Large-scale modelling of ecological data using novel techniques:

Using density-structured models and metabarcoding to investigate the dynamics of agro-ecosystems.

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Statement of contributions:

The majority of modelling, analysis and writing in the research presented within represents the individual work of the candidate. All work was supported, developed and improved by contributions by collaborators, each of whom is acknowledged at the start of each chapter. A breakdown of the major contributions are listed below:

The general introductory and discussion chapters (Chapters one and six), were written by the candidate with editorial advice provided by Professor Robert Freckleton.

The data used in Chapters two and three were collected by a team of researchers including Simon Queenborough, Kirsty Burnett, and Tom Swinfield.

Chapter two is in preparation as a manuscript alongside Chapter three. Model concepts and parameterisations were developed primarily by the candidate, but with support from Dr Matthew Spencer, Dr Dylan Childs, Dr Remi Vergnon, and Dr Shaun Coutts. All modelling and analysis was conducted by the candidate, but with statistical advice from Dr Dylan Childs and Professor Robert Freckleton.

The initial concepts in Chapter three were developed by Dr Remi Vergnon and Professor Robert Freckleton, using models developed by Dr Matthew Spencer. Development, analysis and modelling was conducted by the candidate using models developed in Chapter two.

Chapter four is in preparation as a manuscript, the concepts of which were developed by the candidate and Dr Dylan Childs. All work presented was conducted by the candidate, with advice on modelling provided by Dr Dylan Childs.

Chapter five is in preparation as a manuscript. The initial concept was developed by Professor Robert Freckleton and Dr Dylan Childs. Experimental design, field work, bioinformatics, statistical analysis and the vast majority of the lab work was conducted solely by the candidate.

Declaration by the author:

This thesis contains original work and only includes material published or written by other persons where due reference has been made. The contribution of all collaborators to the research presented within has been clearly stated. I only present work that I have undertaken since starting my PhD and include no content that has been submitted to qualify for any other degree or diploma at another institution. I acknowledge all copyright of the thesis content resides with the copyright holders of that material.

Thesis summary:

There is a discrepancy in the scale at which ecological data is collected and the scale at which we need to understand the dynamics of populations and communities. As environmental conditions and population responses vary over different over time and space, models parameterised with small-scale data can fail to accurately capture the breadth of large-scale dynamics. There is therefore a need to increase the scale of ecological monitoring to provide adequate data for informative modelling.

A central problem is the trade-off between quality and quantity when collecting data, detailed counting of individuals within a population is expensive, time consuming, and limits the scale of ecological research. In this thesis I examine novel methods of generating and analysing data over large scales. The first of these are density-structured models, which forgo counting abundances in favour of simple categorisation of a population at a particular site, and model dynamics as a function of the probability of transitions between categories. In Chapter 2 I develop these models to account for population structure by incorporating hierarchical effects in the parameterisation of transition probability matrices, which define dynamics in these models. I show that hierarchical models provide considerable improvement over non-hierarchical models and suggest several useful parameterisations for future applications. Models that incorporate field-level effects into the cut-point parameters of ordered category logistic regressions demonstrate the best performance.

In Chapter 3 I apply the models developed in Chapter 2 to a national-scale density-structured agricultural weed data set, to examine the effects of spatial heterogeneity and management on weed dynamics. The weed in question, black-grass, is wide-spread, economically damaging, and difficult to control. Through transient analysis, two-step ahead projections and stochastic simulations, I demonstrate that an essential part of cultural weed control- crop rotation- does decrease the severity of weed infestations. Using break crops in rotations dominated by wheat can reduce weed densities and inter-year variability, with some break crops providing greater reductions than others. However, variance

decompositions show that field-specific effects and the initial densities of weeds are greater contributors to the change in weed density than any specific crop-rotation. This suggests that site level factors may obscure or overwhelm the effect of interventions, highlighting the need for large-scale studies of population dynamics such as the one I have undertaken here.

Spatial structure is a major driver of population dynamics, and as such Chapter 4 investigates methods of expanding density-structured models to incorporate spatial information. I developed model parameterisations that incorporate spatial information into density-structured models through inclusion of a simple spatial covariate in the linear predictor of transition probabilities. I show that spatial models perform better than non-spatial counterparts, and the formulations I develop provide modest improvements to the ability of density structured models to capture field-scale spatial structure. Despite relatively modest improvements to model performance, these models demonstrate different dynamics in response to crop-rotation than spatially naïve models, with the contribution to system variances between field-specific factors and managements being far more comparable. This suggests that future predictive applications of density-structured approaches should only consider spatially explicit models when modelling the effects of crop-rotation.

The second technique I investigated in this thesis was metabarcoding, which involves using high-throughput sequencing and molecular taxonomy to simultaneously identify organisms across entire assemblages. This technology has particular promise for arthropod surveys, where traditional methods rely on the dwindling abundance of expert taxonomists, making biodiversity surveys slow, expensive, and often reliant on numerous different individuals. DNA-based identification using metabarcoding can be conducted using environmental samples or bulked samples of the organisms themselves and has been demonstrated to show fast and accurate identification of multiple organisms simultaneously. There has been little critical analysis of the limits of metabarcoding in terms of the number and type of organisms it can detect from bulked samples. I show that using standard pipelines and methodologies metabarcoding can produce taxonomic bias towards certain taxa and exclude others. I go on to demonstrate that pooling (or bulking) samples during DNA extraction reduces community coverage, and PCR produces stochasticity in species detection. I then conduct diversity analyses to assess the impact of landscape features on agricultural arthropod assemblages, however, given that there are obvious issues with barcoding in this manner it is difficult to draw robust ecological conclusions from these data.

Overall this thesis further develops methods for large scale monitoring and modelling of populations and communities. I develop density-structured models to incorporate hierarchical population effects and spatial structure and provide a demonstration of their utility. I also highlight potential problem areas for future metabarcoding studies of abundant and diverse arthropod communities.

Chapter One:
General Introduction

Preamble - Ecological monitoring, modelling and data:

Humankind is overwhelmingly reliant on natural systems for our food and wellbeing (Díaz *et al.*, 2006; Groot *et al.*, 2012; Pomeroy, 2018). Understanding the drivers of variability in these systems is central to the study of ecology, recent history, however, has seen rapid degradation in global ecosystems (Sutherland, 2006). Climate change, pollution, and habitat destruction are rapidly decreasing the ability of natural systems to provide for people (Cardinale *et al.*, 2012). To ensure continued provision of vital ecosystem services, we must manage them effectively, and for this we must have a detailed understanding of how ecosystems will respond to rapid rates of global change.

Our immediate understanding of populations and communities comes from direct observation; empirical studies of natural systems are the foundation of biological science, and small scale experiments are invaluable for ecological research (Whitehorn *et al.*, 2012; Pennekamp *et al.*, 2018). However, as we have built our understanding of ecology, focus has shifted towards statistical models. Models are key tools in and are frequently used for answering complex questions, for example, in the evolution of life-histories (Childs *et al.*, 2003; Ellner and Rees, 2006, 2007), determinants of community structure or population abundances (Freckleton and Watkinson, 1998; Freckleton *et al.*, 2000; Chave, Muller-Landau and Levin, 2002), or the distributions of, and connections between, populations (Hanski, 1998; R P Freckleton and Watkinson, 2002; Guisan and Thuiller, 2005).

Prediction is a key function of ecological models: being able to anticipate changes in the way an ecosystem behaves is essential for effective management. However, for predictions to be useful they must be accurate, and accuracy of predictions themselves are contingent on the quality of data used to parameterise the model. Without sufficient data, models can fail in the face of even slight variability in parameter values, producing erroneous results or high levels of uncertainty (Freckleton *et al.*, 2008; Tredennick, Hooten and Adler, 2017).

In the majority of cases empirical data used to parameterise models are generated over small scales, short durations, and in standardised conditions. Typically, studies of population responses to stimuli are generated in meter scale plots (Queenborough *et al.*, 2011; GarcÍa De León *et al.*, 2014), and studies that collect detailed demographic data over long time periods are typically tied to focal populations by logistics and practicality (Coltman *et al.*, 1999; Coulson *et al.*, 2001). The overwhelming reality, however, is that natural systems are defined by their diversity and dynamism. Populations and communities vary enormously through time and space, dynamics can be complex or chaotic in even homogenous environments (Blasius, Huppert and Stone, 1999; Robert P. Freckleton and Watkinson, 2002), and these patterns in variability are dependent on scale and environmental context (Levin, 1992; Steffan-Dewenter *et al.*, 2002; Urban, 2005; Chase *et al.*, 2017).

Important management issues play out on land-scape, regional, or even international scales, leaving a conflict between the scale at which we have data, and the scale at which we need to model important phenomena (Lee, 1993; Berkes, 2006; Cumming, Cumming and Redman, 2006; Guerrero *et al.*, 2013). This particularly limits the ability of models to forecast the effects of environmental change on ecosystems whilst accounting for spatio-temporal variability in model parameters and dynamic processes. Reconciling this mismatch is a primary task in ecology and has led to significant effort in finding methods for 'upscaling' the small scale processes to larger spatial and temporal scales (Levin, 1992; Miller *et al.*, 2004; Urban, 2005). Extrapolation can be achieved in numerous ways (e.g. Rastetter et al. 2003; Cipriotti et al. 2016), but the assumption that small scale processes are representative at broader scales are unrealistic (Chase *et al.*, 2017), and can lead to erroneous predictions (Freckleton *et al* 2008). A lack of data and a poor mechanistic understanding of the processes that dictate variability organism responses over different scales will ultimately lead to predictive errors (Tshapa and Bossler, 1992; Ludwig, Wiens and Tongway, 2000; Miller *et al.*, 2004).

The overriding message is that to manage natural systems, management must reflect their inherent variability. For models to help us to manage real-world ecosystems, predictions must be applicable at scale, and good management ultimately requires good data (Stephens *et al.*, 2015; Cadotte *et al.*, 2017). Unfortunately, for studies of abundance, diversity, and demography, data collection is often difficult, expensive and time consuming. The costs of biological surveys combined with the budget limitations of most projects, limit the scale of research, and thus our ability to tackle large-scale problems effectively.

Fortunately, technological innovation has kept pace with the rates of global change. Rapid advances in technology over the past century are now providing promising opportunities to address the mismatch of scales in ecology and gather data over increasingly large areas. For example, increased connectivity and ease of communication has enabled the rise of sophisticated citizen science initiatives (Silvertown, 2009; Dickinson, Zuckerberg and Bonter, 2010) which economises data collection or analysis through crowdsourcing. Remote sensing is now routinely applied, with capture of = environmental data possible from satellite imagery, aircraft, and automated UAVS (Turner *et al.*, 2003; Wiens *et al.*, 2009; Lambert *et al.*, 2018). Next-generation sequencing has revolutionised molecular studies in ecology and evolution, allowing economical access to vast amounts of genetic data (Shendure and Ji, 2008; Metzker, 2010).

Novel methodologies have always been part of the analytical toolbox in ecology, as economical data collection has always been a necessity. Also, many metrics are inherently hard to observe. For example, measuring the exact age of an organism is often impossible, but summarising it into a particular stage category is common practice when studying demography. Technology has opened up new opportunities. For example elusive or rare species require effort to find, species detection and estimations of population size can now be achieved through camera trapping (Burton *et al.*, 2015; O'Connel, Nichols and Karanth, 2015; Hsing *et al.*, 2018), acoustic analysis (Bardeli *et al.*, 2010; Stowell *et al.*, 2018), or through molecular analysis of environmental samples (Taberlet and Coissac, 2012a; Thomsen *et al.*, 2012; Bohmann *et al.*, 2014). In turn, new data collection techniques have facilitated new methods of analysis. Matrix projection models were developed to model demography as a function of discrete stage categories (Leslie, 1948; Lefkovitch, 1965; Caswell, 2001), and have in turn inspired more sophisticated analyses (Childs *et al.*, 2003; Ellner and Rees, 2006).

There is great potential for a synergy between novel methods and technologies to spur a drastic uptake in data, and subsequently provide insightful and sophisticated analysis. The motivation behind this thesis was to begin to address the lack of data in ecology by developing and applying innovative approaches that rely on recent advances in technology.

I use two techniques designed to capture large quantities of data rapidly, and over large scales, to investigate the dynamics of populations and communities. These methods have the potential to address the mismatch in scale between the data available to us, and the scale at which we need to model dynamic processes.

Density-structured models:

The first technique I introduce in this thesis are density structured models, which are designed for rapid collection and analysis of population dynamics. Conventional population models have the form:

$$N(t+1) = N(t)\,\lambda(t)\,f[N(t)]$$

Where N is the number of individuals at times t and $t+1$, λ is the population growth rate and, the function f represents the density-dependence of populations resulting from processes such as competition. Part of the limitation of these models is that they require continuous counts of abundance, which are potentially difficult to collect: based on counts of individuals in defined survey sites, population sizes may be time consuming to estimate, especially when populations are large, or difficult to count accurately over large scales. They also require knowledge of the phenomena that dictate the parameters involving growth rate and density dependence (Freckleton *et al.*, 2011).

Density-structured models forgo time consuming detailing of abundances for a simple categorisation of the state of a population at a given site:

$$N(t+1) = \mathbf{T}N(t)$$

In density structured frameworks, survey areas are divided up into pre-defined sites, and the population is assigned one of a number of density states based on a set of criteria. Here, the state variable, *N*, is a vector of density categories which represent the probability of an observation being in one of the pre-defined states. The dynamic processes are modelled as a function of transition probabilities contained in the matrix **T**, which can be estimated from the observed transitions between states over subsequent surveys.

This approach provides numerous advantages over standard methodologies. Primarily, categorisation rapidly speeds up the data collection process, allowing researchers to collect data over much larger areas. It is also reasonably easy to assess the levels of observation error as surveys are easily repeatable. Numerous studies have demonstrated that the dynamics captured and simulated by density-structured models are comparable to traditional models (Freckleton *et al.*, 2011; Queenborough *et al.*, 2011; Tredennick, Hooten and Adler, 2017). The advantages, however, extend beyond rapidity. Although these models are simple, they have a considerable body of literature underpinning their theory (Caswell, 2001). Analysis is also straightforward: a mechanistic understanding of the intricacies of the dynamics of a particular system is not needed, as it is defined solely by the transition probabilities (Freckleton *et al.*, 2011). The simplicity of these models makes them stable and robust to errors induced by parameter sensitivity that are a concern in many demographic models (Freckleton *et al.*, 2008).

Metabarcoding:

The second technique I use is known as metabarcoding (MBC), which uses next-generation sequencing technology to provide large amounts of data on community composition. Conventional biodiversity surveys are limited by a phenomenon known as the ‘taxonomic impediment’, which results from our imperfect knowledge of biodiversity and our reliance on the dwindling availability of taxonomic expertise (Cody and Rodman, 2003; Giangrande, 2003; Wilson, 2004). Studies of diversity and community dynamics are again limited by the difficulty in collecting adequate data. Morphological identification is slow, expensive, and often requires multiple experts for full coverage of communities.

With the molecular revolution in biological science, it has become possible to identify organisms through genetic data (Hajibabaei *et al.*, 2011; Taberlet and Coissac, 2012b; Cristescu, 2014). Next-generation sequencing has enabled the sequencing of vast amounts of genetic information at increasingly affordable costs. DNA-barcoding refers to the practice of molecular identification of organisms through short gene sequences taken from organisms or environmental samples, and Metabarcoding therefore refers to the sequencing of ‘barcodes’ from multiple organisms simultaneously. Identifying organisms in this way decouples research from the reliance on slow, unreliable and expensive methods of identification. MBC therefore facilitates rapid surveys and accurate identification over large scales.

Agricultural systems:

Agricultural systems are particularly important when considering the impacts of global change and the relevance of large-scale monitoring. Agriculture is a leading cause of biodiversity loss worldwide (Tilman *et al.*, 2001), with the declines in many important species being linked to intensification (Bengtsson, Ahnström and Weibull, 2005; Brussaard *et al.*, 2010; Woodcock *et al.*, 2016, 2017). However, agriculture is also reliant on natural systems for provision of ecosystem services that support production (Altieri, 1999; Zhang *et al.*, 2007; Aizen *et al.*, 2009), meaning food security is inherently linked to diversity. In the context of climate change this makes them particularly vulnerable. Rapid environmental degradation threatens both agricultural production and the systems it relies on. Nevertheless, without dramatic change to the consumption habits of society, large-scale industrial agriculture will remain our primary source of food for the foreseeable future (Tilman *et al.*, 2002).

At the outset, monitoring and anticipating changes in agro-ecosystems may seem relatively simple, as monocultures of crops normally make up the majority of organisms. However, as we need to produce a lot of food, agricultural systems are widespread by necessity, and as a result are exposed to considerable environmental variation (Tscharntke *et al.*, 2005). Balancing agricultural production with conservation is a major goal for ecologists, which is essential for continued food security (Tilman *et al.*, 2011), but to do so we must have a thorough understanding of the dynamics of agro-ecosystems at the relevant scales.

Unfortunately, many monitoring efforts are poor (Kleijn *et al.*, 2001; Kleijn and Sutherland, 2003). Agri-environment schemes aimed at preserving biodiversity often do not provide enough data to protect vital characteristics of ecosystems, and most information on processes affecting production are derived from small field-scale experiments (Metcalfe et al., 2018; Nordmeyer, 2006; Uchida, Hiraiwa, & Ushimaru, 2016). In time, this will lead to poor management with detrimental effects on both biodiversity and food production. To maintain ecological integrity and ensure continued provision into the future, the scale of our monitoring has to expand. The two technologies we discuss in detail above have great potential for improving our understanding of agricultural populations and communities over the scales needed for effective management.

Agriculture and density-structured models:

Climate change induced range shifts mean that agricultural systems are under increasing threat from invasive species in the form of agricultural pests (Ziska *et al.*, 2011). Pests are defined as organisms that are detrimental to human health or wellbeing, often in terms of damage to agriculture. Within this subset are agricultural weeds, which can be extremely destructive and expensive to control (Freckleton *et al.*, 2000; Rew and Cousens, 2001; Jones *et al.*, 2005). Most empirical studies of weed dynamics and the impacts of controls are derived from extremely small scales (Metcalfe i., 2017; Metcalfe et al., 2018; Queenborough et al., 2011). For us to fully understand weed populations and their response to management in variable environments, we have to be able to measure them across the full range of conditions they exist in. Density structured models are an effective tool for this challenge allowing surveys to take place over large scales. In one of the first applications, Taylor & Hastings (2004) demonstrate how they can be used to plan optimal control strategies for invasive weeds. In this thesis I aim to further develop density-structured models to account for broad-scale spatial patterns and use them to investigate the management implications of the dynamics of an agricultural weed.

Agriculture and metabarcoding:

Arthropods are key components of agro-ecosystems, providing essential services such as pollination, nutrient cycling and pest control (Klein *et al.*, 2007; Van Veen *et al.*, 2008; Aizen *et al.*, 2009; Isaacs *et al.*, 2009). The focus of crop management is on health and yield, but interventions aimed at tackling problematic pests can have negative effects on many organisms other than those they target (Tscharntke *et al.*, 2005; Henle *et al.*, 2008;

Woodcock *et al.*, 2016, 2017). Such management is thought to be responsible for observed declines of biodiversity and ecologically important taxa. To understand agricultural arthropod diversity, its causes, consequences, and how widely observed declines may affect production, we must track it at the relevant scales.

The vast majority of insect diversity surveys in agriculture however, are lacking in scale, taxonomic coverage, and resolution. Almost all surveys focus on a few indicators or ecologically important taxa over a few sites, whilst ignoring the vast majority of organisms (e.g. Steffan-Dewenter et al. 2002; Giangrande 2003; Westphal et al. 2008; Rader et al. 2013; Uchida & Ushimaru 2014; Uchida et al. 2016). As such, our knowledge of agricultural diversity is biased and incomplete, we lack knowledge of how the diverse array of species respond to changes in land-use and management. MBC provides a mean to track entire communities in their entirety, accurately, and over large-scales (Yu *et al.*, 2012; Ji *et al.*, 2013). In this thesis I aim to asses MBC as a technique for assessing diverse arthropod assemblages over large scales and use molecular diversity data to investigate how land-use affects diversity.

Aims and objectives:

Chapter 2 – Developing hierarchical Bayesian density-structured models.

Density-structured models have the potential to provide large scale empirically backed estimates of population dynamics. However their applications so far have been limited to relatively isolated populations (Taylor and Hastings, 2004; Freckleton *et al.*, 2011; Mieszkowska *et al.*, 2013). Structure is common in ecology, with environmental variation leading to differing responses and effects across groups with various different levels. Hierarchical modelling is a statistical method that allows group-level variance to enter population models (e.g. Wu & David 2002; Zipkin et al. 2009; Cafarelli et al. 2017). Implementing hierarchy in density-structured models will increase their potential for large-scale modelling, allowing them to account for variable environmental factors. In this chapter I parameterise and test hierarchical density structured models with a view to finding the best candidate for future density structured analyses.

Chapter 3 – Using density-structured models to investigate weed population dynamics.

In Chapter three I apply the models parameterised in Chapter two on a large-scale data set of black-grass (*Alopecurus myosuroides*) densities, to investigate national-scale population dynamics and methods of control. I look at how an essential part of weed control, crop-rotation, affects black-grass densities. I simulate density-structured dynamics for a variety of cropping systems, to understand the variability in black-grass dynamics as well as the best rotational strategies for control.

Chapter 4 – Incorporating spatial information into density structured models.

Spatial structure is a key determinant of population dynamics (Durrett and Levin, 1994), but so far density-structured models have only been applied as simple descriptors of populations without consideration of internal spatial structure. In Chapter 4 I further develop density-structured models to incorporate spatial information, a key determinant of population dynamics in all systems. I test a variety of models and investigate the effect that spatial structure has on dynamics. I look at how to incorporate the effect of neighbour density on state to state transition probabilities to account for different spatial processes at the field-scale. I then compare dynamics between spatially naive and explicit models under a variety of crop rotations.

Chapter 5 – Using metabarcoding for assessing arthropod diversity in agro-ecosystems.

MBC has much potential to gather an abundance of diversity data across large scales. However, most ecological applications have been small scale proof-of-concepts or addressing highly localised problems. In Chapter 5 I assess the utility of MBC for large scale assessment of agricultural arthropod communities. I apply MBC to bulk arthropod samples collected across the UK during the course of this PhD. I assess the community coverage provided by bulk extraction of DNA, by comparing species detection across levels of replication & sequencing depth. I then compare detection success and accuracy with morphological identification. Finally, I investigate how landscape features affect the community composition and biodiversity of these communities.

Datasets:

1) *RELU weed data.*

The black-grass data I analyse in the first three data chapters comes from rural economy and land use (RELU) surveys conducted as part of a NERC study on weed distributions across UK arable farms. The data were collected between 2007 and 2011 and consist of density-state observations for 1416 field-level surveys across counties where weed infestations are typically severe, including Oxfordshire, Bedfordshire, Cambridgeshire, Norfolk, Lincolnshire, Nottinghamshire, and Yorkshire. We analyse the density-state observations for a single weed, black-grass (*Alopecurus myosuroides)*, which is particularly destructive and a growing concern for farmers. Black-grass can have severe effects on yields and control is becoming increasingly difficult due to evolution of multiple herbicide resistances. (Moss and Clarke, 1994; Freckleton *et al.*, 2017; Hicks *et al.*, 2018). To control herbicide-resistant black-grass across the entirety of its range, it is necessary to assess the impact of cultural control over a range of environmental conditions. This survey collected data over a range of crop-rotations, an essential part cultural weed control, and allows large-scale analysis of the effect of rotation on weed density.

2) *Arthropod diversity data*:

The molecular diversity data I analyse in the final data chapter was funded by a NERC biomolecular analysis facility (NBAF) support grant and processed in the NBAF facility in Sheffield. I collected this data using over a three-year period in consecutive pan-trapping surveys from 27 UK arable farms. We use a region of the COI gene to assign taxonomies and molecular operational taxonomic units (mOTUs), to each unique sequence. I analyse this data alongside data on landscape variables derived from the CEH land cover and crop cover maps.

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Chapter Two:

Developing Bayesian models for hierarchical density-structured modelling.

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Abstract:

Populations show highly variable responses across time and space in the face of environmental heterogeneity. Understanding how and why populations change is central to the study of ecology, and population models are key tools for understanding dynamics. However, without adequate data, models can often fail to produce accurate or useful results. Density-structured models provide a route to rapid and large-scale modelling and monitoring of populations, but so far, their use has been limited to small scale proof-of-concept or relatively localised applications without consideration of broad-scale environmental heterogeneity. Developing these models to account for hierarchical effects that act across widely-distributed populations will increase their value for understanding large-scale population ecology. In this article we parameterize and test hierarchical density-structured models using a large-scale dataset of agricultural weed density. We demonstrate they provide a significant improvement in capturing population dynamics over non-hierarchical models across three different crop rotations. Leave-one-out cross validation shows that models that incorporate hierarchical effects in model cut-point parameters provide superior predictive performance, and posterior predictive checks demonstrate they better describe the state of field-scale populations.

Introduction:

Ecological populations often exist over large spatial scales, and as a result can be subject to a wide range of environmental conditions. Following perturbation, these populations can exhibit an equally large number of responses in both time and space (Wiens 1989, Levin 1992, Lundberg 2000, Coutts 2016). Managing large-scale populations in stochastic environments requires detailed knowledge of environment-driven spatio-temporal dynamics, whether the focus is the balancing natural resources and conservation (Tscharntke *et al.*, 2005; Flesch and Steidl, 2010), or eradicating problematic species (Freckleton *et al.*, 2000; Bianchi, Booij and Tscharntke, 2006; Ziska *et al.*, 2011). Considering the current rate and scale of environmental change (Sutherland 2006, IPCC 2013), large scale monitoring is an especially important part of modern ecology. However, gathering data of sufficient quality to encapsulate both the full range of population responses, and environmental drivers, is as challenging as it is important.

A detailed understanding of the range of responses in a population is essential, as the effects of focal variables can often be offset or masked by the enormous variance inherent in a population itself (e.g. Freckton et al 2017). Most approaches to studying population dynamics use local population density as the state variable, meaning large scale data collection is required to capture population responses in their entirety. Unfortunately, there is an inherent trade-off between scale and detail when collecting data. Large scale studies are expensive, time consuming, and although they may produce a large quantity of data, errors can be large (Wallinga *et al.*, 1999; Freckleton *et al.*, 2006). On the other hand, intensive but smaller scale studies may produce higher quality data, but often fail to encompass the full range of conditions large populations are subject to (Forman, 1995; Miller *et al.*, 2004).This trade-off limits both the scale of ecological monitoring, as well as the potential to effectively assess population dynamics and inform management strategies. As key tools in ecology, population models are limited by this quantity-quality trade-off, as large scale heterogeneity in both population responses and the environment can make it difficult to obtain data to accurately estimate variance in key parameters (Freckleton et al 2006, Freckleton et al. 2008).

Density-structured models are a method of generating and modelling large scale data whilst also permitting accurate prediction of population dynamics (Taylor & Hastings. 2004, Freckleton et al 2011, Queenborough et al 2011, Miezchowska et al. 2013). Instead of collecting a continuous measure of abundance, density-structured approaches involve discretizing population counts into ordinal density 'states', and modelling future population sizes as a function of transition probabilities between these categories. The advantage of this method is that it facilitates rapid data collection over large scales, as time consuming enumeration of abundances is dropped in favour of a categorization of the state of a population in a survey unit. Despite their apparent simplicity, density-structured models have several advantages over conventional approaches for some purposes. They enable large scale surveys to capture the full range of population responses, whilst retaining the potential to yield accurate representations of current and future abundances (Tredennick et al. 2016), and unlike demographic models, they are robust to numerical instability resulting from environmental heterogeneity (Freckleton *et al.*, 2008).

Although densty-structured models represent a promising technique for addressing large-scale problems, the use of these models has been limited, and previous approaches have been narrow in their exploration of possible methods of analysis. Most density-structured models have all used some variation on multinomial logistic regressions to model transition probabilities between density states, with fitting methods varying between studies. For example Freckleton et al. (2011) use a maximum likelihood approach using Vector Generalised Additive Models (VGAMS), whilst Mieszkowska et al. (2013) use a baseline-category logit model within a bayesian framework. A limitation of these methods, however, is that transition probabilities are modelled as multinomial, or *un-ordered*, responses. Modelling data in this way ignores information about the natural ordering of density states and any impact on inference is lost. Moreover, multinomial analysis requires that transition probabilities from each possible source state to each possible destination state are estimated individually. This can lead to a potential explosion of parameters: for n states, there are n^2 such probabilities. Ordinal regression methods on the other hand account for the natural ordering of density categories, and simplify the parameterisation of transition probabilities within a single model.

Accounting for inherent structure within populations, generated by variation in environmental drivers as well as variance intrinsic to populations themselves, is an essential step in modelling populations in hetereogenous environments. A powerful approach for modelling structured data are hierarchical models, also commonly known as mixed effects models, which been used extensively ecology (Myers and Worm, 2003; Bolker *et al.*, 2009; Cressie *et al.*, 2009; Zipkin, DeWan and Andrew Royle, 2009; Cafarelli *et al.*, 2017). Hierarchical modelling involves accounting for effects that act upon groups defined at various levels which reflect structure in a population. For example, incorporating group-level effects into a model could account for variation between, individuals within a single population (Buckley, Briese and Rees, 2003), variation between geographically separate sub-populations (Myers and Worm, 2003), or individual species within communities (Zipkin, DeWan and Andrew Royle, 2009). Previous density-structured approaches have modelled ‘global’ population responses as a function of a number of environmental covariates. However, hierarchical effects have yet to be incorporated into density-structured frameworks to account for the contribution of geographic and environmental variation on population dynamics.

Accounting for the natural ordering of density states and the inherent hierarchy of environmental effects acting upon a population are two clear opportunities for increasing the utility of density-structured models for large-scale ecological problems. Here we develop such methods for analyzing the population dynamics of arable weeds. These are a major concern for global agriculture, food security and conservation (Mack *et al.*, 2000). Infestations are often detrimental to yields, expensive in terms of control, and can have numerous negative indirect effects on management (Jones *et al.*, 2005; Walker *et al.*, 2005; Popp, Peto and Nagy, 2013). Importantly, these costs are becoming compounded by the evolution of herbicide resistance in many systems worldwide (Heap 1997, Hicks et al. 2018). Managing populations of weeds is a difficult problem, partly, due to the large scale over which populations exist. Populations are often subject to a large range of environmental conditions, including variations in soil, climate, and crop varieties, and can exhibit extreme levels of variation (Wallinga *et al.*, 1999; Freckleton and Stephens, 2009; Lima, Navarrete and González-Andujar, 2012). Hierarchical density-structured modelling of arable weed populations could be especially useful in addressing a suite of problems for which demographic approaches are unstable in the face of variability (Freckleton et al 2008.)

In this paper we identify and test a set of models that incorporate group-level effects to account for inter-population variability on a national-scale weed density dataset collected between 2007 and 2010. We develop and test alternative parameterizations of a hierarchical ordered category logistic regression with the aim finding of the best model to describe field-level population dynamics of black-grass. We show, through out-of-sample cross-validation and a series of posterior predictive checks, that hierarchical models perform better in terms of one-step-ahead field-level prediction than non-hierarchical models. We demonstrate various models which account for field-level population variance through hierarchical effects with comparable predictive performance.

Methods:

Study system & Survey.

Data on the density of black-grass (*A. myosuroides*) were collected in a series of repeated surveys from 2007-2010. This data set is culmination of 1007 field-scale surveys from 427 fields across 48 arable farms. The density-structured survey method, described in detailed in Queenborough et al. (2011), involves repeated surveys of individual fields to map the black-grass densities and how they change through time. Each field is divided up into a set of 20m x 20m survey quadrats, or 'sites' (predefined using a GPS system). Researchers walk the fields recording the densities of each site as one of five categories: absent (A), low (L), medium (M), high (H), or very high (VH). These categories were chosen based on previous surveys, and this method has been critically evaluated to demonstrate high within and between observer repeatability (Freckleton et al. 2011, Queenborough et al. 2011).

Because crop rotation is an integral part of arable weed dynamics and a common method of control (Zacharias and Grube, 1984; Liebman and Dyck, 1993; Melander, Rasmussen and Bàrberi, 2005), we select data from the three most common rotations, wheat to wheat, wheat to oil seed rape (OSR), OSR to wheat, to test our models on a variety of cropping systems with different dynamics.

Modelling density-structured data.

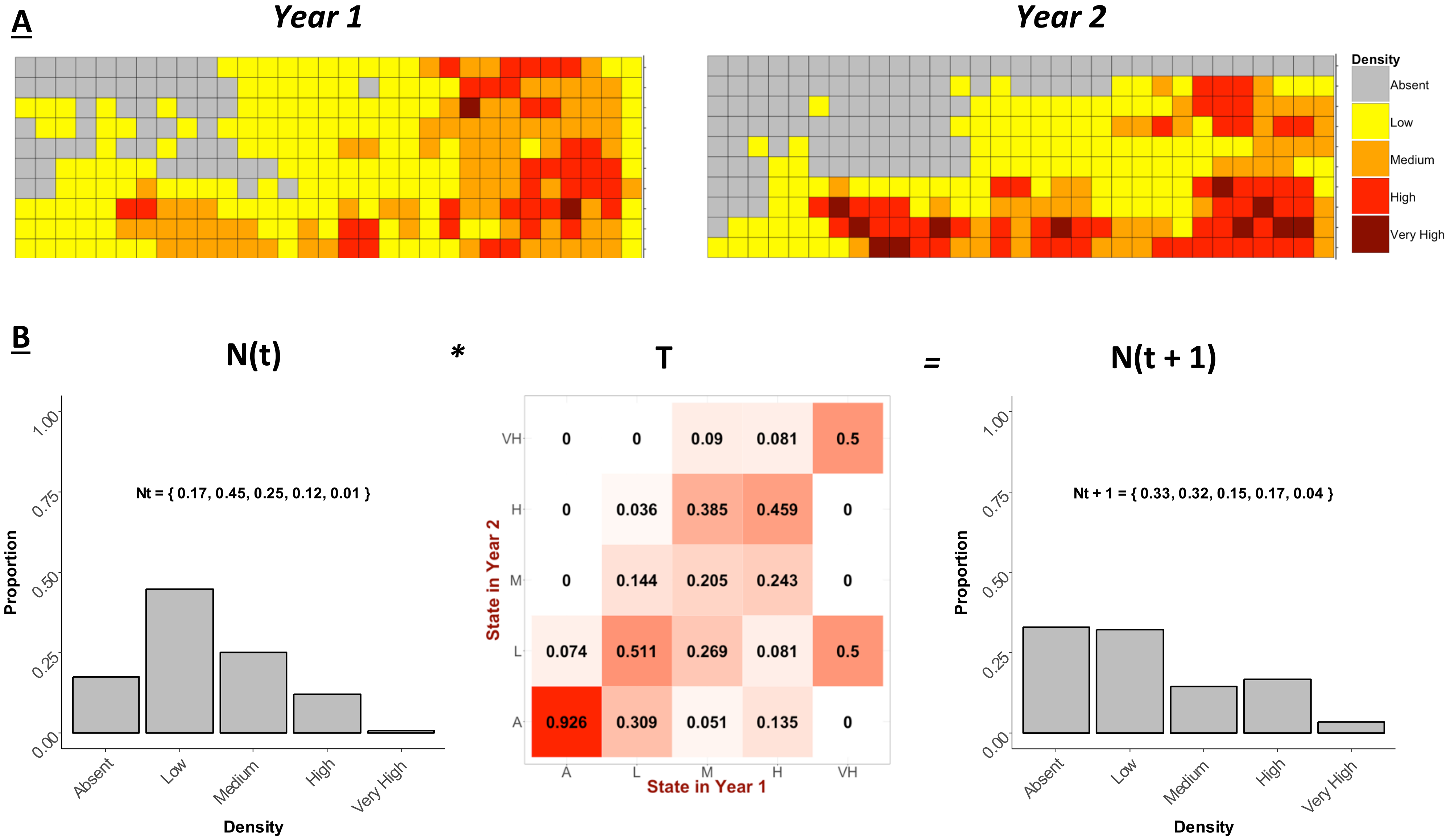
A typical density-structured model has the structure:

$$\mathbf{N}(\mathrm{t}+1) = \mathrm{T}.\mathbf{N}(\mathrm{t}) \qquad (1)$$

Where **N** is an ordered vector of the distribution of density states at time t, and **T** is a $k\ x\ k$ matrix of transition probabilities, where k is the number of density states:

$$\mathrm{T}=\begin{pmatrix} p_{11} & \cdots & p_{1K} \\ \vdots & \ddots & \vdots \\ p_{K1} & \cdots & p_{KK} \end{pmatrix} \qquad (2)$$

Diagonal entries of **T** represent probabilities (p) that a site in a given site will remain in that state for the next survey, and off diagonals represent the transition between states between years. For example, p_{11} is the probability that a site in state 1, will remain in state one, and p_{21} is the probability that a site in state 1 will transition to state 2. By using the first order Markov chain model in equation (1), these models can be used to predict future density state distributions. A more detailed explanation of density-structured models can be found in Freckleton et al (2010). The relationship between field-scale surveys, density state distributions and transition matrices is illustrated in figure 1.



State in Year 2	A	L	M	H	VH
VH	0	0	0.09	0.081	0.5
H	0	0.036	0.385	0.459	0
M	0	0.144	0.205	0.243	0
L	0.074	0.511	0.269	0.081	0.5
A	0.926	0.309	0.051	0.135	0

Figure 1. Maps of black-grass density states in a single field over consecutive surveys (A), with the corresponding density state distributions for each year, and the transition probability matrix for the observed sets of transitions (B). The histograms in (B) represent the proportion of the field in (A) that is occupied by each density state in surveys 1 & 2, the vectors in bold represent the precise values. The transition matrix displays the observed probability of transition between density states in year 1 (x-axis) and year 2 (y-axis). The numbers in each cell represent the probabilities, with the darker colours representing higher probability of transition. The markov chain is displayed above (B) to illustrate the relationship between each component.

Ordered Category Logistic Regressions.

The transition matrix, T, defines the population dynamic processes in these models. Previous approaches have estimated transition probabilities for density-structured models using multinomial regression (Taylor & Hastings 2004, Freckleton et al 2010, Queenborough et al 2011, Freckleton et al 2017). These methods treat the density states as discrete unordered factors. However, ordered category logit models are arguably better suited for analysis of systems with categorical variables with a natural ordering, such as density states.

In an ordered category model, the probability of observing a certain category, *k,* at site *i* is expressed in terms of a real-valued latent variable that reflects the true (unobserved) value. In the simplest form of this model (equation 3), the linear predictor, η_i, is constructed from the row-vector of *J* site-specific explanatory variables, x , and the unknown column-vector parameter β. β_{ij} is therefore the effect of explanatory variable x_{ij} on η, at site *i.* The constraint $\beta_{i1} = 0$ is enforced to allow identifiability, a common practice in logistic regressions (Agresti 2002, p271 – 273).

$$\eta_i = \sum_{j=1}^{J} x_{ij}\, \beta_{ij} \qquad (3)$$

The ordering of categories in this model is then enforced through a set of *K-1,* (Where *K* is the total number of categories) 'cut-point' parameters, c. These cut-points have an inherent ordering; $c_1 < c_2 < c_3 < c_4$. Although η is unobserved, we categorise outcomes according to the following rules, where Θ_{ik}, is the logit of the probability of observing state *k* at site *i.*

$$\Theta_{i1} = 1 - (\eta_i - c_1)$$

$$\Theta_{ik} = (\eta_i - c_{k-1}) - (\eta_i - c_k) \qquad (4)$$

$$\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots$$

$$\Theta_{iK} = \eta_i - c_{K-1}$$

We can then calculate the probability of an observation Yi being in state k, through the inverse logit function.

$$Pr(Yi = k) = \frac{e^{\Theta_{ik}}}{1+\sum_{k=1}^{K-1} e^{\Theta_{ik}}} \qquad (5)$$

The non-linear transformations of the parameters in these models can make model output difficult to interpret. Fortunately, the effect of changes in model covariates on the linear predictor can be easily visualized by thinking about the probability distribution of η in relation to the model cut-points. Figure 2. illustrates how changes in the construction of η will affect the probability, $\Theta,$ of observing density state k. The value of Θ can be thought of as the integral of the density of η that lies between the corresponding cut-points. Panels show how changes the location of the linear predictor η (due to changes in covariates), change the probability of observing a particular category. As there is considerable uncertainty when it comes to estimating parameters in these models with non-orthogonal observational data, we employ a Bayesian framework using the probabilistic programming language Stan (Stan core development team 2017) to allow flexibility in parameterization and to account for this uncertainty.

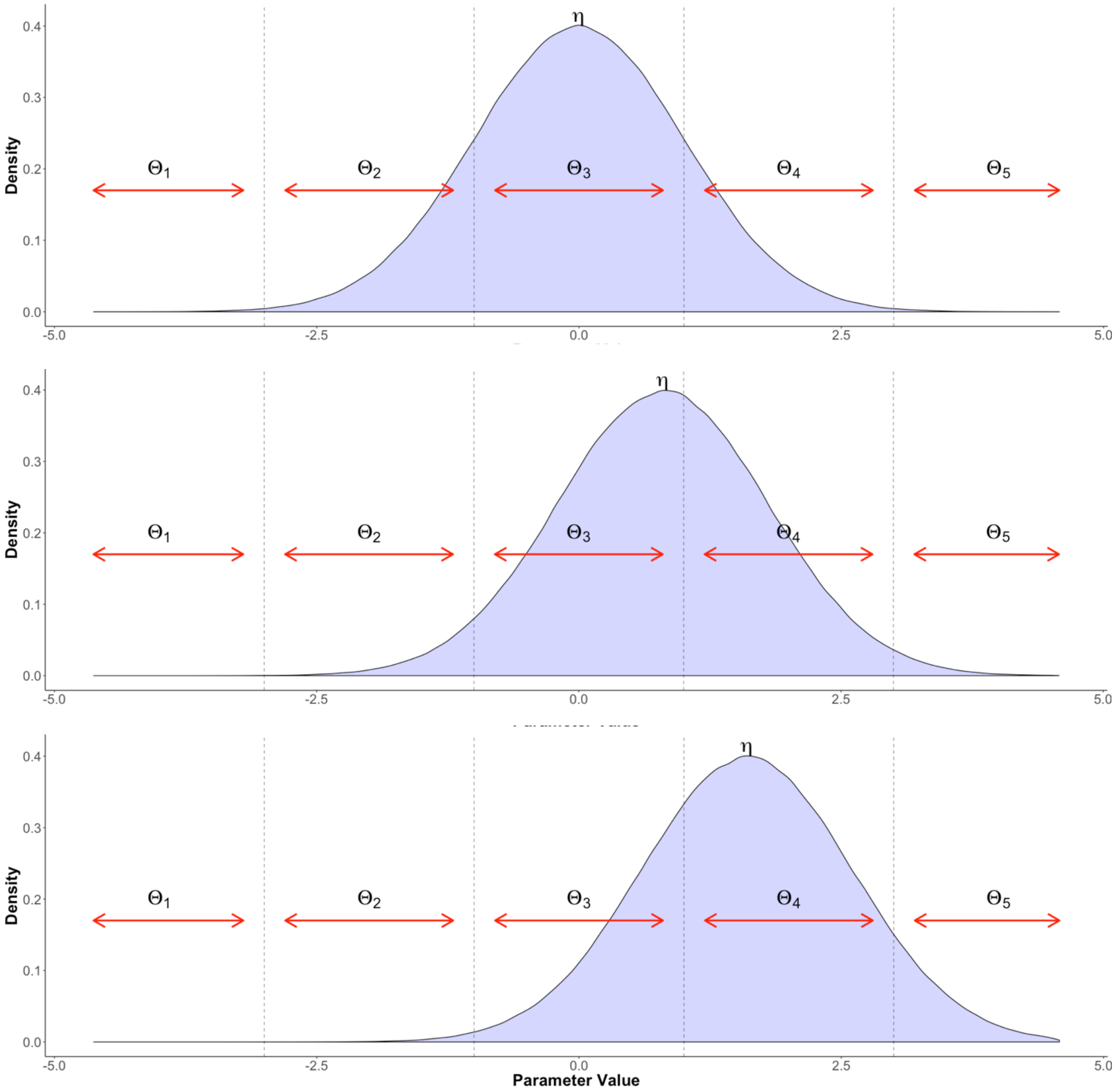


Figure 2. The distribution of the unobserved parameter η *for a single observation with changes in model covariates. The distribution of the linear predictor* η*, is in blue and global model cut points are represented by dashed grey vertical lines. Logits of state transition probabilities are denoted by* Θ*, the red arrows illustrate the part of the probability distribution of* η*, where the integral of which is equal to the logit of the transition probability. Panels illustrate how changes in the location of* η*, (as a result of changes in model covariates), change the logit of transition probabilities to different outcome states.*

We construct a series of ordered category logistic regressions to compare alternative formulation for constructing the transition probabilities to model population dynamics in a hierarchical density-structured framework. A. *myosuroides* occurs country-wide in the UK, yet sub-populations exist and are measured within individual fields. Some processes will affect all populations in a similar manner, however each field-level population will simultaneously be subject to a variety of different local environmental drivers and will display differing responses. Models II-IV (summarized in table 1.) all take different approaches to modelling the hierarchy present in our dataset.

Model	**Description**	**Linear predictor**	**Cut-points**
I	Global / non-hierarchical	$\eta_i = x_{ij}\beta_{ij}$	Global
II	Field-level intercept	$\eta_i = x_{ij}\beta_{ij} + \gamma_{if}$	Global
III	Field-level source state effect	$\eta_i = x_{ij}(\beta_{ij} + \gamma_{ijf})$	Global
IV	Field-level cut-points	$\eta_i = x_{ij}\beta_{ij}$	Field-level
V	Field-level cut-points & intercept	$\eta_i = x_{ij}\beta_{ij} + \gamma_{if}$	Field-level

Model I is the baseline formulation presented in equations (3) - (5). Our general model incorporates the effect of source (i.e. density state of site *i* at time *t*) state as covariates $x_{i1} \dots x_{i5}$. The linear predictor η_i is therefore a function of the source state j at site *i*. Modelling the dynamics in this manner allows us to construct the transition probability matrix for any given set of sites, as source state is recorded in the matrix *x*. Model I is used as a reference for comparison and has no hierarchical components, but we use this formulation as a baseline for all subsequent models.

Model II – Field-level intercept.

The simplest implementation of a model that accounts for the between field variance involves including a field-level effect in the construction of the linear predictor. The scalar intercept term γ_f represents the field level effect on the linear predictor within field *f*. Here the cut-point parameters $c_k \dots c_{K-1}$ remain as in equation (4):

$$\eta_{if} = \sum_{j=1}^{J} x_{ij}\,\beta_{ij} + \gamma_f \qquad (5) \quad \text{Model II}$$

γ_f was drawn from a vague prior, a normal distribution with mean 0 and standard deviation σ_f. Where σ_f is the hyper-parameter for the standard deviation for field effect γ_f, itself drawn from a half-cauchy distribution with 5 degrees of freedom.

$$\gamma_f \sim N(0, \sigma)$$

$$\sigma \sim Cauchy\,(0{,}5) \qquad (6)$$

The population wide effect of source states 1-4, $\beta_{j+1} \dots \beta_J$, were drawn from independent normal distributions, of mean 0 and standard deviation 10:

$$\beta_j \sim N(0{,}10) \qquad (7)$$

Cut-point parameters were given a wide uniform prior:

$$c \sim unif(0{,}10) \qquad (8)$$

Model III – Field-level source state effects.

The logical extension of this model is to allow more flexibility in the construction of the linear predictor by allowing the source-source state effect to vary between fields. The global effect of source state β_{ij} and global cut-points c_k ... c_{K-1}, remain as in model II. The addition to this model is vector γ_{jf} which represents the effect of source state j in field f on η. As the source state effect β_{ij}, is the parameter that allows us the construct transition matrices, allowing this to vary between fields aims to account for the various drivers that affect changes in black-grass densities between surveys:

$$\eta_i = \sum_{j=1}^{J} x_{ij} \left(\beta_{ij} + \gamma_{jf}\right) \qquad (9) \quad \text{Model III}$$

The population wide effect of source states 1-4, β_{ij+1} ... β_{iJ}, were again drawn from independent normal distributions, of mean 0 and standard deviation 10:

$$\beta_j \sim N(0{,}10) \qquad (10)$$

The field-level source state effects, γ_{jf} were drawn from a multivariate normal prior with dimension 5, for each source state.

$$\gamma_{jf} \sim MVN(\mu, \Sigma) \qquad (11)$$

Where μ is a K length vector of 0's, and Σ, is a K dimensioned covariance matrix. We induce a prior on Σ through $\Sigma = S.\Omega.S$ (Barnard, McCulloch and Meng, 2000) where S is the diagonal matrix of the standard deviations of each component of Σ, σ_k, and Ω is the corresponding correlation matrix of Σ. Within Stan this is parameterized in terms of cholesky decompositions of the covariance matrix for efficiency and numerical stability (Stan development team 2017). Hence, we use the recommended combination of a half-cauchy prior on the standard deviations and LKJ distribution (Lewandowski, Kurowicka and Joe, 2009) as a prior on the cholesky factor of Ω. Where $\Omega=\mathbf{L}\mathbf{L}^{\mathbf{T}}$; $\mathbf{L}$ is the lower-triangular matrix of Ω, and $\mathbf{L}^{\mathbf{T}}$ its transpose.

$$\sigma_k \sim Cauchy\ (0{,}5)$$

$$\mathrm{L} \sim LKJ(\mathrm{H}) \qquad (12)$$

The hyper-parameter (H) in this case is set to 1, which reflects a uniform distribution on the correlation matrix.

Model IV – Field-level cut-points.

Many approaches to random effects modelling utilize the approach we have outlined above, accounting for group level variance by including a term for random effects in the construction of the linear predictor. In an ordered category logistic regression an alternative approach is to allow cut-points, which control the conditional probability of an observation being in state *1:K*, to vary between each group. This can be implemented by allowing for each field, *f*, to have its own set of cut-points, as outlined below.

$$\Theta_{i1f} = 1 - \left(\eta_i - c_{1f}\right)$$

$$\Theta_{ikf} = \left(\eta_i - c_{k-1f}\right) - \left(\eta_i - c_{kf}\right) \qquad (13) \quad Model\, IV$$

……………………………..

$$\Theta_{iKf} = \eta_i - c_{K-1f}$$

Within this framework, however, it is necessary to ensure that the ordering of the cut-point parameters remains intact, i.e. $c_1 < c_2 < c_3 < c_4$ must be true for a given field. This can be achieved by re-parameterization of the cut-points themselves (Tutz and Hennevogl, 1996; Hartzel, Agresti and Caffo, 2001). Ordering is handled by mapping the ordering constraints onto the differences between cut-point parameters:

$$c_k \begin{cases} c_1 & if\ k = 1, and \\ \log\left(c_k - c_{k-1}\right) & if\ 1 < k \le K \end{cases} \qquad (14)$$

Group-level cut-points can then be implemented by using hierarchical priors on the parameter space between model cut-points and fixing the first group-level cut-point parameter c_{1f}:

$$c_{1f} \sim N(0,1)$$

$$c_{kf} - c_{k-1f} \sim N(\mu, \sigma) \qquad (15)$$

The first group-level cut-point, c_{1f}, is given a standard normal prior whilst distance between subsequent cut-points are given normal priors with a mean of μ and standard deviation σ.

The mean for all cut-point distances μ is given a wide normal prior with a mean of 0 and standard deviation of 10. The standard deviation of cut-point distances σ is given a half-cauchy prior with mean of 0 and 5 degrees of freedom.

$$\mu \sim N(0,10)$$

$$\sigma \sim Cauchy(0,5)$$

Here the linear predictor and associated prior distributions are identical to the formulation in model I. We can illustrate the effect of field-level cut-points on the transition probabilities Θ in the same way we have above. Figure 3. illustrates how changes in cut-points on the value of η_i for a model with a global linear predictor.

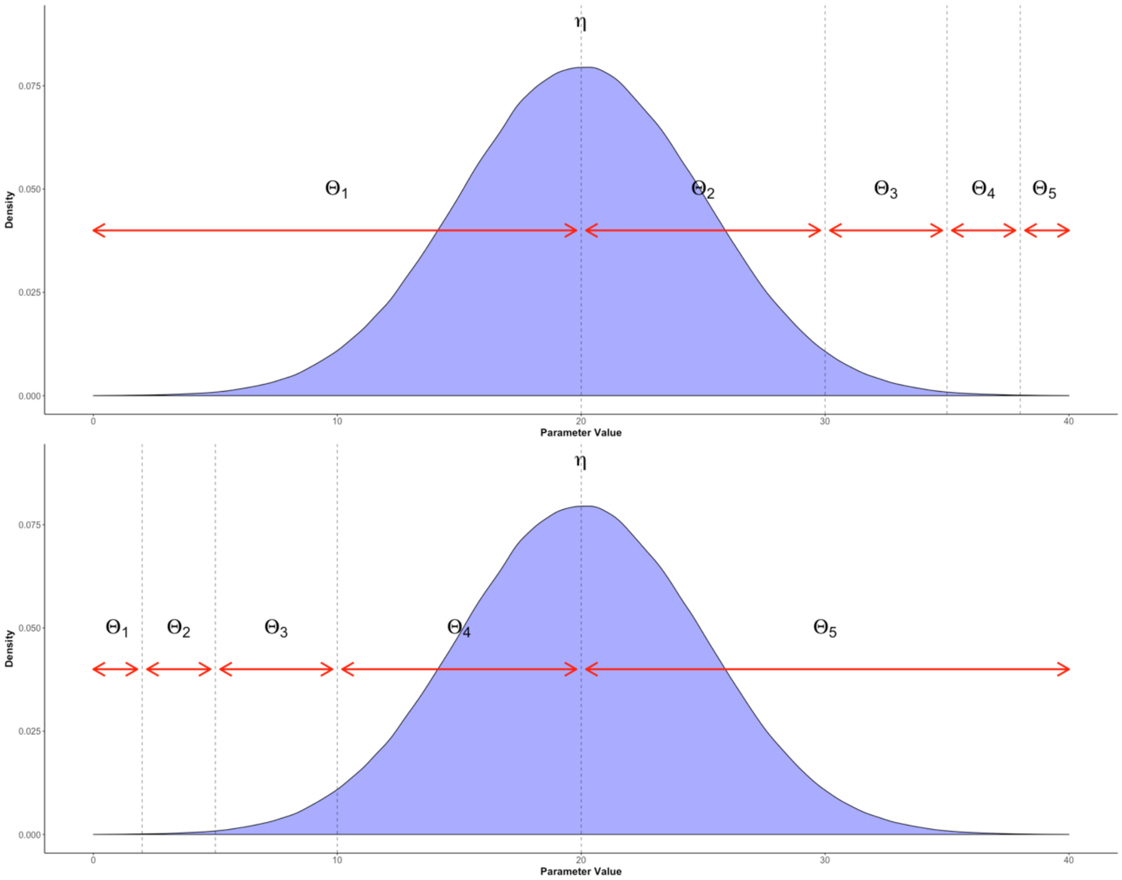


Figure 3. Illustration of the effect on Θ from a model with hierarchical cut-points. The distribution of the linear predictor η is in blue, whilst random cut-points are grey vertical lines. Logits of density state transition probabilities are denoted by Θ, the red arrows illustrate the part of the probability distribution of η where the integral of which is equal to the logit of the transition probability. Panels illustrate the distribution of η between sets of cut-points for different fields.

Model V – Field-level cut-points and field level intercept.

The final model, Model V, is a combination of the above, with both random cut-points and a random intercept included in the linear predictor. As such the linear predictor is the same as in Model II, and the cut-point parameters are the same as in model IV.

Model Fitting

All models were fitted using adaptive Hamiltonian MCMC, implemented in Stan version 2.17 (Stan Development Team 2017), interfaced with R (R version 3.4.0, R core development team 2017) through the package Rstan (Version 2.17.3, Stan development team 2017). All Models were run with 4 independent chains over 3000 iterations each with a 1000 iteration adaption period. Trace plots were inspected to assess mixing and potential convergence problems, of which no evidence was found. Scale reduction factors were calculated to assess if increased iterations would improve mixing, all of which were close to 1, suggesting iteration number was providing optimum efficiency.

Assessing predictive performance and posterior checks.

To assess model performance across all rotational subsets we use leave-one-out cross validation implemented in the 'loo' R package (Vehtari, Gelman & Gabry 2016), and WAIC as a measure of relative predictive error (Watanabe 2012). We also visualize model performance via graphical posterior predictive checks. We simulate field scale density distributions from posterior distributions and compare them to the observed distributions in each corresponding field, to assess model fit. We compare full distributions mean density states, and a measure of continuous abundance calculated by fitting a gamma distribution to the categorical density state distribution (Freckleton et al 2011).

The log of field-scale population density can be approximated from fitting a gamma distribution to a stable density state distribution in the form:

$$gamma(x; \alpha, \beta) = \frac{\beta^{\alpha}}{\Gamma(\alpha)} x^{\alpha-1} e^{\beta-x}$$

Where this distribution is defined by two key parameters, the shape (α) and rate (β). The mean of this distribution is α / β, and variance is α / β^2. If the distribution of density states 1:K, predicted or observed, was **s,** then the numerical mean of **s** **(**α / β) can be approximated by finding the values of α and β that best fit the stable density distribution. The above fits were optimised using the 'optim' function in R (R Development Core Team 2017).

Results

Models that incorporate field-level effects into estimating transition probability have better predictive accuracy than our non-hierarchical model (Figure 4). The lower row of Figure 4 removes model I to allow clearer comparison within the set of hierarchical models. Here LOO cross-validation provides the most support for models that incorporate hierarchical effects through cut-point parameters, however they all provide similar levels of predictive accuracy. The hierarchical cut-point models (Models IV & V) provide the best estimates, while models that only incorporate hierarchical structure in the linear predictor fare slightly worse than group-level cut-point models, whilst there is no difference between in the field-level cut-point model and the combined linear predictor/ cut-point model. Rotational subsets of our data all display the same order of model preference, suggesting that each model performs similarly under different cropping systems.

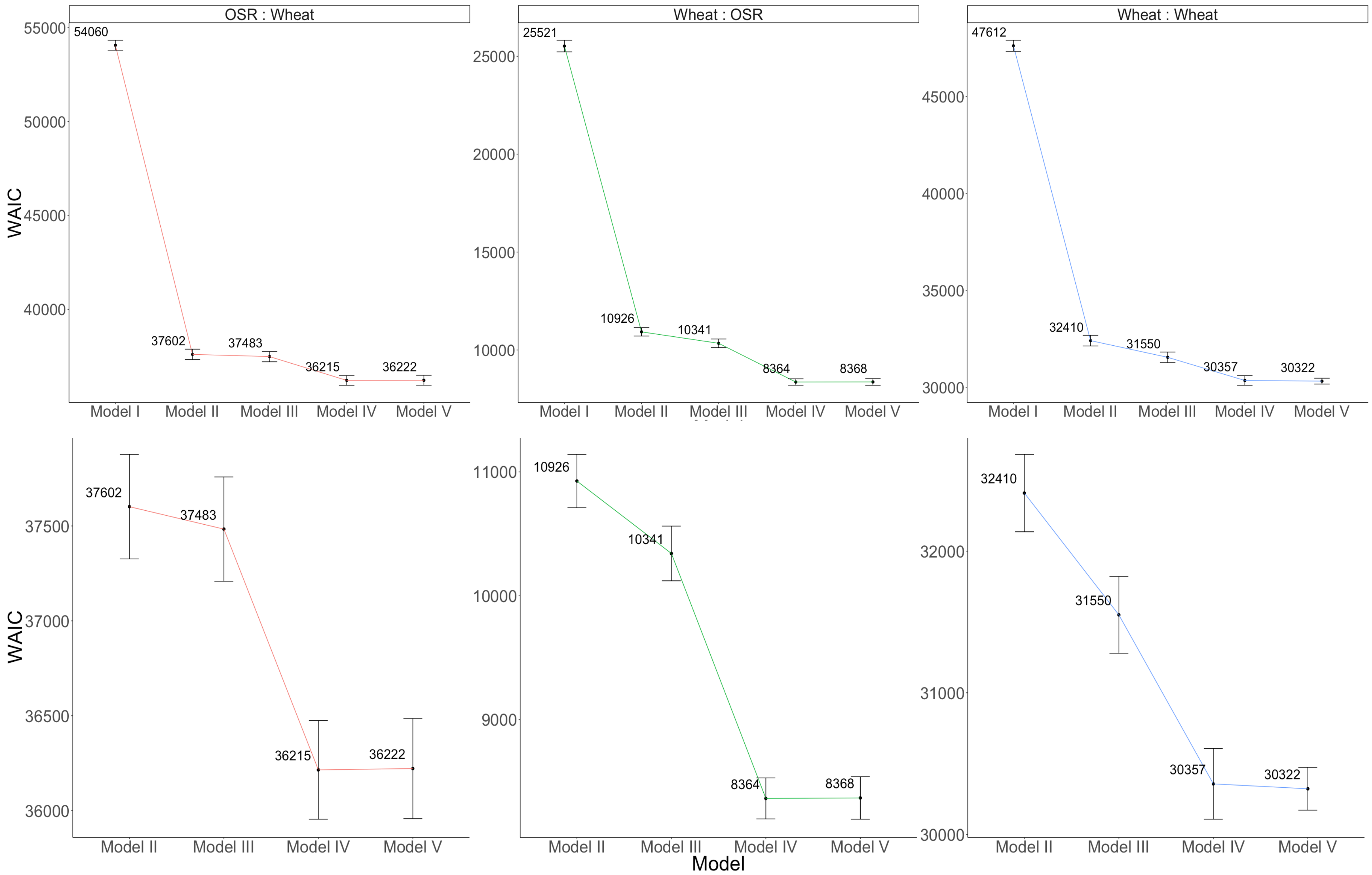


Figure 4. WAIC scores for each model and rotational subset. Top row compares all models, the second row removes model I for closer comparison of hierarchical models. Vertical bars are the standard errors around each WAIC estimate.

Overall observed vs predicted outcomes for each model (figure 5), show that higher density states are more difficult to predict accurately for all models, but models II-IV do considerably better than model I. Despite relatively poor point-wise predictive performance, all hierarchical models capture field-scale density state distributions well. Observed and model predicted density state distributions for 5 example fields that rotated from wheat to wheat are shown in figure 6. These fields were selected to demonstrate performance over a range of different field scale density-state distributions. These examples illustrate that our non-hierarchical model provides less accurate predictions than models that have hierarchical structure, as median density frequencies are further away from observed values and 90% density intervals are wider. Adding field-level effects to the linear predictor (Model II & III) visibly reduces model error in these examples and median density frequencies are relatively accurate for most distributions. Implementing the hierarchical effects through model cut-points provides a further increase in accuracy, with predicted density state distributions matching observed values in most cases. Although there is considerable inaccuracy from all models when predicting the distribution in the final field (column 5 of figure 6.), all hierarchical models correctly predict the shift to a larger proportion of higher density states, whilst Model I does not.

Figure 7, illustrates the difference between predicted and observed field-scale mean density states (top row) and mean log density per survey quadrat (bottom row). It again is apparent that hierarchical models provide better predictions than our baseline model, and although slight improvements are seen from models IV & V compared to II & III, all hierarchical models have similar performance in that in all case the prediction error and 90% density intervals are close to zero. There are, however, noticeable differences in the predictive accuracy of the non-hierarchical models between rotational subsets. Variance in predictive error is much higher in fields rotating from wheat or OSR into wheat, than from wheat into OSR. The lower Spearman's rank correlation coefficient associated with the predictions of Model I is also accompanied by a tendency to overestimate field-scale density. This is not the case with the hierarchical implementations, with all models displaying smaller error distributions and higher correlations between observed and predicted densities. Moreover, looking at the difference between overall predicted vs observed density-state distributions it is apparent that hierarchical cut-point models perform better than those that incorporate field-level effects in the linear predictor (Figure S1).

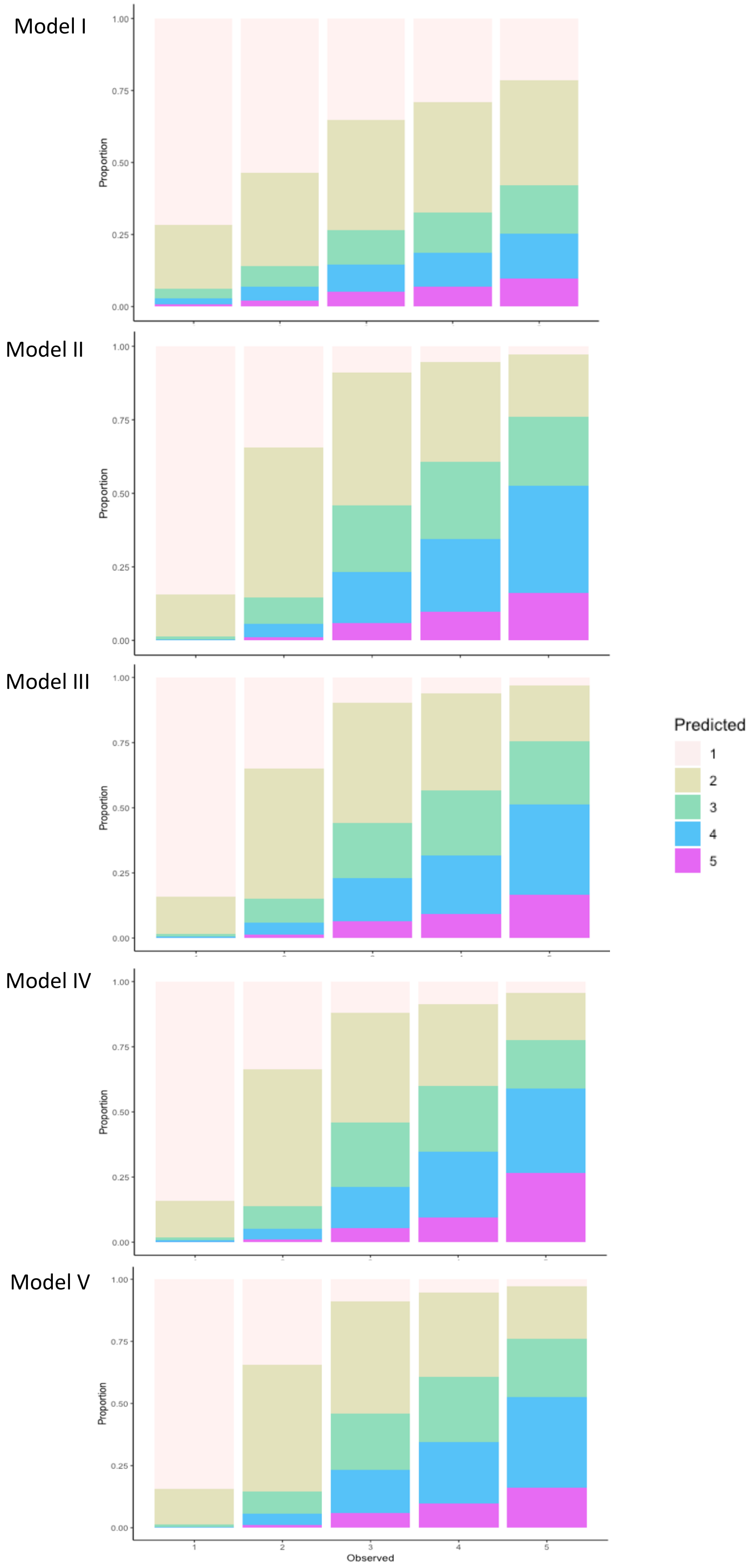


Figure 5. Predicted vs observed density states for each model in the wheat:wheat subset. Quadrat level transition probabilities were computed over the entire set of posterior samples, these probabilities were then sampled 1000 times to produce a distribution of density state predictions. Each bar represents the entire set of predictions for each observed density state, and each coloured sub-bar represents the proportion of those predictions in each category.

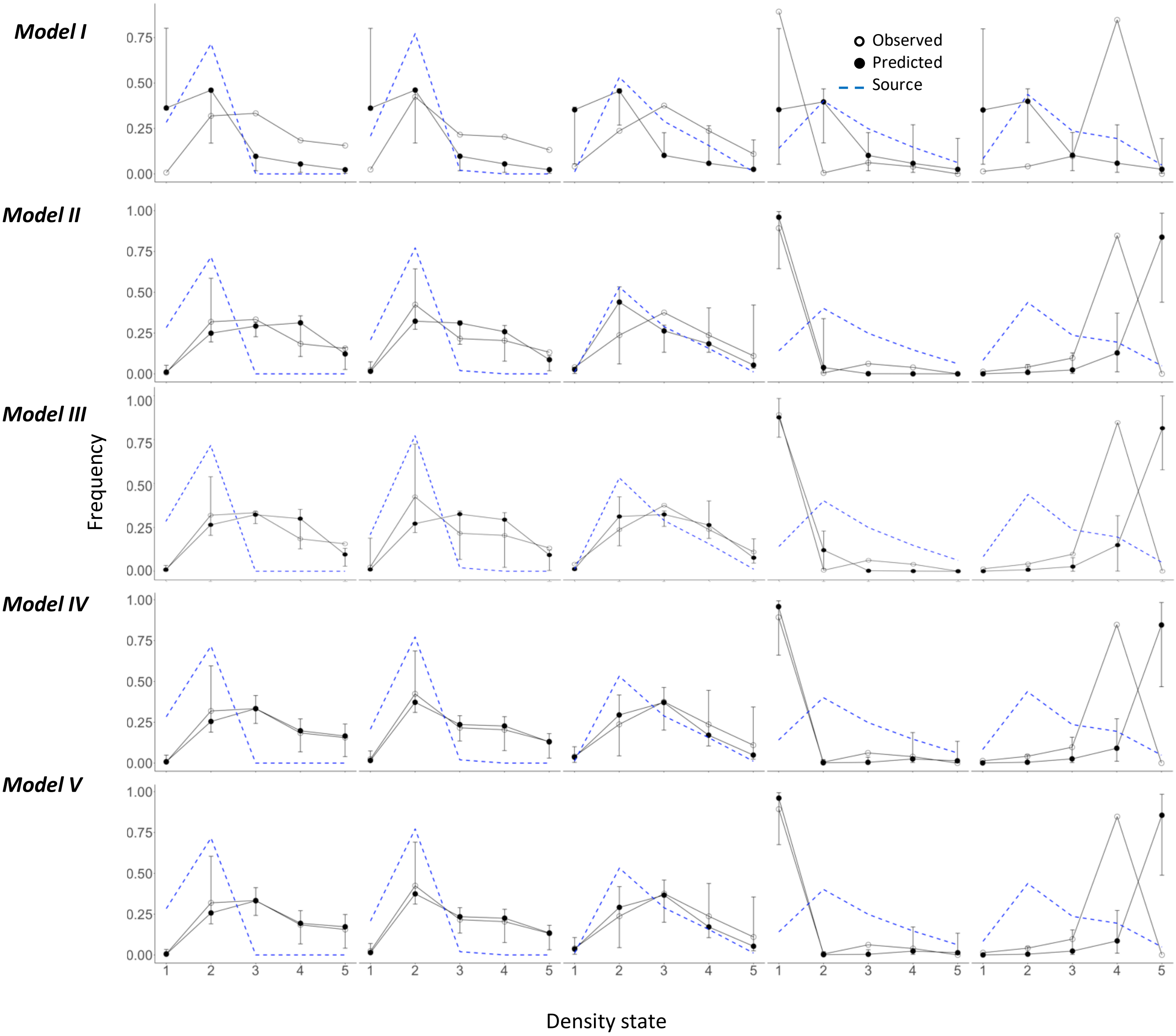


Figure 6. Examples of field-scale observed vs predicted density state distributions in the wheat to wheat rotational subset. Black points represent are median predicted densities, vertical bars are 90% density intervals, and hollow points are the observed distribution for that field. Dashed blue lines represent the source state distribution. Columns represent individual fields, whilst rows represent each of our five models.

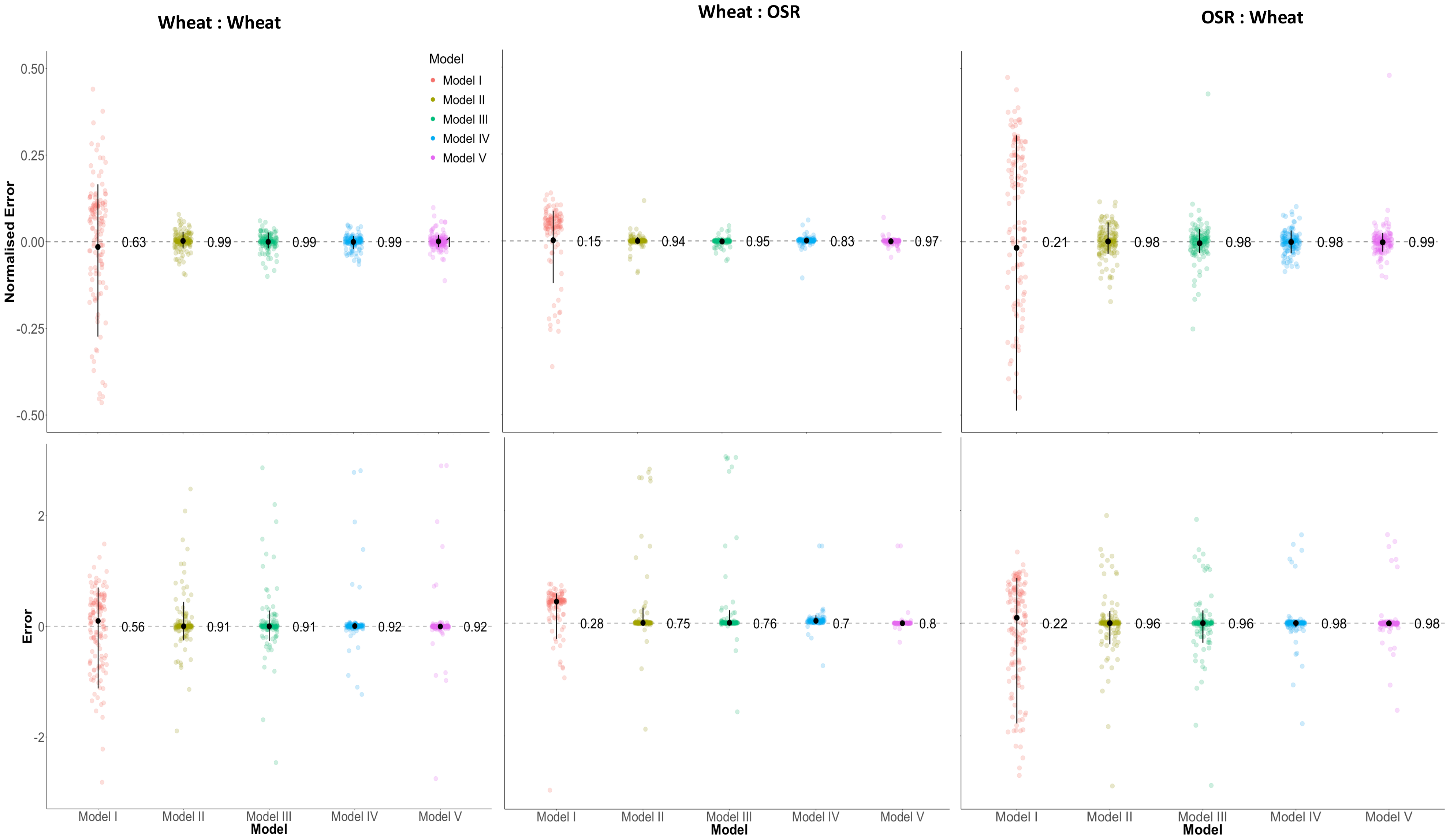


Figure 7. Field scale errors for mean density state (top row) and a continuous measure of density from a fitted gamma distribution (bottom row), by rotational subset. Black dots are median errors and vertical bars are 90% density intervals. Numbers next to each median are the spearman’s rank correlation coefficients between predicted and observed densities. Individual coloured points represent difference between observed and predicted values for an individual field.

Discussion:

It has previously been argued that density-structured models offer the potential to provide accurate measurement and prediction of population dynamics over large scales (Taylor & Hastings 2004; Freckleton et al. 2011; Queenborough et al. 2011; Mieszkowska et al. 2013; Tredennick et al. 2017). Consideration of environmental heterogeneity is an essential step towards understanding large scale population dynamics. Above we have implemented several hierarchical models designed to account for different sources of environmental and population heterogeneity. We demonstrate that a non-hierarchical approach based on a simplistic global model does not provide adequate field-scale predictions: this is to be expected given the enormous spatio-temporal variance in the dynamics of arable weeds (Freckleton and Watkinson, 1998, 2002; Freckleton and Stephens, 2009; Hicks *et al.*, 2018). Previously, most approaches to modelling density-structured data have treated density-state distributions as a multinomial response variable and have ignored the natural ordering of the data. Above we have implemented a simple improvement, in terms of efficiency and straightforwardness of parameterization, by using ordinal regression methods. Previously such models may have been harder to apply, but, as we demonstrate above, with modern probabilistic languages flexible implementations of these models are now straightforward and formulations much simpler.

Hierarchical models are a powerful approach when accounting for environmental or population heterogeneity and are capable of tackling problems with multiple layers of complexity (Wu & David 2002, Zipkin et al. 2009, Cafarelli et al. 2017). As density-structured models are inherently empirical approaches to understanding large scale dynamics of populations they naturally encapsulate large amounts of information on population and environmental variation. We have shown that adapting a density-structured framework to incorporate the hierarchical structure of a populations was both relatively simple and improved predictive power. Poor predictive performance for higher density states for all models may seem a cause for concern, yet all hierarchical models capture field-level density state distributions well, which are the primary input for density-structured models. It is important to note that although we assess predictive performance at the field level, the benefit of improved power will mainly be felt at the landscape-scale. Group-level effects are unmeasurable in advance, and the field-level components of this model probably could not be used to accurately predict the dynamics of a new field. However, as we have measured the variance in parameters across a large range of fields and environmental conditions, predictions of regional dynamics will be achievable. An obvious area for future research is therefore making these models better at field-scale prediction, which may be achievable by including more comprehensive covariate data. For example soil type, weather and climate all affect black-grass dynamics (Colbach and Sache, 2001; Colbach *et al.*, 2006; Metcalfe *et al.*, 2018), and including this information may increase model performance.

One clear criticism of modelling group-level effects in terms of model cut-points is that it deviates from the philosophy of using latent variable analysis for problems of this nature (Agresti 2002, p10-21). Latent variable models are useful tools for describing patterns in systems where the defining processes are complicated and difficult to observe directly. These models are popular in ecology as they allow inference for systems with complex interacting dynamic process and structures (Arhonditsis *et al.*, 2006). In most cases (and in models II,III & V presented above), the effects of environmental or biological factors are used to construct a linear predictor. Although not a true 'latent-variable', this component models the effects of underlying biological processes on the response variable. The key advantage of this approach is that it allows conceptualization of how biological processes contribute to the outcome. Incorporating hierarchical effects into the variables that produce the linear predictor allows specific statements to be made about the biological processes that vary between populations.

Modelling hierarchy as a function of the model cut-points makes it less obvious which biological processes are responsible for the variation. Shifts in relative cut-point positions essentially signify the same thing as a shift in the linear predictor; a change in the probability of an observation to transition to another density state. In models II & III, the global cut-points are directly tied to the definitions of density states, which in turn are tied to processes involved in the ecology of the weed, or the sampling process. Although density-structured models are generally robust to census error, density-state definitions are not static; they will vary through over time, space and observer. As hierarchical cut-point models are more flexible than models that account for field specific effects in the linear predictor, they may also account for more variation in observation error. Transition probabilities will also be linked to the condition of the crop or weeds (i.e. its competitive ability), both of which are features that might be missed by less flexible models. How variation in the observation process and more subtle aspects of weed ecology affect outcome in hierarchical cut-point formulations for these models should be considered when fitting these models in future applications. However, the major defence in response to this criticism is that density-structured models are inherently empirical and designed to be applied to real world problems. Model IV has better accuracy, comparable efficiency (Figure S2), and fewer parameters than other versions of the model, and as such will provide more useful information to managers about the state of current and future populations

Implications for future large-scale surveys:

Density-structured models have demonstrated their utility in addressing questions about large-scale population dynamics. These models offer improvements over many mechanistic or demographic models, which are often limited to small scales and are unreliable in the face of variation (Freckleton *et al.*, 2008, 2011; Queenborough *et al.*, 2011). With the addition of hierarchical effects, the potential utility and accuracy of these models can be expanded even further. Density-structured studies have the ability to capture population dynamics over scales much greater than the areas surveyed by more conventional approaches, and as such encompass a large degree of environmentally driven variation in population responses. Improving model prediction of when this variability will arise in population dynamics will allow better understanding of how and why populations change, but also how to manage them.

The dataset we analyze in this study is a good example of how density-structured models that account for population hierarchy will increase the landscape-scale predictive power of such frameworks. Our analyses demonstrate how variability in the environment a population can result in large prediction errors, but when field-level effects on transition probability are included, error margins are drastically reduced. Accounting for hierarchy demonstrably improves prediction accuracy of density-structured models in the face of environmental variation. In future, hierarchical density-structured models will allow us to understand and predict how individual sub-populations will respond to the multitude of interacting environmental variables over large scales. Hopefully, this approach will enable managers to apply localized interventions to address the specific drivers and responses of a particular population.

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Appendix:

Results & Discussion:

The field scale errors are broken down into errors for each destination density state in Figure S1. Here the error is the difference between the observed and predicted proportion of a field occupied by each density state. Predictive errors are noticeably higher in the Model I with wider density intervals and average field-scale errors diverging from zero. The models which implement a hierarchy through field-level effects in the linear predictor display lower levels of error than Model I, but there is not much discernable different between the two. Models IV & V are noticeably more accurate, with most of the error distributed close to zero. Most of the error is contained in the lower density states, with all models having lower error in density states 4-5. Figure S2. illustrates the differences in efficiency between models, in the terms of number of effective samples per second of sampling time. Model II (i.e. the simplest hierarchical model) is the most efficient, whilst model IV (the best performing model) is the second most efficient.

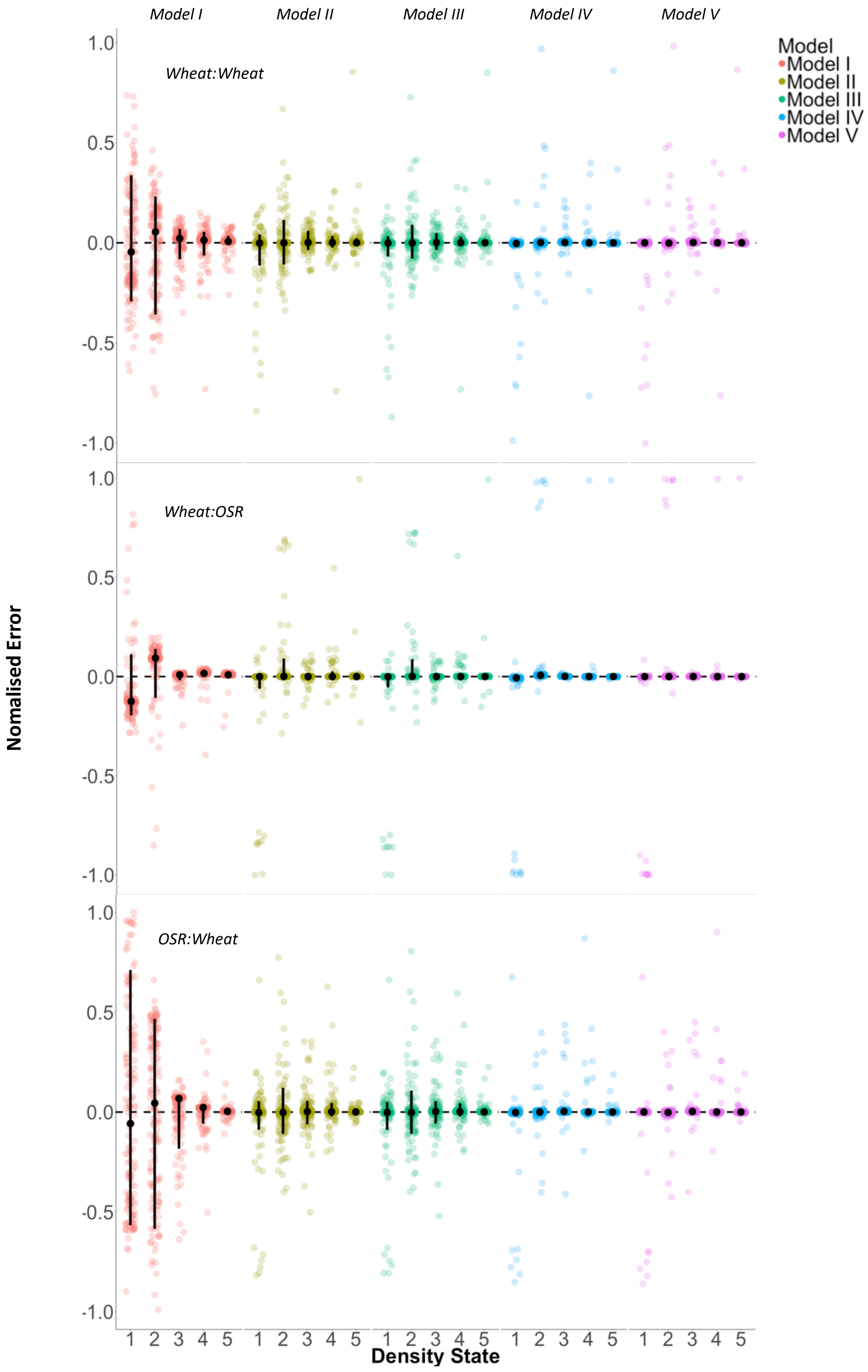

Figure S1. Field-scale error distributions by rotational subset. Black dots are median field-scale differences between predicted and observed frequencies of each density state (1:5), vertical bars are 90% density intervals. Coloured points represent the difference in observed vs predicted proportion of a density state in individual field. Colours highlight the across-state error distributions for each of our 5 models.

Despite the numerous advantages for using a Bayesian framework for hierarchical density-structured modelling, these kinds of models are notorious for being computationally intense and having long run times. Aside from predictive performance an important criterion for the ‘useful-ness’ of a model is its ability to produce predictions in a timely manner, as such we have presented the relative efficiencies of each model which are a useful metric from which to judge utility. From this it is apparent that in terms of effective samples per second that our simplest hierarchical model (Model II) is the most efficient, as in it produces the most effective samples relative to run time. In cases where practitioners may have limitations in terms of computational power we suggest that this model could be used in lieu of one of the more complicated versions, at only a slight loss in predictive power.

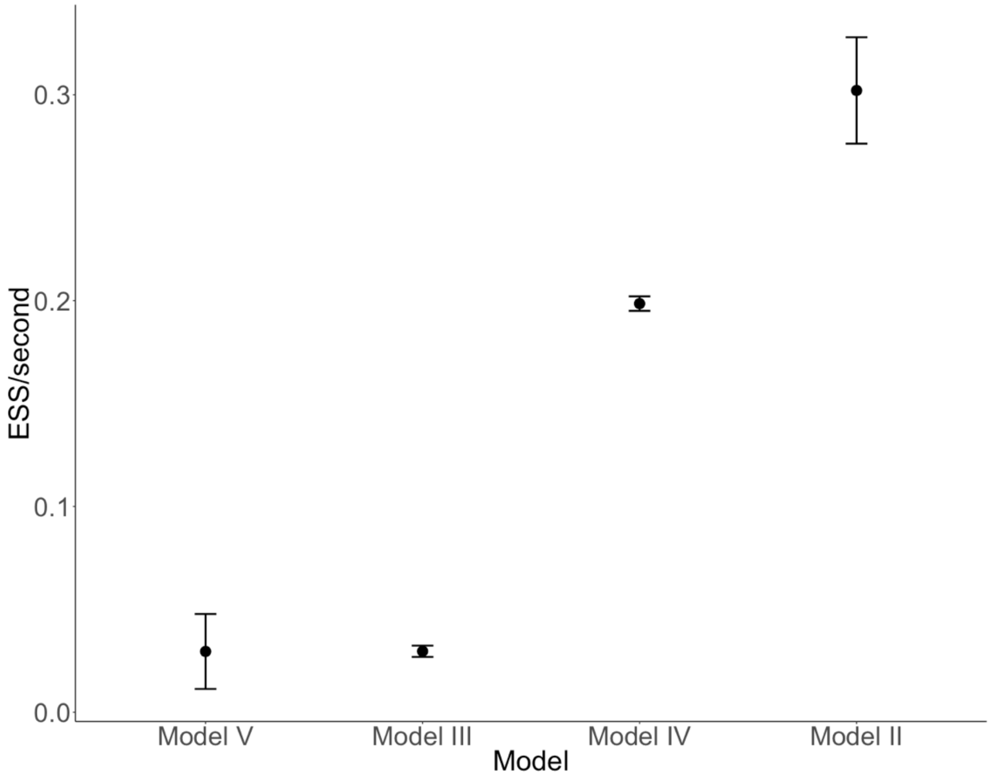

Figure S2. Model efficiencies in terms of effective samples per second of sampling time. Points are the mean value across all parameters and vertical bars standard errors. Models II and IV demonstrate better efficiencies.

Chapter Three:

Using density-structured models to investigate the landscape-scale dynamics and control of *Alopecurus myosuroides.*

Robert M. Goodsell, Shaun Coutts Matthew Spencer, Remi Vergnon, Simon Queenborough,Tom Swinfield, Dylan Z. Childs, Robert P. Freckleton.

Abstract:

Weeds are widely distributed, exhibiting varied population dynamics in response to a wide range of environmental conditions. Most studies of responses to managements, however, are derived from localized experiments under a very small range of environmental conditions. To limit the damage done by agricultural pests, we must understand how they respond to managements over the relevant scales and under a broad range of conditions. Unfortunately, traditional methods of gathering data and modelling population dynamics are expensive and limit the scale of ecological studies. Density-structured models are a method that facilitate large-scale assessment of populations via rapid surveys in an analytically simple framework. In this study we apply these models to study the population dynamics of an agricultural weed in response to a common control method: crop rotation. We analyse transient dynamics, short term and stochastic simulation experiments to assess the effect of different crop rotations on the dynamics of black-grass in a UK wide dataset. We identify several rotational strategies that, on average, reduce weed densities, as well as their temporal variance and autocorrelation. We demonstrate that rotations with high wheat dominance exhibit the most severe infestations, and rotations such as beans and potatoes have lower weed densities. However, the prevailing result is that localized field-specific effects contribute the most to the change in density over time, with rotation contributing comparatively little overall. Our results highlight the need for modelling large scale population dynamics whilst accounting for local scale variability in the drivers of weed dynamics.

Introduction:

The dynamics and drivers of population change vary at multiple scales (Gurevitch et al 2016, Chase et al. 2017), consequently, populations that are distributed over landscapes or regions are often of interest to ecologists (Levin, 1992). Due to their large distributions, these populations are invariably subject to a large range of environmental conditions, yet populations are typically studied in a single location where conditions are relatively static (Miller *et al.*, 2004). The combination of large-scale distributions combined with fine scale heterogeneity makes it difficult to draw general inferences about population dynamics when our empirical understanding is limited to local populations (Urban 2005; Coutts et al. 2016).

Pests are organisms detrimental to human wellbeing, and as the most important have wide distributions, the dynamics of these organisms has to be considered at large spatial scales and in the context of environmental heterogeneity (Mack *et al.*, 2000). An area of particular interest and relevance to large-scale population monitoring is the persistence and spread of agricultural weeds. Weeds are widespread problems by definition, with populations causing enormous economic loses and damaging biodiversity over large spatial scales (Wiese *et al.*, 1997; Tilman *et al.*, 2001; Walker *et al.*, 2005). Population models are key tools that allow the inference and prediction of range expansions of pests and have seen extensive use in weed ecology (Buckley, Briese, & Rees, 2003; Colbach et al 2005; Freckleton & Stephens, 2009; Freckleton & Watkinson, 1998; Freckleton et al. 2000; MacDonald & Watkinson, 1981). However, modelling weed dynamics is hindered by the difficulties in collecting detailed demographic data to parameterize accurate models (Cousens, 1995), and demographic models may become numerically unstable when highly variable data are used for parameterization (Freckleton et al. 2008).

Density-structured models are particularly suited to tackling these problems (Taylor & Hastings 2004). Assessing population dynamics at any scale requires intensive data collection, but there is a tradeoff between the amount of data an observer can collect and its quality: high quality data collection is time consuming and costly, whilst less thorough methods may produce data with large errors (Freckleton *et al.*, 2006). Density-structured models are an alternative to detailed local-scale surveys that can generate accurate population assessments and predictions over large scales, and are robust to errors from rapid, course resolution density surveys (Taylor & Hastings 2004, Freckleton et al. 2011, Queenborough et al. 2011, Mieszkowska et al. 2013, Tredennick et al. 2017, Freckleton et al. 2017).

Various factors influence the abundance of weeds in agro-ecosystems. These include climatic variables (Lima, Navarrete and González-Andujar, 2012; GarcÍa De León *et al.*, 2014), the state & management of soils (Metcalfe et al., 2017), and direct control interventions (e.g. Buhler 1999, Chauvel et al. 2001; Batlla Benech-Arnold 2003, Moss et al. 2007). Empirical measures of the impact of environmental drivers and weed control strategies are typically derived from meter scale study plots or individual fields (Buhler, 1999; Metcalfe *et al.*, 2017, 2018). Indeed, previous attempts at modelling weed dynamics have looked at relatively localized populations (e.g. Rees et al. 1996, Freckleton et al. 2000, Buckley et al. 2003), or have parameterized models based on small scale experimental data (Gonzalez-Andujar & Fernandez-Quintanilla 1991, Colbach et al. 2005, Colbach et al. 2006). In such studies, where the range of environmental conditions are narrow, predictions are difficult to extrapolate to larger areas. In the real world, these problems are spread over extremely large scales. To understand the impact of the various interacting combinations of management and environmental conditions, expanding the scale of empirical studies of weed population dynamics is essential. Density-structured models allow rapid data collection as well as analytically robust modelling of dynamics. And promise to allow examination of effectiveness of interventions over much larger areas than traditional methods.

Predicting densities of weeds is vital for understanding the effect of management. However, this is challenging given the huge spatial and temporal variability in population dynamics (Cardina *et al.*, 1997). We apply density-structured models to a multi-year survey that included over 1400 field-level assessments of black-grass densities. Black-grass is one of Europe's most economically damaging weeds (Moss, et al. 2007); it is widespread, inflicts significant yield penalties and is becoming increasingly hard to manage with cost-effective herbicides (Hicks *et al.*, 2018). Due to this diversifying management options is necessary to continue control, with increasing emphasis needed on non-chemical options. In this article we focus on the impact of crop rotation on weed populations, as it is an integral part of weed dynamics and central to cultural control strategies. To our knowledge this study and data set, which covers a total survey area of approximately 50 km^2, represents one of the largest studies of weed dynamics to date.

Using this data set within a density-structured framework we examine the effects of different rotations on weed densities that are directly estimated from large scale empirical data. We set out to investigate which rotations provide the best control for black-grass, as well as the spatio-temporal variability in management outcome. We examine the transient dynamics of black-grass populations using stable-state projection, short term effects using two-step-ahead predictions and long-term effects using stochastic models. Our results support the current consensus that crop rotation helps alleviate weed infestations, and our empirically supported models suggest some effective rotations for black grass control. However, on average, the effect of even the most effective crop rotation is small compared to environmentally driven between-field differences in black grass density.

Methods:

Study system & Survey:

The data set used in this study was collected as part of weed survey between 2007 and 2010, during this period the surveys recorded black-grass densities from over 400 fields across 72 UK arable farms in Bedfordshire, Cambridgeshire, Lincolnshire, Norfolk, Oxfordshire and Yorkshire. Data collection involved repeated surveys of individual fields across this four year period, to map changes in black-grass densities. Fields were divided up into a series of 20x20m survey quadrats or 'sites'. Observers walked the tramlines of each field and recorded black-grass densities in each quadrat as one of five discrete states; absent, low, medium, high or very high. This method produces an accurate description of black-grass densities as well as high inter/intra observer repeatability (Queenborough et al 2011).

Density-structured models:

Our density-structured models have the structure:

$$\boldsymbol{N}(t+1) = \mathrm{T}_{\mathrm{fr}}.\boldsymbol{N}(t) \qquad (1)$$

Where **N** is an ordered vector of the distribution of K density states at time *t*. This can be viewed as either the proportion of a given population occupied by each state, or the probability that a given site is in a particular state. T_{fr} is a $K\ x\ K$ matrix of transition probabilities parameterized from observed transitions in a field in a particular rotation, e.g. a field rotating from wheat into barley.

$$\mathrm{T_{fr}} = \begin{pmatrix} p_{11} & \cdots & p_{1K} \\ \vdots & \ddots & \vdots \\ p_{K1} & \cdots & p_{KK} \end{pmatrix} \qquad (2)$$

Entries p_{ij} are probabilities that a site in a state j will transition to state i. Diagonal entries of $\mathrm{T_{fr}}$ represent probabilities that an observed site will remain in the same state for the next survey, and off diagonals represent the probability of transition between states. For example p_{11} is the probability that a site in state 1, will remain in state 1, and p_{21} is the probability that a site in state 1 will transition to state 2. Equation (1) therefore represents a first order Markov model that can be used to predict future density state distributions.

Population dynamics under different rotations can be modelled by changing the rotation specific transition matrix at a specific time step in the Markov model:

$$\boldsymbol{N}(t+1) = \mathrm{T_{fr1}}.\boldsymbol{N}(t) \qquad (3)$$

$$\boldsymbol{N}(t+2) = \mathrm{T_{fr2}}.\boldsymbol{N}(t+1)$$

Where $\mathrm{T_{fr1}}$ is the field-level matrix containing the transition probabilities for the first half of the rotation (e.g. wheat to barley), $\mathrm{T_{fr2}}$ is the field-level matrix for the second half (barley to wheat), and $\boldsymbol{N}(t)$ is the initial density distribution.

The asymptotic dynamics of a two-step rotation, analogous to running the Markov chain continuously, can be studied via net transition matrices which are defined as the product of two component matrices:

$$\mathrm{T_{net}} = \mathrm{T_{fr1}}.\mathrm{T_{fr2}} \qquad (4)$$

A detailed explanation of density-structured models can be found in Freckleton et al (2010). The relationship between field-level surveys, density distributions and transition matrices is summarized in figure 1, Chapter 2.

Analysis of rotational controls:

To investigate the effect of different rotations on black-grass densities we use hierarchical density-structured models parameterized for each of the rotations summarized in Table 1. We parameterized field-specific transition matrices for each field observed in a rotation. For example, we generated 16 field-level transition matrices using observed density state transitions from the 16 fields rotated from barley into wheat, and 38 of wheat into barley. Transition probabilities are a function of the change (or stasis) in density states between years, so the observed density states in two consecutive years can be used to parameterize T_{fr} for a given field.

Table 1. Observed sets of rotations in the weed survey dataset, with number of surveyed fields and individual quadrats in each subset.

First crop	Second crop	No. fields	No. observations
Barley	Barley	16	3135
	OSR	17	3580
	Sugar beet	10	2043
	Wheat	7	1468
Beans	Wheat	29	5890
OSR	Wheat	145	29937
Potatoes	Wheat	13	2352
Sugar beet	Barley	11	2782
	Wheat	30	6320
Peas	Wheat	12	2508
Wheat	Barley	38	6969
	Beans	36	7660
	OSR	121	23370
	Potatoes	9	1698
	Sugar beet	28	5286
	Peas	17	3637
	Wheat	143	28518

Transient dynamics:

As the sum of ***N*** will always equal 1, the dominant eigenvalue in T is always 1. Density state transition models will therefore converge on a stable density structure (Caswell, 2001). A general approach to studying the transient dynamics of these models is to compare the stable structures and the rates of convergence of different models. To investigate the transient dynamics of particular cropping systems we compare net transition matrices for each two-step rotation. For each rotation we generate each possible permutation of net matrix from each possible combination of component matrices. For each of these matrices we then produced two summary statistics that allow us to examine the dynamics of a particular cropping system.

We calculate the stable density structure for each net matrix, from the ratio of the first and second eigenvectors of a net transition matrix (equation 5):

$$\boldsymbol{S} = \frac{v_1}{v_2} \qquad (5)$$

Where v_1 is the first eigenvector and v_2 the second of the net matrix. ***S*** is therefore the field-level distribution in terms of proportion of the field in each density state. We calculate the mean density state to summarise the stable density structure for each net matrix. This is calculated from the proportion of a population occupied by each state multiplied by the integer value (i.e. 1-5) of each state category. The rate of convergence to this structure is governed by the relationship between dominant and subdominant eigenvalues (equation 6).

$$\boldsymbol{P} = \frac{\lambda_1}{\lambda_2} = \lambda_2 \qquad (6)$$

Where λ_1 is the dominant eigenvalue (which is always 1 as row probabilities all sum to 1) and $\lambda_2$is the second largest eigenvalue. **P** is the 'damping-ratio' and gives a measure of sensitivity in the face of perturbation, or the rate at which a population will reach its stable density structure. The higher this ratio, the slower the convergence.

Short-term projections: two-step rotations:

Examining short-term dynamics allows understanding and prediction of tangible outcomes under each cropping system. Using the component matrices from each rotation from table 1, we constructed models for all possible wheat rotations. We made 2-step-ahead projections from winter wheat to an intermediate, or break crop, and then back to winter wheat (i.e. one full rotation), using the markov model in (3). We take the mean density state of the final density distribution $\boldsymbol{N}(t + 2)$, as the outcome. These projections were generated for each possible combination of field-level matrices, for three initial starting densities (representing typically low, middling and high levels of black-grass). Thus, for the wheat: barley: wheat example, there would be $16 * 38 = 608$ outcomes for each starting density, and therefore $608 * 3 = 1824$ outcomes in total. We compare the average outcome of each rotation from each initial starting density, as well as the relative change compared to winter wheat.

We use a transient life table response (LTRE - Caswell 1989), to analyse the variance in model outcomes due to local environmental effects (or field identity), initial densities and, rotation. This method uses the change in black-grass density from the two-step projections above as a response variable in a linear mixed effects model (e.g. Freckleton et al. 2017), to account for variance in population structure and intrinsic dynamics. We estimate the variance components associated with the field identity (i.e. the permutation of field-level matrices used in the projection), the initial density distribution, and the rotation applied to that field, to evaluate the relative contributions of each to the overall variance of the system.

Long-term dynamics: stochastic projections:

Finally, we model the stochastic dynamics of black-grass under a range of different cropping systems to allow us to examine the overriding effects of wheat dominance (i.e. the proportion of winter wheat in rotation), and type of break crop, on black-grass density. These alternative rotational options are summarized in Table 2. Stochasticity is implemented by random selection of a component matrix for a particular step in a rotation, projecting the density state forward with the markov model in (3), then calculating the mean density state at each time-step. All models were started at the average density distribution across all fields and projected for 10000 time steps to ensure convergence. Across time series we then compare means, variances, and coefficients of autocorrelation, for a series of rotations with differing break crops, levels of wheat dominance and rotational diversity (summarized in table 2). From this we can examine rotational effects on black-grass density, variability and autocorrelation - which can indicate whether observed densities are likely to persist.

Table 2. Cropping systems included in the stochastic simulations. Includes the length of the rotation in years, the number of years a field spent in a break crop and the wheat dominance (proportion spent in wheat) of a rotation. Rotation denotes the sequence of wheat crops (W) and break crops (B), where B1 & B2 represent the first and second break crops in systems with two different break crops.

Break Crop	Length	No. of Break Years	Rotation	Wheat Dominance
Barley	1	1	B : B	0
	6	4	W : B : B : B : B : W	0.33
	5	3	W : B : B : B : W	0.4
	4	2	W : B : B : W	0.5
	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
Beans	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
Beet	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
OSR	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
Peas	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
Potatoes	3	1	W : B : W	0.66
	4	1	W : W : B : W	0.75
	5	1	W : W : W : B : W	0.8
Barley : Beet	2	2	B : B	0
	6	4	W : B1 : B2 : B1 : B2 : W	0.33
	5	3	W : B1 : B2 : B1 : W	0.4
	4	2	W : B1 : B2 : W	0.5
	5	2	W : W : B1 : B2 : W	0.6
Barley : OSR	4	2	W : B1 : B2 : W	0.5
	5	2	W : B1 : B1 : B2 : W	0.6
Cont. Wheat	1	0	W : W	1

Parameterising transition matrices:

To account for spatial variability and allow us to investigate the effects of individual rotations, we implement a field-level hierarchical approach to generating transition matrices. We construct hierarchical ordered category logistic regressions in terms of a real-valued latent variable that reflects the true (unobserved) density.

The response variable y_{if}, is the outcome density at site i in field f and is an ordinal variable on a scale $1, \dots, K$. We model probability of observing a category k as a function of a linear predictor η_i. The linear predictor is constructed from the row-vector of *J* site-specific explanatory variables x and the unknown parameter column-vector β:

$$\eta_i = \sum_{j=1}^{J} x_{ij}\, \beta_{ij} \qquad (7)$$

In (7), β_j is the effect of explanatory variable x_{ij} on η , at site *i.* We use the density state observed at site *i* in the previous year as covariates β_1 through to β_5 to account for the effect of current density state on the density next year, and the constraint $\beta_1 = 0$ is enforced to allow identifiability (Agresti, 2002, p271 – 273).

In a typical ordered category logistic regression, the ordering of categories are enforced through a set of *K-1* 'cut-point' parameters, c, which have an inherent ordering, i.e. $c_1 < c_2 < c_3 < c_4$. We introduce hierarchical field-level effects to account for between population variance into the model through these cut-point parameters, such that c_{1f} is the first cut-point for field f and c_{4f} the final cut-point. Outcomes are categorized according to the following rules:

$$\Theta_{i1f} = 1 - (\eta_i - c_{1f})$$

$$\Theta_{ikf} = (\eta_i - c_{k-1f}) - (\eta_i - c_{kf}) \qquad (8)$$

………………………………..

$$\Theta_{iKf} = \eta_i - c_{K-1f}$$

where Θ_{ikf}, is the logit of the probability of observing state *k* at site *i* within field *f*. We can then calculate the probability of an observation y_{if} being in state *k*, through the logistic function:

$$Pr\left(y_{if} = k\right) = \frac{e^{\Theta_{ikf}}}{1+\sum_{k=1}^{K-1} e^{\Theta_{ikf}}} \qquad (9)$$

It was assumed that each observation was drawn from a categorical distribution:

$$y_{if} \sim categorical\left(\Pr\left(y_{if}\right)\right) \qquad (10)$$

Prior specification:

We employ a Bayesian framework using the probabilistic programming language Stan (Stan core development team 2017) for its flexibility. We use the following prior specifications. The population-wide effects of source states 1-4, β_{j+1} ... β_J, are drawn from independent normal distributions, of mean 0 and standard deviation 10:

$$\beta_j \sim N(0,10) \qquad (11)$$

To ensure that ordering of the cut-point parameters remains intact, i.e. $c_1 < c_2 < c_3 < c_4$. ordering constraints are enforced on unordered vectors (Tutz & Hennevogl 1996; Hartzel et al. 2001), by mapping these constraints onto the differences between cut-point parameters:

$$c_k \begin{cases} c_1 & if\ k = 1, and \\ \log\left(c_k - c_{k-1}\right) & if\ 1 < k \le K \end{cases} \qquad (12)$$

Modelling the group-level effects was implemented by imposing priors on the difference between model cut-points and fixing the first group-level cut-point parameter c_{1f}.

$$c_{1f} \sim normal(0,1)$$

$$c_{kf} - c_{k-1f} \sim normal(\mu, \sigma) \qquad (13)$$

The first group-level cut-point, c_{1f}, is given a standard normal prior whilst distance between subsequent cut-points are given normal priors with a mean of μ and standard deviation σ.

The mean cut-point distances across all fields, μ, is given a wide normal prior with a mean of 0 and standard deviation of 10. The standard deviation of cut-point distances σ is given a half-Cauchy prior with 5 degrees of freedom.

$$\mu \sim normal(0,10)$$

$$\sigma \sim cauchy(0,5) \qquad (14)$$

Results:

Transient dynamics:

Average transition matrices for each of the modelled wheat rotations in table 1 are presented in figure 1. There are clear trends even from a simple visual inspection of these matrices. Fields of continuous winter wheat exhibit higher probability of transition into higher density states than continuous barley (Figure 1, row 1). For example, an absent quadrat in continuous wheat has a 0.28 probability of being occupied by low densities of black-grass in the next year, whilst rotating from barley to barley the same transition is 0.11. A similar trend is apparent in matrices that model rotations *into* wheat (Figure 1, row 2). For example, there is an 0.18 probability that a patch of ‘very high’ density black grass will remain in that state when rotating from peas to wheat. Conversely, rotations into an intermediate crop (Figure 1 row 2) show the bulk of the transition probability into lower density states.

There is a great deal of diversity both between and within rotations. Fields of continuous winter wheat have higher probability of transition to higher density states than rotations that involve a break crop, but there are also differences between break crops themselves. Higher density states are more probable in barley & peas, whilst in beans, sugar beet and potatoes they are less likely. These differences in transition probability are reflected in the resultant stable density distributions (Figure 2b). Equilibrium density states for continuous winter wheat are higher and more variable than in rotations that include break crops or continuous barley. Using barley as a break crop results in the highest equilibrium density for rotated systems, whilst potatoes have the lowest. Continuous barley produces higher equilibrium densities than all rotations using break crops except those that use continuous barley.

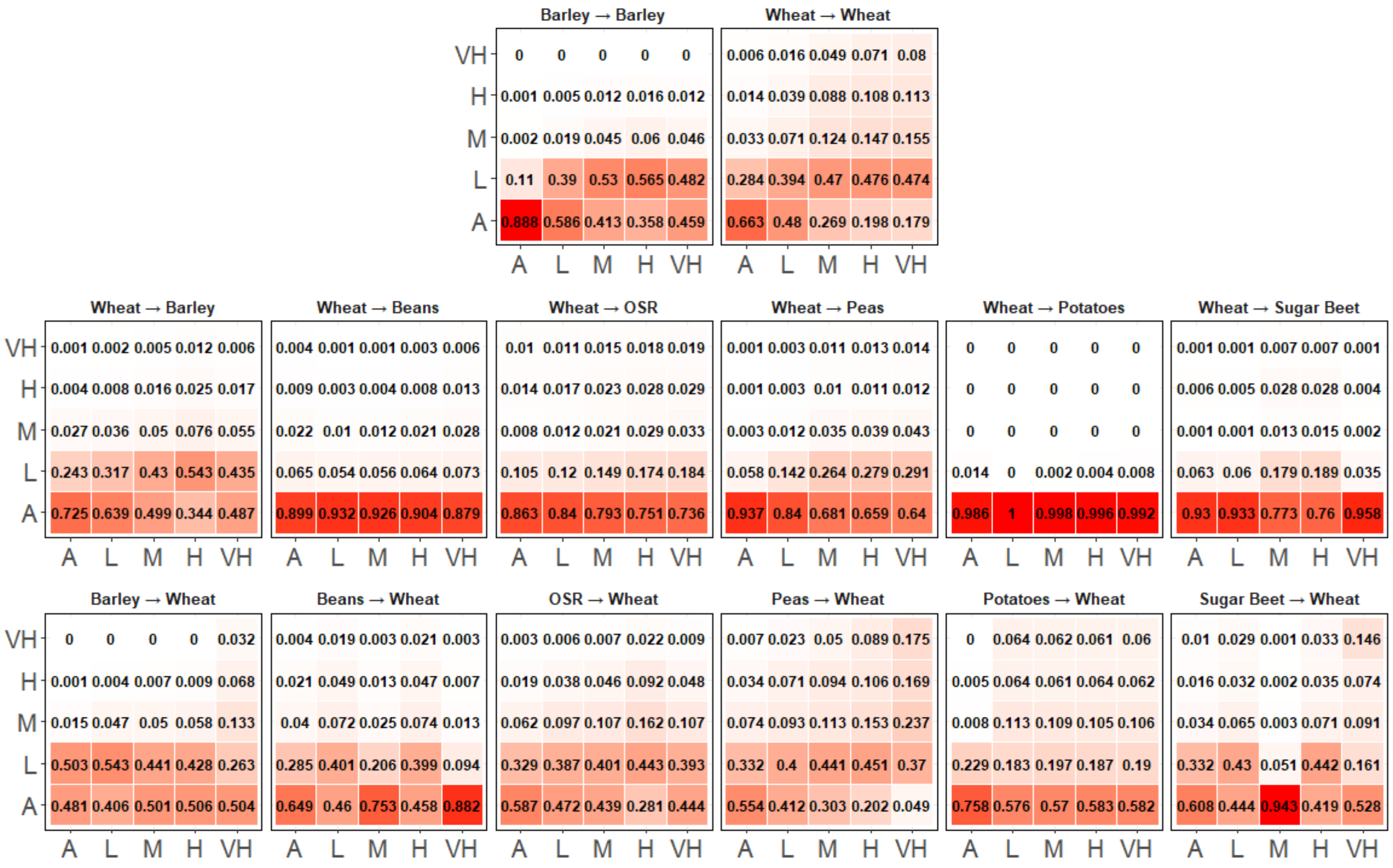

Figure 1. Across-field average transition probability matrices for example crop rotations . Matrices display the probability of transitioning from a state in year 1 (x axis) to any other state in year 2 (y axis). The darker the colour the higher the probability of transition. Numbers in each cell are the estimated probabilities. The first row are the matrices for continuous barley and wheat, the second row are matrices for rotations out of wheat, and the final row are rotations are rotations back into wheat.

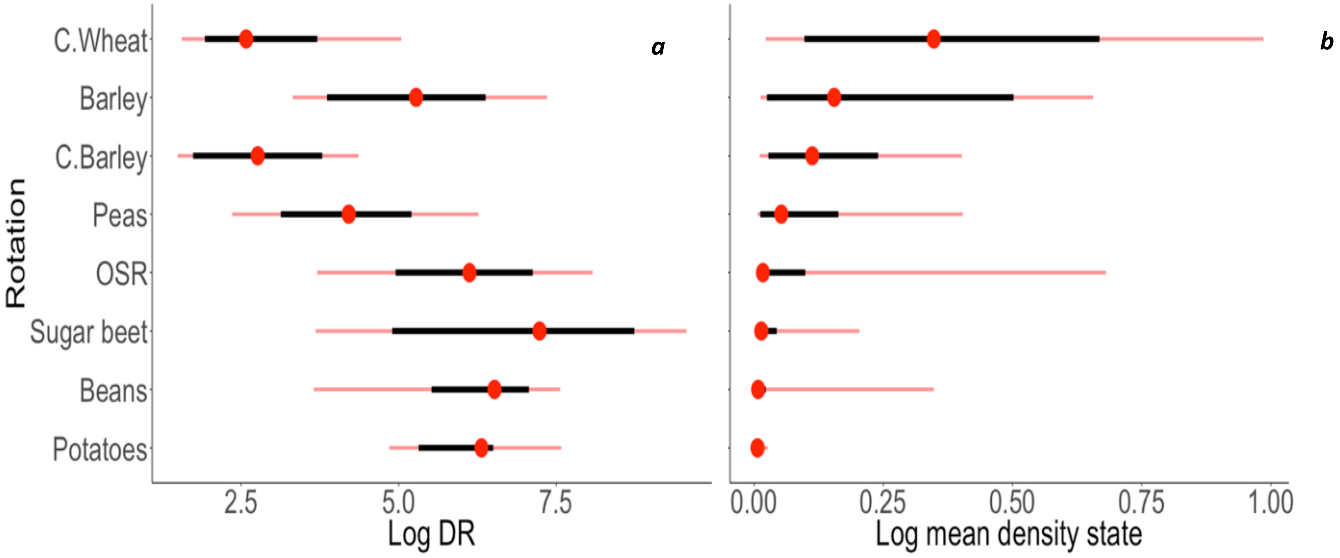

Figure 2. Results from analysis of transient dynamics using damping ratios and mean density states from stable state distributions . Distribution of field level log damping ratios (a) and mean density states (b) for each rotation, and continuous crops of barley and wheat (C.barley & C.wheat). Red points are the median value across all permutations of net matrices, red and black bars are 90% and 50% density intervals respectively.

Differences in field-scale damping ratios between different rotational strategies are also evident (Figure 2a) . Populations of black-grass that have been subjected to a rotation demonstrate higher damping ratios and therefore slower convergence on stable state distribution. As low damping ratios give an indication of convergence 'speed' to its stable density structure, higher values suggest sensitivity to perturbation. Paired with low equilibrium densities (as in the case of sugar beet, beans, OSR and potatoes), this can suggest that populations are easier to reduce or eradicate. As continuous wheat systems have low damping ratios paired with generally high stable density structures, this suggests that these systems allow the rapid growth and establishment of weed populations.

Short term dynamics: two-step rotation:

After two-step rotations, field-scale mean density states differ between, but are highly variable within each rotation (figure 3a). Continuous wheat and, to a lesser extent, continuous barley show sensitivity to the initial density, but some rotations seem to be invariant to initial state, for example rotation into OSR produces the same outcome regardless of initial conditions. The changes in mean density state relative to continuous wheat also vary with initial density distribution (Figure 3b). At low densities, beans and sugar beet offer low reductions and OSR and barley offer next to no reduction in final densities, whilst rotating to peas increases densities. This trend attenuates with higher starting densities with all rotations, except into peas, offering considerable reduction in relative density state at the highest initial density. As wheat is the only rotation that shows sensitivity, this will be driven by the effect of initial conditions on wheat, and not by the effect of particular break crops.

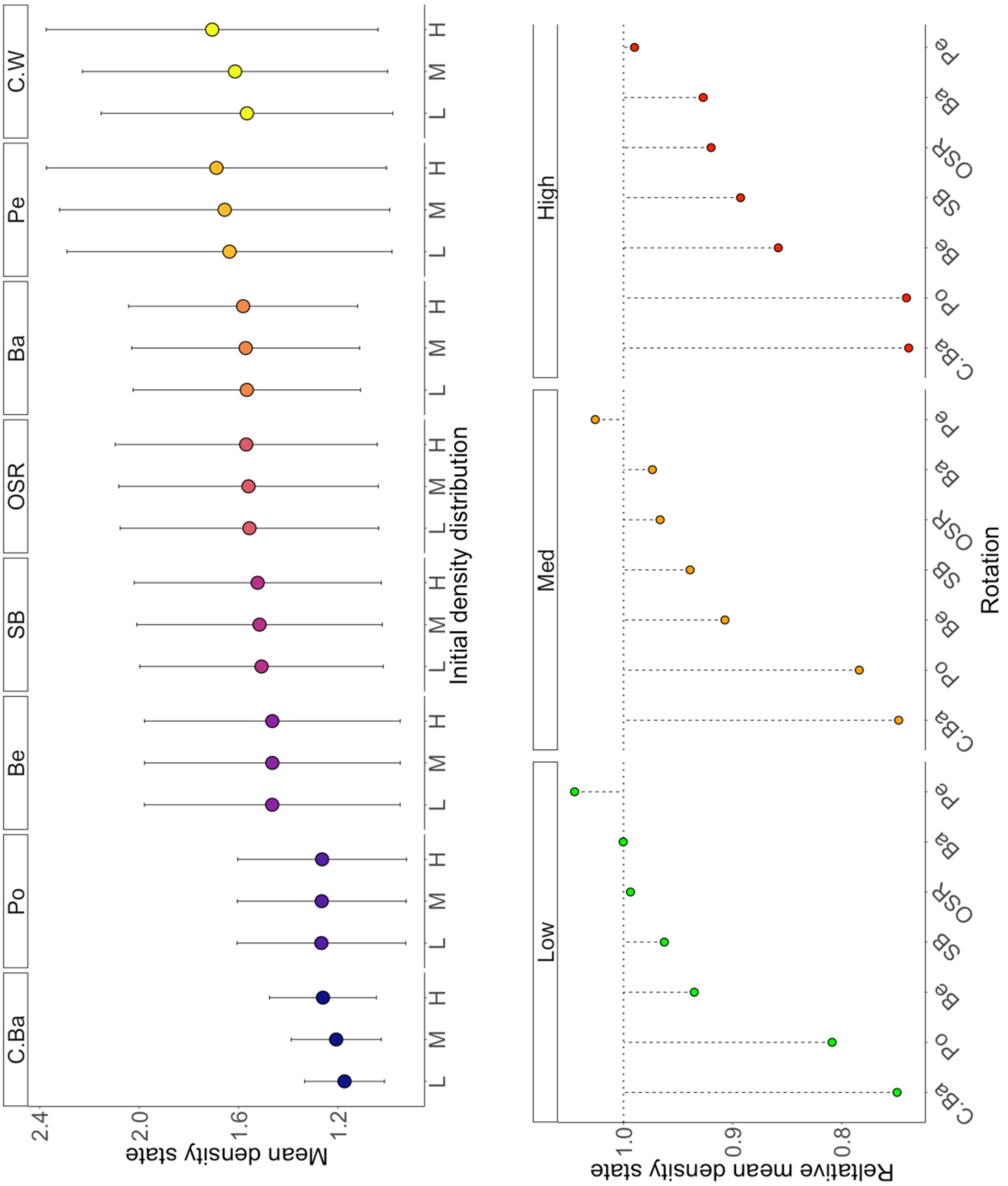

Figure 3. Mean density state (a) & mean density state relative to continuous winter wheat (b) for model projections under rotations from wheat into potatoes, beans, sugar beet, OSR, barley and peas, as well as continuous barley (Po, Be, SB, OSR, Ba, Pe, & C.Ba), for three initial densities (low medium and high).

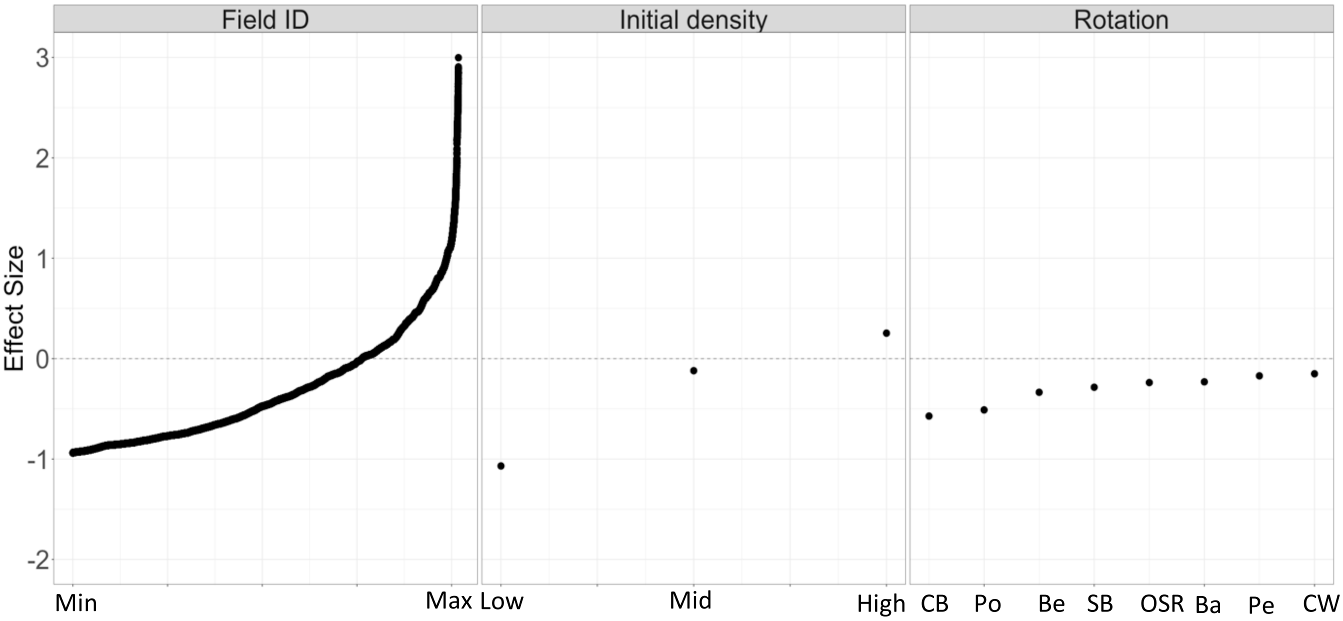

Figure 4. Contribution to the variance in the difference between initial and final densities by Field ID / matrix origin (first panel), initial density (second panel) and rotational control (third panel). Each point represents the size of the effect that variable had on the change in density.

The role of large-scale spatial heterogeneity, i.e inter-field scale variation, in the outcome of management is presented in figure 4. The figure illustrates the variance in change in density due to the identity of the pair of matrices used in the model (Field ID), the initial density conditions, and each of the rotations. Using a linear mixed effects model we estimated the variance contributions of each of these as 0.513, 0.118 and 0.0804, with a residual variance of 0.287. Within these two-step rotations it is clear that field ID (i.e. the field specific conditions) has the largest contribution to the final density state. The initial density contributes a reasonable proportion, whilst the actual management intervention, rotation, contributes the least.

Long-term dynamics: stochastic projections:

Stochastic projections of densities generate high variability between years (Figure 5) and also between cropping system. Continuous winter wheat has a higher mean and variance in density state compared to continuous cropping of continuous barley or continuous barley to sugar beet rotation. Introducing break-crops into a rotation has a varied effect, with variance & mean density ranging from almost as high as continuous winter wheat (in the case of peas & OSR), to much lower (e.g. rotation to potatoes). Variability, however, remains high throughout all break-crops, and increases with the larger amounts of winter-wheat in a rotation.

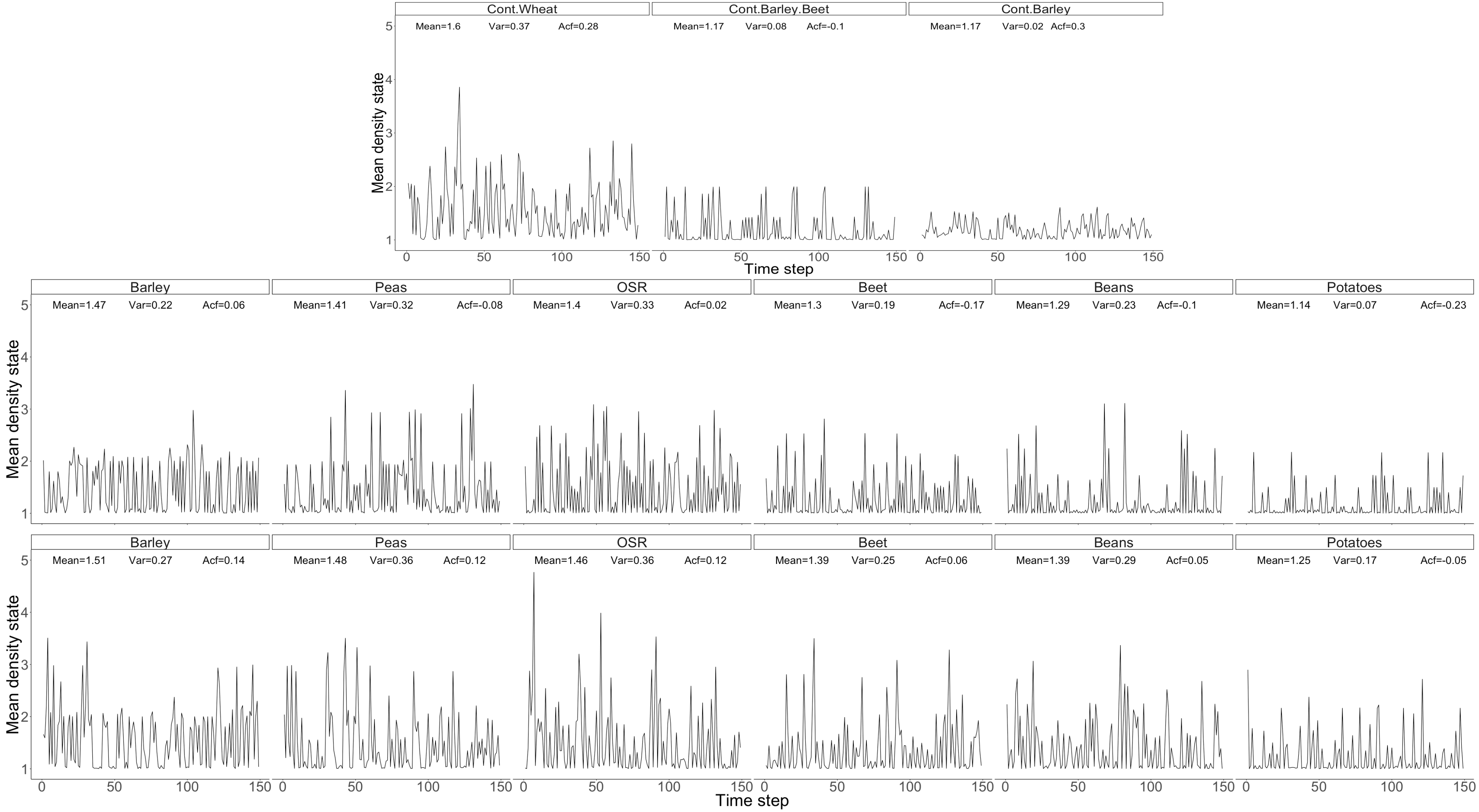

Figure 5. Example time series for stochastic model projections for continuous wheat & non-wheat rotations, rotations (First row), 3 year wheat rotations with a single break crop (second row) and 4 year wheat rotations with a single break crop (third row). Models were run for 10,000 iterations and the final 150 displayed above. Numbers at the top of each plot represent the across time-series mean, variance and autocorrelations for the full 10,000 iterations.

There are significant additive effects of winter-wheat dominance and break crop on both mean density state (*Linear model, Adj* $R^2 = 0.9431$, $F=43.64$, $df=7,11$, $p<0.001$) and variance (*Linear model, Adj* $R^2 =0.7506$, $F=8.739$, $df=7,11$, $p<0.001$). Reducing the proportion of winter wheat in a rotation decreases overall black-grass density and between year variability (Figure 6 a & b). Although there are no significant relationships between wheat dominance, break crop and autocorrelation (Figure 6, c), the pairings of outcome density and autocorrelation are worth consideration. Low densities paired with high autocorrelation suggests that the rotation is effective in a continual suppression of black-grass populations. However single break-crop rotations (winter-wheat proportions 0.66 – 0.8) display interesting dynamics. In these rotations, decreasing wheat dominance decreases auto-correlation, shifting from positive (density is positively correlated with density in the previous year) to negative autocorrelation. The implication of this is that higher densities in these rotations may persist while lower densities may not.

Figure 7 illustrates the effect of break crop on outcome density from single break-crop systems aggregated over all rotation lengths and levels of wheat dominance. There is noticeable variation in the effect of break crop on outcome density, between year variance and autocorrelation. Using potatoes as a break crop produces significantly lower overall black-grass density, sugar beet and beans slightly higher, and barley, OSR and peas at comparably high levels. Potatoes again also produce significantly lower between year variability, beet, barley and beans produce higher levels of variance. OSR and peas produce the highest levels of between year variability in black-grass densities. Similar trends are evident in the relationship between break crop and autocorrelation. Potatoes are the only crop that produces almost consistent negative autocorrelation, barley and OSR produce consistently positive autocorrelation between years, whilst beans, beet, and peas vary around zero. The implications for rotation specific densities are therefore mixed, the high densities found in barley and OSR are likely to persist, whilst the low densities in potatoes are not.

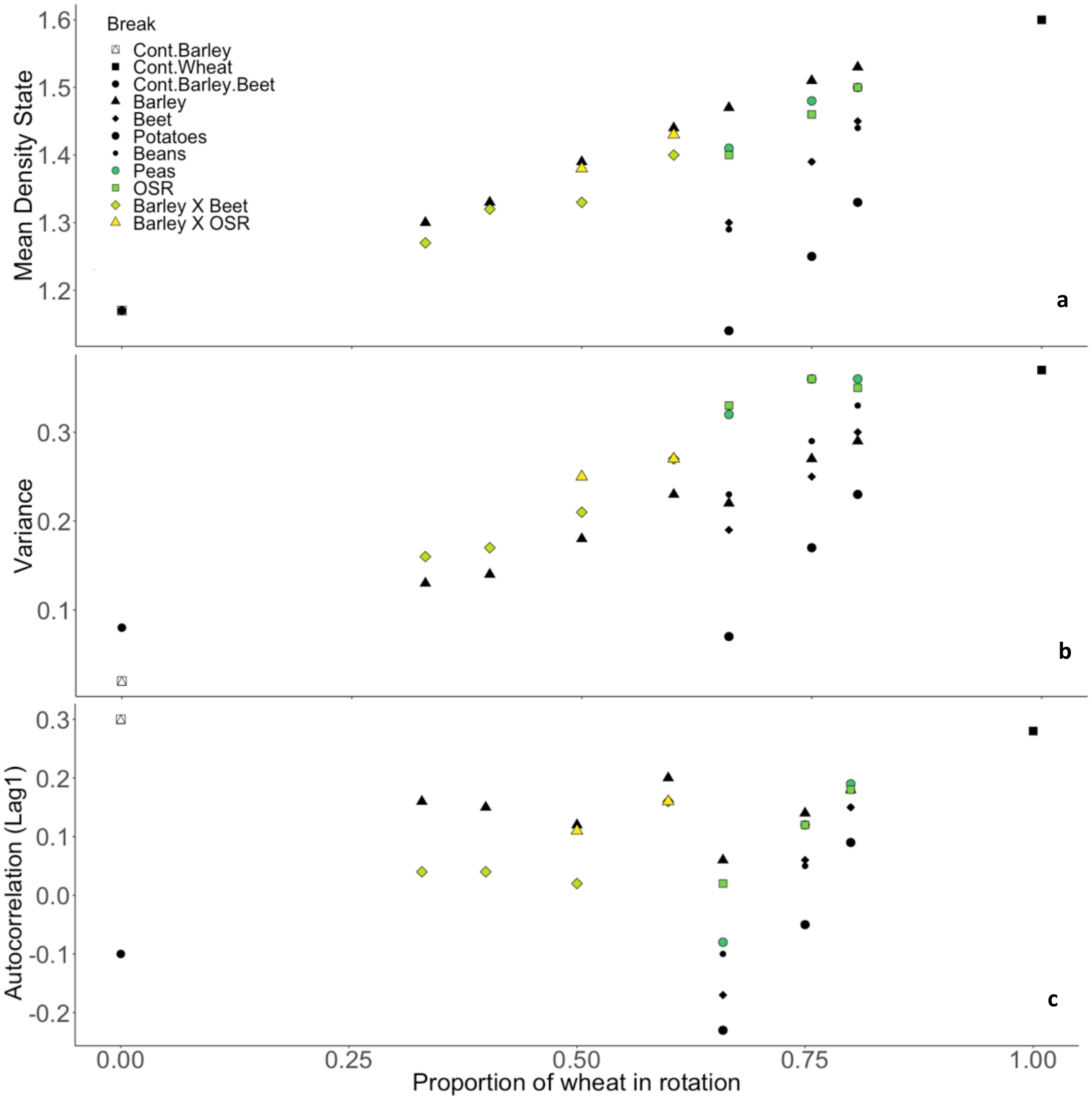

Figure 6. Relationship between the proportion of wheat in a rotation and the mean density state (a), variance (b), and Lag-1 cutocorrelation (c), across stochastic model projections. Individual points represent time-series averages for a model under with a specific break crop/rotation at a specific proportion of winter wheat. Colours and shapes of each point represent the break-crop or combination of break crops used in each model.

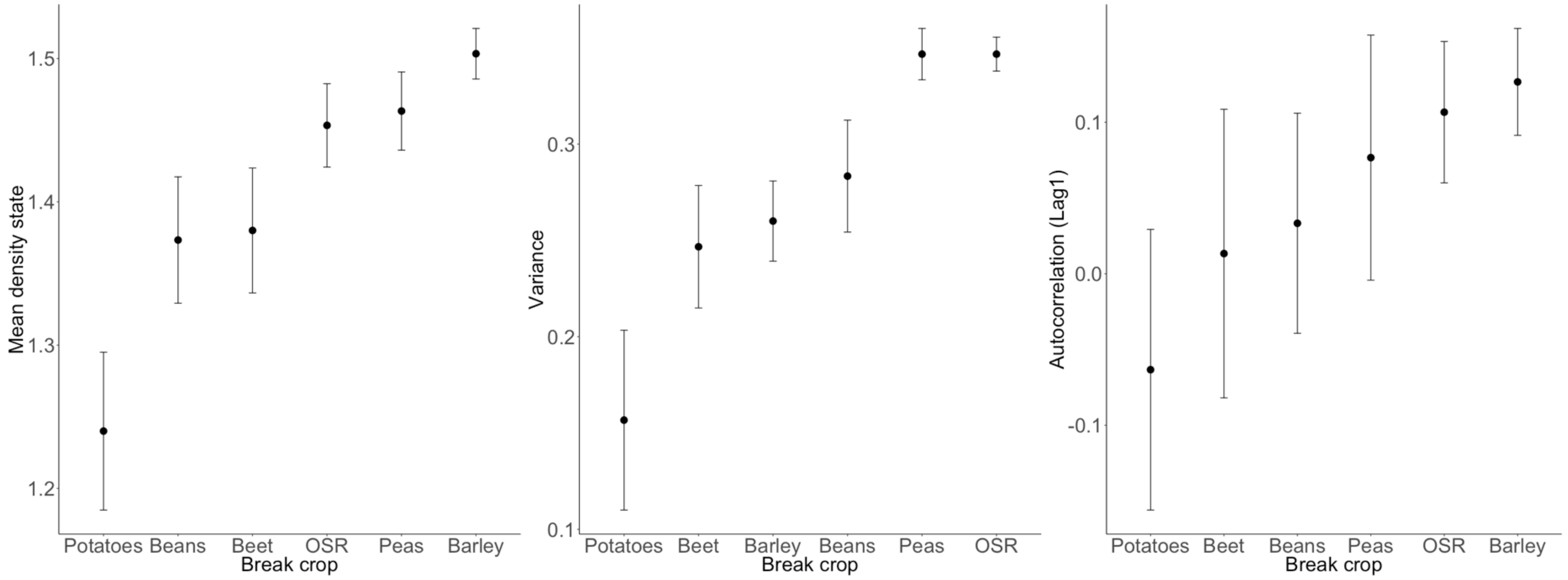

Figure 7. Mean density states, variance and Lag-1 autocorrelation in stochastic models with different break crops. Only models with a single break year were included. Vertical bars represent two standard errors.

Discussion:

Weed populations are inherently variable and there is a significant body of literature dedicated to understanding the causes and consequences of their variability (Freckleton and Watkinson, 1998, 2002a; Gonzalez-Andujar and Hughes, 2000; Freckleton and Stephens, 2009). Monitoring and predicting highly variable dynamics is challenging, but the first step to tackling the issue is capturing the range of responses exhibited by a population. Large scale monitoring is needed to gather all the important variation in the effect of interventions or control strategies. Our hierarchical density-structured approach captures high inter-population variability across a national scale; results from stable-state projections, two-step-ahead analysis and stochastic models all demonstrate that weed density is highly variable within all the cropping systems we consider. Field-scale variation can have various origins; for example persistent seedbanks, soil type, and local climatic variables will all substantially impact on black-grass populations throughout the season (E.g. Colbach et al. 2006, Metcalfe et al. 2018). Importantly though, the large contribution of field-level effects on total system variance suggests that rotational controls could have little impact compared to environmental drivers at a particular site.

The high variation in black grass-density between fields, regardless of rotation used, has implications for both large scale modelling and management of black-grass. High levels of variability suggest that in some cases, any benefit from an applied control may be overwhelmed by site-specific effects positively influencing black-grass growth (Freckleton et al., 2017). Without the necessary data to model the drivers of the site-level effects of black-grass dynamics, it becomes extremely difficult to predict outcomes for individual populations. Here we only consider one-aspect of population dynamics in agricultural systems – rotation, while there are many more that could improve model predictions and tease apart the contributions to site-level variance. Including more information on site-level factors could only improve our understanding of the dynamics in these systems.

Despite high levels of variability, we still provide evidence for the effectiveness of rotation for reducing weed infestations. In our models, cropping continuous wheat results in high density, highly variable infestations that are likely to persist (due to high autocorrelation). Winter wheat is well known to be particularly susceptible to black-grass (Hicks et al. 2018) as managing black-grass in wheat directly is difficult due to overlapping germination profiles, meaning control is limited by the risk of damage to the crop itself (Thurston 1964). We show that rotation decreases not only the average density of black-grass, but also its variability and autocorrelation, suggesting that weed populations will be more predictable and less likely to persist in rotated systems. The benefits of rotational controls are widely appreciated in the literature and have various modes of action (Zacharias & Grube 1984, Liebman & Dyck 1993). Primarily, rotation is allows opportunities to apply controls without risking damage to crops. As black-grass emergence usually occurs during autumn (Thurston 1964, Moss 1990) rotating into a spring crop (known as spring cropping) is often cited as an effective control measure.

Spring cropping can reduce black-grass abundance by facilitating targeted herbicide application, seed bed preparation, and cultivation during a period where the field is empty of crop, but still during the germination period of the weed (Moss & Clarke 1994, Chauvel et al. 2001, Moss et al. 2007, Lutman et al. 2013). Indeed, many of the cropping systems with the lowest black-grass densities from our analyses include spring crops. For example, broad-leaf crops such as sugar beet, beans and potatoes (which provide the highest density reductions in our models) will almost invariably be planted in spring and will also be resistant to grass-specific herbicides. The larger reductions in density seen from potatoes may well be due to a combination of features; as well the benefits seen from spring cropping, potatoes require additional cultivation through 'hilling', which may destroy seedlings that were missed during the initial round of control (Eberlein *et al.*, 1997).

Control can also be achieved through direct and indirect competition for resources. Competitive cultivars such as barley or OSR can suppress weed populations through rapid accumulation of biomass, exclusion from nutrients and sunlight (Nicholas 1991, Christensen 1993), or other biotic interactions such as allelopathy (Wu *et al.*, 2001). We see reductions in density from crops often cited as competitive cultivars, namely OSR and barley. Compared to continuous wheat there are noticeable reductions in density, but these reductions are generally lower than most alternatives. Some of the benefit of competitive cultivars, however, comes from resistance to yield penalties rather than reduction of seed return (Andrew, Storkey and Sparkes, 2015), which may account for their popularity despite continued abundance of black-grass.

The well documented benefits of rotation mean the relationship between wheat dominance and black-grass density in a system is relatively intuitive: the lower proportion of wheat in a rotation, the more opportunity there is to use spring cropping or competitive cultivars as control. However, as winter wheat is the most valuable crop in the UK, the value of particular rotations needs to be considered in terms of economics. Rotations that reduce the prevalence of wheat will reduce income, and this economic loss will have to be balanced against the potential loss of continued infestation or the viability of alternative crops.

With the evolution of multiple herbicide resistances across the UK and Europe (Hicks et al 2018), the value of rotational control is only likely to increase. The surveys we use in this study were carried out between 2007 and 2010, just three years after a new selective herbicide in winter wheat (Atlantis) was released in 2003. Evolved resistance to Atlantis and older herbicides means that UK farms have no effective chemical treatments that target black-grass (Hicks et al 2018). The broad-spectrum herbicide glyphosate is currently used in conjunction with spring cropping to effectively control black-grass between rotations (Hicks et al. 2018). However, there is considerable concern that repeated use of glyphosate will lead to resistance in black-grass, especially as it has driven resistance in several other agricultural systems (Sammons and Gaines 2014). The cultural components of rotational control are therefore becoming increasingly relevant and show promise for reducing the reliance on herbicides (Travlos, 2012). Although modern agriculture is heavily reliant on pesticides, reducing pressure on them as the main provider control has multiple benefits. Reducing reliance will reduce the destruction of biodiversity (Beketov *et al.*, 2013; Woodcock *et al.*, 2016), as well as preserving efficacy for continued use in integrated control strategies (Harker and O'Donovan, 2013).

Density-structured models are extremely capable of evaluating large scale dynamics of spatially expansive populations and our application shows how rotational control can help manage the widespread infestations of one of Europe's most damaging weeds. However, an important limitation of these models is that they only provide summary descriptions of field-level populations. Within field dynamics and demography and spatial structure are extremely important in dictating larger scale patterns in weed abundance (Cardina *et al.*, 1997; Freckleton and Watkinson, 2002b). Improving this approach to include information on spatial interactions and lifecycles will be an important step for extending utility of density-structured models for improved landscape scale prediction and more targeted managements. Fortunately, the nature of density-structured frameworks makes approaching these questions relatively simple.

Monitoring, understanding and predicting large scale population dynamics and their variation is an essential step for effective pest management. Above we have demonstrated that density-structured models can directly tie empirical observations to management predictions for spatially extensive and variable populations. In doing so, we show that several rotational management strategies decrease the infestation of an economically damaging weed, but all these systems demonstrate extreme inter-field variance in the degree of control. Developing integrated controls that are effective over a wide range of environmental conditions is necessary to limit the detrimental effects of destructive weeds. Landscape assessments of dynamics such as ours are the first step in understanding population responses to interventions and achieving effective management over large scales.

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Chapter Four:

Determining the influence of within-population structure on *Alopecurus myosuroides* dynamics using density structured models.

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Abstract:

Within-population spatial structure is linked to all drivers of organism abundance and is a fundamental part of population dynamics at larger scales. Economically important weed populations are often characterised by their patchy distributions within fields but modelling their spatial interactions can often be difficult due to the lack of adequate data. Density structured models allow large scale empirical data to be collected in a framework that is amenable to modelling the effects of within-population spatial structure. In this study we develop and test spatially explicit density-structured models that attempt to account for different spatial processes at the field scale. We incorporate spatial information into the parameterisation of field-level transition matrices, via a covariate describing the density of the neighbours of a focal quadrat. We build a series of these models in an attempt to account for field-scale spatial processes that may affect population dynamics, each are implemented by applying different configurations of weights upon neighbouring quadrats. We show that point-wise predictive performance (calculated via LOO cross validation), is better in spatially explicit models but there is no difference between different spatial parameterisations. Using custom posterior predictive checks, we then demonstrate that spatial models only slightly improve on non-spatial models in terms of description of field-scale spatial structure and mean density state. However, using simulations spatial models demonstrate drastically different population dynamics in response to crop-rotation, with more comparable effects of field-specific effects and crop rotation on overall system variability.

Introduction:

Agricultural weeds are an increasingly problematic aspect of large-scale population ecology, being costly both environmentally and economically (Swinton et al. 1994; Wiese et al. 1997; Hicks et al. 2018). There is wide recognition of the importance in spatial structure on the dynamics of weed populations (Cardina et al. 1997; R. Freckleton & Watkinson 2002b; Nordmeyer 2006; Gonzalez-andujar et al. 2018), and diverse spatial interactions and complex dynamics can even occur in environmentally homogenous systems like arable fields (Freckleton & Watkinson 2002). At the scales relevant to pest control, however, environments are both highly variable and complex (Forman 1995). Given the importance of spatial interactions in population dynamics (Levin 1992; Legendre 1993), they must be considered in the context of environmental variability that already hinders management of damaging weed populations (Wallinga et al. 1999; Urban 2005; Freckleton & Stephens 2009; Coutts et al. 2016).

In general, empirical and theoretical frameworks tend to focus on large scale interactions between separate populations (Durrett & Levin 1994; Dunning et al. 1992; Hanski 1998; Blasius et al. 1999; R. Freckleton & Watkinson 2002b; Wintle et al. 2005), but an important aspect of weed populations is the 'patchiness' observed at within fields (Cardina et al. 1997; Rew & Cousens 2001; Holst et al. 2007). The meso-scale dynamics evident at this level are distinct from the processes that drive broader scale distributions or small scale population self-structuring (Cardina et al. 1997; Somerville et al. 2017;

Gonzalez-andujar et al. 2018). Moreover, this spatial structure has consequences for weed management, as high density patches can cause severe yield loss (Hicks et al. 2018), and accounting for spatial structure can increase the efficiency of control (Bennett & Pannell 1998; Nordmeyer 2006; Metcalfe et al. 2017). Spatially explicit models are necessary to predict, monitor and manage these problems, however, we currently lack an empirical foundation for modelling field scale structure in widely distributed populations.

Typically, data on abundance are difficult and expensive to collect over large scales. Density structured models expand the potential scale of population monitoring through inexpensive, rapid surveys, and analytically simple modelling (Taylor & Hastings 2004; Queenborough et al. 2011). These models provide accurate assessment and prediction of population trends, as well as being robust to errors commonly associated with demographic models (Freckleton et al. 2011; Mieszkowska et al. 2013; Freckleton et al. 2017). Further, placing density structured models in a hierarchical framework allows models to account for variability in abundances over large scales (Wu & David 2002; Chapter 2). So far, however, these models have only been analysed as non-spatial descriptors of whole-field population dynamics. Importantly, the survey process naturally captures within-population spatio-temporal information; sampling locations are divided up into defined quadrats which map changes in density from year to year. As the data from surveys are spatially structured, in principle they can be analysed in a spatially explicit context; Incorporating spatial information into density-structured models provides a convenient way to model and eventually link, patch, field, and regional scale dynamics.

Dynamic models of weed populations have proved useful tools for improving understanding and management (Freckleton & Watkinson 1998; Freckleton et al. 2008; Freckleton & Stephens 2009). Spatial structure in weed populations is well known to be characteristic of population dynamics at the field level (Paice 1998; Rew & Cousens 2001; Metcalfe et al. 2017), and there are numerous different spatial processes that can affect the density of weeds across a field. Dispersal from the plant itself will generally be isometric and limited to its immediate vicinity, but other processes such as ploughing or harvest can directionally spread seed (Somerville et al. 2017). However models of weed dynamics typically neglect spatial information (Holst et al. 2007), and empirical measures are normally only derived from a few locations without consideration of large scale environmental heterogeneity (Buhler 1999; Travlos 2012; Metcalfe et al. 2017; Metcalfe et al. 2018). Scale-dependencies are rife within ecology (Urban 2005; Steffan-Dewenter et al. 2002; Chase et al. 2017), and understanding the interaction between spatial processes and environmental heterogeneity will be vital for effective pest control. Density-structured models offer a means to model the interaction between field scale spatial dynamics and management, parameterised with real-world observations of weed density gathered from a range of environmental conditions.

In this study we have two objectives. I) To Parameterise and test density-structured models that account for the different processes influencing spatial structure of weeds in arable fields. II) To Compare predictions of density-structured dynamics from spatial and non-spatial models for populations subjected to different managements to quantify and understand how important spatial processes are for population dynamics. We show that spatial density structured models perform as well as non-spatial models when summarising the state of field-level populations but are better in terms of observation-level predictive performance and capturing spatial structure. We demonstrate that spatial structure plays an important role in the dynamics of the weed *Alopecurus myosuroides,* and its response to rotational management.

Methods:

Study system & Survey:

Weed density state data was collected between 2007 and 2010 and surveys recorded black-grass densities from over 400 fields across 72 arable farms. Individual fields were repeatedly surveyed across this four year period to map changes in black-grass densities. Before each survey, fields were divided up into a series of 20 x 20m quadrats or 'sites'. Observers then walked the tractor wheelings of each field and recorded black-grass densities in each quadrat as one of five discrete states: absent, low, medium, high or very high. This method produces an accurate description of black-grass densities as well as high inter/intra observer repeatability (Freckleton et al 2011, Queenborough et al 2011).

Density structured models:

A density-structured model has the structure:

$$\mathbf{N}(\mathrm{t}+1) = \mathrm{T}.\mathbf{N}(\mathrm{t}) \qquad (1)$$

Where **N** is an ordered vector of the distribution of k density states at time *t.* This can be interpreted as either the proportion of a given population occupied by each state, or the probability that a given site is in a particular state. T is a k x k matrix of transition probabilities:

$$\mathrm{T} = \begin{pmatrix} p_{11} & \cdots & p_{1K} \\ \vdots & \ddots & \vdots \\ p_{K1} & \cdots & p_{KK} \end{pmatrix} \qquad (2)$$

Diagonal entries of T represent probabilities (*p*) that a site in a given site will remain in that state for the next survey, and off diagonals represent the transition between states. For example, p_{11} is the probability that a site in state 1, will remain in state 1, and p_{12} is the probability that a site in state 1 will transition to state 2. Given an initial distribution of density states, the resulting first order Markov model in equation (1) can be then used to predict future density state distributions. A detailed explanation of density structured models can be found in Freckleton et al (2011), and the relationship between field-level surveys, density distributions and transition matrices is summarized in figure 1 Chapter 1.

Parameterising transition matrices: non-spatial model:

We generated field-specific transition matrices using hierarchical ordered category logistic regression. We model the probability of observing a certain category k at survey quadrat i, as a function of a real-valued latent variable η_{if}. In equation 3, this linear predictor is constructed from the row-vector of J site-specific explanatory variables x, and the unknown parameter column-vector β. β_{ij} is therefore the effect of explanatory variable x_j on η , at site *i.*

In the non-spatial formulation of this model, explanatory variables x_1 through to x_5 are the current density state of quadrat i, and parameters β_{i1} through to β_{i5} are the effect of current density state on density next year. The constraint $\beta_{i1} = 0$ is enforced to allow identifiability (Agresti 2002, p271 – 273). We model variability in population level (i.e. field-level) black-grass dynamics through the parameter γ_f , which accounts for field-level effects in attaining different density states:

$$\eta_{if} = \sum_{j=1}^{J} x_{ij}\, \beta_{ij} + \gamma_f \qquad (3)$$

A set of *K-1* cut-point parameters, c, enforces the ordering of density state categories, they have the inherent ordering $c_1 < c_2 < c_3 < c_4$. We categorise the probability of observing a density state according to the relationship between eta and the cut-points as in (4), where Θ_{ikf}, is the logit of the probability of observing state *k* at site *i* within field *f*:

$$\Theta_{i1} = 1 - (\eta_i - c_1)$$

$$\Theta_{ik} = (\eta_i - c_{k-1}) - (\eta_i - c_k) \qquad (4)$$

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$$\Theta_{iK} = \eta_i - c_{K-1}$$

We can then calculate the probability of an observation *Yif* being in state *k*, through the inverse logit function:

$$Pr(Y_{if} = k) = \frac{e^{\Theta_{ikf}}}{1+\sum_{k=1}^{K-1} e^{\Theta_{ikf}}} \qquad (5)$$

Spatial density structured models:

To investigate the spatial dynamics of black-grass populations, we fitted a series of models that incorporate the density of weeds surrounding a focal quadrat into estimating the probability of observing density states in subsequent surveys. We calculate spatial covariates, *S*, as a function of the density states in the neighbourhood of a focal quadrat (Figure 1B). Each quadrat within this neighbourhood has a location, *l* (1-8), and a density state, *x*, valued 1-5 respective to ordering. The spatial covariate for a specific configuration can then be calculated through a series of weights, *w*, assigned to each location within the neighbourhood, so that:

$$S_i = \sum_{l=1}^{8} w_l \, x_{il} \qquad (6)$$

Where w_l is the weight assigned for location *l* and the total of all weights is constrained to sum to one over the neighbourhood (see table 1). x_{il} is the density state value at location *l*, within the neighbourhood of observation *i,* and S_i is the spatial covariate for observation *i.* Figure 1 illustrates the relationship between the neighbourhood locations, density states and weights during the calculation of a spatial covariate.

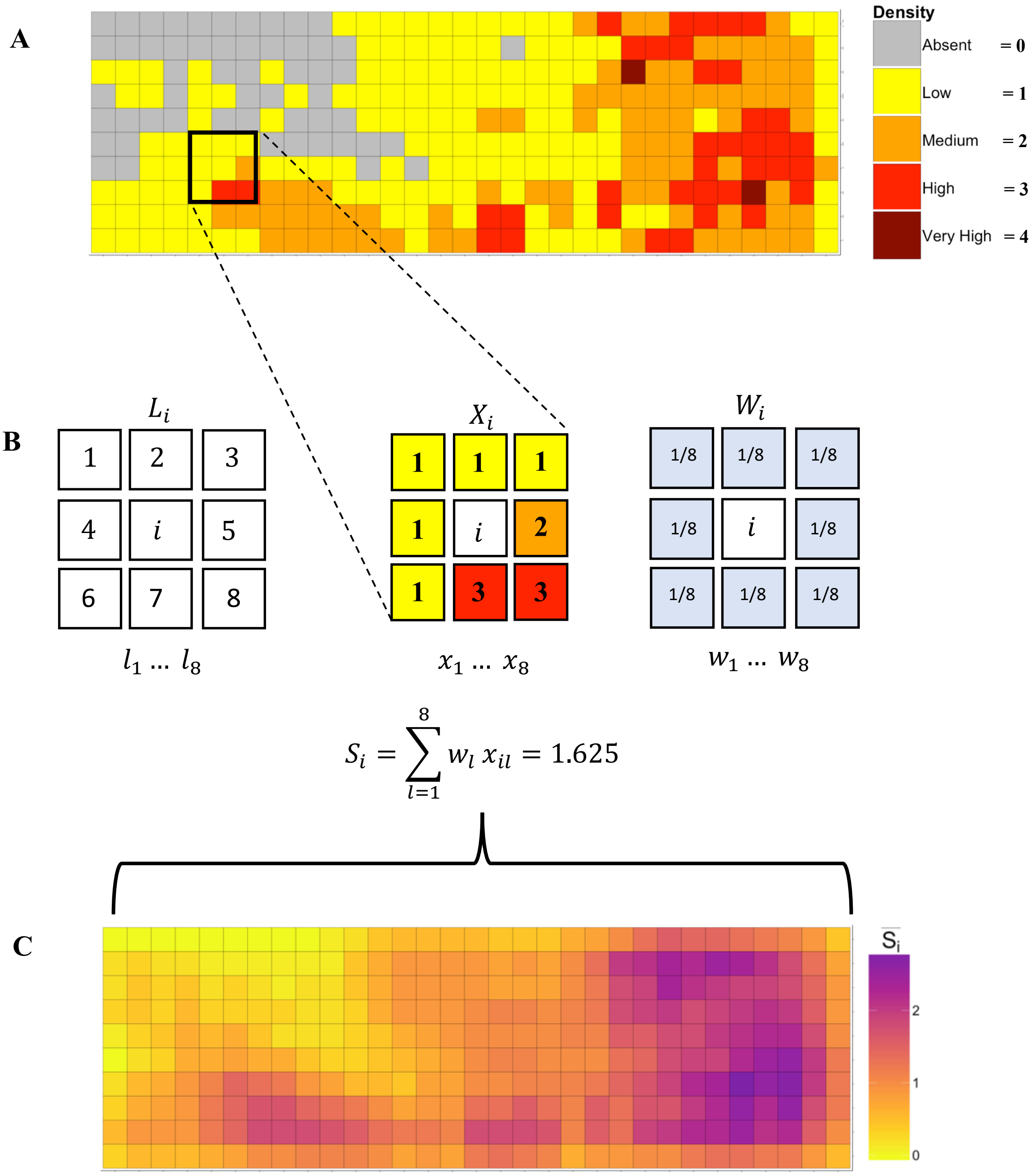
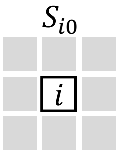
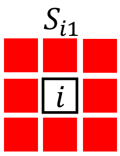
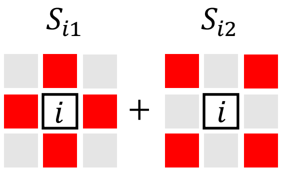
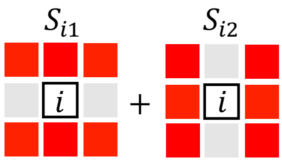
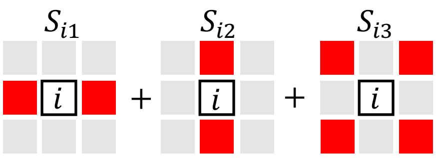


Figure 1. Calculating the spatial covariate for a single observation using an isometric configuration of location weights. (A) The distribution of density states within a field (B) The neighbourhood and locations for a focal quadrat (L_i), the density states (X_i) and weights (W_i) for each location. (C) A heatmap of the spatial covariate calculated over the entire field in (A).

There are numerous different spatial processes that can affect the density of weeds within each quadrat, most of which will be related to seed dispersal. As black-grass seed is shed around the plant before the maturation of the crop, any effect on density will be isometric, and limited to a locality. However, dispersal can also be directional, for example seed can be spread large distances across a field via harvest or ploughing. To account for the different spatial processes affecting transition probability at the quadrat scale, we costruct models with different spatial covariates. Each covariate uses a different configuration of weights (w_l), and allows different parts of the neighbourhood to vary in their contribution to the probability of observing a particular density state next year (Table 1).

Table 1. The suite of models with the configuration and value of weights for each spatial covariate.

Model	**Configuration**	$\boldsymbol{w_l}$
Naive		S_{io} ⬜ = 0
Model I		S_{i1} 🟥 = 1/8
Model II		S_{i1} 🟥 = 1/4 ⬜ = 0 S_{i2} 🟥 = 1/4 ⬜ = 0
Model III		S_{i1} 🟥 = 1/6 ⬜ = 0 S_{i2} 🟥 = 1/6 ⬜ = 0
Model IV		S_{i1} 🟥 = 1/2 ⬜ = 0 S_{i2} 🟥 = 1/2 ⬜ = 0 S_{i3} 🟥 = 1/4 ⬜ = 0

We incorporated spatial covariates into the linear predictor:

$$\eta_{if} = \sum_{j=1}^{5} x_{ij}\,\beta_{ij} + \sum_{c=1}^{C} S_{ic}\,\beta_{ic} + \gamma_f \qquad (7)$$

Where *C*, is the total number of spatial covariates (or different configurations of weights for the neighbourhood of *i*), S_{ic} is the spatial covariate value for observation *i,* within configuration *c,* and β_{ic} is the effect of spatial configuration *c* on the density state in quadrat *i* next year.

To model different spatial processes, each model in table 1 introduces flexibility in the ways different components of the neighbourhood can contribute to the density of the focal quadrat in subsequent years.

Model I.

The first of these models, *Model I* is the simplest model where disperssal is assumed to be uniform around the focal quadrat. Here the entire neighbourhood contributes equally to η_i, producing a single spatial covariate S_{i1}.

Model II.

As the majority of the contact between the focal and surrounding quadrats will be between non-diagonal neighbours, these components may have a potentially greater influence on future density state. We can allow for more flexibility in contributions of the diagonal and non-diagonal components of the neighbourhood to the linear predictor by seperating these components, calculating different covaraites for each and modelling their effects seperately. In our second model, we model the effects of adjacent (S_{i1}) and diagonal (S_{i2}) surrounding density states on the linear predictor, by introducing individual terms for each.

Model III.

The distribution of black-grass seeds within a field is influenced by management, with practices such as ploughing and harvest spreading seed throughout the field (Somerville et al. 2017). Typically fields are travelled along the longest axis, which means that seed spread will likely be greater longitudinally. Future density states may therefore be influenced more heavily by quadrats perpendicular to the longest axis. To account for this possibility, our third model incorporates covariates from the means of perpendicular (S_{i1}) and parallel (S_{i2}) neighbouring density states.

Model IV.

Our fourth model is a combination of models II & III, allowing for varying contributions from surrounding quadrats that are perpendicular (S_{i1}) or parallel (S_{i2}) to the longest axis, as well as those that are diagonal to the focal quadrat (S_{i3}). This seperates effects of longitudinal seed dispersal from the potentially limited effect from diagonally adjacent quadrats.

Prior specification:

We employ a Bayesian framework using the probabilistic programming language Stan (Stan core development team 2017) for ease and flexibility of parameterization. The population wide effects of source state and spatial covariates, $\beta_{j+1} \ldots \beta_J$, were drawn from independent normal distributions, of mean 0 and standard deviation 10:

$$\beta_j \sim N(0,10)$$

The field level effect on probability of observing a particular state, γ_f was drawn from a vague normal prior, with mean 0 and standard deviation σ. Where σ is the hyper-parameter for the standard deviation for field effect γ_f, itself drawn from a half-Cauchy distribution with 5 degrees of freedom.

$$\gamma_f \sim N(0, \sigma)$$

$$\sigma \sim Cauchy\ (0,5)$$

Cut-point parameters were given a wide uniform prior:

$$c \sim unif(0,10)$$

Model fitting:

We compare the predictive performance of these models using approximate leave-one-out cross validation and WAIC using the 'loo' R package (Vehtari, Gelman & Gabry 2016). We use a series of posterior predictive checks to visualize and compare field-level outcomes across models derived from one-step-ahead projections. Across models, we compare field-level differences between observed and predicted mean density states, and differences in predicted and observed spatial autocorrelation.

Simulation experiments:

To assess the impact of spatial structure on density structured dynamics we compare spatially naïve models to our 'best' spatial model for three rotations in our data. We simulate spatially-explicit density structured dynamics for each rotation in a hypothetical field with 20 x 20 quadrats. As the transition probabilities for each quadrat are conditional on their unique neighbourhood densities, the model becomes:

$$\mathbf{N_{ifr}}(\mathrm{t}+1) = \mathrm{T_{ifr}}.\mathbf{N_i}(\mathrm{t}) \qquad (8)$$

Where $\mathbf{N_i}(\mathrm{t})$ is the initial density state in quadrat *i* of the simulated field. $\mathrm{T_{ifr}}$ is the transition matrix for an observation in quadrat *i,* conditional on its neighbourhood density, and parameterized from the linear predictor estimated for field *f* in rotation *r* (equation 7). $\mathbf{N_{ifr}}(\mathrm{t}+1)$, is therefore the resultant density state in the next year. Each field in each rotation is therefore represented by a set of 20^2, transition matrices.

Using this model we made two-step projections for three rotations: continuous, wheat to OSR, and wheat to sugar beet. Rotations were simulated by changing the set of matrices that model quadrat level transitions at each time step. For example when $t = 1$, the set of matrices could model transitions for a specific field rotating from wheat to OSR, and when $t = 2$, OSR to wheat. Within each rotation we made projections for every permutation of fields, i.e. all field-level matrix sets parameterized for the first step of a rotation (e.g. a field rotating from wheat to OSR), would be paired with every possible combination of field-level matrix sets in the second step of the rotation (OSR to wheat). All projections were started from the same initial density distribution and spatial layout, which represented the average field-level conditions across all three rotations. For each projection we calculate the mean density state of each field, then compare the distribution of these field-scale measures across rotations, and between spatial and non-spatial models.

To assess the contributions of rotation and local environmental effects to the overall system variance we use a transient life-table response experiment (LTRE – Caswell 1989). This uses the change in black-grass density in the two-step projections above as the response variable in a linear mixed effects model (Freckleton et al. 2017). We compare the effect sizes of field identity (i.e. the matrix set combination for a projection) and each rotation within and between spatial and non-spatial models.

Results:

Model fitting:

WAIC scores from approximate leave-one-out cross validation are summarised in figure 3. The spatially naïve model performs considerably worse (lower values indicate better performance) than models that incorporate a covariates for the surrounding density of the focal quadrat. There is not, however, any difference in predictive performance between any of the spatially explicit models.

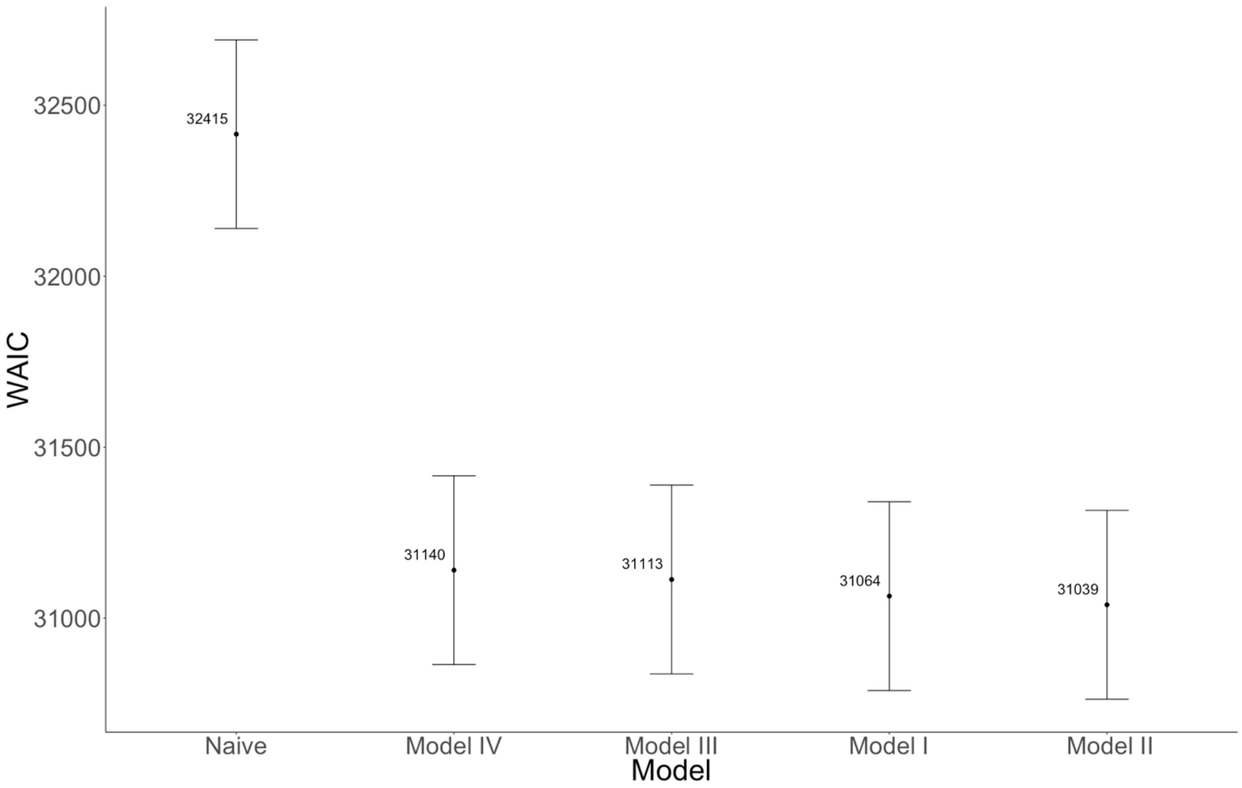


Figure 3. Widely applicable information criterion (WAIC), calculated via leave-one-out cross validation from the Loo R package. Vertical bars are one standard error and text next to each point are the exact WAIC scores to the nearest integer.

There is little difference between models in terms of predicted and observed field-scale mean density state (Figure 4). Each point on this figure represents the difference between the observed and predicted mean density state for a particular field, or field-scale prediction error. Positive values represent cases where the model overestimated the mean density state of a given field and negative values represent underestimation. All models perform similarly well, with the majority of field scale errors being confined between +/-10% of the observed value. The first two spatially explicit models perform slightly worse than the naïve model in terms of root mean square error (RMSE), with more of a tendency to overestimate mean density state. At this level there is little to differentiate the accuracy of spatially explicit models. Similar results are seen in terms of observed vs predicted density-state distributions (Figure S1), and Moran’s I measure of spatial autocorrelation (Figure S2).

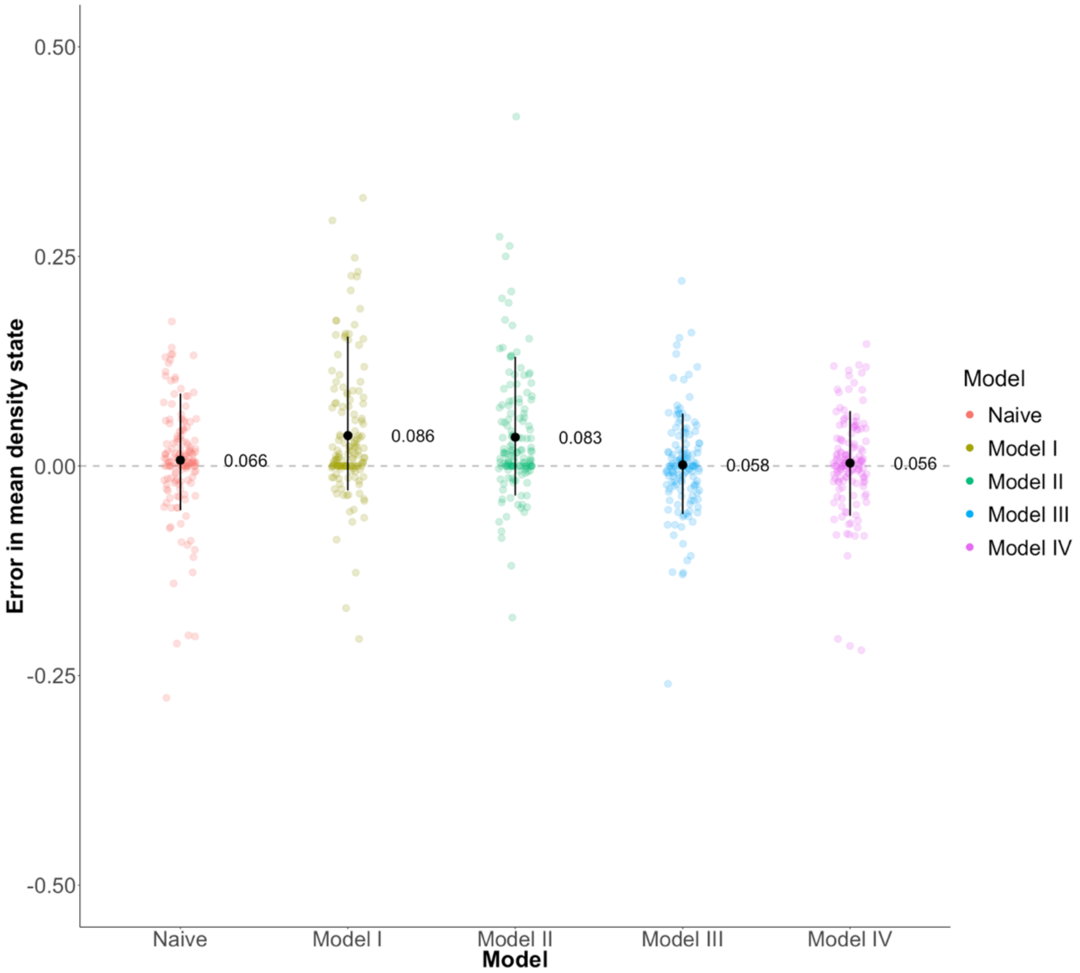


Figure 4. Field-scale differences in observed vs predicted mean density states for each model. Black points represent the median difference in mean density state across all fields, and vertical bars are 90% quantile intervals. Individual coloured points represent the difference between the predicted and observed mean density state for an individual field. Positive values represent model overestimation of current density state, negative values represent underestimation

Figure 5 displays field scale maps of density state distributions for three example fields, picked to display the range of predictive performances by each model. The first row displays the observed density state outcome, and the subsequent rows show predictions for each spatial model. It is clear that in some cases the spatially explicit models perform considerably better in predicting the patchy distribution of black-grass distributions (Field 1), than the naïve model – which predicts diffuse patterns. However, there are also numerous situations where there are no discernible differences between the predictions of the spatial and non-spatial models. Again, there is little difference between the spatial models.

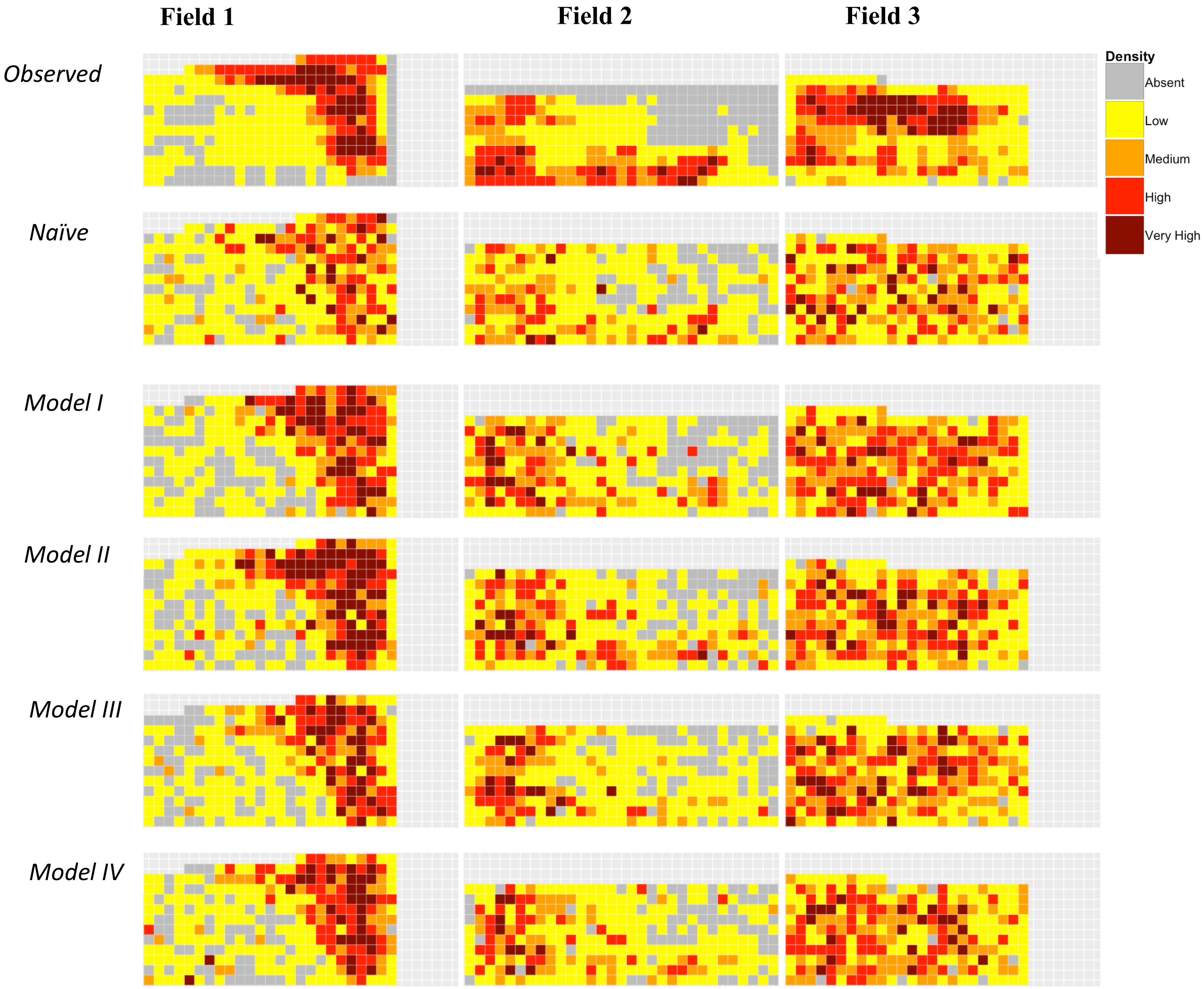


Figure 6. Maps of black-grass densities in three example fields. Density states are indicated by colour; grey = absent, yellow = low, orange = medium, red = high, dark red = very high. The first row shows the observed density distribution in each field, and the second shows the density-state predictions from the spatially naïve model. Subsequent rows show predicted density states from models I-IV.

Predictive performance of models decreases with spatial autocorrelation (Figure 7). Root mean square error (RMSE) of observed and predicted field-scale mean density states increases with higher spatial autocorrelation. All models again perform similarly, with the increase in error with spatial autocorrelation slightly lower in models III & IV. Overall, however spatial models better recover the spatial structure of the observed data, with higher correlations between observed and predicted spatial autocorrelation in spatially explicit models (Figure 8).

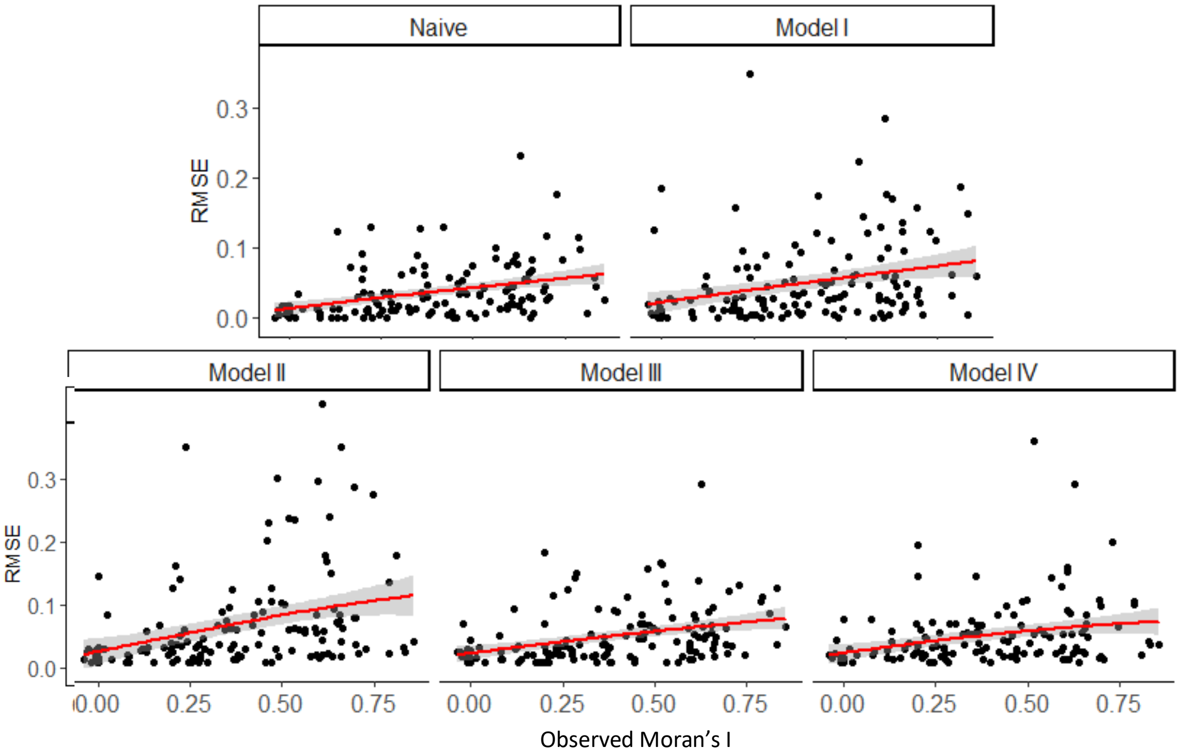


Fig 7. Model specific relationships between root mean square error for field-level mean density state and the observed Moran's I measure of spatial autocorrelation. Model predictive performance is lower for fields with higher spatial autocorrelation.

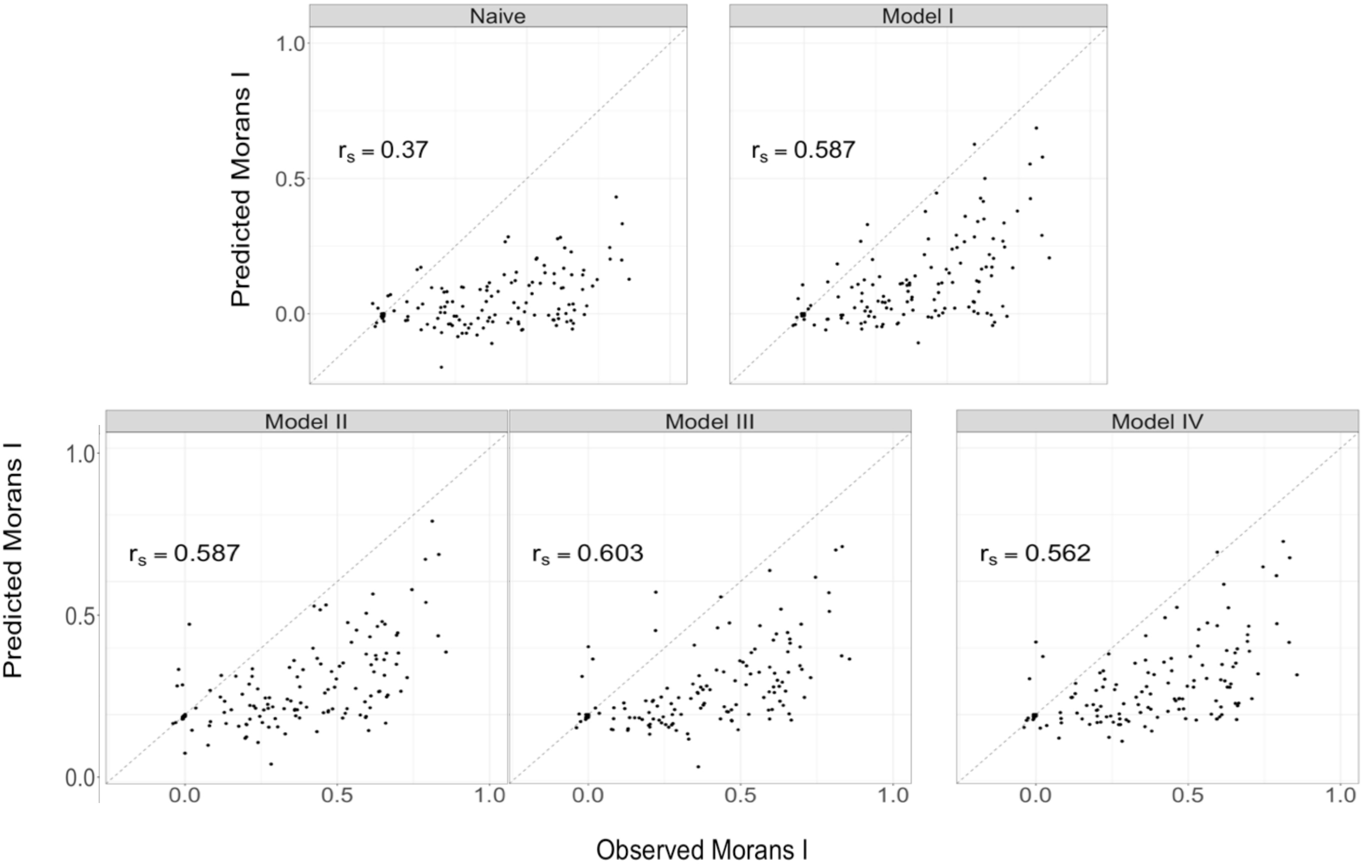


Figure 8. Observed vs predicted field-scale values of Morans I for each model. Numbers on each plot are spearman's rank correlation coefficient. Spatially explicit models better capture the spatial structure of a field, with a larger proportion of points being closer to the dashed 1:1 line.

Simulation experiments:

For ease of parameterisation, all simulations were run using *Model I*. Equilibrium densities under continuous wheat are much higher when spatial information was incorporated into the model (Figure 9). Moreover, the effect of rotation produces slight reductions in weed density in naïve models, but this effect becomes much more pronounced in spatially explicit models. The distribution of mean density states is wider, and the mean of equilibrium states is higher in spatial models than their naïve counter parts, but the densities in wheat are comparatively much higher than in systems rotation to OSR or beet. Stochastic time-series display a similar trend (Figure S3).

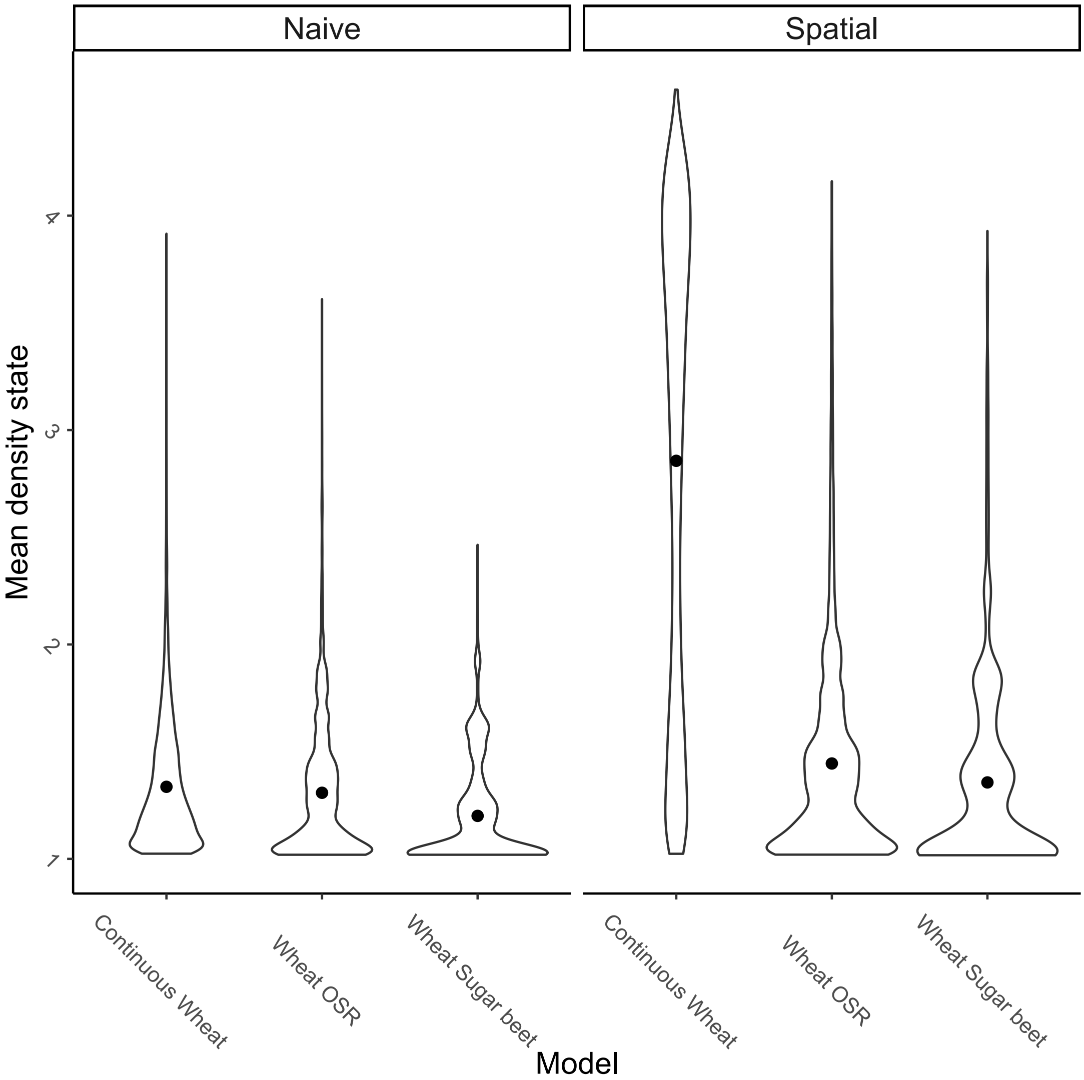


Figure 9. Distributions of mean density states for naïve and spatially explicit models. Distributions are from mean density states for each permutation of field-level matrices projected two time steps, from wheat, to a break crop, and back to wheat. Black points represent the median value for each distribution.

Examining the contributions to overall system variance of rotations and local environmental effects (i.e. field specific effects) reveals that crop rotation explains more variation in the change in density in spatial models (Figure 10). In spatial models, the variance contributions of field identity and rotation are 0.27 and 0.43 respectively with 0.30 residual variance. Naïve models have field identity and rotation variance contributions of 0.81 and 0.07 and a residual variance of 0.12. In naïve models there is a steep gradient towards the end of the field ID curve, meaning that in the absence of positive feedbacks caused by spatial structuring, a large proportion of variance in black-grass density is due a few fields where environmental conditions led to high densities. In spatially explicit models, the curve is more linear but steeper overall, as positive feedbacks can occur and overcome conditions which limit the spread of black-grass in a field. Rotation in spatial models, however, inhibits feedbacks from self-structuring, and contributes to system variance by reducing densities compared to continuous wheat. The Rotational effect sizes are much lower in naïve models, hence the greater impact of field specific effects.

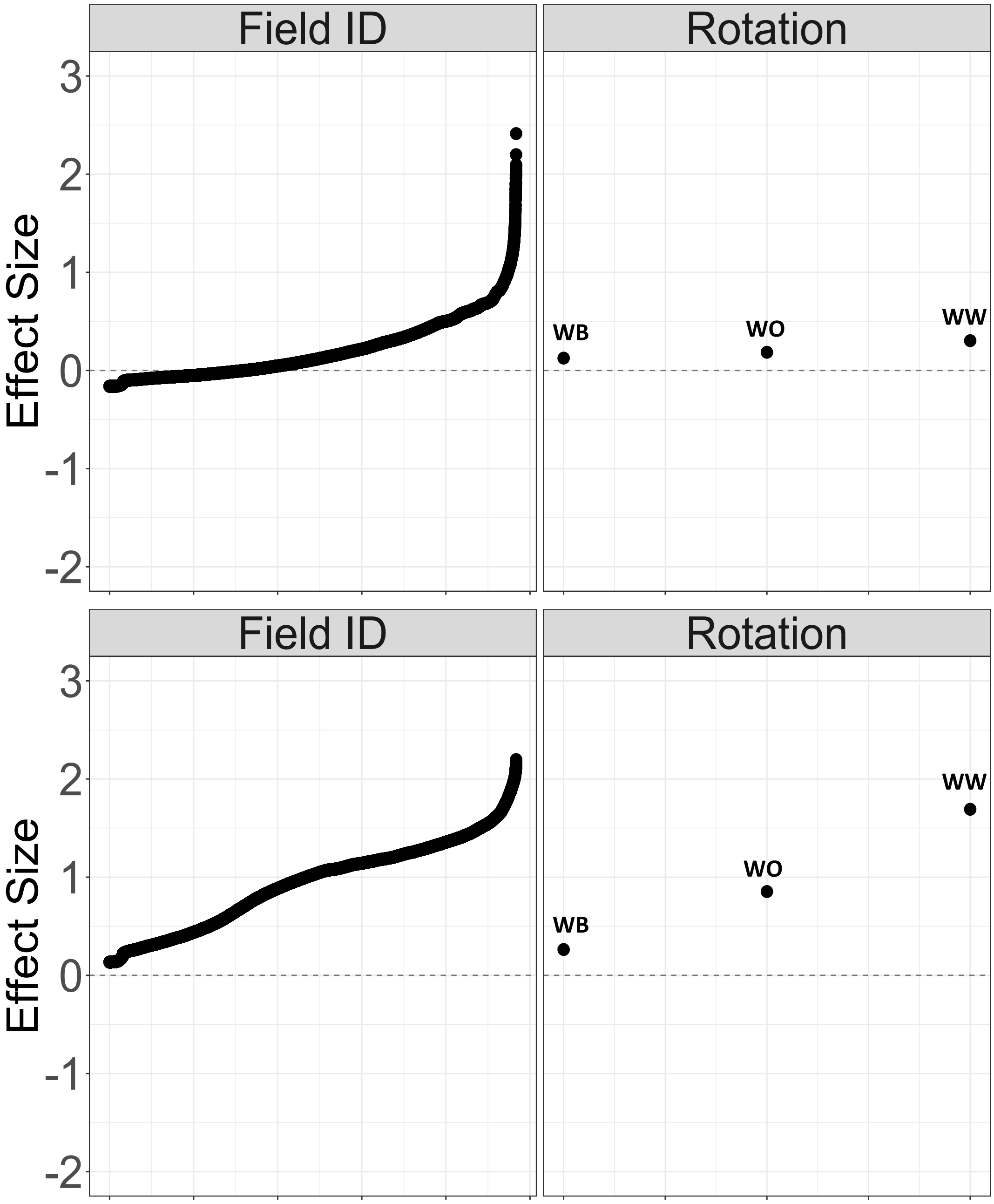


Figure 10. Contribution to variance in the difference between initial and final densities from Field ID (i.e. the combination of matrices made for that projection) and rotational control, for spatially naïve (top row) and explicit (bottom row) models. Each point represents the contribution to the change in density of each different variable, e.g. a point in the rotation panel represents the contribution to the change in density from a particular rotation.

Rotations are labelled; Continuous wheat = WW : Wheat -> OSR = WO; Wheat -> Beet = WB.

Discussion:

Including spatial information into density-structured models is important for assessing the dynamics of black-grass infestations over large scales. Within-population structure is intrinsically linked to almost all drivers of organism abundance such as dispersal, competition, predation, and reproduction and is a fundamental driver of larger-scale population dynamics (Blasius, et al 1999; Nathan and Muller-landau 2000). As we show using density-structured simulations, it is necessary to include spatial structure in these models as it has important consequences for weed dynamics. Cropping continuous wheat in spatially explicit models leads to severe infestations, whilst rotation reduces density considerably. Conversely, non-spatial simulations show little difference between densities in rotated systems or those in continuous wheat. The two sets of models, spatial and non-spatial, have drastically different outcomes in terms of management decisions, with rotation being an extremely effective control in spatially explicit systems, but less so in those that do not consider spatial self-structuring.

Under our naïve model, increases in density will only affect transition probabilities of a focal quadrat without any impact on those in close proximity. Allowing neighbourhood density to contribute to the density of a focal quadrat allows seed dispersal to neighbouring patches to affect weed density in subsequent seasons. Without the spatial interaction between patches of weeds, there is no positive feedback from spatial structure and naïve models predict much lower levels of black-grass abundance. Rotation may have a marginal effect in these models, but when spatial information is included the benefits become much more apparent. The likely cause of reduction is through a break down in self-structuring, which reduces positive feedbacks resulting high density patches of weeds. The modes of action of rotation are numerous, but the primary benefit is desynchronization of the germination profiles of black-grass and the crop, allowing a window for chemical or cultural control (Liebman & Dyck 1993; Moss & Clarke 1994; Chauvel et al. 2001; Melander et al. 2005; Colbach et al. 2006; Freckleton et al. 2017). Introducing more opportunity for control will reduce densities at regular intervals and reduce the positive feedback from higher seed density and dispersal.

Above we have highlighted the consequences of spatial interaction between patches of weeds subject to a range of environmental drivers and managements. Complexity and variability are apparent in our study, with fields in rotations of continuous wheat displaying a wide range of densities. Climatic, or other management effects are likely to play an important part in the variability we observe and, given that the required data are available, will be easy to incorporate to an expanded analysis. However, there are limitations to the spatial models we present above which also need consideration. Although incorporating spatial information improves fit, benefits are modest, and patterns do not capture the true spatial pattern of weed distribution. Moreover, model fit is poorer when there is higher spatial autocorrelation within a field, suggesting that these models do not adequately capture spatial structure. There are numerous factors that may be limiting these models to accurately predict spatial patterns across varied situations. Firstly, the neighbourhood effect kernels we use here are relatively simplistic and assume that only directly adjacent patches will influence the density of the focal quadrat. The reality is that seed can spread over large distances and through various mechanisms within a field (Colbach & Sache 2001). It may be model fits could be further improved by alternative kernels that model these processes in a more sophisticated manner. For example, Doxford & Freckelton (2012), use several different models to investigate the spread of plants across the UK. The *Phalanx spread* model they use in part of their analyses uses neighbourhood kernels from multiple time steps in the estimation of colonisation probability.

The way in which we model the dynamics of spatial processes in this application may also contribute to the limitations of these models. Density structured approaches are inherently probabilistic; dynamics are modelled as a function of the transition matrices which dictate the change in density states between years. Here we modelled spatial dynamics as a function of the *mean* transition probability for an observation in a field conditional on a set of neighbour densities. However, we do not consider how spatial interactions may affect the *variance* in responses between patches. The use of 'mean-field' approaches to summarise dynamics by taking the mean values of parameters through time or across space, is common in ecology (e.g. O'Dwyer & Chisholm 2014). Moreover, they have seen frequent use in spatial modelling (Morozov & Poggiale 2012) as they can simplify computationally intense and complicated problems. There are parallels to be drawn between mean-field models, and density structured models, with both reducing complexity of a problem by simplifying dynamic processes. However the former have drawn criticism as they ignore important information when modelling spatial dynamics; incorporating moment-closures that better encapsulate spatial patterns can improve the accuracy of spatially explicit models (Murrell et al. 2004).The discussion of how spatial processes are modelled has been mostly either in continuous space or with continuous measures of population abundance, but modelling dynamics as discrete systems is an area of research that would potentially further improve density-structured approaches (Durrett & Levin 1994).

Another notable limitation of these models is that they assume the existence of a seedbank across the simulated field. Seed banks form an integral part of plant population dynamics and are often included in population models (MacDonald & Watkinson 1981; Queenborough et al. 2011). They allow populations to persist through time and can decouple dynamics from the above-ground life cycle of the plant. This means that they can remain unaffected by managements applied the visible part of the population, for example post-emergence pesticides or cultural controls (Buhler 1999). Despite the relative ease of modelling, seed banks are much harder to observe and spatial distributions hard to estimate before emergence (Gonzalez &

Ghermandi 2012). As density structured frameworks are intrinsically empirical, this poses problems for implementation of seed banks into modelling weed dynamics.

If long time series of repeated surveys are available, then it may be possible to simply infer the presence of a seedbank by looking at absence/presence of blackgrass in a particular quadrat. However, the unobserved nature of the seedbank in these systems means that inclusion of an additional 'hidden' state may be the best option. Hidden state Markov models (Baum & Eagon 1966; Louvrier et al. 2018), are a set of models within ecology that can account for 'missing-ness' in empirical data describing dynamic processes. They have seen frequent use in modelling animal movement (e.g. Langrock et al. 2012), but are amenable to integration into the Markov process in density structured frameworks. Accounting for seedbanks will begin to improve predictions for limited scale infestations of fields, and potentially expand the uses of density structured models through allowing study of invasion dynamics and spatial patterning under a wide variety of scenarios.

To conclude, incorporating spatial information into density structured frameworks is both simple and beneficial to model performance, but it has important consequences for predictions of population dynamics. We recommend that future applications of density structured frameworks should take spatial information into account when assessing large-scale population dynamics. However, there are clear limitations to this approach, with predictions often failing to capture the patchiness of weed distributions. There is a need to better account for within-field demography as well as population structure to be able to fully capture and accurately predict the range of dynamics displayed by weeds in arable systems.

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Appendix:

Methods:

Model fitting:

Two additional posterior predictive checks were made, the first compares density state distributions within each field for all models. The second compares differences in observed and predicted Morans I.

Simulations:

Stochastic projections of black-grass density are made through the Markov model in (8), where the set of quadrat level matrices for a given field, T_{ifr}, are randomly selected from all available matrices in a given rotation. The model is projected over 10,000 time steps to ensure convergence, and we then compare the time series of spatially explicit and naïve models.

Results & Discussion:

In terms of field-level errors across the entire density distributions, all models again are comparable in figure S1. Most displaying almost identical error patterns, with the highest level of error in low and absent density states and higher density states have lower error distributions. All models show a tendency to under-predict the presence of absent states but over predict occupied states, again there is little difference between spatially explicit models.

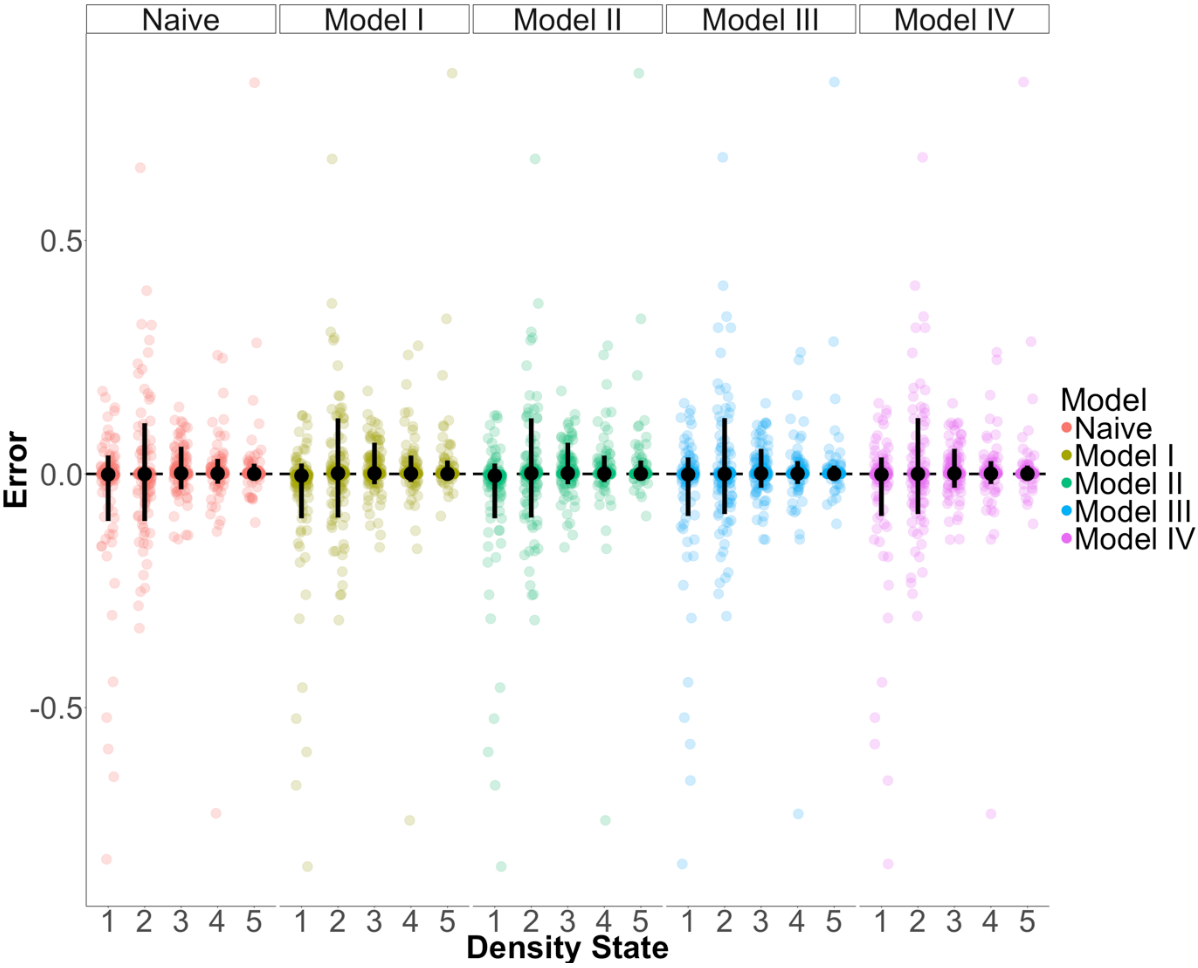

Figure S1. Field-scale differences in density state distributions for each model. Density states 1-5 represent states 'absent' to 'very high' respectively. Black points and vertical bars represent the median difference between observed and predicted density state proportions and 90% quantile intervals. Individual coloured points represent the difference in predicted and observed density state proportion for a density state within a single field.

There are slight improvements in the ability of spatial models to capture spatial autocorrelation (Figure S2) with median values closer to 0 and lower RMSE, but again there is little to distinguish between spatially explicit models. All models systematically under-estimate the level of autocorrelation.

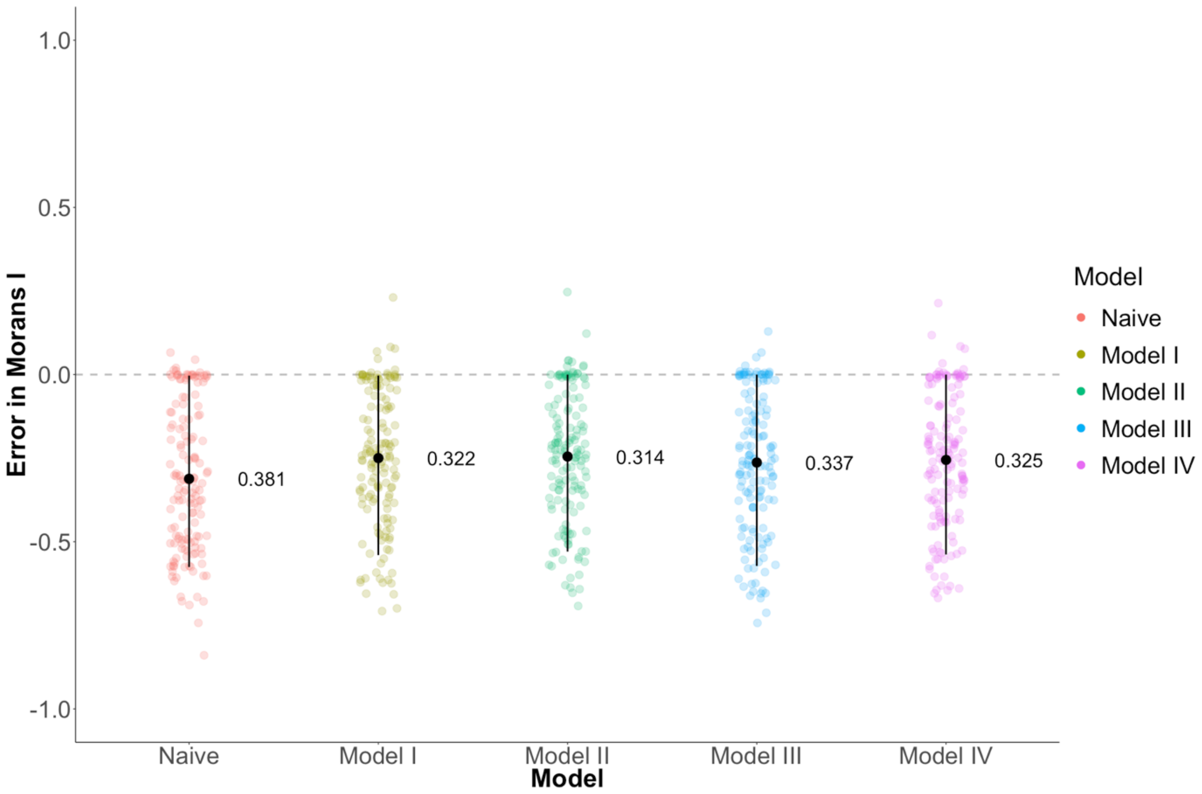

Figure S2. Field-scale differences in the observed vs predicted Morans I statistic for spatial correlation for each model. Black points represent the median difference between predicted and observed Morans I statistic across all fields, and vertical bars are 80% quantile intervals. Individual coloured points represent the difference between the predicted and observed Morans I for an individual field. Positive values represent model overestimation of current density state, negative values represent underestimation.

Figure S3 provides a closer look at the stochastic dynamics of spatial and naïve models. Inspection of naïve models reveals that all rotations will tend towards lower density states at equilibrium. Continuous wheat in a spatially explicit system has a much higher equilibrium density, even when perturbations push it towards lower densities it will tend back to a mean density state of around 4. Spatially explicit systems with a break crop included in the rotation will show very similar dynamics to spatially naïve models.

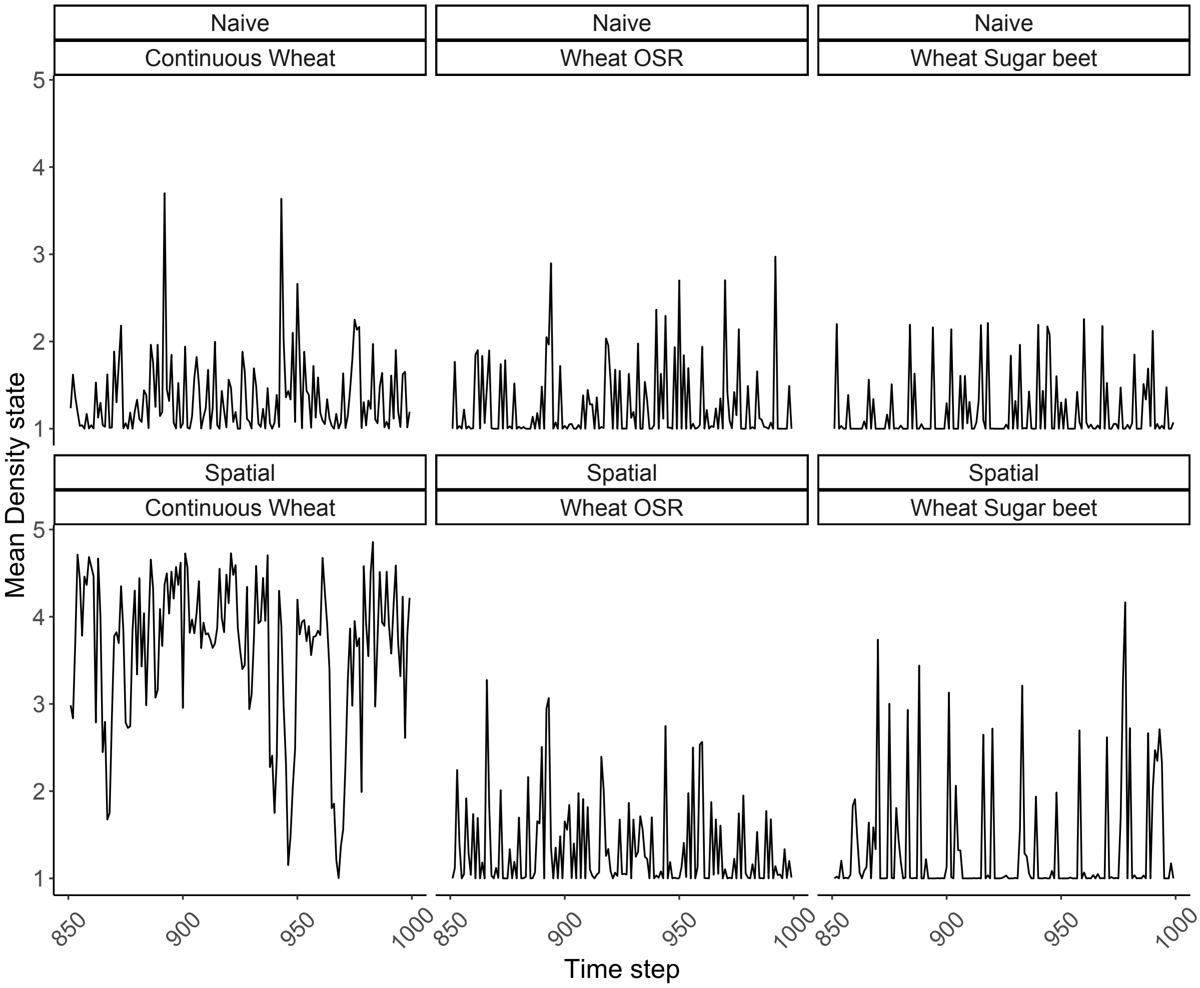

Figure S3. Example time series for rotations for each naïve and spatially explicit model. The last 150 time steps are shown for clarity.

Chapter Five:

Assessing metabarcoding as a means to understand the drivers of arthropod diversity in agro-ecosystems.

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Abstract:

Managing agricultural invertebrate biodiversity is essential to maintain agricultural productivity and prevent environmental degradation. Arthropods are an integral part of productive agro-ecosystems, but functionally important communities are in decline worldwide. Implementing large scale monitoring schemes is therefore a priority but this is typically hindered by the expensive nature of standard survey techniques. Through mass sequencing of communities, metabarcoding (MBC) provides a potential means of cost-effective monitoring over large scales. One of the major selling points of metabarcoding is simultaneous identification of organisms through extraction of bulked samples. However, there have been few studies that assess the limit to which bulk MBC can provide accurate and reliable community data. We undertook a three-year national-scale invertebrate survey to investigate the utility of high-throughput metabarcoding as a tool for assessing agricultural arthropod communities, and the impacts of land use on their composition and diversity. We show that bulked DNA extraction results in lower detection rates across our samples and can result in poor and even stochastic detection of taxa in our samples. Taxonomic bias in our pipeline is present, with many important organisms from Apidae being excluded from our samples entirely. We conduct diversity analyses (that demonstrate no apparent associations between our detected taxa and landscape features) however due to the issues we highlight with this pipeline we cannot draw robust ecological conclusions from these data.

Introduction:

Biodiversity allows ecosystems to provide invaluable services to mankind (Cardinale *et al.*, 2012). Agricultural production depends on arthropod communities, which are an essential component of biodiversity in agro-ecosystems. These organisms provide essential services, such as pollination, nutrient cycling, and pest-control (Losey & Vaughn, 2006; Aizen *et al.*, 2009; Isaacs *et al.*, 2009), but the intensification of agriculture destroys the communities that it so heavily depends on (Tilman *et al.*, 2001; Woodcock *et al.*, 2016, 2017; Tsvetkov *et al.*, 2017). As diversity supports agricultural productivity, designing agricultural economies that support biodiversity is essential to balance conservation and food production (Altieri, 1999; Zhang *et al.*, 2007; Tilman *et al.*, 2011). Understanding what drives arthropod diversity is therefore important if we are to build productive and sustainable agricultural economies.

When managing the diversity and function of agro-ecosystems, landscape context matters (Tscharntke *et al.*, 2005; Fiedler, Landis and Wratten, 2008; Phalan *et al.*, 2011). In principle we understand the relationship between landscape-scale dynamics and their implications for management (Clough, Kruess and Tscharntke, 2007; Haaland, Naisbit and Bersier, 2011; De La Fuente *et al.*, 2014; Uchida and Ushimaru, 2014; Uchida, Hiraiwa and Ushimaru, 2016). However, despite appearances these systems are dynamic and complex (Steffan-Dewenter *et al.*, 2002; Tscharntke *et al.*, 2005; Chase *et al.*, 2018). To understand how to conserve biodiversity we have to capture the intricacies of environmental dynamics, and for this, long term, large scale, and thorough monitoring frameworks are necessary.

Effective management is wholly reliant on our ability to accurately and rapidly measure changes in the environment. In general however, ecological monitoring efforts are lacking (Kleijn & Sutherland, 2003), and without accurate information to inform managment they will often fail to protect biodiversity (Kleijn et al, 2001). Many of the inadequacies in current monitoring programs stem from problems with standard methodologies that limit the scale of ecological surveys. Standard identification of organisms is reliant on the availability of taxonomic expertise (often requiring multiple taxonomists) and the data often have low taxonomic resolution due to difficulty in distinguishing related species (Giangrande, 2003). These techniques are expensive and time consuming by nature, making it difficult to capture the information necessary for well-informed management.

Recent advances in next generation sequencing and molecular techniques have enabled rapid collection of diversity information through analysing short gene sequences, collected either from environmental samples, or en-masse from organisms themselves (Taberlet and Coissac, 2012; Yu *et al.*, 2012; Ji *et al.*, 2013; Bohmann *et al.*, 2014). Known as metabarcoding (MBC), this technology can provide rapid and high-resolution identification of organisms, without the need for taxonomic expertise. One of the major selling points of MBC is its ability to track entire arthropod assemblages simultaneously, which is achieved through bulk DNA extraction and the use of 'universal' primers that target a diverse array of organisms (Leray *et al.*, 2013; Brandon-Mong *et al.*, 2015). Universal MBC pipelines are a promising development for biological monitoring, and have now been applied to a wide range of problems (Thomsen *et al.*, 2012; Ji *et al.*, 2013; Yang *et al.*, 2014; Fritz *et al.*, 2018).

Despite the obvious promise of MBC, there is no unifying framework for the mass survey of arthropods, and there are numerous points during the survey process that might influence the detection of different species. For example, most studies pool samples during DNA extraction, but there is evidence to suggest that pooling reduces detection success (Mata *et al.*, 2018). Studies also generally neglect to report how much sample biomass was DNA extracted from, which could influence rates of detection and species richness estimates. To ensure good community coverage practitioners suggest having multiple replicates during amplification (Douglas Yu, Personal communication), but the vast majority of applications use only one (Andersen *et al.*, 2012; Thomsen *et al.*, 2012; Ji *et al.*, 2013; Quéméré *et al.*, 2013; Schmidt *et al.*, 2013; Mollot *et al.*, 2014; Oliverio *et al.*, 2018). Moreover, numerous studies demonstrate detection bias towards certain taxa during bulk sequencing (Yu *et al.*, 2012; Zhou *et al.*, 2013; Brandon-Mong *et al.*, 2015; Ficetola *et al.*, 2015; Mata *et al.*, 2018), but the extent of biases and their limitations are rarely considered in applications using standard pipelines. Furthermore, sequencing depth per sample (i.e. the number of reads per sample) will affect detection probability, but some validation studies for universal primers use sequencing depths far in excess of what is economical for large scale studies (Brandon-Mong *et al.*, 2015), and performances have yet to be tested with realistic constraints.

It is not entirely clear what the limits are for high-throughput MBC in terms of community coverage, and there has been little discussion of detection under the restrictions of an economical large-scale survey. Considering the potential for MBC to reveal patterns of arthropod diversity in agricultural systems, we had two objectives. Firstly, we wanted to test the utility of high-throughput MBC for revealing agricultural arthropod diversity over large scales. We undertook a three-year national-scale survey, collecting 729 bulk invertebrate samples from 27 UK arable farms, and sequenced them under a standard cost-effective protocol. We compared detection rates of samples extracted using two levels of pooling to examine whether mass extractions accurately captured community composition and species richness. We then assessed whether total community complexity or biomass affected detection probability and to assess potential detection biases we compared molecular and morphological identification in a subset of insects in our samples. Secondly, we aimed to use this data to investigate the drivers of the structure and biodiversity of detected communities in intensively farmed agricultural habitats. We investigated the relationships between community composition and species richness with the abundance of broadleaf woodland and all non-arable habitat, as well as the diversity of crop cover. We demonstrate that using standard laboratory techniques, a set of regularly used universal primers shows poor detection rates and fails to detect ecologically important taxa from Apidae in our samples. In our molecular dataset we find no visible trends in diversity or community composition that could be explained by the three landscape features we consider.

Methods:

Survey:

Our study was conducted over a set of 27 UK arable farms, spanning from Oxfordshire to North-Yorkshire. We conducted pan-trapping surveys (Westphal *et al.*, 2008) over the course of a three year period (2015–17), with surveys taking place between June and August in each year. A single trap consisted of triplicate sets of blue, yellow and white bowls coated in UV reflective paint (Sparvar Leuchtfarbe, Spray-Color GmbH, Merzenich, Germany), attached to wooden stakes and filled with 400ml of water and a drop of odour-less detergent. Replicate traps were placed at vegetation height on north-facing hedgerows in two to three fields per farm (depending on farm size) with a separation of between 200–300m. During each survey, traps were emptied once every 24 hours over a three day and contents were stored in 50ml centrifuge tubes with 100% ethanol until DNA extraction. Over the three repeated surveys, we collected 729 trap-samples in total.

Sample preparation, PCR protocol and Illumina sequencing of COI amplicons:

Each trap-sample worth of specimens was prepared for DNA extraction by drying at 56°C overnight. In each sample we sorted material for extraction by removing two fore-legs of specimens equal to or larger in size than a honey bee (workers of *Apis mellifera,* ~10–15mm), or using the whole specimen if smaller. These sorted samples were then pooled over each season so that each pool consisted of three days' worth of invertebrates from the same trap within a given survey year. Pooled samples were frozen in liquid nitrogen and homogenised in a pestle and mortar, reducing the number of samples for extraction to 422 (including extraction blanks and negative controls). We then took 50mg or 10% of this homogenate by weight (whichever was larger), or the entire sample if under 50mg. We added 50mg of each extract to 1ml of digsol solution (tris-HCL 30 mM ph 8, EDTA 10 mM, and 0,4% SDS) and 20µl of proteinase K (20mg/ml = 9:1) in a 2ml Eppendorf. Multiple digestions were prepared for the 6 samples that had over 50mg of extract, with the sample being divided equally between Eppendorf tubes. The homogenate was then incubated at 56°C overnight in a rotary oven. DNA was extracted from the supernatant using ammonium acetate and quantified using the FluoStar Optima micro-plate flourometer. Samples were then diluted to between 10–15 ng μl^{-1} for amplification.

422 samples were amplified using PCR with the degenerate primers mlCOIintF & dgHCO2198 (Leray *et al.*, 2013; Brandon-Mong *et al.*, 2015). This primer pair was chosen as it showed the highest amplification success out of several pairs tested on DNA mixtures of 80 invertebrate taxa from 11 orders (Brandon-Mong *et al.*, 2015). The Illumina sequencing library was prepared using a two-step PCR process, following Campbell *et al.* (2015). Firstly the DNA was amplified using primers with the target COI sequences and adapters added to the 5' end (adapters are italicised; 5' to 3'):

mlCOIintF: *TCTACACGTTCAGAGTTCTACAGTCCGACGATC*GG WACWGGWTGAACWGTWTAYCCYCC

dgHCO2198: *GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT* TAAACTTCAGGGTGACCAAARAAYCA

Each sample was amplified in a 20 µl reaction volume containing 2µl of DNA template, 10µl of Qiagen PCR Master Mix (Qiagen, Manchester UK), 4µl of each primer (2ng per µl) and 4µl of ddH_20. In negative controls we replaced the DNA template with ddH_20. We used the following cycling profile of 95°C for 2 minutes, 16 cycles of 95°C for 15 seconds; 52°C for 30 seconds; 72 °C for 2 mins and a final extension of 72°C for 10 min.

The PCR products were separated on a 1% agarose gel stained with ethidium bromide, and amplicon sizes were compared to a 100bp ladder (ThermoFisher Scientific, Paisley, UK) to assess amplification success and potential contamination in negative controls. PCR products were re-quantified on the fluorimeter and diluted as necessary to ensure equal quantities were added to the second PCR. Unique multiplex-identifier tags were attached to each sample using a second 10µl PCR containing, 5µl of Qiagen PCR master mix, 1µl of the forward and reverse Ilumina Multiplex Identifier (MID) indexed primers with sequence complementary to the adapters used in the first PCR (1um), 1µl ddH_20 and 4µl of template. The reaction conditions were initial denaturation at 95°C for 15 minutes; 10 cycles of 98°C for 10 seconds, 65°C for 30 seconds, 72°C for 30 seconds; final extension of 72°C for 5 minutes. Product concentrations were again quantified with a fluorimeter.

Samples were randomised and selected to be pooled in either of two libraries for sequencing. Equal concentrations of each sample were pooled into groups of 8, with pools having approximate concentration of 15ng μl^{-1}. PCR products were purified using a bead clean-up using AMPure XP PCR purification kit protocol (Beckman Coulter Genomics; Austria). We selected two purified pools at random to analyse on a Tape Station (Agilent Technolies, USA), to inspect the distribution of product sizes and check for presence of primer dimer. A Blue Pippin (Sage Science Inc,. Beverly MA), was used for final size selection of our target sequences and to remove primer dimer. We made serial dilutions of each pool and quantified them using qPCR with a KAPA Library Quantification kit (Kapa Biosystems). The products were compared against kit standards and normalised to 4nM for sequencing. Each library was run using a 500 cycle kit (2x 250bp paired-end reads) on the Miseq Desktop Sequencer (Illumina, San Diego CA), samples were randomly divided between these two runs to provide adequate depth and to account for any 'run effect'.

Validation:

To assess whether our extraction method gave full taxonomic coverage we retained un-pooled homogenate from 45 trap-samples (i.e. 15 pooled samples) and extracted and sequenced each of these individually. After these un-pooled extractions, each sample was pooled and extracted following the protocol for the rest of the samples above. In each un-pooled sample we extracted an equivalent amount of material and sequenced these samples on the same run as the corresponding pooled samples. We also sent a set of Apidae specimens to an expert taxonomist to assess whether our pipeline was providing accurate representation of communities. The sequence of steps for these validation sets is detailed in figure 1.

Bioinformatic analysis:

We used a bioinformatics pipeline established at the NERC Biomolecular Analysis Facility at the University of Sheffield, validated for biodiversity metabarcoding and diet analysis. Analyses were performed on 'Iceberg' and 'Sharc', the high performance computing clusters at the University of Sheffield, UK. Trimmomatic v0.32 (Bolger, Lohse and Usadel, 2014) was used for quality filtering of paired end reads (Min. quality score of 20 in a 4bp sliding window) and to remove Illlumina adapter sequences. Reads under 100bp in length were discarded. FLASH (Magoč and Salzberg, 2011) and Mothur (Schloss *et al.*, 2009) were used to align sequences, match reads to our primer sequences, and remove the primer sequence from the aligned COI read. Low frequency sequences (i.e. fewer than 10 copies) and chimeric sequences were removed using Usearch v9.2.64 (Edgar, 2010), and the remaining sequences clustered into mOTUs at 97% similarity. Unique COI mOTUs were matched to reference sequences in the NCBI GenBank nucleotide database using BLAST (Altschul *et al.*, 1997), and assigned a taxonomic unit with at least 97% certainty. MEGAN (Huson *et al.*, 2007) was used against the NCBI taxonomic framework for mapping and visualisation of the BLAST results, using the top 2% of bitscores in the LCA assignment. A custom R script was used to filter out non-arthropod reads and low frequency (< 30 reads) mOTUs.

Statistical & Ecological analyses:

All statistical analyses were run in R version 3.4.4 (R core development, 2018). We analyse community composition and biodiversity metrics against three environmental variables, i) the proportion of broadleaf woodland within a 1km radius of each farm, ii) the proportion of all non-arable land cover and iii) an index of rotational complexity calculated from the diversity of land cover of different crops. We use the Shannon-Wiener diversity function to calculate a measure of rotational complexity that takes into account diversity as well as evenness:

$$H' = -\sum_{i=1}^{i=c} p_i \ln p_i \qquad (1)$$

Where c is the number of different crops in a 1km radius from the survey site, and p_i is the proportion of total cover for crop i. Land cover and crop cover statistics were derived from the CEH Land Cover and Crop maps (LCM 2015). As these values were derived from a 1km radius from the centre of each farm we aggregated samples for each farm over each survey year, so each individual sample consisted of the detected mOTUs in three sets of sequence data. Each sample in our statistical analyses therefore represented the community of a survey farm within a particular survey season.

Ordination:

We use non-metric multidimensional scaling (NMDS) to visualise patterns in community composition with regard to gradients in our three land use variables, and multidimensional fuzzy set ordinations to evaluate sensitivity in community structure to these variables. Fuzzy set ordination (Roberts, 1986, 2008, 2009) is an ordination method that directly incorporates information of environmental variables into calculation of ordination coordinates. The algorithm (implemented in the *fso* package in R) uses community composition to create similarity metrics between all communities. Simultaneously fuzzy set membership (on a [0,1] interval) is assigned on the basis of the value of a communities environmental variable in relation to the entire sample. This membership is then used to weight the similarity metrics of community samples. A positive correlation between the observed value of an environmental variable and the weighted fuzzy set membership μ, indicates sensitivity in community composition to that variable. We also used multivariate generalised linear models in the *mvabund* package in R, (Wang *et al.*, 2012; Warton, Wright and Wang, 2012) fitted to absence/presence measures to further examine effects of our land cover variables on community composition. For our ordination analyses we removed singleton species (i.e. species only observed once in the entire data) as per (Ji *et al.*, 2013), but these are retained for the alpha diversity analyses below.

Regression models:

As well as analysing the drivers of community structure, we examine the drivers of insect diversity. We look at three indices of diversity; overall mOTU richness as a proxy for species richness, incidence based measures of Chao's index (Chao, 1984) and the Jack-knife index (Zahl, 1977), with the latter two both accounting for detection bias against rare species and sampling frequency when calculating species richness. We use generalised linear mixed effects models using *lme4* (Bates *et al.*, 2014) to analyse trends in diversity with land cover variables. We incorporate group-level intercept terms for year in these models to account for differing environmental effects over each season.

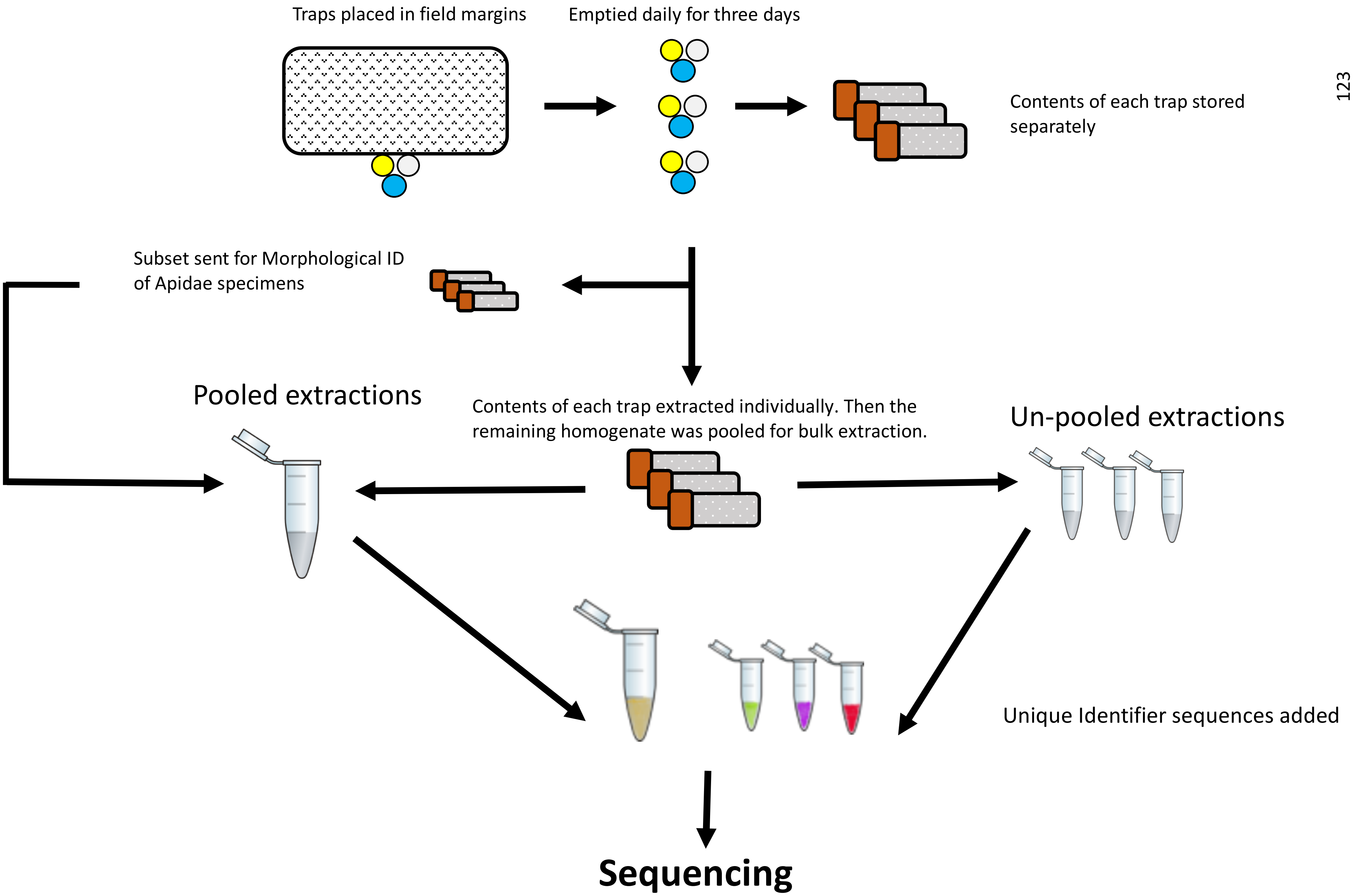

Figure 1. Diagram of the extraction routine for morphological and un-pooled validation sets.

Results:

General description:

Our sequencing runs yielded 2,176,215 post quality control reads from our COI barcode that could be assigned to arthropods. In this MBC dataset we retrieved a total of 368 insect mOTUs clustered at 97% identity, 366 of these were identified down to order, 274 to family, 209 to genus and 130 to species level. The breakdown of taxon-level assignments by region is displayed in table 1. There was representation of five insect orders within our dataset, including Diptera, Hymenoptera, Coleoptera, Lepidoptera & Hemiptera. The vast majority of our mOTUs were Dipteran, making up 80% of the total mOTUs in our sample.

Table 1. The number of mOTU designations at each taxonomic level in total and by each survey

Region	Class	Order	Family	Genus	Species
Total	368	366	274	209	130
Bedfordshire	140	139	105	85	56
Cambridgeshire	96	95	74	59	42
Lincolnshire & Nottinghamshire	202	201	158	126	76
Norfolk	103	102	80	68	49
Oxfordshire	124	122	97	77	51
Yorkshire	150	148	117	98	64

Figure 2. visualises the alpha diversity in our dataset, as well as the main groups represented down to genus level. This illustrates how the majority of our mOTUs were from Diptera, primarily from families Syrphidae and Anthomyiidae. This figure also visualises the frequency at which each taxon was found across our samples, with mOTUs from many Dipteran families being ubiquitous, whilst other orders were less well represented. Hymenopteran families Andrenidae & Halictidae, were also common, as were Cantharids in Coleoptera.

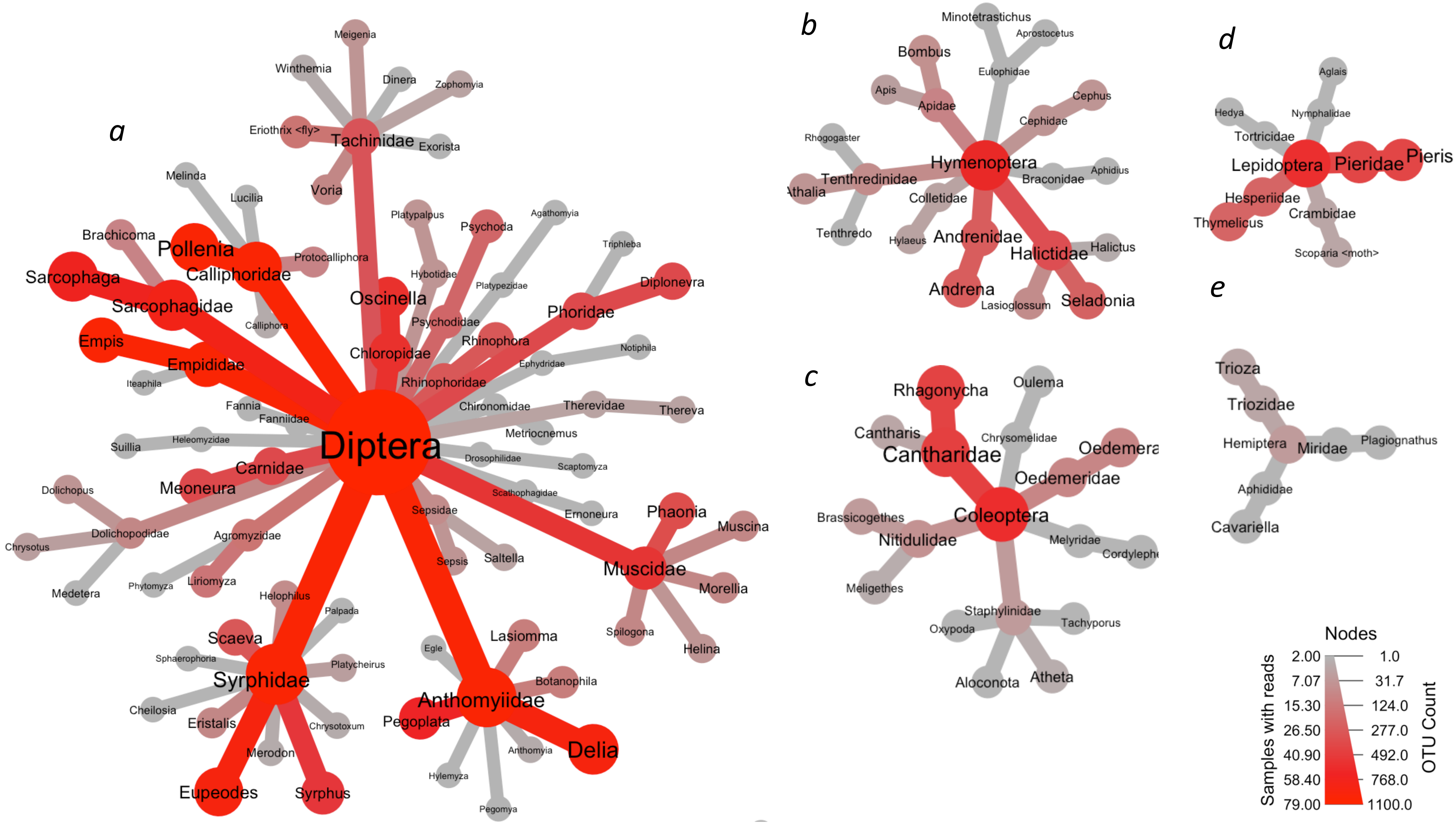

Figure 2. Visualisation of the composition of the MBC data set by order. Trees represent all mOTUs in each of the five Insect orders represented in our samples, Diptera (a), Hymenoptera (b), Coleoptera (c), Lepidoptera (d), and Hemiptera (e). Taxa are displayed down to genus level for clarity. Intensity of colour represents the frequency of occurrence of a particular taxon across our survey farms, the size of each node represents the number of times mOTUs were detected in that taxon.

Comparison of pooled and un-pooled mOTU counts (Figure 3A) reveals pooling during extraction results in the detection of fewer taxa (*Generalised linear mixed effects model, df = 1, F value = 47.6, P<0.001).* Although some samples detected identical or near identical assemblages, coverage of pooled samples was poor, only picking up 37% of the mOTUs detected in the corresponding un-pooled extractions on average, spatial replicates increased this figure to 50% (Figure S1).. Figure 3B illustrates the number of unique mOTUs found in pooled and un-pooled extractions, i.e. the number that were only found in either set. Although on average un-pooled samples had more unique mOTUs, pooled extractions still contained a considerable number of mOTUs unrepresented in the equivalent un-pooled samples. This difference in mOTU detection was not be explained by the total pooled sample weight (i.e. the dry mass of the insects caught in a trap over a 3-day period),but had a strong negative relationship with the OTU number detected by un-pooled extractions, which is an indicator of overall community complexity (Figure 4). Similarly there was also no association between mOTU richness and sample weight (Figure S2). In our morphological validation set, detection of Apidae species was very poor compared to morphological identification (Figure 5). Morphological identification was able to identify a total of 22 species across 20 samples, whereas our MBC pipeline only detected a single mOTU in 2 samples (corresponding to *Apis mellifera*).

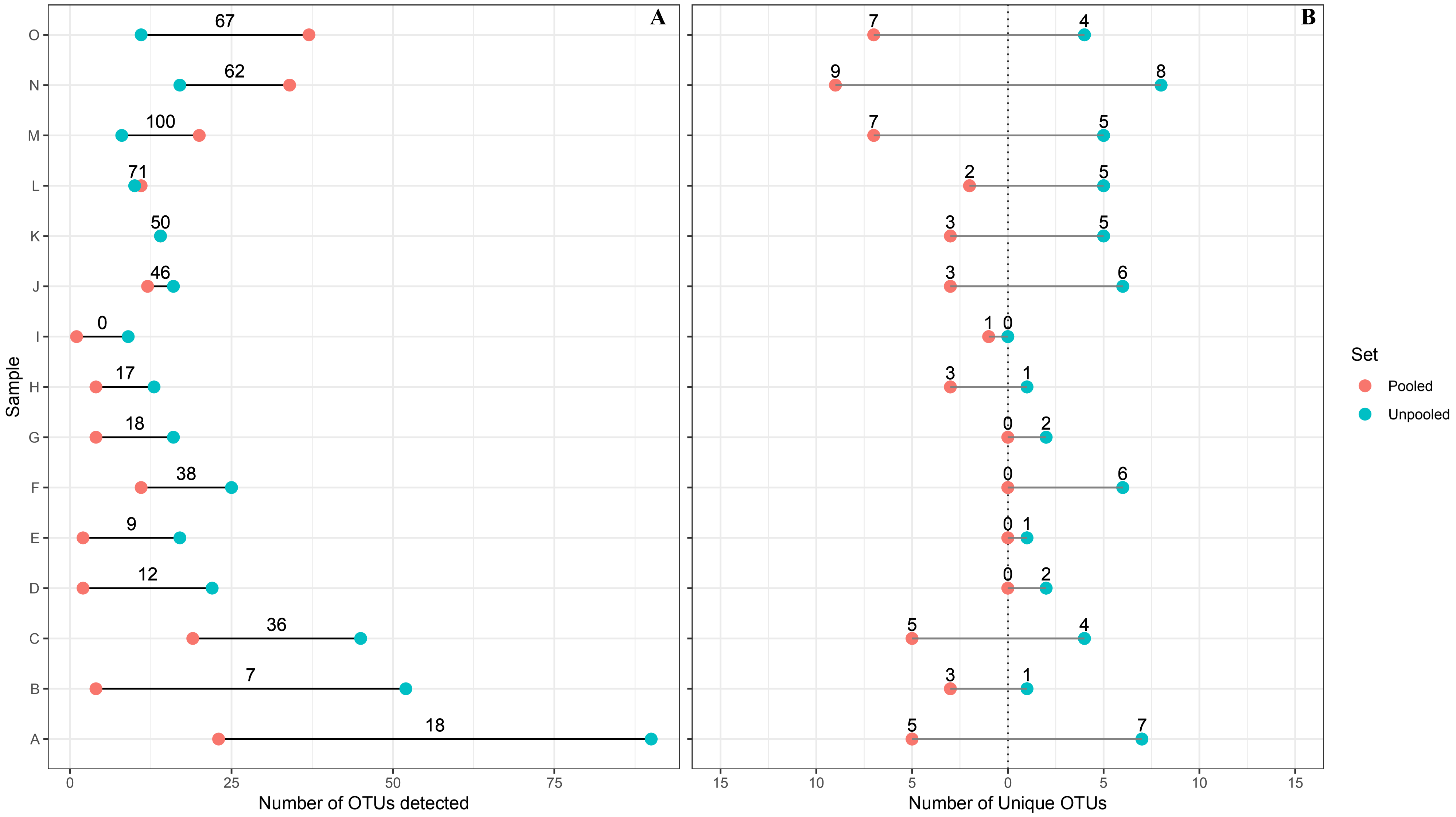

Figure 3. LEFT: The number of mOTUs detected in samples that were pooled (red) and un-pooled (blue) before extraction. Numbers above the horizontal lines represent the percentage of mOTUs in the un-pooled samples that were detected in the pools. RIGHT: The number of mOTUs unique to each pooled (red points) and un-pooled (blue points) sample.

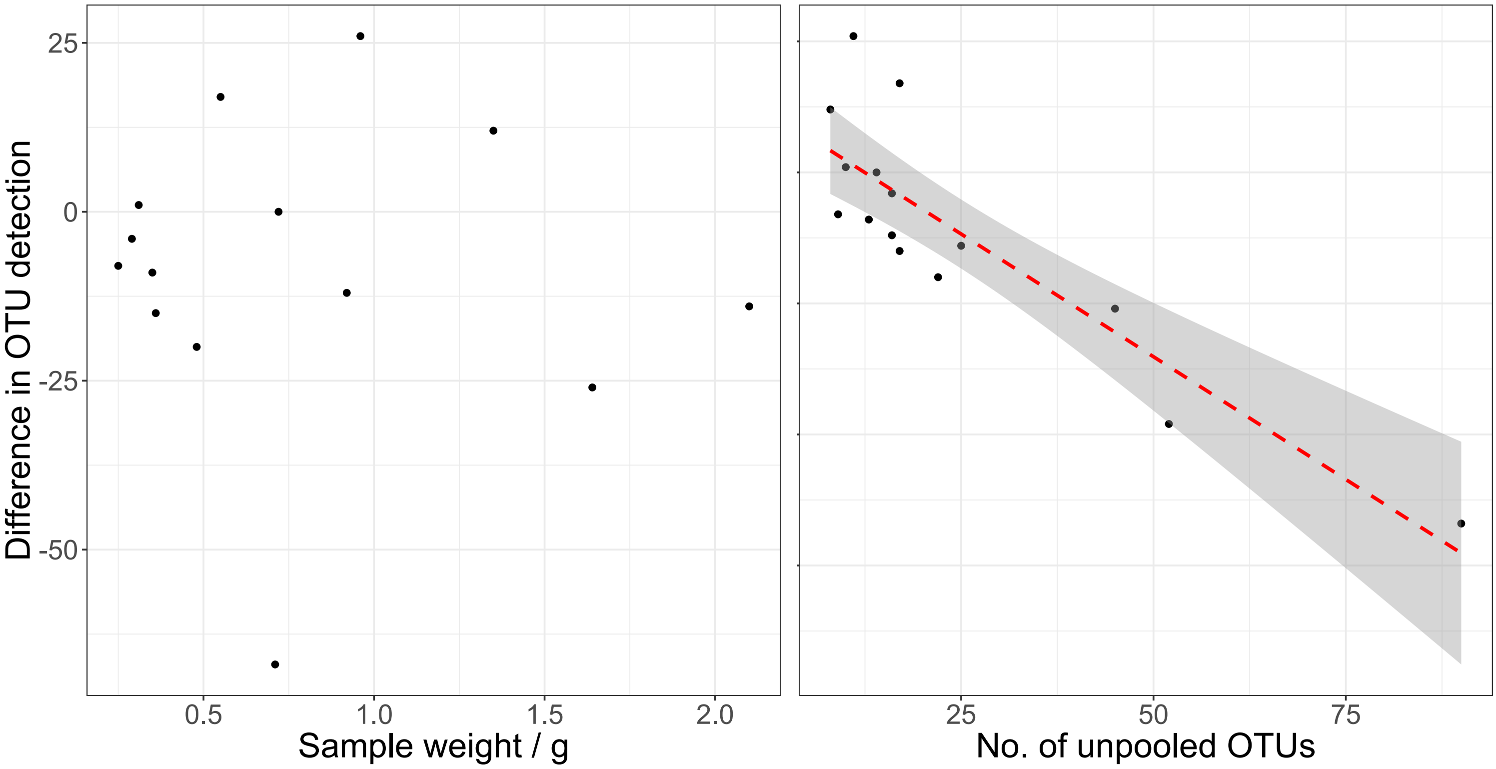

Figure 4. The relationship between the difference in mOTU detection and pooled sample weight (Left) and sample complexity (Right). There was a significant negative relationship between the number of mOTUs missed by pooled samples, and the number of mOTUs in un- pooled samples (Linear model, Adjusted R-squared = 0.7782, F=23.81, df=11, P<0.001), but no such relationship between mOTU detection and pooled sample weight (P=0.588).

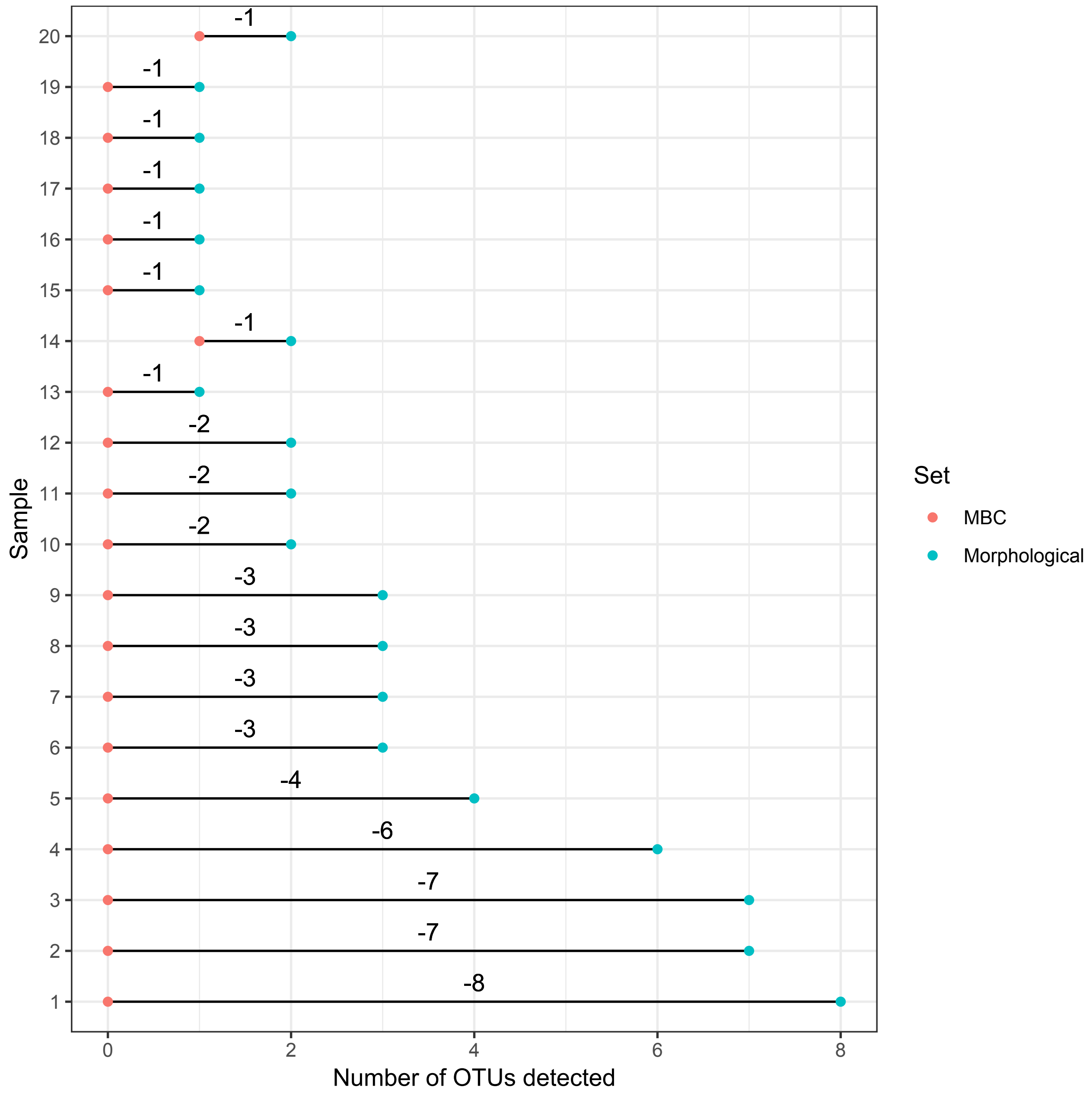

Figure 5. The difference between number of Apidae OTUs detected by our MBC pipeline (red), and by a specialist taxonomist (blue). Numbers above each line are the difference in OTU/Species number between each set.

We intended to investigate whether community composition drove the likelihood of failed detections. However, as so few non-dipteran taxa were detected in even un-pooled extractions this makes robust statistical inference difficult on this data. It is likely that amplification bias was so severe that we failed to detect many of organisms present even with multiple extractions (Figure S3).

Dissimilarity ordination (Figure 6) shows little differentiation in community composition of shared mOTUs by region. Mapping the topography of environmental variables to these ordinations also did little to visualise potential predictors of the variance in community composition (Figure 7.) with generalised additive models returning non-significant smooth terms (P>0.05). There are no clear patterns to their arrangement, nor is there any explanatory power given by region or environmental variables.

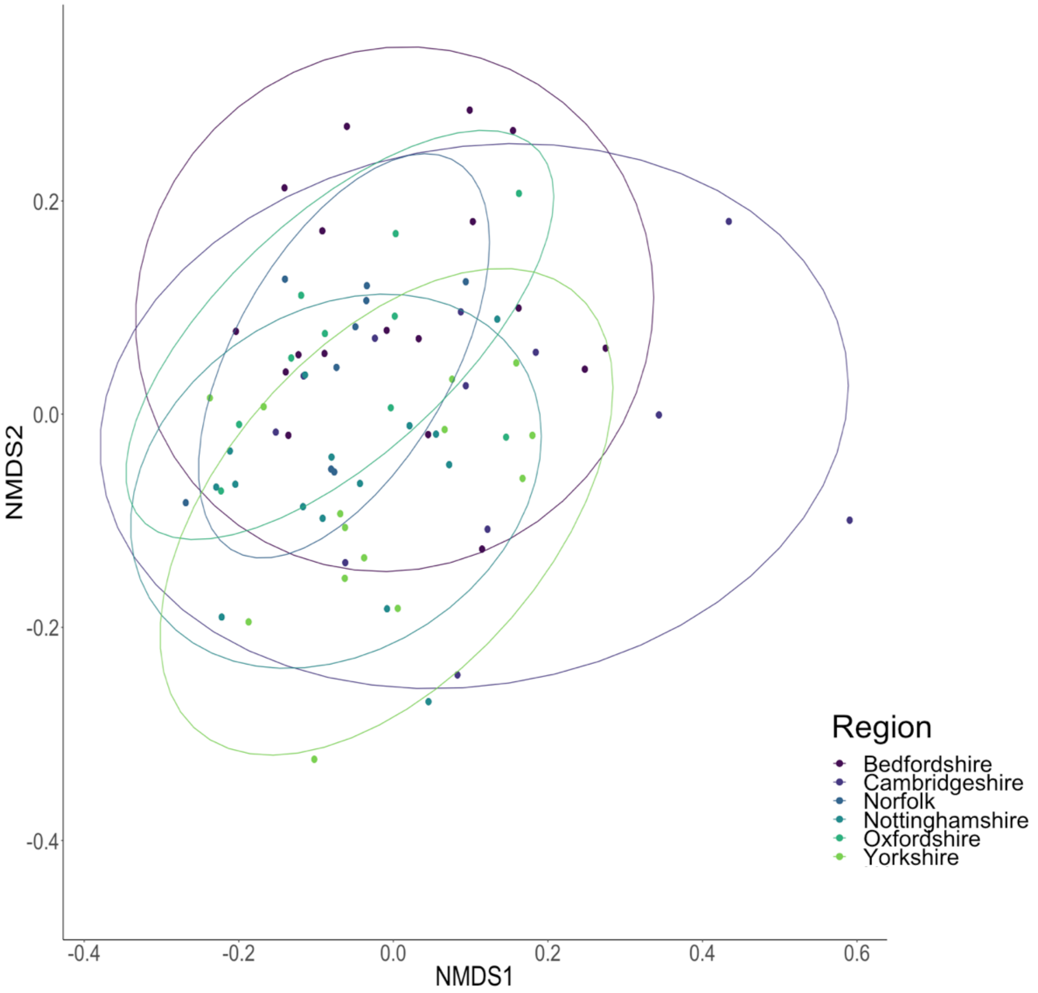

Figure 6. Non-metric multidimensional scaling (NMDS) ordinations of presence absence measures. Points are communities pooled across farms within each season, ellipses are 95% confidence intervals of centroids for each survey region (Okansen et al 2012).

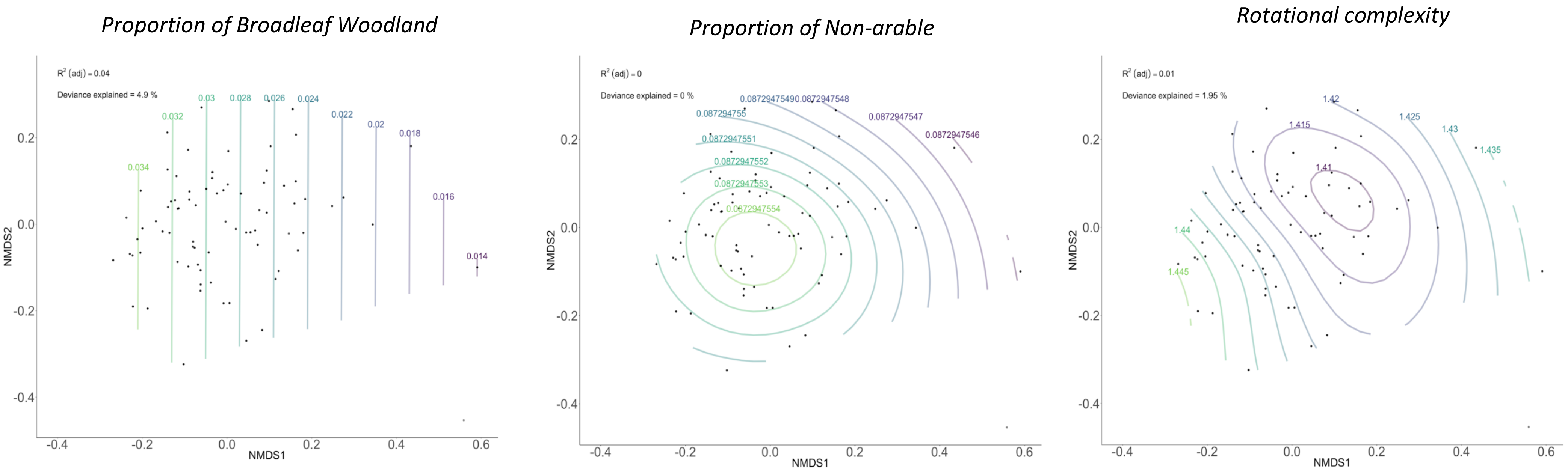

Figure 7. Non-metric multidimensional scaling (NMDS) ordinations of presence absence measures. Points are communities pooled across farms within each season, contours are calculated from environmental variables using a GAM in 'ordisurf'. Adjusted R squared values and percentage deviance explained difference in composition of each community explained by each variable are displayed in the top left. The numbers next to the contours are the predicted values of each environmental variable given sample position in ordination space. All smooth terms were non-significant (P>0.05) and are only displayed to demonstrate lack of ability to explain variation in community composition.

Fuzzy set ordinations (Figure 8) provide weak evidence that community structure is sensitive to the proportion of Broadleaf Woodland and our index of rotational complexity. Statistically significant but very weak positive correlations can be observed between fuzzy set membership and the real values of the the proportion of broadleaf woodland (r_s *= 0.28, df = 76 P =0.017*) and rotational complexity (r_s *= 0.24, df = 74, P = 0.049*), indiciating that these variable may have a small impact on community strucure. There was no such trend with the total proportion of non-arable habitat (r_s *= -0.07 , df = 75, P=0.51*). However, multivariate generalised linear models fitted to absence/presence measures (*manyglm & many.anova* -Wang et al 2012), did not corroborate these results , with no significant effects on community composition found for prorportions of broadleaf woodland (*wald value = 11.53,df = 76, P=0.53*), non-arable (*wald value = 11.90, df = 75, P=0.64*), or rotational complexity (*wald value = 12.98, df=74, P=0.25*).

There were also no significant effect of any landscape features on raw mOTU richness (*Generalised linear mixed effects model, df = 1, F value = 1.4, 0.0332, 1.48, P>0.05*), chao's index (*df = 1, F values = 0.33, 0.005, 0.5172, P>0.05*) or the jackknife index (*df = 1, F value = 0.94, 0.08, 0.262, P>0.05*). Figure 9 illustrates the high variance observed in diversity between farm-scale samples, yet the absence of any impact of the surrounding landscape features on the diversity of assemblages.

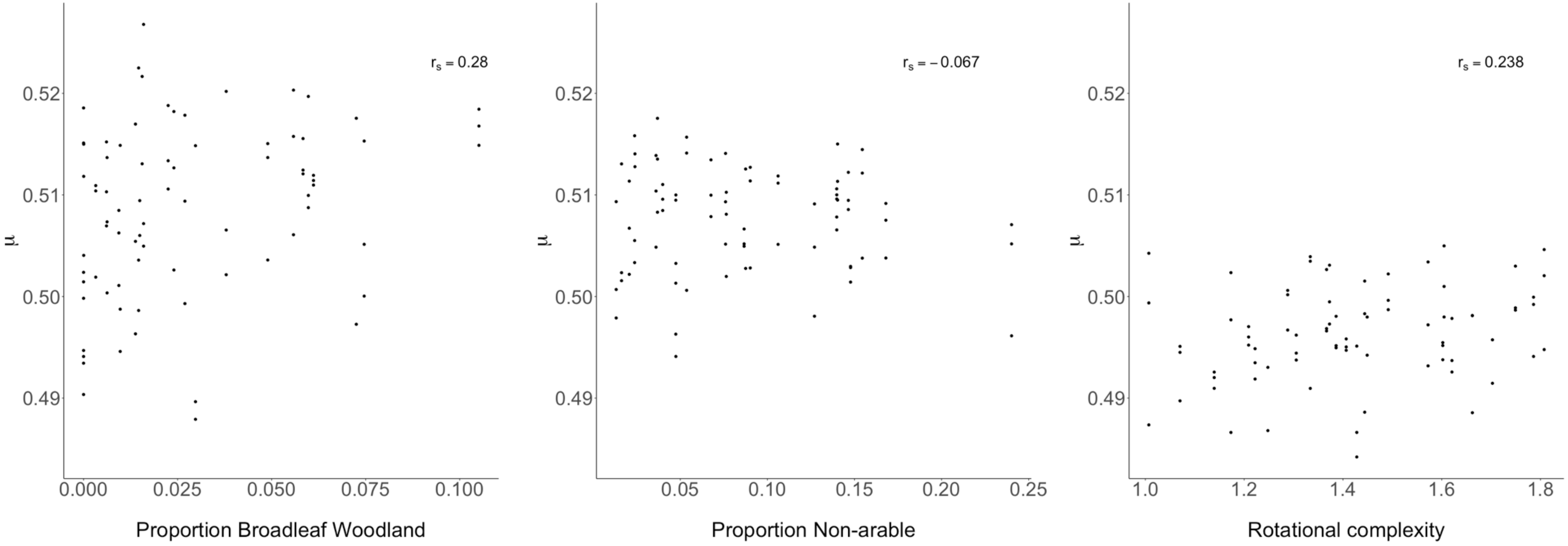

Figure 8. Fuzzy set ordinations for communities against real values environmental variables. Fuzzy set membership, μ *represents the gradient of similarity between plots along the environmental gradient, and is displayed on the y axis. The actual value of the environmental variable is displayed on the x axis. Positive correlations between* μ *and the actual variable represent sensitivity in the composition of a community to perturbations in the environmental variable. P values are derived from permutation tests.*

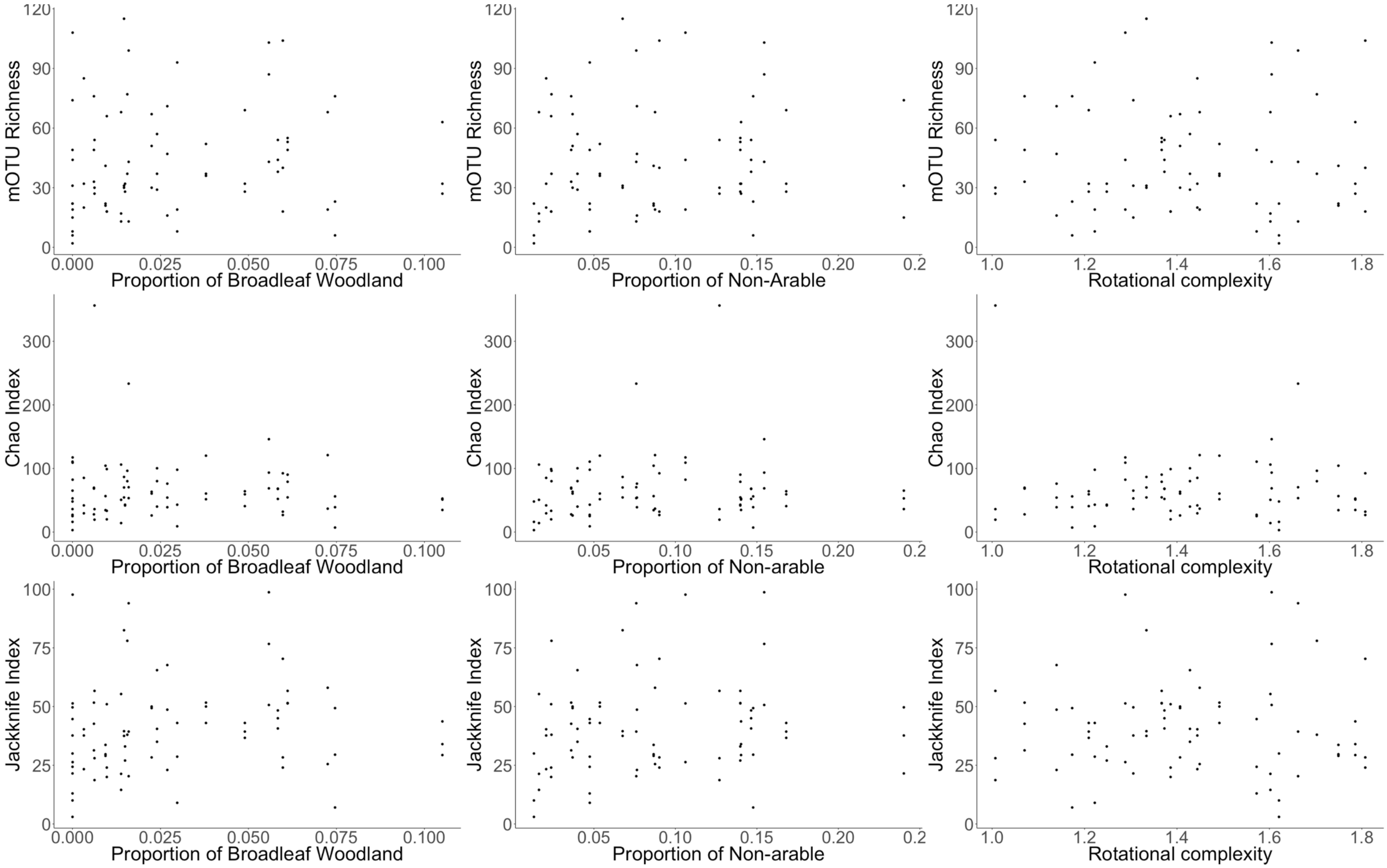

Figure 9. Relationship between three metrics of diversity, (mOTU counts, Chao's index and the Jackknife index) and the three environmental variables. Generalised linear mixed effects models were run to examine the effect of all environmental variables on diversity indeces, all of which had non-significant (P>0.05) terms.

Discussion:

Previous barcoding studies have showcased the use of MBC across a variety of taxa and environments (Yu *et al.*, 2012; Ji *et al.*, 2013; Leray *et al.*, 2013; Brandon-Mong *et al.*, 2015; Oliverio *et al.*, 2018). In this study we find that at large scales and high-throughputs, detection success is highly variable and much lower than expected. Pooling samples for extraction is justifiable on the basis of cost effective species detection and is advised by many (Yu *et al.*, 2012; Ji *et al.*, 2013; Cristescu, 2014). Here we show it likely comes at the cost of vastly reduced coverage due and stochasticity in mOTU detection. Moreover, previous studies have demonstrated that MBC performs as well as, or better than expert taxonomists (Ji *et al.*, 2013), however we demonstrate the opposite, as morphological identification of Apidae specimens vastly outperformed molecular identification.

These issues are indicative of an underlying problem with extraction and amplification, resulting in low probability of detecting target sequences that represent the entire community. A possible explanation is that, despite size selective sorting of samples and the fact that most samples were extracted from over 50% of available biomass (unpublished data), our sub-sampling of a minimum of 10% by sample weight was too conservative, and we did not extract DNA from all organisms in the sample. If this were the case, we may expect detection failures to scale positively with higher sample weight, but as we have shown, the degree of detection failure is not related to biomass. It is also unlikely that primer availability was a limiting factor as we had to remove excess primer dimer before qPCR. Detection failure does, however, increase with un-pooled OTU abundance, suggesting that detection failure is a function of community complexity. With this being the case it seems more likely that the poor performance of our pipeline was due to insufficient sequencing depth and primer biases towards certain taxa.

Primer sequences can exhibit a binding preference for certain targets, resulting in biased amplification towards taxa that have a higher binding site affinity (Yu *et al.*, 2012; Deagle *et al.*, 2014; Piñol *et al.*, 2015). The exponential rate of target amplification in PCR can mean any bias results in drastic overrepresentation of taxa compared to true abundances. Numerous studies have now reported low detection rates for Hymenoptera (Yu *et al.*, 2012; Zhou *et al.*, 2013; Brandon-Mong *et al.*, 2015), so it is perhaps unsurprising that these taxa were discriminated against in our pipeline. The topic of affinity bias is a concern to advocates of MBC (Ishii and Fukui, 2001; Sipos *et al.*, 2007; Piñol *et al.*, 2015; Krehenwinkel *et al.*, 2017; Mata *et al.*, 2018), and numerous suggestions have been made to reduce its impact. Despite taking the majority of these, including maximising template concentration, using degenerate primers, lower annealing temperatures, and providing biological replication, our community coverage is still poor.

Affinity bias is likely to be compounded by high abundances of specimens towards which primers exhibit affinity bias. Although the universal primer set we use in this study has been validated across diverse communities (Leray *et al.*, 2013; Brandon-Mong *et al.*, 2015), demonstrations of utility were across taxonomic orders and not within. Binding site similarity will likely correlate with taxonomic similarity of species, meaning that if a community contains an abundance of similar organisms with a high affinity for the primer sequence, the average competition for primer molecules will be higher, and less similar taxa will be discriminated against at a higher rate. In this case, its probable that the abundance of Diptera combined with a high affinity for our primer sequence meant they dominated the sample after amplification. So far, validation of 'universal' pipelines has been done with contrived communities that do not reflect the compositions of real-world assemblages (Brandon-Mong et al 2015). The problem of affinity bias with regards to large communities with multiple levels of diversity is an important one, and it is necessary to test these technologies over more diverse communities.

Poor detection due to subsampling and bias introduced by primer preference are justification for deeper sequencing and technical replicates during PCR (Ficetola *et al.*, 2015). Although we have considerable biological replication during our study, technical PCR replicates are something that our study, like many others, neglected. The discussion of suitable sequencing depth for bulk MBC applications in the literature is limited. We modelled our sequencing strategy around that of Ji et al (2013), who study a similar set of organisms, and as such we adopted a similar level of sequencing depth and similar universal primers. These primers were validated in Brandon-Mong et al (2015), where they report high detection rates but report over half a million post-QC reads were devoted to a single community. This vastly outweighs our sequencing strategy of around two million runs for 422 samples. However, as commercial MiSeq runs can cost upwards of £2000, adopting a deep sequencing strategy with multiple PCR replicates per sample to ensure adequate coverage, is far from cost-effective.

Uneven sequencing depth and extraction biomass across samples, may justify multiple levels of rarefaction to accurately estimate species richness (see figure S1 – Appendix).Nonetheless, this is something that numerous studies reject on the grounds that amplification bias means that some low frequency detections will be discarded (Ji *et al.*, 2013; McMurdie and Holmes, 2014). Additionally, some advocate adding taxa specific primers to increase detection chance for low-frequency taxa (Parducci *et al.*, 2013), and one study has shown how more thorough size selective sorting can increase taxonomic coverage (Elbrecht, Peinert and Leese, 2017). Both of these however, require more sequencing, more time spent on extraction and additional replication. Spending resources on time consuming size selective sampling, technical PCR replicates, and extra sequencing depth to achieve adequate coverages conflicts with the promise espoused by some that MBC can provide high-coverage, rapid, and cost-effective taxonomic survey. There needs to be a concerted effort to identify the limits to MBC detection rates in a variety of scenarios, questions involving the relationship between primer bias, community composition, complexity, detection probability and sequencing depth should be included in future work into the efficacy of MBC.

Despite stochasticity in detection and a clear bias against Apidae*,* our MBC survey still detects a considerable number of taxa across a national scale survey. Our surveys detected a wide range of taxa, but most representation was from Diptera. The emphasis in surveys of agricultural biodiversity has overwhelmingly been on species from Hymenoptera, due to their importance for pollination (Klein *et al.*, 2007; Garibaldi *et al.*, 2014). The majority of agricultural surveys generally neglect the diversity of Diptera, likely due to difficulties in morphological identification, and when they are included studies usually focus on just a few families (e.g. Jauker & Wolters, 2008).

Diptera are also some of the most diverse and abundant agricultural taxa (Howlett *et al.*, 2009) and have important roles in supporting ecosystem function through pollination (Jauker and Wolters, 2008; Rader *et al.*, 2013), and natural pest control (Pfister *et al.*, 2017). Despite the aforementioned short-comings of this survey the diversity and frequency of these taxa across our samples is likely to be a reasonable approximation of the communities in UK agro-ecosystems. The role of flies as pests is well studied, with an abundance of literature on controlling damage to important agricultural crops (Miller and Cowles, 1990; Vanninen, Hokkanen and Tyni-Juslin, 1999; Dosdall *et al.*, 2000; Jyoti *et al.*, 2001; Soroka *et al.*, 2004). Moreover, some of the most widely distributed species in our MBC survey are damaging agricultural pests, for example *Delia platura, D. antiqua* and *D. florensis* (common pests of beans and onions) were almost ubiquitous across our samples. Molecular methods promise to increase the rapidity and scale of pest detection and monitoring, as they are capable of identifying diverse, but hard to distinguish species in bulked samples.

Previous research has demonstrated that habitat availability and diversity has positive effects on arthropod biodiversity in agricultural systems (Tscharntke *et al.*, 2005; Diekötter *et al.*, 2008; Diekötter & Crist, 2013; Hendrick *et al.*, 2014), as well as ecological function (Altieri et al, 1999; Gurr *et al.*, 2003). Despite detection of a large array of taxa, we find no such associations with our environmental metrics and measures of community structure or diversity. The majority of arthropod studies focus on a few well studied taxa (namely specific families within *Hymenoptera*, *Lepidoptera & Coleoptera*), which are under-represented in our data. Surveys that do focus on the taxa we detect are few in number, and often focus on effects of local habitat features adjacent to arable fields (Andersen, Sjursen and Rafoss, 2004; Grégoire Taillefer and Wheeler, 2010). The few landscape scale studies that investigate the diversity of flies, generally find that diversity and community composition is invariant to crop cover and availability of semi-natural habitat, or only have weak, and highly variable associations across different scales (Josso *et al.*, 2013).

It is necessary to acknowledge the apparent homogeneity of our communities in context. This study represents one of the largest molecular diversity studies in the UK, and has identified a large quantity of organisms to a high resolution, the quantity and quality of data we provide here is far in excess of those that could be identified in a morphological study with the same resources. Despite finding no associations between land-use and diversity, this still has implications for conservation of the taxa we detect. In this instance, conserving broadleaf woodland, creating rotational complexity, and overall habitat diversity are clearly not priorities. The range of the environmental drivers included in our study sites may seem narrow compared to some studies that assess landscape scale affects (Steffan-Dewenter *et al.*, 2002), but these values represent the typical landscape scale variation present in intensively farmed UK agricultural systems (LCM 2015). Improvements to biodiversity may be seen at the higher ends of these scales, but the economic feasibility of increasing the availability of broadleaf woodland, for example, is extremely low.

MBC is a promising technique for expanding biodiversity monitoring, but large scale and high throughput pipelines are likely to miss key taxa and give inaccurate representations of communities. Fine-scale dynamics play an important role in maintaining diversity and ecological function, and molecular methods have a clear role in improving our understanding of these dynamics. Despite the flaws we highlight, MBC can still outperforms standard techniques in terms of both resolution and scale, but only with careful planning. Pipelines should take into account the composition of communities before sampling, including primers for important taxa, conduct multiple PCR replicates, and size selective sorting. Despite their power MBC surveys should still be approached with caution, as standard and cost-effective pipelines are likely to cause bias when assessing diverse communities with high abundances.

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Appendix:

Methods:

We assess the sampling efficiency in our validation set as a function of sequencing depth using rarefaction curves. Although there is debate about whether this is appropriate, and is not included many studies (Ji *et al.*, 2013; McMurdie and Holmes, 2014), we include it here to investigate whether higher sequencing depth was responsible for high species detection rates. We also compare un-pooled extractions to biological replication over their respective survey farms to see whether biological replication increased detection rates. Finally we investigate whether detection failures differed among taxonomic order.

Results & Discussion:

Biological replicates detected 50% of mOTUs present in our un-pooled subset (Figure S1), which suggests that spatial replication can increase the chances of successful detection. Even so there is clear stochasticity in detection success, as some extractions at a single location were still picking up more species than those spread over a larger area We also found no significant relationship (*Linear model, adjusted* R^2*= -0.041, F=0.49 df=1,12, P=0.50*) between overall sample weight (i.e. pooled sample weight) and the mOTU richness for un-pooled extractions (Figure S2).

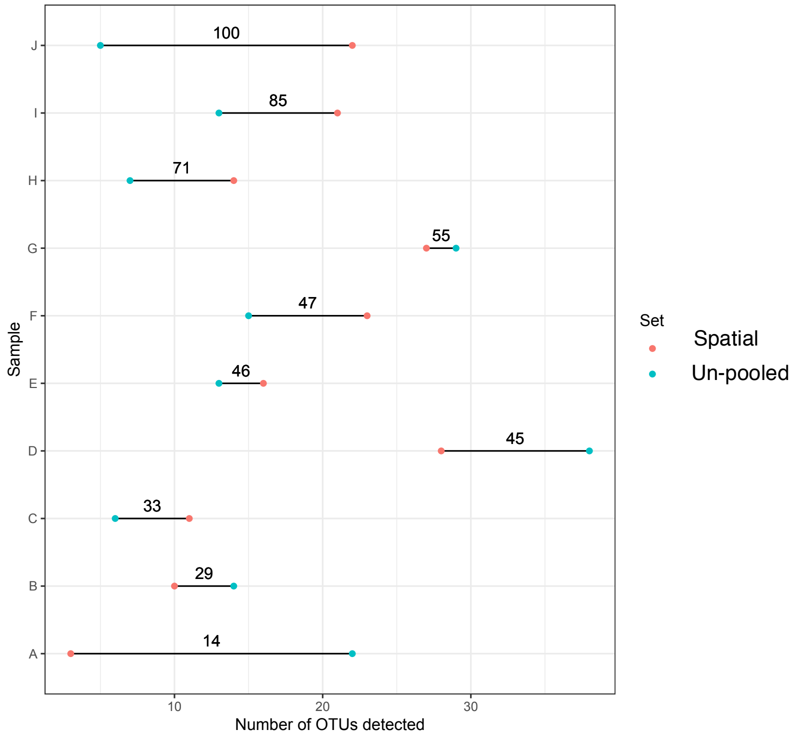

Figure S1. The number of mOTUs detected by all farm scale spatial replicates within a single season (red) and un-pooled extractions for a single trap on the same farm. Numbers above each horizontal line represent the percentage of mOTUs found in un-pooled samples that were represented in our spatial replicates.

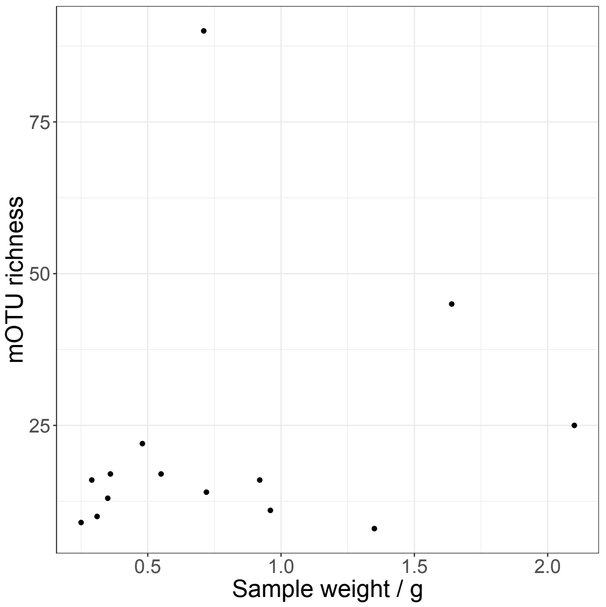

Figure S2. The relationship between sample weight and total mOTU richness of un-pooled extractions.

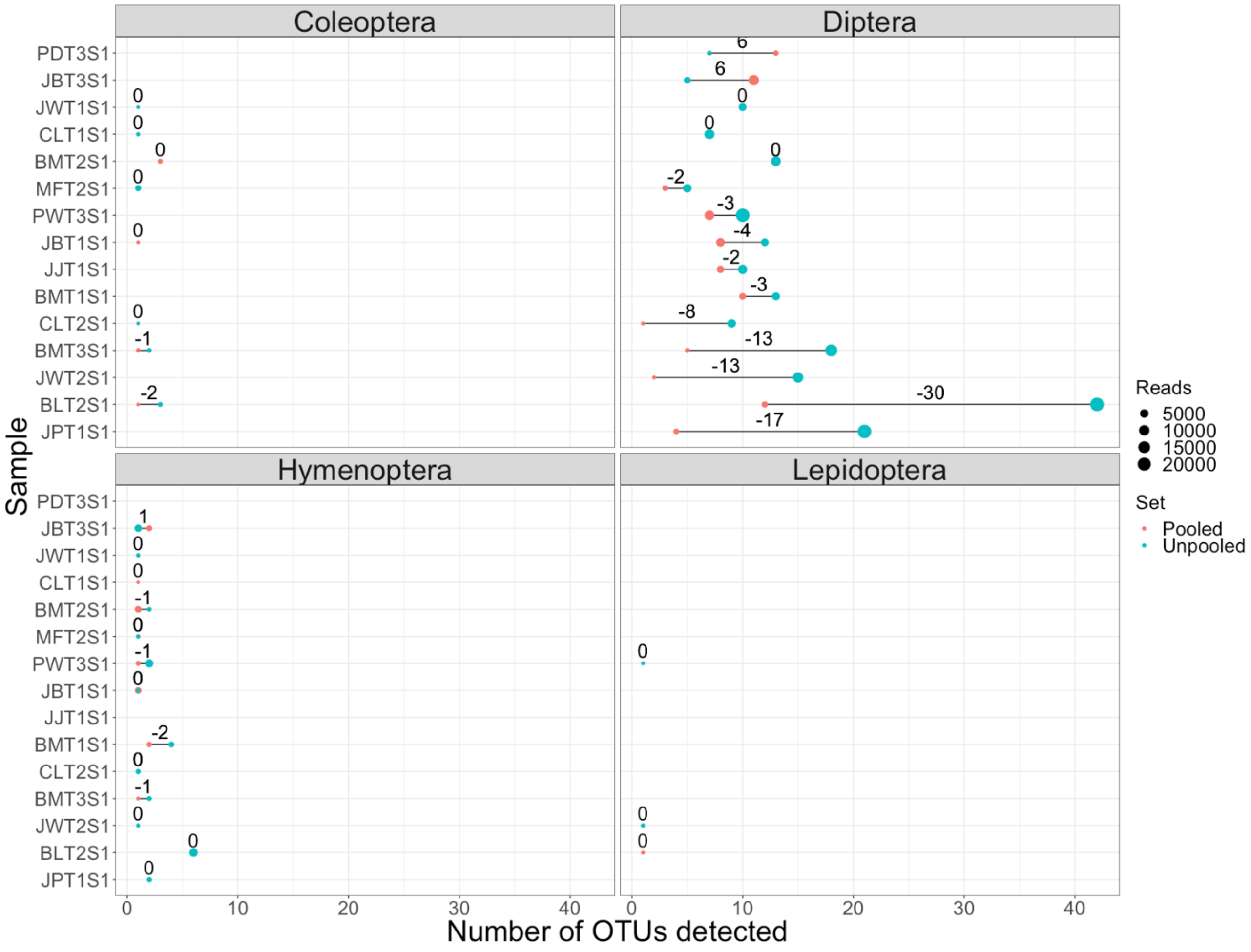

Figure S3. The difference between pooled and un-pooled mOTU detection numbers by insect order. Point size represents total read number for pooled and un-pooled samples. Numbers above the horizontal lines represent the difference between un-pooled and pooled samples.

Chapter Six:
General Discussion.

Technology can help address the mismatch between scale and quality of ecological data. In this thesis I have demonstrated that two novel methods, density-structured modelling and metabarcoding, have real potential for providing large-scale and high quality data. In the introduction to this thesis I laid out the research I present in each chapter and the questions I aimed to address:

Chapter 2 – Developing hierarchical Bayesian density-structured models.

In my first data chapter I aimed to implement hierarchical density structured models and examine their utility for future applications. As population dynamics in density-structured models are defined by transition probabilities, a central issue is how to account for population structure in the transition matrices of these models. In this chapter I evaluate several models which account effects in population structure via parameterising hierarchical ordered category logistic regressions. I evaluate models that incorporate hierarchical effects via incorporating intercept terms into the linear predictor, via model cut-point parameters, and a combination of both, against a baseline non-hierarchical model. Using leave-one-out cross validation and a series of posterior-predictive checks I demonstrate that all hierarchical models perform much better than non-hierarchical counterparts. Models that incorporate hierarchical effects via cut-point parameters provide superior predictive performance (in terms of LOO cross validation), and better fit the data, in terms of difference between the field-scale observed and predicted density-state distributions. These models all perform well across data gathered from a range of cropping systems in which population dynamics show considerable variation.

Chapter 3 – Using density-structured models to investigate the dynamics of weed populations.

In Chapter 3 I aimed to estimate the impact of differing rotation strategies on national-scale black-grass densities through examining measures of transient dynamics and simulation experiments. I reveal the efficacy of a series of rotations in controlling black-grass densities in context of high geographic variability. Using a combination of analysis of transient dynamics, two-step ahead, and stochastic simulations I demonstrate that black-grass exhibits a range of dynamics under different crop rotations. Increasing the proportion of winter wheat in a rotation increases densities and relative to winter wheat most rotations reduce black-grass densities under low, medium or high starting densities. The exception to this rule are rotations including peas which can increase the severity of an infestation at low or medium initial densities compared to continuous wheat. However, using variance decompositions of our two-step model projections, we show that by far the largest contributor to variance in the change in weed density are the origin of the matrices used to parameterise the models. This suggests that field-specific conditions are the principal driver of black-grass dynamics across our study system. This result highlights the need for large-scale assessment of management interventions, as well as consideration of variability within managements when assessing their impact on population dynamics.

Chapter 4 – Incorporating spatial information into density structured models.

In Chapter 4 I set out to investigate ways of incorporating spatial information into density-structured dynamics, how spatial models differ from naïve ones, as well as how spatial information affects dynamics in the context of crop rotation. Firstly, I built models that incorporated spatial information through consideration of the neighbour densities of a focal observation. Each of these models calculates a spatial covariate from a kernel across the direct neighbours of a focal observation, with each model using a different configuration of weights across this kernel. Comparison of these models with a baseline non-spatial model reveals they provide considerable improvement in terms of point-wise predictive error (calculated from LOO cross validation), but only slight improvements in the ability of these models to better describe spatial patterning within a particular field. Additionally there was very little difference between spatial models parameterised with different configurations of neighbour density weights. However, there was a considerable difference in the dynamics of weeds between spatial and non-spatial models. In Chapter three I demonstrated the small impact of crop rotation compared to field-specific effects, in spatially explicit models, the relative weed densities between rotated and un-rotated systems are dramatically different and matrix origin is comparable to rotation in terms of its contribution to total system variance.

Chapter 5 – Using metabarcoding for assessing arthropod diversity in agro-ecosystems.

In Chapter 5 I wished to investigate the level of community coverage from metabarcoding using standard protocols, as well as assess the relationship between diversity and land-use. As one of the major selling points of metabarcoding is the retrieval of community information from bulk DNA extractions I tested the effect of different levels of bulk extractions on community coverage. I show that pooling samples during extraction reduces community coverage considerably with bulk extractions resulting in poor or even stochastic detection. All samples were biased towards specific taxa, with many important organisms from apidae being excluded from sample coverage. I performed diversity analyses on these data, from which I found no associations between community composition or species richness and the prevalence of landscape features such as broadleaf woodland or diversity in crop-cover. However due to the problems with the method I highlighted it is not possible to draw any robust ecological conclusions about community composition and landscape features from this molecular data.

Implications for modelling agro-ecosystems:

Monitoring and modelling agro-ecosystems using novel methods reveals important features of population and community dynamics that would be neglected by smaller-scale studies. In Chapters two to four, the main conclusion we can make is that managing the weed *Alopecurus myosuroides,* or other pests, requires acknowledgement of the variables that drive the variability in dynamics over large scales. The fact that that rotational management contributes little to the overall density of weeds compared to local conditions, may be surprising (and worrying) for farmers. The evidence from experimental trials strongly suggest that successful control would be achieved from numerous rotations (Zacharias and Grube, 1984; Liebman and Dyck, 1993; Chauvel *et al.*, 2001; Melander, Rasmussen and Bàrberi, 2005; Moss, Perryman and Tatnell, 2007), but, in many cases, the extreme inter-field variability we observe will offset and mask the effect of these interventions. To be able to plan comprehensive management, we need thorough understandings of how populations respond to specific conditions.

The application of density structured models presented in this thesis allows us to model dynamics over an impressive scale: we can capture the variance in dynamics of populations across this landscape, but we cannot attribute the causes of variance to specific drivers. Without this information it is difficult to plan individual managements which is one of the ultimate goals of large-scale modelling of weed populations. Climatic variables such as rainfall and temperature, as well as other idiosyncrasies in management, will undoubtedly have an important role in driving the observed variability in weed density (Freckleton *et al.*, 2000; Colbach *et al.*, 2006; Lima, Navarrete and González-Andujar, 2012; Metcalfe *et al.*, 2017), and there is already research underway to investigate how these will influence density-structured dynamics.

When it comes to preserving biodiversity, its clear that studies need to expand in two ways. First, to include the full range of organisms present in agro-ecosystems, and second conduct research into their effects on agricultural productivity. Diptera made up the majority of the species in our samples, yet they are under-represented in the literature in terms of study of prevalence and function, mainly due to difficulty in accurate identification. With sufficient planning and development, MBC will be an excellent tool for expanding our knowledge of the role that these organisms play in agro-ecosystems. As I identify several important pest species across the survey locations, it may be particularly useful for early detection and tracking of pest species or disease vectors (Bohmann *et al.*, 2014; Davy, Kidd and Wilson, 2015).

Implications for developing methods for large scale monitoring:

In Chapter three I highlight why expanding the scale of monitoring and modelling must be a priority for ecologists. Populations are highly variable over large scales, the primary contributors to this variability however, are not the managements routinely applied to reduce weed densities, but spatial-heterogeneity. This high variability really accentuates a point that is frequently made in the ecological literature: local measurements of key variables are not large enough for robust predictions of dynamics or the effect of management. In order to make accurate predictions over the appropriate scales our monitoring efforts must expand.

In the case of this study, dissecting the roles of the numerous factors that vary over our study sites will be an important step in being able to make predictions and inform management on the scale of individual farms or fields. More generally, however, my results demonstrate that for a full understanding of large-scale population dynamics, monitoring must provide more than just information on populations: data on the drivers of change must be gathered alongside. Large scale monitoring programs must therefore be multi-faceted, and reliant on more than a single technique. Indeed, the RELU weed data survey I analyse in this thesis was underpinned by extensive social research on management data. Combinations of technologies or methods must be employed to gather comprehensive data on populations and their drivers, a relevant example would be using environmental data obtained through remote sensing with density structured models to model the effects of climate on weed abundance.

It is also important to consider the current limitations of promising technologies, as there are always issues facing transition from a successful proof of concept study to a successful application. My fifth Chapter covers the use of metabarcoding for diversity surveys, and highlights the need for thorough validation before application. I show that even using standard practices in a specialised facility, that MBC often fails to detect all species present in a community. Despite a thorough review of the literature, I could find little evidence of work done to address issues surrounding adequate levels of technical replication, sequencing depth, impacts of the level of community diversity, primer bias, and multiple levels of subsampling. All of the above can limit high-throughput and large-scale applications like ours without proper consideration. These issues obviously need to be addressed for MBC to become a main-stay of diversity monitoring and this result highlights a general need for thorough frameworks to be developed before techniques are applied. A major problem is that the literature is very positive about the potential of MBC,but does not hold enough information on the limitations of the technology at scale. Although the problems above are specific to MBC, this will likely hold true for most technologies scaling from small proof of concept to large-scale long-term studies.

It is necessary that the failures of studies are discussed in terms of the way in which the scientific community disseminates research. In science, bias is endemic and self-propagating. Publication bias, where positive messages and results are disproportionality propagated (Lortie *et al.*, 2018), can in turn drive authors to spin journal articles in favour of a positive message. Bias may also be introduced by enthusiasm for new technologies that exhibit potential, causing people to ignore negative findings. For example, a search on google scholar reveals that references to Neural Networks in the literature have increased dramatically over the past few years. However, there is evidence to suggest that much simpler regression models perform as well or better than their far more sophisticated counterparts (Matloff and Mohanty, 2018). Bias, whatever its origin, holds particular relevance for research into developing new technologies, as studies describing failures, and the conditions that led to them, may be either left unpublished, or failures glossed over to reflect a positive message. For future users wanting to apply these technologies, limitations are as relevant as potential, and without consideration of both, any ability to inform effective management will be compromised.

Future work:

Aside from further development of existing technologies and applications in new systems, there are numerous avenues of future research that stem from this thesis. At the start of this project, the research goals I laid out were very different from what eventually materialised. The project was initially aimed completely around developing novel MBC pipelines in tandem with quantitative methods of analysis. Unfortunately, these plans never came to fruition and the project changed direction to encompass a broader theme. However, these goals are still relevant and worth consideration.

MBC can provide amounts of data far in excess of what is achievable for traditional surveys within a similar timescale and budget. Pipelines for generating this data have developed quickly, increasing the quality of molecular data from sources (Zhou *et al.*, 2013; Bálint *et al.*, 2014; Brandon-Mong *et al.*, 2015; Tang *et al.*, 2015). Analysis of the data, however has remained relatively static, almost all studies do not progress beyond some version of ordination (Littlefair *et al.*, 2018), mixed effects models (Edwards *et al.*, 2018; Littlefair *et al.*, 2018; Nakagawa *et al.*, 2018), or in some cases generalised multivariate models (Ji *et al.*, 2013).

Part of the lack of ambition in analysis of MBC data is likely due to the lack of familiarity with statistical models that are suitable for type of data that MBC generates. Biases in amplification and sequencing mean MBC produces only absence-presence measures and is incapable of producing accurate data on abundance (Ishii and Fukui, 2001; Sipos *et al.*, 2007; Amend, Seifert and Bruns, 2010; Piñol *et al.*, 2015; Krehenwinkel *et al.*, 2017). There are, however, an abundance of sophisticated methods of analysing absence-presence data in ecologically relevant ways. Foremost among these are occupancy models, which model the probability of observing particular species at a particular site, or given a set of covariates (Bailey, MacKenzie and Nichols, 2013; Dextrase, Mandrak and Schaefer, 2014; Woodcock *et al.*, 2016; Louvrier *et al.*, 2018). Although they require careful planning of surveys, these models are well suited to MBC datasets, especially given the extensions that can be made to model multiple species simultaneously (Enzie, Ichols and Eamans, 2009; Green, Bailey and Nichols, 2011; Woodcock *et al.*, 2016).

As with studies of weeds, large scale models of agricultural arthropod diversity would be extremely beneficial for balancing productivity and conservation. For such an end, there is scope for getting density-structured models and MBC to meet in the middle, providing empirically backed models of diversity dynamics. Moreover, there is also potential to parameterise density-structured models with estimates of rank or relative abundance from MBC datasets, allowing large-scale modelling of population dynamics for multiple species. With arthropod abundance measures in a density-structured framework, lots of relevant questions are then easily approachable. For example, I had plans to examine the relationship between weed density, pesticide applications and diversity, the central theory being that heavy application of herbicides to control weeds could reduce resource availability of pollinator populations.

Part of the scope of the initial project was to investigate ways of obtaining more accurate information on abundance than MBC is currently capable of. Obtaining abundance data is difficult due to primer biases, however there has been considerable progress in gleaning more and more accurate density information from read numbers. Affinity bias can be partially corrected for using amplification factors (Krehenwinkel *et al.*, 2017), and accounting for it statistically was one of the aims I had considered at the outset of the project. Factors such as concentration of particular base-pairs, annealing temperature, and binding site affinity could all in theory be used to better predict the level of preferential amplification. There are models serve a similar purpose for gene expression in RNA sequencing (Li, Jiang and Wong, 2010; Roberts *et al.*, 2011; Jones *et al.*, 2012), however, PCR is inherently stochastic and with higher levels of community complexity, the more difficult this will be.

The more likely route to accurate estimates of abundance from molecular data will be from PCR free methods. Metagenomic approaches do not require amplification of barcode regions for sufficient detection, and instead rely on the sequencing of entire genomes. De-coupled from the biases introduced via PCR, shotgun sequencing of mitochondrial genomes has demonstrated that relative abundance measures are achievable (Tang *et al.*, 2015). Due their expense, and level of bioinformatics expertise required, these approaches are currently out of reach for most molecular ecology studies, but with the drastically decreasing cost of sequencing, they will likely become much more prominent.

Large scale population monitoring and modelling are crucial for preserving natural systems in the face of rapid global change. Continued development of methods of data collection and analysis are essential for the large-scale management of important ecosystems. However, it is important that methods are rigorously tested, and the limitations fully discussed in the literature. Unifying frameworks underlying the best practice for application of technologies must be established before they will be able to make meaningful impacts on management.

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