures with the same grazing treatment (e.g. in breeding density, Chapter 3, Figure 3.1). Moreover, the effects of grazing intensity on plant diversity have been shown to vary with altitude (Speed *et al.* 2013), and the effects of grazing intensity on soil uptake of carbon can vary with climate and precipitation (McSherry & Ritchie 2013). On sites with special conservation concern, other site specific measures and regulations of grazing intensity could be applied, such as average sward height (Diack *et al.* 2000) or utilisation levels by livestock adapted to site-specific vegetation (Pakeman & Nolan 2009) rather than stocking density.

It is also likely that some species (e.g. many wader species) are more limited by high predation pressure than local land use (Fletcher *et al.* 2010; Roos *et al.* 2018), even after attempts to control predators (Calladine *et al.* 2014). As the UK has a high density of mesopredators compared to many other European countries, and predator control is costly, further research is needed on management that naturally results in a decrease of predation pressure on ground-nesting birds (Roos *et al.* 2018).

6.2 Observed differences of short- and long-term effects

The arthropod and breeding meadow pipit surveys carried out in the later sampling period (2015-16) showed some important differences compared with the earlier sampling period (2003-5) while other effects remained more consistent, or were less prominently changed than other local factors, such as the increased predation pressure across all treatments (Chapter 3). For example, total arthropod mass was high in ungrazed plots in both the early sampling year (2005) and later sampling year (2015) while extensively sheep grazed plots had a similarly low total mass as intensively grazed plots in the early year but much higher mass than ungrazed plots in the later sampling year (2015) (Chapter 2, Figure 2.3). This suggests that treatments that are more similar to each other, such as different grazing treatments compared to commercial (intense) or no grazing changes, may take longer to show visible differences. The difference between more similar treatments may also be masked by inter-annual variation, and a dataset based on more consecutive years would be needed for differences in breeding conditions to be observed. Prior to analysis, a general increase in the magnitude of treatment effect between the early and late sampling periods

was predicted. Although the meadow pipit fledgling output (Chapter 3, Figure 3.3) appears to have changed from no effect to a weak effect during the sampling periods, this result was not significant and no other similar trends were observed. Instead, the changes observed, such as in arthropod total mass mentioned above, did not occur as predicted. This suggests that grazing experiments such as the present study site may be very important for predicting the long-term effects of a range of trophic levels (see Evans *et al.* 2015). During the course of the experiment, shrubs have appeared in the ungrazed plots and to some extent in extensively grazed plots. Another decade of continued treatments may see the development of trees and other new elements such as more predators, with potentially stronger effects on breeding meadow pipits and other breeding bird species.

6.3 The potential of using DNA-based techniques and nutrient availability estimates of faecal samples for avian science

Several new questions can be answered using DNA-based techniques for diet assessments alongside nutrient intake estimates. For example, information on both diet and nutrient concentration intake may make it easier to highlight the cause of altered fledging conditions. Therefore, studies evaluating these two methods in a captive, controlled environment could result in useful tools for avian conservation research. It is, however, clear from this study (Chapter 4) that more reference DNA sequences are needed for groups such as Diptera to reliably analyse upland bird diets to a species level using molecular tools. Although there is a potential for primer bias in metabarcoding of a wide range of species (Clarke *et al.* 2014) that may benefit from more purpose-optimised primer design (Elbrecht & Leese 2017), some level of method-bias (Moreby & Stoate 2000) or level of uncertainty (Oosten 2016) is often present in morphological identification methods as well. Molecular, DNA-based methods for prey identification may also be at risk of contamination, both during sampling and sample processing. However, the error rates when studying diets through, for example, visual observations may be harder to estimate without comparing the applied method to other methods, while error rates (i.e. potential contamination) using barcoding of prey species may be estimated by sequencing blank extractions. Improving and subsequently applying diet and nutrient intake assessments more widely will be useful for an increased understanding of the habitat sensitivity of avian species and suitable restoration strategies. For example, it is only in a species' natural environment, with accurate energy and nutrient

needs, that we can understand how a shortage of certain nutrients affect individual vitality and, ultimately, population demography and distribution.

To better explain the mechanisms behind grazing pressure on meadow pipit breeding conditions, the impacts of diet diversity and specific prey groups in the diet of both adult and nestling birds on breeding output ought to be tested. However, the results found here suggest that breeding success is increasingly limited by predation in this field site and identifying the main predators there, and how they are linked to grazing management, may be more important in this situation. As the effects of extensively grazed and ungrazed plots on vegetation have had time to develop, more bird species are present in the experiment plots than what was observed when the experiment commenced and the plots where almost solely inhabited by meadow pipits (unpublished data from territory mapping). For example, several nests of black grouse *Tetrao tetrix*, a red listed species in the UK, have been encountered in the ungrazed plots and nests of Whinchats *Saxicola rubetra* and Stonechats *Saxicola rubicola* are encountered in the extensively grazed plots in the later sampling period. This could have negative consequences for meadow pipits due to competition of territories with other insectivorous species (Orians and Willson 1964), but seems to be positive for local bird diversity and the abundance of other bird species of conservation concern. The benfits of habitats containing a mosaic of these management types, from intensively grazed to ungrazed areas, may therefore be more pronounced after long-term management, although that question remains to be investigated.

It is obvious that the field of DNA-based methods for studies of trophic interactions, such as prey of insectivorous birds, is advancing rapidly. However, few articles highlight any technical difficulties or limitations, which is necessary for avoiding repetition of similar failures and improving the field in a cost-efficient way. Moreover, little is known about the nutritional consequences of altered arthropod diets, and hence their importance on avian reproduction and survival. Effects on nutrient intake during the nestling stage may not be apparent immediately but may instead be shown on annual recruitment or future breeding output. Further studies should therefore focus on applying nutritional and prey identification analyses of diets simultaneously on studies of both nestling condition and annual survival rates.

6.4 Conclusions

The strong declines of British upland bird populations (Balmer *et al.* 2013) requires large efforts in terms of research and management. The results presented here suggest that it may be straightforward to detect management (i.e. grazing intensity) effects on a specific variable or within a specific time period (e.g. a few consecutive years) but when analysing several factors during a longer time period, treatment effects on breeding conditions appear more multifaceted. Therefore, a range of aspects, such as foraging conditions, predation pressure and the scale of space and time of management alterations should be considered when examining the impacts of land-use on breeding bird populations. New tools such as species-level, DNA-based diet assessments may help in these predictions. Even if established metabarcoding and sequencing methods are available, there is still an important amount of work left in building reference collections that match all generated sequences with prey species or families. This means that taxonomists still have an important role in developing diet assessment techniques, while new skills will be required in bioinformatics to handle the large output of sequences generated from increasingly efficient, high-throughput sequencing methods. Together with large-scale management experiments, DNA-based tools could provide a mechanistic, ecological network-approach for understanding how species of conservation concern respond to habitat modifications.

6.5 References

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I. & Fuller, R. (2013). *Bird Atlas 2007-11: The Breeding and Wintering Birds of Britain and Ireland*. BTO Books, Thetford.

Benton, T.G., Vickery, J.A. & Wilson, J.D. (2003). Farmland biodiversity: is habitat heterogeneity the key? *Trends Ecol. Evol.*, 18, 182–188.

Bonari, G., Fajmon, K., Malenovský, I., Zelený, D., Holuša, J., Jongepierová, I., *et al.* (2017). Management of semi-natural grasslands benefiting both plant and insect diversity: The importance of heterogeneity and tradition. *Agric. Ecosyst. Environ.*, 246, 243–252.

Buchanan, G.M., Grant, M.C., Sanderson, R.A. & Pearce-Higgins, J.W. (2006). The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis*, 148, 615–628.

Buchanan, G.M., Pearce-Higgins, J.W., Douglas, D.J.T. & Grant, M.C. (2017). Quantifying the importance of multi-scale management and environmental variables on moorland bird

abundance. *Ibis*, 159, 744–756.

Calladine, J., Critchley, C.N.R., Baker, D., Towers, J. & Thiel, A. (2014). Conservation management of moorland: a case study of the effectiveness of a combined suite of management prescriptions which aim to enhance breeding bird populations. *Bird Study*, 61, 56–72.

Clarke, L.J., Soubrier, J., Weyrich, L.S. & Cooper, A. (2014). Environmental metabarcodes for insects: in silico PCR reveals potential for taxonomic bias. *Mol. Ecol. Resour.*, 14, 1160–1170.

Clark, M.E. & Martin, T.E. (2007). Modeling tradeoffs in avian life history traits and consequences for population growth. *Ecol. Modell.*, 209, 110–120.

Coppedge, B.R., Fuhlendorf, S.D., Harrell, W.C. & Engle, D.M. (2008). Avian community response to vegetation and structural features in grasslands managed with fire and grazing. *Biol. Conserv.*, 141, 1196–1203.

Critchlow-Watton, N., Dobbie, K.E., Bell, R., Campbell, S.D.G., Hinze, D., Motion, A., *et al.* (2014). *Scotland's State of the Environment Report*. Scotland's Environment Web.

Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., *et al.* (2008). The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.*, 45, 279–287.

Dennis, P., Young, M.R. & Gordon, I.J. (1998). Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. *Ecol. Entomol.*, 23, 253–264.

Diack, I.A., Burke, M., Peel, S. & Others. (2000). Grassland management for nature conservation: towards a consistent approach to sward measurement and description. In: *Grazing management: the principles and practice of grazing, for profit and environmental gain, within temperate grassland systems. Proceedings of the British Grassland Society Conference held at the Cairn Hotel, Harrogate, 29 February-2 March 2000.* BBSRC Institute of Grassland and Environmental Research, pp. 155–156.

Douglas, D.J.T., Evans, D.M. & Redpath, S.M. (2008). Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland: Capsule Foraging sites with low vegetation height and density, but with high arthropod biomass, are selected. *Bird Study*, 55, 290–296.

Elbrecht, V. & Leese, F. (2017). Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Front. Environ. Sci. Eng. China*, 5, 11.

Evans, D.M., Redpath, S.M. & Evans, S.A. (2005). Seasonal patterns in the productivity of Meadow Pipits in the uplands of Scotland. *J. Field Ornithol.*, 76, 245–251.

Evans, D.M., Villar, N., Littlewood, N.A., Pakeman, R.J., Evans, S.A., Dennis, P., *et al.* (2015). The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. *Ecosphere*, 6, 42.

Fletcher, K., Aebischer, N.J., Baines, D., Foster, R. & Hoodless, A.N. (2010). Changes in breeding success and abundance of ground-nesting moorland birds in relation to the experimental deployment of legal predator control. *J. Appl. Ecol.*, 47, 263–272.

Fuhlendorf, S.D., Harrell, W.C., Engle, D.M., Hamilton, R.G., Davis, C.A. & Leslie, D.M., Jr. (2006). Should heterogeneity be the basis for conservation? Grassland bird response to fire and grazing. *Ecol. Appl.*, 16, 1706–1716.

Homberger, B., Duplain, J., Jenny, M. & Jenni, L. (2017/5). Agri-evironmental schemes and active nest protection can increase hatching success of a reintroduced farmland bird species. *Landsc. Urban Plan.*, 161, 44–51.

Hovick, T.J., Elmore, R.D., Fuhlendorf, S.D., Engle, D.M. & Hamilton, R.G. (2015). Spatial heterogeneity increases diversity and stability in grassland bird communities. *Ecol. Appl.*, 25, 662–672.

Kaspari, M. & Joern, A. (1993). Prey Choice by Three Insectivorous Grassland Birds: Reevaluating Opportunism. *Oikos*, 68, 414–430.

Littlewood, N.A. (2008). Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.*, 1, 151–160.

Low, M., Arlt, D., Eggers, S. & Pärt, T. (2010). Habitat-specific differences in adult survival rates and its links to parental workload and on-nest predation. *J. Anim. Ecol.*, 79, 214–224.

Magrath, R.D. (1991). Nestling Weight and Juvenile Survival in the Blackbird, Turdus merula. *J. Anim. Ecol.*, 60, 335–351.

McSherry, M.E. & Ritchie, M.E. (2013). Effects of grazing on grassland soil carbon: a global review. *Glob. Chang. Biol.*, 19, 1347–1357.

Moreby, S.J. & Stoate, C. (2000). A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study*, 47, 320–331.

Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001). Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *J. Anim. Ecol.*, 70, 730–738.

Newton, I. (2004). The recent declines of farmland bird populations in Britain: an appraisal of

causal factors and conservation actions. *Ibis* , 146, 579–600.

Oosten, H.H. van. (2016). Comparative Breeding Biology of Three Insectivorous Songbirds in Dutch Dune Grasslands. *Ardea*, 104, 199–212.

Orians, G.H. & Willson, M.F. (1964). Interspecific Territories of Birds. *Ecology*, 45, 736–745.

Pakeman, R.J. & Nolan, A.J. (2009). Setting sustainable grazing levels for heather moorland: a multi-site analysis. *J. Appl. Ecol.*, 46, 363–368.

Pearce-Higgins, J.W. & Grant, M.C. (2006). Relationships between bird abundance and the composition and structure of moorland vegetation. *Bird Study*, 53, 112–125.

Roos, S., Smart, J., Gibbons, D.W. & Wilson, J.D. (2018). A review of predation as a limiting factor for bird populations in mesopredator-rich landscapes: a case study of the UK: Predation on UK birds. *Biol Rev*, The Birds of the Western Palearctic, 63, 10.

Sanz, J.J. (2002). Climate change and breeding parameters of great and blue tits throughout the western Palaearctic. *Glob. Chang. Biol.*, 8, 409–422.

Speed, J.D.M., Austrheim, G. & Mysterud, A. (2013). The response of plant diversity to grazing varies along an elevational gradient. *J. Ecol.*, 101, 1225–1236.

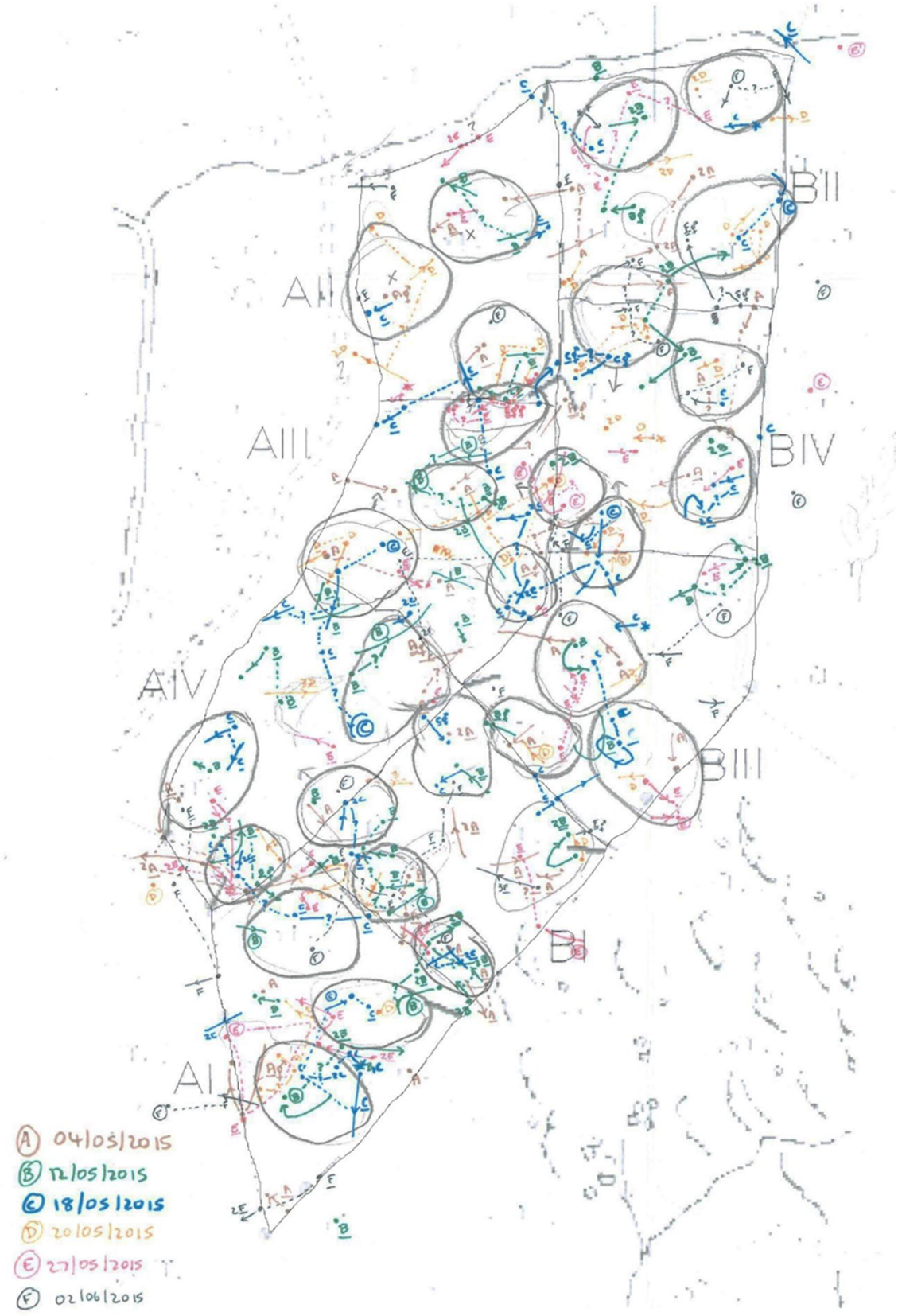
Vandenberghe, C., Prior, G., Littlewood, N.A., Brooker, R. & Pakeman, R. (2009). Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic Appl. Ecol.*, 10, 662–670.

Villar, N., Lambin, X., Evans, D., Pakeman, R. & Redpath, S. (2013). Experimental evidence that livestock grazing intensity affects the activity of a generalist predator. *Acta Oecol.*, 49, 12–16.

Whittingham, M.J. (2011). The future of agri-environment schemes: biodiversity gains and ecosystem service delivery?: Editorial. *J. Appl. Ecol.*, 48, 509–513.

Whittingham, M.J. & Evans, K.L. (2004). The effects of habitat structure on predation risk of birds in agricultural landscapes. *Ibis* , 146, 210–220.

Appendix A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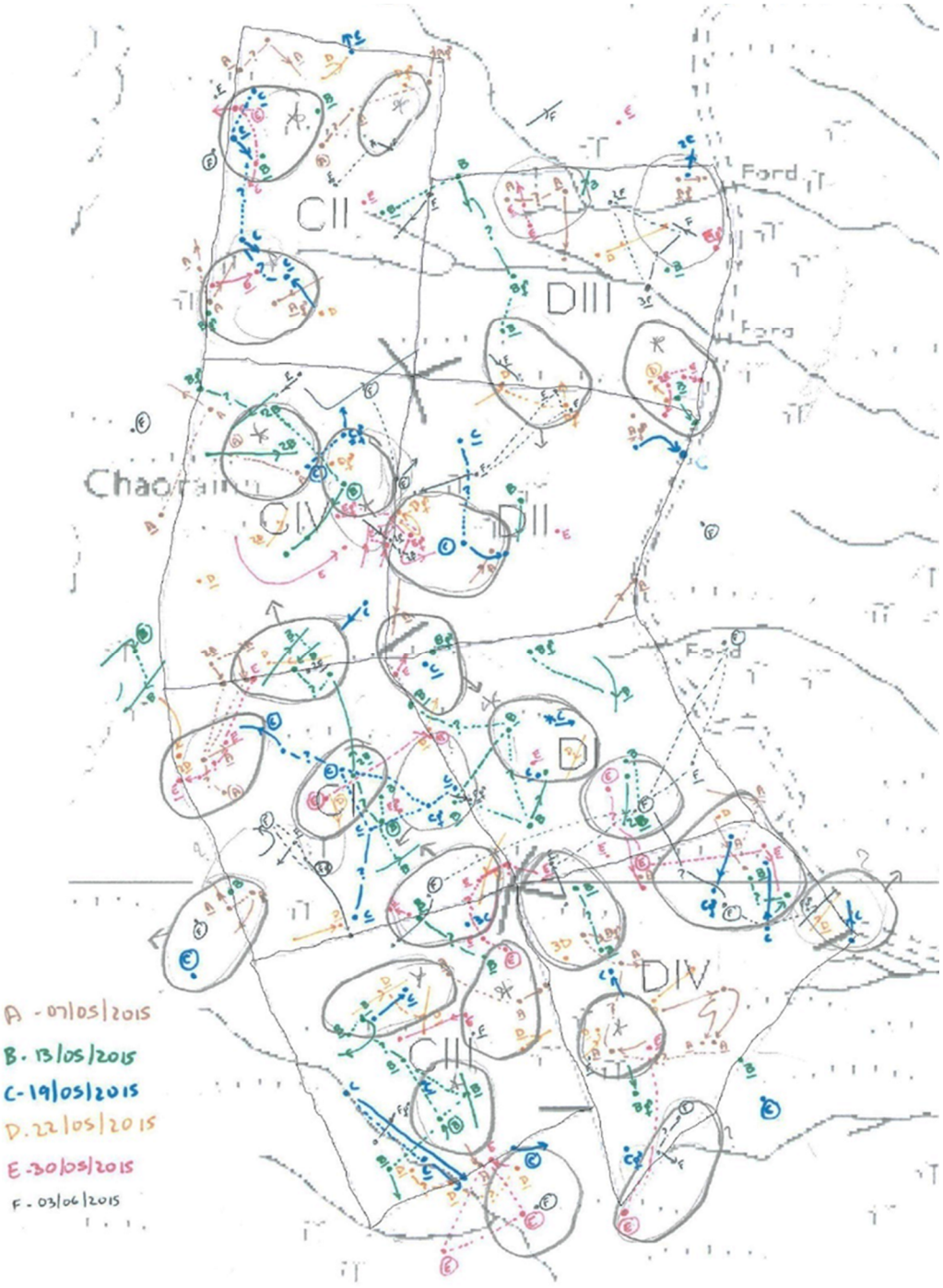
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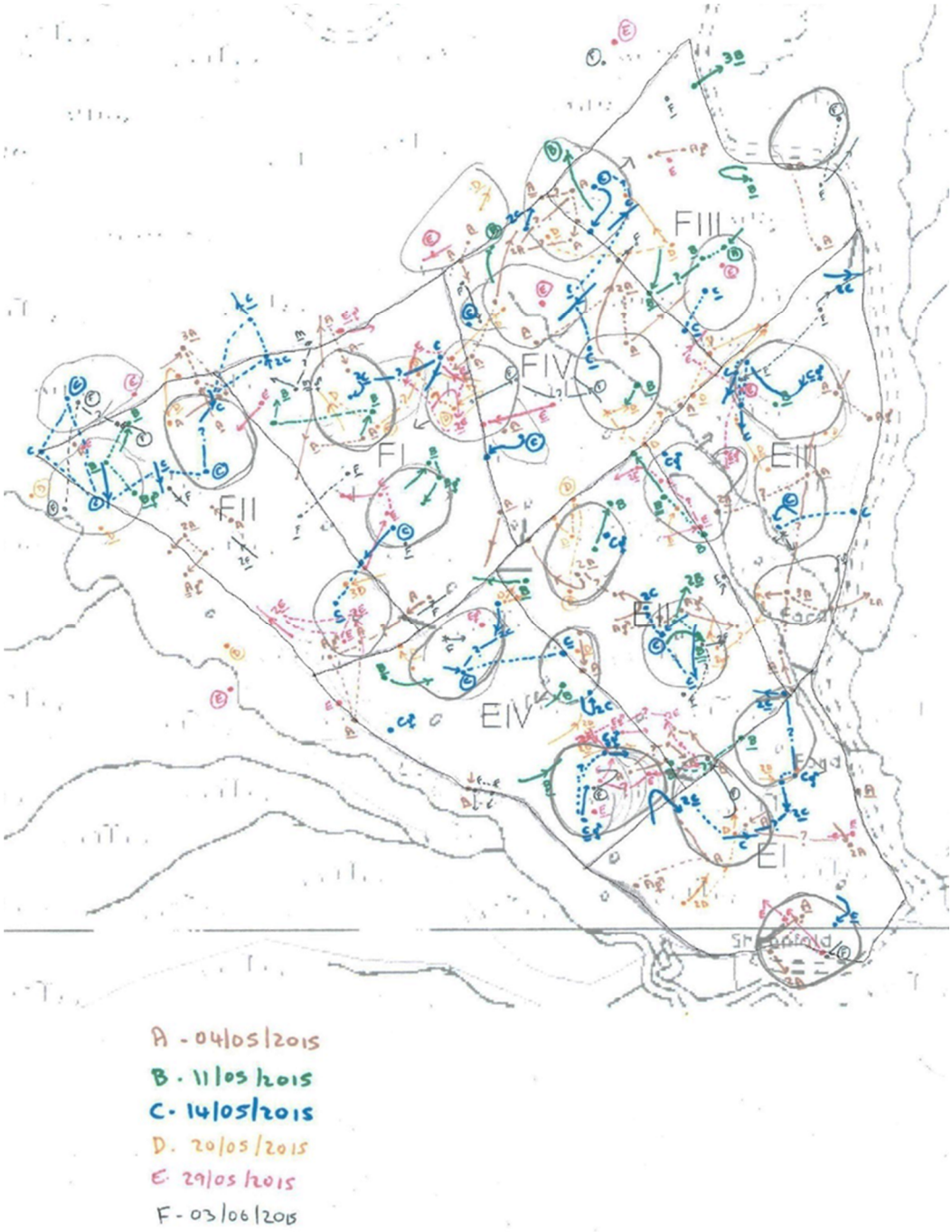

Supplementary Figure S.1. Example maps of meadow pipit territory mapping for estimates of breeding density in the three experiment blocks: “AB” (above), “CD” (middle) and “EF” (below). Territory mapping for these maps was carried out in 2015 on the dates listed below each map. Letters and colour used for each date corresponds to observations marked on the map with the same letter and colour. Circled letters indicate singing and underlined letters indicate alarm calls. Dashed lines between observations indicate two different individuals observed simultaneously, unbroken lines indicate movement of the same individual and numbers before letters indicate number of individuals observed together (when observing more than one). The approximate, estimated area of each breeding territory has been circled.

		Est.	SE	X^2	p value	Marg. R^2	Cond. R^2
Breeding density (log)	Distribution: *Normal*						
Final - Fixed	Treatment*Sampling Period			1.88	0.597	0.18	0.41
w/o interactions	Treatment			**20.15**	**< 0.001**		
	Treatment II	-0.305	0.075				
	Treatment III	-0.003	0.075				
	Treatment IV	-0.134	0.075				
	Sampling Period	-0.133	0.109	1.67	0.196		
Final - Random	Year/Block						
Clutch size	Distribution: *Normal*						
Final - Fixed	Treatment*Sampling Period			0.90	0.825	0.09	0.09
	scale(Hatch date)	3.063	0.884	**12.92**	**< 0.001**		
	scale(Hatch date^2)	-3.028	0.883	**12.67**	**< 0.001**		
	No. of Territories	0.032	0.034	0.82	0.360		
w/o interactions	Treatment			0.36	0.940		
	Treatment II	0.056	0.123				
	Treatment III	0.056	0.107				
	Treatment IV	0.050	0.111				
	Sampling period	-0.229	0.086	**6.68**	**0.010**		
Final - Random	Year + Block						
Hatch date	Distribution: *Poisson*						
Final - Fixed	Treatment*Sampling Period			2.06	0.559	0.08	0.15
	No. of Territories	0.009	0.004	**4.09**	**0.043**		
w/o interactions	Treatment			**13.01**	**0.005**		
	Treatment II	0.053	0.016				
	Treatment III	0.009	0.014				
	Treatment IV	0.009	0.015				
	Sampling Period	0.034	0.025	1.51	0.220		
Final - Random	Year + Block						
No. of Fledglings	Distribution: *Negative Binomial*						
Final - Fixed	Treatment*Sampling Period			5.71	0.127	0.23	0.30
	scale(Hatch date)	2.315	1.383	2.88	0.090		
	scale(Hatch date^2)	-2.404	1.383	3.01	0.083		
w/o interactions	Treatment			2.63	0.452		
	Treatment II	0.217	0.177				
	Treatment III	0.183	0.168				
	Treatment IV	-0.002	0.174				
	Sampling Period	-0.555	0.224	3.62	0.057		
Deleted	No. of Territories	-1.013	0.065	0.04	0.844		
Final - Random	Year + Block/Plot						

Continued on following page.

		Est.	SE	X^2	p value	Marg. R^2	Cond. R^2
Est. Fledglings/Plot	Distribution: *Gaussian*						
Final - Fixed	Treatment*Sampling Period			3.893	0.273	0.187	0.264
w/o interactions	Treatment			5.869	0.118		
	Treatment II	-1.443	1.553				
	Treatment III	1.832	1.522				
	Treatment IV	-1.294	1.491				
	Sampling Period	-3.591	1.862	**4.250**	**0.039**		
Deleted	scale(Hatch date)	-0.552	0.569	0.602	0.438		
	scale(Hatch date^2)	-1.713	13.072	0.001	0.976		
Random	Year + Block/Replicate/Plot						
Egg-stage Nest survival	Distribution: *Binomial*						
Final - Fixed	Treatment*Sampling Period			6.70	0.082	0.19	0.19
	No. of Territories	-0.157	0.289	0.29	0.588		
	scale(Hatch date)	-0.950	0.363	**7.39**	**0.007**		
w/o interactions	Treatment			**10.07**	**0.018**		
	Treatment II	3.418	1.130				
	Treatment III	1.221	0.967				
	Treatment IV	1.574	0.977				
	Sampling Period	-3.191	0.825	**16.66**	**< 0.001**		
Deleted	scale(Hatch date^2)	-2.642	7.790	0.12	0.731		
Final - Random	Year/Plot/Nest						
Nestling-stage nest survival	Distribution: *Binomial*						
Final - Fixed	Treatment*Sampling Period			0.85	0.838	0.00	0.00
	scale(Hatch date)	9.920	10.825	0.03	0.854		
	scale(Hatch date^2)	-9.783	10.797	0.55	0.460		
w/o interactions	Treatment			0.37	0.946		
	Treatment II	-0.366	1.308				
	Treatment III	-0.035	1.328				
	Treatment IV	-0.685	1.309				
	Sampling Period	-0.337	0.937	0.13	0.721		
Deleted	No. of Territories	-0.303	0.436	0.49	0.483		
Final - Random	Year/Replicate + Nest						

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		Est.	SE	X^2	p value	Marg. R^2	Cond. R^2
Overall Nest survival	Distribution: *Binomial*						
Final - Fixed	Treatment*Sampling Period			4.65	0.199	0.16	0.827
	No. of Territories	-0.359	0.268	2.33	0.127		
	scale(Hatch date)	9.324	6.968	1.30	0.255		
	scale(Hatch date^2)	-9.813	6.950	1.50	0.221		
w/o interactions	Treatment			2.54	0.469		
	Treatment II	0.860	0.996				
	Treatment III	0.914	0.877				
	Treatment IV	-0.236	0.912				
	Sampling Period	-3.344	0.817	**8.99**	**0.003**		
Final - Random	Year + Plot/Nest						

Supplementary Table S.1 Results of generalized linear mixed models shown as χ^2 - and p - values of variables included in the model of each breeding parameter. Independent effects of Treatment and Sampling period variables were tested in a separate model with the same final structure as with interactions. Final model indicates the model after model selection. Variables were removed sequentially, except when testing for the interaction between Treatment and Sampling Period; in that case, the linear terms for Treatment and Sampling Period were always kept in the model and the interaction was always removed when testing the effects of Treatment and Sampling Period separately. "Random" states the structure of random variables and "Deleted" shows variables deleted during model selection. "Distribution" shows the probability distribution applied to the model. Estimate and Standard error of estimates are provided for Treatments using Treatment I as a baseline, for Sampling period by using the early period as a baseline, and for all integer variables. Marginal r^2 shows the proportion of variance explained by fixed effects only and conditional r^2 shows the variance explained by fixed and mixed effects of final models. Treatment: factor with four grazing treatments: I) 9 sheep per plot, II) 3 sheep per plot, III) 2 sheep per plot and, during 3 weeks each autumn, 2 cows with suckling calves and IV) ungrazed. Sampling period: factor as time period with either early (immediate and one year after the grazing treatments commenced) or late (12 and 13 years after the grazing treatments commenced) of the same management in each plot. Hatch date: Julian date of hatching. Note that estimate of hatch date is on a rescaled level. Territories: number of breeding meadow pipit pairs per plot. Eggs: No. of eggs in each clutch.

		Est	SE	χ^2	p value
Clutch size					
	Sampling period	-0.256	0.102	**6.179**	**0.013**
	Treatment			0.371	0.946
	scale (Rain)	0.001	0.049	0.001	0.976
	scale (Temperature)	< 0.001	0.066	< 0.001	0.996
	Territories	0.026	0.030	0.774	0.379
	scale (Hatch date)	3.229	0.862	**13.916**	**< 0.001**
	scale (Hatch date)^2	-3.189	0.862	**13.580**	**< 0.001**
Est. Fledglings/Pot					
	Sampling period	-1.498	1.305	1.423	0.233
	Treatment			5.506	0.138
	scale (Rain)	-0.609	2.124	0.540	0.467
	scale (Temperature)	1.722	0.646	**6.623**	**0.010**
	scale (Hatch date)	-0.797	0.571	2.097	0.148
	scale (Hatch date)^2	-3.299	13.125	0.071	0.790
Egg-stage nest survival					
	Sampling period	-2.100	0.912	**5.859**	**0.016**
	Treatment			**8.468**	**0.037**
	scale (Rain)	-0.864	0.399	**4.595**	**0.032**
	scale (Temperature)	0.224	0.503	0.197	0.657
	Territories	-0.378	0.269	2.051	0.152
	scale (Hatch date)	-1.161	0.369	**11.197**	**< 0.001**
	scale (Hatch date)^2	-7.343	7.557	0.990	0.320
Overall nest survival					
	Sampling period	-2.049	0.878	**6.186**	**0.013**
	Treatment			2.716	0.438
	scale (Rain)	-0.355	0.496	0.512	0.474
	scale (Temperature)	0.833	0.485	3.005	0.083
	Territories	-0.527	0.269	3.019	0.082
	scale (Hatch date)	11.318	6.967	2.745	0.098
	scale (Hatch date)^2	-12.054	6.972	3.123	0.077

Supplementary Table S.2 Generalized linear mixed models of meadow pipit clutch size, estimated number of fledglings produced per plot, egg-stage nest survival and overall survival where weather variables have been included. The table presents model estimates, standard error, $\chi 2$- value, and p-value of each variable tested by one model per response variable. The four response breeding variables included are those in which a significant effect of sampling period was found, and a cause for this difference due to inter-annual weather differences were sought to be excluded. “Rain” and “Temperature” are numerical variables showing the average rain (precipitation) per month during the months of May, June and July (i.e. the meadow pipit breeding season) in the region of Scotland, UK where the study site is located. Variables preceded by “scale” indicates rescaled variables for better model convergence. The estimate for sampling period states the change between the first and second sampling period. Remaining variables are described in Supplementary Table S.1.