Quantifying the effects of biodiversity on food web structure: a stable isotope approach

Submitted by

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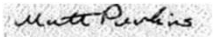
I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

Signature : 

Abstract

Food web structure is of underlying importance to ecological functions and processes. Whilst it is understood that a range of biotic and abiotic factors affect structure, relatively little is known of the role of biodiversity *per se* in structuring food webs. In this thesis I utilise novel multi-dimensional estimates of food web structure based on stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to quantify structural responses to changing community diversity. I additionally investigate methodological aspects of sample preparation and stable isotope quantifications of food chains. Using an arthropod prey-predator system, in chapter 2 I demonstrate that tissue selection and lipid extraction are important methodological procedures for deriving accurate $\delta^{15}N$ and $\delta^{13}C$ signatures. In chapter 3 I test the utility of $\delta^{15}N$ to quantify food chain length, and $\delta^{13}C$ to trace primary energy sources through to end consumers. Bayesian resampling of variance in sample means for plant and arthropod food chains produces robust isotopic estimates that match known food chain length well despite some error variance, and estimates of $\delta^{13}C$-range that trace trophic transfers. Chapter 4 represents a change in system from lab to field as I determine $\delta^{15}N$ and $\delta^{13}C$ signatures for plant and invertebrate species within three grassland communities representing a gradient of biodiversity. Quantifications of community bivariate isotopic space using isotopic metrics revealed that greater taxonomic richness increased both diversity of resource space exploited and overlap in resource space. These results therefore suggest that loss of diversity affected structure through altering relative patterns of niche partitioning in resource exploitation amongst community members. In chapter 5, I additionally find evidence that grassland management mediated changes in food web compartmental structure that were associated with differences in generalist invertebrate predator feeding habits. Taken together, these findings develop and demonstrate the utility of isotopic approaches to quantifying food web structure, and provide evidence of important mechanisms by which biodiversity affects food web structure. I conclude that the preservation of natural food web structure and trophic dynamics are further reasons for halting loss of biodiversity.

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Author's Declaration

Funding for this research was provided by the Food and Environment Research Agency (FERA), an Agency of the UK government. Additional consumable supplies used to aid data collection for Chapters 4 and 5 were supplied by Rothamsted Research at North Wyke.

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For chapters 4 and 5, taxonomic identification of invertebrates to Order level was conducted by MJP, with higher taxonomic level identification undertaken by Joe Ostoja-Starzewski at FERA.

Data analysis was conducted by MJP with considerable input from Stuart Bearhop, Robbie McDonald and Frank van Veen.

All chapters in this thesis were written by MJP with comments and editing provided by Stuart Bearhop, Robbie McDonald and Frank van Veen. Guidance throughout the PhD across all aspects of work was provided by Stuart Bearhop, Robbie McDonald and Frank van Veen.

CHAPTER 1

GENERAL INTRODUCTION

1.1 The importance of food web structure

Understanding causes and underlying mechanisms of food web structuring continues to be a fundamental agenda for future ecological research as the consequences of structure are profound. For instance food web structuring is of significance in explaining patterns of diversity we observe in nature (Rooney & McCann 2012), consequences of extinctions and invasions (Dunne *et al*. 2002; Srivastava & Bell 2009) and how communities are likely to respond to human induced habitat alteration (Schindler *et al*. 2010) and climatic change (Petchey *et al*. 1999; Hansson *et al*. 2013). Additionally, structuring of food webs is of underlying importance to our understanding of the 'emergent' properties of biological communities, including the stability of ecosystems (McCann & Rooney, 2009; Rooney & McCann 2012), their functions (Cardinale 2011; Thompson *et al*. 2012) and the services they provide (Settle *et al*. 1996; Cardinale 2011). Given the sustained declines in biodiversity and erosion of habitats globally (Estes *et al*. 2011), it is of increasing importance to understand how food web structure may mediate trophic responses, particularly to better predict likely cascading impacts on ecosystem stability, function and services.

The underlying relationship between structure and stability, function and services can be better understood with reference to an example; it has been shown that food web structure may consist of relatively discreet energy channels or compartments embedded within a larger food web (Scheu 2001; Kardol & Wardle 2010; Rooney & McCann 2012) that may each contain myriad trophic connections yet relatively fewer that couple the compartments (Scheu 2001; Kardol & Wardle 2010; Rand *et al*. 2012). Species coupling such compartments may potentially exert disproportionate effects on food web structure by catalysing energy transfer throughout the larger food web (Scheu 2001; van Veen *et al*. 2008; McCann & Rooney, 2009). Predator mediated coupling is considered particularly important given generalist predator's mobile and adaptable foraging links spatially and temporally distinct energy channels (McCann & Rooney 2009; Rand *et al*. 2012; Rooney & McCann 2012). As there is an inherent asymmetry in the interaction strengths and turnover speeds of

abundance between energy channels, predator coupling has the important effect of dampening variability from perturbations in both the predator populations (through alternate prey) and prey populations (predators move to other prey / suppress competitively dominant prey), conferring stability to populations and the overall food web (Kondoh 2006; McCann & Rooney 2009; Rooney & McCann 2012). In systems where structure is simplified to single energy channels of fewer trophic interactions, system stability (Dunne & Williams 2009) and maintenance of functions and services (Settle *et al*. 1996; Bell *et al*. 2008) can be reduced. Food web structure is therefore crucial to our understanding of how communities function.

In many instances (as cited above), quantifications of food web structure have examined structure as an explanatory variable, yet conversely other studies have quantified structure as a response to environmental variables, such as ecosystem size (Post *et al*. 2000), changing diversity (Rooney & McCann 2012), disturbance (Sabo *et al*. 2010), spatial and temporal variability (McCann & Rooney 2009) and extinction scenario (Haddad *et al*. 2009). Importantly some studies have gone further to link structure as both response and an explanatory variable and test how the effect of such biotic and abiotic environmental variables on community structure in turn affect ecosystem functions and processes (Petchey *et al*. 1999; Macfadyen *et al*. 2009; Estes *et al*. 2011). Thus food web structure is fundamentally important in affecting ecological patterns and functions, yet is itself determined by a range of biotic and abiotic variables.

1.2 The influence of biodiversity on food web structure

In seeking to understand mechanistic causes of food web structuring it is important to firstly note that biotic and abiotic variables affect structure and ultimately ecosystem functions and processes via modifying patterns of community diversity, for example in terms of species richness and evenness (Petchey *et al*. 1999; Macfadyen *et al*. 2009; Estes *et al*. 2011). Thus any given measure of

food web structure will inherently be dependent to some extent upon the identity and number of species in a community and their relative abundances and trophic relationships. For instance, fundamental ecological theory and empiricism shows that competition, succession, extinction and population dynamics all affect community structure, although such effects are indirect; structure is affected via the effects of such dynamics modifying patterns of diversity (Thebault *et al*. 2007; Schrama *et al*. 2012; Petchey *et al*. 1999; McCann & Rooney 2009, respectively). Importantly, therefore, achieving a mechanistic understanding of the causes of food web structure firstly requires discerning how diversity affects structure, yet to date few studies have sought to determine effects of diversity on food web structure directly (Rooney & McCann 2012).

Studies have quantified diversity and structure simultaneously in many contexts, however many of the measures of structure utilised (such as biomass, connectivity, network structure) are strongly influenced by dominant species effects (Melian *et al*. 2009; Anderson & Sukhdeo 2011). Consequently, less dominant or rarer species contribute relatively little to the value of structure and are in effect redundant. Often the emphasis in dominant- species sensitive measures is on singular or subsets of species rather than the wider community, and specifically how their interactions affect structure (Pocock *et al*. 2012 and references therein). Such studies vary in approach, from determination of keystone or ecosystem engineer species effects on diversity (Anderson & Sukhdeo 2011; Carey & Wahl 2011), through to sophisticated ecological network analyses of interaction strengths (Melian *et al*. 2009). Such approaches are particularly well tailored to identifying underlying mechanisms of energy flow and ecosystem functions which are often attributed to dominant species effects (Hooper *et al*. 2005; van Veen *et al*. 2008; Anderson & Sukhdeo 2011). Whilst such measures have been insightfully used to infer community dynamics (Dunne *et al*. 2002; van Veen *et al*. 2008), they may have limited capacity to uncover effects of diversity *per se* on food web structure, or of diversity on differing aspects of food web structure. In seeking to improve our

understanding of how diversity can affect food web structure it would therefore be constructive to consider additional measures of structure that are inclusive of all species, both dominant and rare.

In this respect, it may be useful to consider the range or distribution of species effects within a community rather than the total effects. For instance, considering a range of feeding modes or niches occupied by community members makes dominant species effects redundant, as arguably rare species should have an 'equal' probability of occupying niches at range limits and thus contributing to overall community niche distribution. Regardless, rare or dominant terminology and effects become redundant when considering how diversity may affect such structural measures, thus potentially providing new insights into community structure.

1.3 Food web structure and stable isotopes

Whilst a range of methods have been utilised to quantify food web structure they share in common a need to characterise the connections between species. This may range from simpler qualitative connectance webs based on binary (present / absent) interactions (Melian *et al*. 2009; Anderson & Sukhdeo 2011) or quantitative webs in which interactions are weighted by strength (van Veen *et al*. 2008; MacFadyen *et al*. 2009). Determination of feeding relationships between species is therefore integral to quantifying trophic structure, and traditional methods include gut-content analysis, faecal analysis and behavioural observations. These methods can be laborious, however, and may not reflect variation in digestibility and assimilation of source items. Furthermore, if limited in their collection in space and time, such methods may lead to over or under representation of source contributions (Bearhop *et al.* 2004). Similarly, to produce quantitative weighted trophic webs, extra measures of abundance and frequency of interactions may be required which can also be laborious and subject to their own caveats.

Increasingly, stable isotope ratios of nitrogen (N^{15} : N^{14}, termed $\delta^{15}N$) and carbon (C^{13} : C^{12}, termed $\delta^{13}C$) in consumer tissues are utilised to provide a temporally and spatially integrated construct of dietary niche (Bearhop *et al.* 2004), with $\delta^{15}N$ and $\delta^{13}C$ of consumer proteins reflecting the proteins of their food sources (DeNiro and Epstein 1978, 1981). As different tissue types in each organism have differing turnover rates (Hobson & Clark 1992; Bearhop *et al.* 2002), appropriate tissue sampling for $\delta^{15}N$ and $\delta^{13}C$ analysis provides an 'average' of an organisms diet over the temporal spread represented by the tissue, thus avoiding caveats associated with stomach content analysis or behavioural observations. Typically, enrichment in $\delta^{15}N$ of 2.5‰ to 3.4‰ is observed from diet to consumer (Post 2002, Vanderklift and Ponsard 2003, Caut *et al.* 2009), allowing determination of an organism's trophic level (Vander Zanden *et al.* 1997, Vander Zanden and Rasmussen 1999, Post 2002) and overall food chain length (Cabana and Rasmussen 1996, Vander Zanden and Fetzer 2007). Conversely, enrichment in $\delta^{13}C$ is much smaller between diet and consumer (Post 2002, Caut *et al.* 2009), and as basal sources often differ in their $\delta^{13}C$ values, $\delta^{13}C$ can be utilised to trace prey – consumer connections or food chains (Post 2002). Hence change in $\delta^{15}N$ and $\delta^{13}C$ from source to consumer as described (termed trophic discrimination factors and represented as $\Delta\delta^{15}N$ or $\Delta\delta^{13}C$), is the mechanism that crucially underpins the positioning of individuals, populations and species relative to one another in bivariate isotopic space (typically with $\delta^{15}N$ on a y-axis and $\delta^{13}C$ on an x–axis). For instance, food chain length is calculated as:

$$\lambda + (\text{nitrogen range} / \text{average } \Delta\delta^{15}N)$$

where λ is minimum trophic position and nitrogen range is mean difference between trophic levels of maximum and minimum $\delta^{15}N$ (Vander Zanden *et al.* 1997, Post 2002, Layman *et al.* 2007a). In this manner, isotopic measures of food chain length have been used to good effect to elucidate ecological patterns concerning factors such as ecosystem size, disturbance and productivity (e.g. Post *et al.* 2000, Takimoto *et al.* 2008, McHugh *et al.* 2010).

Whilst the use of $\delta^{15}N$ and $\delta^{13}C$ in ecological studies emerged some time ago (e.g. DeNiro and Epstein 1978), it is the more recent innovations in applications of isotopic data that has realised the potential of isotopic approaches to quantify food web structure (Layman *et al.* 2007a; Jackson *et al.* 2011; Layman *et al.* 2011). Layman *et al*. (2007a) proposed the use of 6 metrics that quantify differing aspects of food web structure based on the Euclidean distances between member species of communities in isotopic space. Briefly, these 6 measures are:

1. $\delta^{15}N$ range (NR) : Distance between species of min and max $\delta^{15}N$, providing for a measure of trophic length of the community and subsequently used to calculate food chain length;

2. $\delta^{13}C$ range (CR) : Distance between species of min and max $\delta^{13}C$ providing for an estimate of diversity of basal resources;

3. Total area (TA): Delineated by a convex hull around peripheral species, this quantifies the area of all species in bivariate isotopic space, thereby providing an estimate of total trophic niche diversity of community;

4. Mean distance to centroid (CD): Average distance of each taxa to bivariate centroid, providing a measure of average spacing of taxa and thus trophic niche diversity;

5. Mean nearest neighbour distance (MNND): Mean of distances to each taxa's nearest neighbour in bivariate space providing an estimate of density of taxa packing, and thus a measure of functional redundancy;

6. Standard deviation of nearest neighbour distance (SDNND): Provides a measure of evenness of spatial density and packing in bivariate isotopic space.

Such measures are increasingly utilised to study food web structure; Layman *et al*. (2007b) utilised measures of TA to show niche reduction across populations of a generalist predator subjected to varying severity of habitat degradation and loss of prey diversity. Similarly Quevedo *et al*. (2009) quantified change in TA across fish populations, whilst Okuzaki *et al*. (2009) contrasted TA

and NR estimates between humus and litter sub-communities in forests, and Cooper & Wissel (2012) used all six metrics descriptively to characterise Prairie lake communities. Despite the suitability of such stable isotope approaches, no studies to date have tested how biodiversity affects food web structure. Additionally, recent innovations in the analysis of isotopic data have provided practitioners with more robust tools to calculate trophic structure metrics (Jackson *et al*. 2011). Utilising Bayesian inference, these analytical approaches improve accuracy of population and community trophic metric estimates through resampling of variance in mean $\delta^{15}N$ and $\delta^{13}C$ of raw values, effectively propagating variation in raw data as quantified uncertainty in subsequent metric outputs. These developments have thus further improved the accuracy of isotopic quantification of trophic structure and catalysed their uptake amongst ecologists.

1.4 General overview of study systems

In order to utilise stable isotope approaches to test how biodiversity affects food web structure I employed two separate study systems: a controlled laboratory system and natural field system. In both instances I worked with terrestrial plant and invertebrate communities; much previous research using isotopic measures of trophic structure has focused on aquatic systems, often only utilising subsets of invertebrate communities, or emphasising vertebrates, such that invertebrates and terrestrial systems are isotopically understudied (Martinez del Rio *et al*. 2009; Boecklen *et al*. 2011).

My laboratory system was based around natural 4 tier food chains that were assembled within the laboratory and animals were fed under strictly controlled conditions. Food chains were:

Plant → Aphid (herbivore) → Hoverfly (predator) → Parasitoid (secondary predator)

I employed 3 different versions of this food chain by varying the primary producer; two strict laboratory food chains were based on either a C3 photosynthetic pathway (wheat *Triticum aestivum*) or a C4 pathway (maize *Zea mays*), enabling separation of the plants on a $\delta^{13}C$ axis and thus broadening the generality of any observed patterns (Vialatte *et al*. 2006). A third analogous food chain was collected from the wild and utilised nettle plants (*Urtica dioica*) and nettle aphids (*Microlophium carnosum*) in addition to the same hoverfly and parasitoid species. These wild species were largely obligate feeders and thus represented an analogous wild food chain with which to compare the laboratory food chains. I used these food chains in Chapters 2 and Chapter 3 to provide a controlled setting for methodological tests of sample preparation, and trophic dynamics of $\delta^{15}N$ and $\delta^{13}C$, respectively.

My second system was a field study and focussed on wild grasslands. These studies included all above-ground plant and invertebrate species. To provide a gradient of grassland biodiversity with which to test hypotheses about community structure using stable isotopes, I used 3 different grassland types based on decreasing sward height and variation: natural meadows, intensive cattle pastures and golf-course fairways. All samples were collected *in situ* for subsequent laboratory preparation and analysis, and the data is used in Chapter 4 and Chapter 5. This work was conducted at Rothamsted Research farm, Devon, UK.

1.5 Thesis structure

Chapter 1 – In this chapter I have provided a broad overview of the importance of studying biodiversity effects on food web structure, with reference to previous work in this area, as well as detailing the application of stable isotope approaches for this purpose.

Chapter 2 – In this chapter I investigate methodological approaches to sampling insects prior to stable isotope analysis (SIA). Given a lack of clarity about sample processing prior to SIA, and a scarcity of laboratory studies validating suitable methods for invertebrates, I assess how tissue selection and lipid extraction may affect estimates of $\delta^{15}N$ and $\delta^{13}C$ and subsequently derived trophic discrimination factors.

Chapter 3 – Using well replicated 4-tier food chains of known trophic structure, in this chapter I progress to investigating the utility of $\delta^{15}N$ and $\delta^{13}C$ to quantify food chain structure. I test dynamics of $\delta^{15}N$ and $\delta^{13}C$ across four trophic levels, in addition to quantifying the accuracy of estimates of Nitrogen Range (NR) to determine food chain length, and estimates of Carbon Range (CR) to trace basal energy sources through to end consumers. NR and CR are calculated using Bayesian resampling procedures that propagate variance as uncertainty in final estimates to improve robustness of these findings.

Chapter 4 – In this chapter I investigate how changing biodiversity alters food web structure. Utilising wild grassland communities spanning three 'levels' of diversity, $\delta^{15}N$ and $\delta^{13}C$ values are determined for all taxa in order to subsequently derive community metrics to characterise effects of diversity on structure. For different facets of diversity, I also test how TA and MNND change with diversity, in order to elucidate mechanistic insights into how species may coexist and the potential role of diversity in driving ecosystem functions.

Chapter 5 – In this chapter, I utilise a range of isotopic techniques alongside other community measures to investigate how community compartmental structure within grasslands may change as a consequence of human landscape management, and determine subsequent effects on predator feeding habits. Placing stable isotope approaches in an applied context in this chapter aims to both

test and demonstrate the potential ubiquity of isotopic techniques to improve ecological understanding of management effects upon diversity, community structure and trophic interactions.

Chapter 6 – I utilise this chapter to synthesise the findings of the previous four chapters, discuss the strengths of their approaches and conclusions, and discern weaknesses to be improved upon. I then discuss broader implications of this work and future research directions.

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CHAPTER 2

Important impacts of tissue selection and lipid extraction on ecological parameters derived from stable isotope ratios

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2.1 Abstract

The nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios of animal tissues can help identify the composition of diets and open up a myriad of ecological applications. However, consumers do not ingest or assimilate all components of food items, and it is not well understood how sampling different tissues of sources and consumers may affect isotopic values ascribed, and thereby how such variation affects derived ecological measures. Utilising a simple prey–predator feeding relationship in insects, we examined isotopic differences in soft, exoskeleton and whole tissues using samples with and without lipid extraction. As a derived ecological measure, we calculated trophic discrimination factors, changes in $\delta^{15}N$ or $\delta^{13}C$ between source and consumer, for the different prey - predator tissue combinations. Lipid extraction did not affect $\delta^{15}N$ values and we found significant tissue differences in $\delta^{15}N$ that varied between prey and predator. Lipid extraction enriched $\delta^{13}C$ values in most instances, and it was only after extraction of lipids that we observed consistent depletion of $\delta^{13}C$ in exoskeleton relative to soft tissues in prey and predator. Isotopic differences between tissue types propagated marked variation in derived ecological parameters. Common sampling practice using whole tissue for prey and predator (whole : whole) resulted in a trophic discrimination factor of 0.48‰ for $\delta^{15}N$, compared with correct factors of 0.97‰ (soft : whole) and 2.18‰ (soft : soft) using prey soft tissue actually ingested by the predator. For $\delta^{13}C$, variation across discrimination factors was less, with whole : whole tissue of -0.14‰, whilst correct factors were -0.55‰ (soft : whole) and -0.04‰ (soft : soft). Our results indicate tissue selection and preparation are important considerations for isotopic studies using arthropods. Lipid extraction is necessary to derive accurate $\delta^{13}C$ values based on proteins, whilst consequences of tissue selection are likely context-dependent: In poorly defined systems where sources are isotopically similar or have larger variance our results indicate tissue selection within sources is important to avoid significant error, whether estimating trophic positions or dietary proportions using mixing models. In such cases we strongly recommend exclusion of source materials not assimilated in consumers.

2.2 Introduction

Stable isotope ratios of nitrogen (N^{15} : N^{14}, termed $\delta^{15}N$) and carbon (C^{13} : C^{12}, termed $\delta^{13}C$) are increasingly used for testing a broad suite of ecological theories. Isotopic data are frequently employed as a characterisation of dietary niche (Newsome *et al*. 2009), with $\delta^{15}N$ and $\delta^{13}C$ signatures in consumer protein allowing inference of prey sources (DeNiro & Epstein 1978; 1981). Typically $\delta^{15}N$ enriches from food source to consumer, and though this enrichment is variable (e.g. 0.6‰ to 5.4‰; Post 2002), average $\delta^{15}N$ enrichment of between 2.5‰ to 3.4‰ (Post 2002; Vanderklift & Ponsard 2003; Caut *et al*. 2009) is commonly used for the estimation of species' trophic positions (Post 2002) and food chain length (Vander Zanden & Fetzer 2007). Conversely, $\delta^{13}C$ typically enriches by <1‰ from diet to consumer (Post 2002; Caut *et al*. 2009), and because $\delta^{13}C$ often varies between basal resources, $\delta^{13}C$ is generally used to trace prey – consumer interactions or food chains (Post 2002). Thus changes in $\delta^{15}N$ and $\delta^{13}C$ between the food source and its consumer, termed discrimination factors (Martinez del Rio *et al*. 2009), can allow for the elucidation of trophic relationships, or for inference of diet-related mechanisms driving ecological or evolutionary processes (Post 2002; Bearhop *et al*. 2005; Vander Zanden & Fetzer 2007). Combining $\delta^{15}N$ and $\delta^{13}C$ as an investigative tool, isotopic dietary information has been instrumental in developing empirical understanding in a number of research areas including trophic relationships (Syvaranta & Jones 2008; Newsome *et al*. 2009), dietary reconstruction using mixing models (Moore & Semmens 2008; Parnell *et al*. 2010), population niches (Bearhop *et al*. 2004; Newsome *et al*. 2007) and community food web structure (Layman *et al*. 2007; Jackson *et al* 2011; Layman *et al*. 2011). However, to improve research in these areas, hypothesis testing requires isotopic data that accurately reflects dietary pathways and so it is important that sampling protocols for providing $\delta^{15}N$ and $\delta^{13}C$ values are appropriate to the questions being tested (Martinez del Rio *et al*. 2009; Boecklen *et al*. 2011).

Many factors are known to affect $\delta^{15}N$ and $\delta^{13}C$ values, some of which are well studied including age, size, diet quality, habitat, season, trophic position, consumer's nutritional state and mode of excretion (Caut *et al*. 2009; Martinez del Rio *et al*. 2009; Boecklen *et al*. 2011 and

references therein) and where appropriate, researchers can control for these factors. One factor that has not been thoroughly considered is the extent to which different tissue types within an individual differ in their $\delta^{15}N$ and $\delta^{13}C$ values. Such tissue differences can occur because of: differing amino acid structure of tissues (Martinez del Rio *et al*. 2009), differing protein turnover rates amongst tissues (Tieszen *et al*. 1983; Arneson *et al*. 2006), differential metabolic routing of nutrients to tissues (Voigt *et al*. 2008), and ontogenetic tissue synthesis coupled with temporal or ontogenetic diet shifts (O'Brien *et al*. 2005).

Tissue specific sampling is routinely practiced for vertebrate studies, including fish (Perga & Gerdeaux 2005), birds (Bearhop *et al*. 2002) and mammals (DeNiro & Epstein 1981; Tieszen *et al*. 1983). In contrast, research has rarely explored tissue sampling of invertebrates (Vanderklift & Ponsard 2003), in particular arthropods (Caut *et al*. 2009; Boecklen *et al*. 2011), which are a major component of most food webs. Tissue specific sampling for arthropods can be problematic and laborious, and current common practice utilises whole tissue (Vanderklift & Ponsard 2003; Mateo *et al*. 2008; Caut *et al*. 2009 and references therein for all). Consequently, most literature estimates of arthropod discrimination factors, utilised to reconstruct diet and calculate subsequent ecological parameters, are based on whole tissue. In reviews of laboratory arthropod studies, Vanderklift & Ponsard (2003) and Caut *et al*. (2009) report 82 estimates of $\delta^{15}N$ discrimination factors from 18 studies, and 73 estimates of $\delta^{13}C$ discrimination factors from 14 studies, in which all but 1 study (5 estimates of $\delta^{15}N$ only) utilised whole tissue.

Arthropod anatomy is crudely characterised as soft internal tissues (broadly composed of proteins, sugars and fats) contrasting with a hardened exoskeleton largely constructed of chitin embedded with protein (scleratin), which in aquatic species also often contains inorganic carbon in the form of $CaCO_3$. Given such differences in tissue composition, in isotopic studies tissue selection is likely to be important because many consumers feed selectively on the prey they capture; >70% predatory terrestrial arthropods ingest soft internal components but not cuticles of arthropod prey (Cohen 1995). Thus the inclusion of exoskeleton, inherent in measures of whole tissue, has the

potential to introduce error into estimates of $\delta^{15}N$ and $\delta^{13}C$ in instances of selective assimilation by consumers.

Few studies have explicitly compared $\delta^{15}N$ and $\delta^{13}C$ in arthropod tissues, especially between soft and exoskeleton tissues (Macko *et al*. 1989; Tibbets *et al*. 2008), and it remains untested how tissue selection may lead to erroneous estimates of subsequent isotopic measures such as discrimination factors, which are key to dietary reconstruction. It is therefore of importance to establish when and whether tissue differences should be considered sufficient to change the way we sample and process arthropods prior to generating isotopic data.

Additionally, to accurately estimate $\delta^{13}C$ of proteins within tissues it is accepted practice to first remove free-lipid contained within. Lipid is naturally depleted in $\delta^{13}C$ (DeNiro and Epstein 1977) and its concentration varies between tissues. The extraction process is well studied but has produced mixed results (Pinnegar & Polunin 1999; Sweeting *et al*. 2006; Bodin *et al*. 2007). Failure to extract lipids can lead to erroneous conclusions (Tarroux *et al*. 2010), but it is currently underutilised (Mateo *et al*. 2008) likely because of the lack of clarity about when it is needed (Post *et al*. 2007). It has been shown that some invertebrates contain significant concentrations of lipid (Meier *et al*. 2000) but to date few studies have considered tissue–specific effects of extraction across multiple tissues (Pinnegar & Polunin 1999; Sweeting *et al*. 2006; Logan & Lutcavage 2008), particularly in arthropods (Bodin *et al*. 2007; Mateo *et al*. 2008 and references therein).

In this study we addressed three questions: 1) How consistent are differences in $\delta^{15}N$ or $\delta^{13}C$ signatures of whole, exoskeleton and soft tissue? 2) How important are tissue - specific differences in $\delta^{15}N$ or $\delta^{13}C$ in explaining differential estimations of discrimination factors for $\delta^{15}N$ and $\delta^{13}C$? 3) How does lipid extraction differentially alter $\delta^{15}N$ or $\delta^{13}C$ signatures of whole, exoskeleton or soft tissue?

2.3 Methods

Herbivorous grain aphids (*Sitobion avenae*) of a common stock and predatory 1st generation larvae of wild-caught hoverfly (*Syrphus vitripennis*) yielded soft and exoskeleton tissue to test for differences in $\delta^{15}N$ and $\delta^{13}C$ between tissue types within each species. We reared aphids on two independent food plants; One based on a C3 photosynthetic pathway (wheat *Triticum aestivum*) and the other on a C4 pathway (maize *Zea mays*), enabling separation of the plants on a $\delta^{13}C$ axis and thus broadening generality of any observed patterns. Aphids were raised under a 16:8 light: dark cycle in 70% humidity. Plants were raised on a common source of homogenised compost and distilled water, and introduced to aphids at 20 days (wheat) or 30 days (maize). Randomly collected aphids of all ages were frozen (-20°C) for later dissection. 15 gravid wild-caught hoverfly females were induced to lay eggs in the laboratory and emergent larvae randomly assigned to feed on either wheat or maize aphids, under a 16:8 light: dark cycle at 70% humidity. Hoverfly larvae entered pupation 8 – 10 days after hatching and after 72 hours pupation were frozen (-20°C) for later dissection. Prior experimentation identified 72 hour pupation as suitable to provide exoskeleton tissue in the form of exuviae, whilst terminating pupation before larval metamorphosis was judged to have sufficiently altered body form and thus potentially caused significant shifts in $\delta^{15}N$ and $\delta^{13}C$ of tissues. Notably, Tibbets *et al*. (2008) showed $\delta^{15}N$ of whole tissue for half pupated Diptera to not differ from larvae. 72 hour pupation for our hoverflies represented < one-third total pupation time.

Tissue Preparation & Lipid Extraction

For each of 15 replicates; 80 pooled aphids were dissected into soft and exoskeleton components to provide enough material for two lipid treatments, one with lipids present (+L) and one with lipids extracted (-L), whilst a separate sample of 80 pooled aphids provided whole tissue for each lipid treatment. For hoverflies, we collected 15 replicates of individual larvae, each of which provided four tissue treatments; having split pupae and separated soft and exoskeleton (exuviae) tissues, for

each larva half of the soft and exoskeleton tissue underwent lipid extraction. Thus there were six tissue treatments for aphids: (1) soft+L, (2) soft-L, (3) exoskeleton+L, (4) exoskeleton-L, (5) whole+L, (6) whole-L; and four tissue treatments for hoverfly (1-4). All samples were dried at 45°C for >48hrs and then homogenised. For samples undergoing lipid extraction, tissue was subsequently immersed in 2:1 Chloroform: Methanol solution for 50 minutes to remove free-lipid, and then left to air dry.

Stable Isotope Analysis

For all samples, 0.5mg ± 0.05 dried material was enclosed in tin capsules. Stable Isotope Analysis (SIA) was conducted at the Food and Environment Research Agency, York, UK. Samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ in a Fisons EA1108 elemental analyser (Carlo Erba Instruments, Milan, Italy), coupled with an Isoprime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Stable isotope ratios are reported in delta (δ) notation where $\delta^{15}N$ and $\delta^{13}C = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Isotope ratios are expressed in per mil (‰) relative to the ratio of international reference standards ($R_{standard}$) which are Atmospheric Nitrogen and Vienna PeeDee Belemnite (VPDB) for nitrogen and carbon respectively. Measures of standards placed throughout samples exhibited acceptable instrument reproducibility of < 0.09‰ (SD) for $\delta^{15}N$ and < 0.18‰ (SD) for $\delta^{13}C$ using collagen standard, insect whole tissue standard (cockroach; *Nauphoeta cinerea*), and sucrose C4 plant standard.

Data Analysis

Analyses were employed separately for aphids and hoverflies using General Linear Mixed Models (GLMM) to test how response variables $\delta^{15}N$ or $\delta^{13}C$ were affected by explanatory variables *food chain* (levels = wheat or maize), *tissue* (levels = soft, exoskeleton or whole), and *lipid extraction* (levels = lipid present (+L) or lipid extracted (-L)). The random effect *replicate* was incorporated to account for non–independence of paired tissue samples (soft+L, exoskeleton+L, soft-L, exoskeleton-

L,) taken from each replicate. Analyses were conducted using R version 2.14.1 (R Development Core Team, 2011) using 'lme' from the *nlme* package (Pinheiro *et al*. 2010). Model simplification used backwards stepwise regression from a maximal model and ANOVA model comparisons to identify non-significant model terms for elimination. Homogeneity of variances and normality of model residuals were checked in all instances.

Differences among tissue types were calculated within each paired sample. For aphids only, whole tissue replicates were paired at random with soft and exoskeleton treatment replicates from which they were independent. For all pairings n=15. Mean ± SD for each tissue comparison was calculated on these 15 pairings.

Mean ± SD of $\delta^{15}N$ and $\delta^{13}C$ discrimination factors were calculated from differences between species (i.e. hoverfly whole – aphid soft), based on bootstrap resampling using all replicate pairings (i.e. 15 x 15, n=225), for each tissue combination. Hoverfly whole tissue $\delta^{15}N$ and $\delta^{13}C$ values were estimated as proportional distances between soft and exoskeleton for each isotope, based on the mass - balance ratio of dry hoverfly exoskeleton to soft tissue (average = 22% : 78% respectively) for each of the 15 replicates, producing average values of -2.3‰ ± 0.5‰ for $\delta^{15}N$ and -31.0‰ ± 0.2‰ for $\delta^{13}C$. This method was validated as suitable through comparing mass – balance calculated aphid whole tissue values (using aphid exoskeleton : soft tissue; average = 49% : 51% respectively) against known aphid whole values and finding no significant difference. Discrimination factors were calculated using wheat food chain, lipid - extracted aphid and hoverfly data only.

Literature Review

In addition to our empirical study, to assess consistency of any differences in $\delta^{15}N$ or $\delta^{13}C$ signatures between whole, exoskeleton and soft tissues, we also conducted a literature review to collate all estimates of isotopic values within these tissues in arthropods. We identified 11 studies reporting 26 estimates of $\delta^{15}N$ and 18 estimates of $\delta^{13}C$ for combinations of soft, whole and exoskeleton (or chitin extract) tissues, across 22 species of arthropods.

2.4 Results

Lipid Extraction & Tissue Selection

Whilst $\delta^{13}C$ models had a greater number of significant terms than $\delta^{15}N$, patterns for either $\delta^{15}N$ or $\delta^{13}C$ were consistent across species (Table 1).

Lipid Extraction

$\delta^{15}N$

Lipid extraction did not effect $\delta^{15}N$ of either aphid or hoverfly tissues (Table 1).

$\delta^{13}C$

For both species, significant interactions between lipid extraction and tissue type (Table 1) indicated tissue-specific effects on $\delta^{13}C$ were dependent upon the application of lipid extraction. Broadly across both species, $\delta^{13}C$ of tissues was significantly enriched by lipid extraction and these differences were large (range 1.3‰ to 2.9‰, Fig. 1, Table 2).

For aphids, all tissues were significantly enriched in $\delta^{13}C$ after the extraction of lipids. A significant interaction between lipid extraction and tissue-type indicated that the effects of lipid extraction differed in strength between tissue types: soft > whole > exoskeleton. This pattern was consistent across food chains (Fig. 1, Table 2). Similarly for hoverflies, a significant interaction between tissue type and lipid extraction showed extraction effects on $\delta^{13}C$ were dependent on tissue type (Table 1). Fig. 1 and Table 2 show hoverfly soft tissue was strongly enriched in $\delta^{13}C$ as a result of the extraction process (average across food chains = 1.9‰) but that hoverfly exoskeleton was not.

For both aphid and hoverfly, whilst overall patterns of tissue response to lipid extraction were consistent for both food chains, the significant interaction between lipid extraction and food

chain indicated the magnitude of tissue $\delta^{13}C$ enrichment following lipid extraction was greater for tissues on the wheat than maize food chain (average enrichment across tissues: wheat = 2.3‰ ± 0.4‰, maize = 1.7‰ ± 0.3‰; Table 2).

Importantly, differences in $\delta^{13}C$ between tissue types were only detectable after lipid extraction (Fig. 1, Table 2).

Tissue Selection

$\delta^{15}N$

In both the aphid and hoverfly models, tissue type and food chain significantly influenced $\delta^{15}N$ (Table 1). There were no significant interaction terms. Tissue effects on $\delta^{15}N$ differed between aphid and hoverflies. For the aphid model, significant but very small tissue effects showed soft tissue $\delta^{15}N$ was 0.1‰ to 0.5‰ less than either exoskeleton or whole tissue (Fig. 1 and Table 2). Conversely, hoverfly soft tissue was significantly enriched in $\delta^{15}N$ relative to exoskeleton and this difference was large, with a mean difference consistent across food chains of 5.2‰ (Fig. 1 and Table 2).

Literature - reviewed estimates of $\delta^{15}N$ consistently showed that soft > whole > exoskeleton or chitin (Table 3). $\delta^{15}N$ of soft tissue was enriched relative to exoskeleton on average by 5.9‰ ± 0.6‰ (1 study, 3 estimates), whilst estimates of whole tissue were enriched relative to exoskeleton on average by 4.1‰ ± 2.5‰ (5 studies, 13 estimates). These findings concur with our hoverfly observations but contrast our aphid observations.

$\delta^{13}C$

Following lipid extraction, across species and food chains consistent significant differences in $\delta^{13}C$ between tissue types were found (Table 1). Soft tissue was significantly enriched in $\delta^{13}C$ relative to exoskeleton, with the magnitude greater in hoverflies (0.6‰ and 2.2‰ for aphids and hoverflies

respectively, averaged across food chains). Additionally for aphids, the pattern of tissue differences in $\delta^{13}C$ was: soft > whole > exoskeleton (Fig. 1, Table 2).

For reviewed studies, patterns of $\delta^{13}C$ between tissue types were inconsistent (Table 3), contrasting with the consistency we observed across aphids and hoverflies.

Tissue – Specific Trophic Discrimination Factors

In our study, differences among source and consumer tissues propagated notable variation in the derived estimates of discrimination factors (Table 4).

$\delta^{15}N$

For common sampling practice, where whole tissue is used for prey and predator (whole : whole), a discrimination factor of 0.48‰ for $\delta^{15}N$ was obtained, compared with correct factors of 0.97‰ (soft : whole) and 2.18‰ (soft : soft) based on prey soft tissue actually ingested by the predator.

For $\delta^{15}N$, overall range in observed discrimination factors for all source – consumer tissue combinations extended from enrichment to depletion (2.18‰ to -3.69‰). The upper and lower boundaries of these discrimination factors were determined by the differences in hoverfly soft and exoskeleton tissues (Table 4).

$\delta^{13}C$

For $\delta^{13}C$, the differences between discrimination factors were less, with common sampling practice tissues (whole : whole) of -0.14‰, whilst correct factors were -0.55‰ (soft : whole) and -0.04‰ (soft : soft).

For $\delta^{13}C$, overall range in observed discrimination factors for all source – consumer tissue combinations also extended from enrichment to depletion (0.56‰ to -2.32‰). The upper and lower

boundaries of these discrimination factors were also determined by the differences in hoverfly soft and exoskeleton tissues (Table 4).

Table 1. Effects of tissue type, food chain and lipid extraction on aphid and hoverfly $\delta^{15}N$ and $\delta^{13}C$ (‰). 4 separate GLMMs were used. Test statistic is chi-sq χ^2 in all instances, with degrees of freedom in brackets. Significant effects are indicated by ***, with $p < 0.001$ in all instances.

Full Model Terms	$\delta^{15}N$ Aphid	$\delta^{15}N$ Hoverfly	$\delta^{13}C$ Aphid	$\delta^{13}C$ Hoverfly
Tissue	13.62 $_{(2)}$***	388.19 $_{(1)}$***	68.05 $_{(4)}$***	277.06 $_{(2)}$***
Food Chain	100.52 $_{(1)}$***	116.93 $_{(1)}$***	297.21 $_{(2)}$***	244.73 $_{(2)}$***
Lipid Extraction	0.02 $_{(1)}$	2.79 $_{(1)}$	482.82 $_{(4)}$***	245.65 $_{(3)}$***
Tissue*Lipid Extraction	0.64 $_{(2)}$	1.60 $_{(1)}$	58.72 $_{(2)}$***	173.68 $_{(1)}$***
Food Chain*Lipid Extraction	0.14 $_{(1)}$	3.70 $_{(1)}$	62.19 $_{(1)}$***	16.07 $_{(1)}$***
Food Chain*Tissue	0.41 $_{(2)}$	0.01 $_{(1)}$	1.47 $_{(2)}$	2.31 $_{(1)}$
FoodChain*LipidExtraction*Tissue	2.51 $_{(2)}$	0.05 $_{(1)}$	3.19 $_{(2)}$	0.01 $_{(1)}$

Table 2. Mean ± SD (‰) of *difference* between tissue treatments for $\delta^{15}N$ and $\delta^{13}C$, based on paired samples *n*=15. Values are given in reference to CAPITALISED tissue. +L = with lipid, -L = lipid removed.

	Aphid		**Hoverfly**	
$\delta^{15}N$ (‰)	Wheat	Maize	Wheat	Maize
Lipid Extraction				
SOFT (-L) - soft (+L)	-0.16 ± 0.67	0.20 ± 0.80	-0.23 ± 0.57	0.14 ± 0.56
EXOSKELETON (-L) - exoskeleton (+L)	-0.03 ± 0.49	-0.02 ± 0.71	0.04 ± 1.97	-0.11 ± 0.55
WHOLE (-L) - whole (+L)	0.33 ± 0.52	-0.11 ± 0.67		
Tissue Differences (post extraction)				
SOFT - exoskeleton	-0.21 ± 0.39	-0.54 ± 0.69	5.22 ± 0.70	5.19 ± 0.80
SOFT - whole	-0.08 ± 0.74	-0.47 ± 1.33		
WHOLE - exoskeleton	-0.14 ± 0.62	-0.07 ± 1.12		
$\delta^{13}C$ (‰)				
Lipid Extraction				
SOFT (-L) - soft (+L)	2.90 ± 0.36	2.02 ± 0.39	2.07 ± 0.18	1.73 ± 0.33
EXOSKELETON (-L) - exoskeleton (+L)	1.94 ± 0.31	1.35 ± 0.28	0.18 ± 0.22	-0.15 ± 0.54
WHOLE (-L) - whole (+L)	2.42 ± 0.33	1.83 ± 0.47		
Tissue Differences (post extraction)				
SOFT - exoskeleton	0.60 ± 0.24	0.54 ± 0.30	2.28 ± 0.25	2.16 ± 0.40
SOFT - whole	0.40 ± 0.38	0.24 ± 0.44		
WHOLE - exoskeleton	0.20 ± 0.27	0.30 ± 0.33		

Table 3. Survey of literature that explicitly tests $\delta^{15}N$ and $\delta^{13}C$ (‰) values across different soft and exoskeleton tissue components in Arthropods.

Ref	Arthropoda Phylum	Class	Common name	Habitat	Tissue 1	Tissue 2	$\delta^{15}N$ Difference (=Tissue 1-2)	$\delta^{13}C$ Difference (=Tissue 1-2)	Notes
Macko *et al*. 1989	Crustacea	Malacostraca	Lobster	Aquatic	Soft (muscle)	Exoskeleton (carapace)	6.67	0.61	b
Macko *et al*. 1989	Crustacea	Malacostraca	Brown Shrimp	Aquatic	Soft (muscle)	Exoskeleton (carapace)	5.46	0.36	b
Macko *et al*. 1989	Crustacea	Malacostraca	Tiger Shrimp	Aquatic	Soft (muscle)	Exoskeleton (carapace)	5.68	0.79	b
Macko *et al*. 1989	Crustacea	Malacostraca	Lobster	Aquatic	Soft (muscle)	Chitin Extract	7.50	1.23	b
Macko *et al*. 1989	Crustacea	Malacostraca	Brown Shrimp	Aquatic	Soft (muscle)	Chitin Extract	5.64	1.57	b
Macko *et al*. 1989	Crustacea	Malacostraca	Tiger Shrimp	Aquatic	Soft (muscle)	Chitin Extract	7.18	1.53	b
Macko *et al*. 1989	Crustacea	Malacostraca	Mantan Shrimp	Aquatic	Soft (muscle)	Chitin Extract	10.68	1.51	b
Montoya *et al*. 1992	Crustacea	Malacostraca	Amphipod	Aquatic	Whole	Exoskeleton (carapace)	3.80	---	
Montoya *et al*. 1992	Crustacea	Malacostraca	Amphipod	Aquatic	Whole	Exoskeleton (carapace)	4.60	---	
Currin *et al*. 1995	Crustacea	Malacostraca	Crab	Aquatic	Soft (muscle/gills)	Whole	1.0 / 0.5	-0.5 / -1.2	b
Yokoyama *et al*. 2005	Crustacea	Malacostraca	Ghost Shrimp A	Aquatic	Soft (muscle)	Whole	1.20	-1.00	*b
Yokoyama *et al*. 2005	Crustacea	Malacostraca	Ghost Shrimp A	Aquatic	Whole	Exoskeleton	3.30	-3.80	*b
Yokoyama *et al*. 2005	Crustacea	Malacostraca	Ghost Shrimp B	Aquatic	Soft (muscle)	Whole	0.90	-0.70	*b
Yokoyama *et al*. 2005	Crustacea	Malacostraca	Ghost Shrimp B	Aquatic	Whole	Exoskeleton	3.50	-1.60	*b
Perga 2010	Crustacea	Branchiopoda	*Bosmina*	Aquatic	Whole	Exoskeleton	---	-0.80	**c
Perga 2010	Crustacea	Branchiopoda	*Daphnia*	Aquatic	Whole	Exoskeleton	7.90	1.40	**c
Perga 2011	Crustacea	Branchiopoda	*Daphnia*	Aquatic	Whole	Exoskeleton	9.00	---	**
DeNiro & Epstein 1978	Uniramia	Insecta	G'hopper/Beetle/Bug	Terrestrial	*Diet*	Chitin Extract	---	-0.1 to -0.7	b
DeNiro & Epstein 1981	Uniramia	Insecta	Grasshopper	Terrestrial	*Diet*	Chitin Extract	6.60	---	
DeNiro & Epstein 1981	Uniramia	Insecta	Milkweed Bug	Terrestrial	*Diet*	Chitin Extract	8.60	---	
Webb *et al*. 1998	Uniramia	Insecta	Locust (on diet A)	Terrestrial	Soft (muscle)	Chitin Extract	6.80	1.50	a
Webb *et al*. 1998	Uniramia	Insecta	Locust (on diet B)	Terrestrial	Soft (muscle)	Chitin Extract	12.00	0.90	a
Gratton & Forbes 2006	Uniramia	Insecta	Beetle	Terrestrial	Soft (various)	Exoskeleton (various)	---	+0.3 to -0.8	b
Tibbets *et al*. 2008	Uniramia	Insecta	Silkworm Moth	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	1.30	---	
Tibbets *et al*. 2008	Uniramia	Insecta	Wax Moth	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	1.70	---	
Tibbets *et al*. 2008	Uniramia	Insecta	Tobacco Moth	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	-1.00	---	
Tibbets *et al*. 2008	Uniramia	Insecta	Butterfly	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	0.50	---	
Tibbets *et al*. 2008	Uniramia	Insecta	Flesh Fly	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	3.90	---	
Tibbets *et al*. 2008	Uniramia	Insecta	Beetle	Terrestrial	Whole (larvae)	Exoskeleton (exuviae)	-1.20	---	

* Non Acid Washed estimates used; ** Exoskeletons obtained by rinsing with KOH.

To the best of our knowledge, prior to SIA of $\delta^{13}C$; a) lipid extraction stated as having been conducted; b) lipid extraction not stated c) correction factor applied.

Table 4. Mean ± SD (‰) discrimination factors for $\delta^{15}N$ and $\delta^{13}C$ (calculated as hoverfly predator – aphid prey) for all prey : predator tissue combinations. All values based on bootstrap resampling (n=225). Common sampling practice whole tissue (whole : whole) discrimination factor is denoted *. Correct factors based on prey soft tissue actually assimilated by predators in our experiment are marked with **(soft : whole) and ***(soft : soft). Values based on lipid extracted wheat food chain data.

Tissue combination			Discrimination factor	
Aphid tissue	Hoverfly tissue		Mean $\delta^{15}N$	Mean $\delta^{13}C$
Soft	Soft	***	2.18 ± 0.97	-0.04 ± 0.27
Whole	Soft		1.69 ± 0.83	0.36 ± 0.29
Exoskeleton	Soft		1.84 ± 0.76	0.56 ± 0.25
Soft	Whole	**	0.97 ± 0.92	-0.55 ± 0.26
Whole	Whole	*	0.48 ± 0.78	-0.14 ± 0.28
Exoskeleton	Whole		0.63 ± 0.70	0.05 ± 0.24
Soft	Exoskeleton		-3.2 ± 1.06	-2.32 ± 0.36
Whole	Exoskeleton		-3.69 ± 0.94	-1.91 ± 0.37
Exoskeleton	Exoskeleton		-3.55 ± 0.87	-1.72 ± 0.34

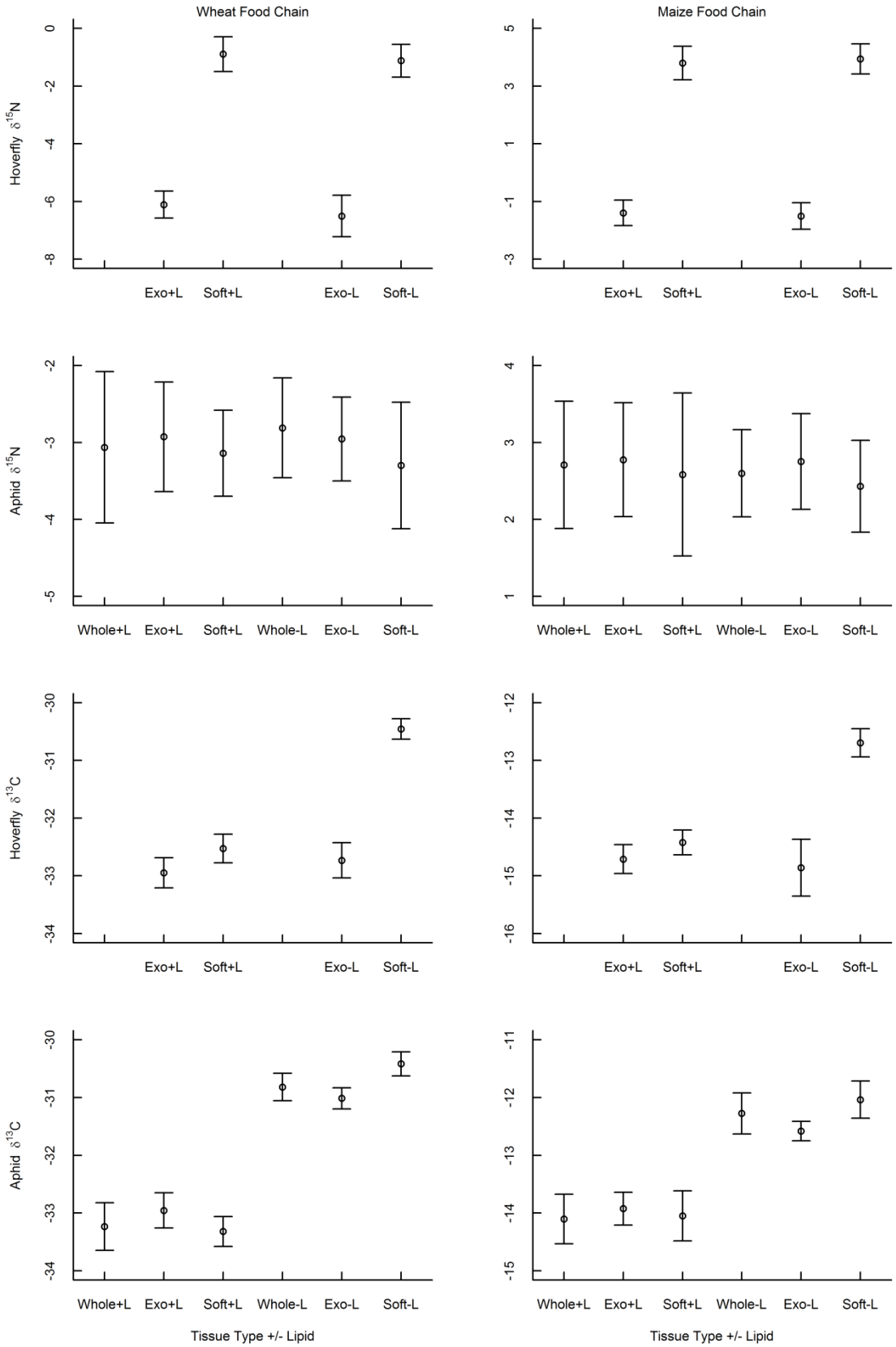

Figure 1. Mean ± SD (‰) of $\delta^{15}N$ and $\delta^{13}C$ of different tissue types and lipid treatments, by species and food chain. +L = with lipid, -L = lipid removed. *n* = 15 for all. Exo = exoskeleton.

2.5 Discussion

We measured for the first time tissue - specific trophic discrimination factors in an arthropod predator - prey system and, perhaps not surprisingly, our results show that estimates of trophic discrimination factors can be markedly affected by tissue selection. In our literature review we additionally identify strong evidence that large tissue differences are frequent across arthropod taxa, suggesting that tissue effects on trophic discrimination factors such as we have demonstrated may also be frequent. Tissue differences as shown in our findings may explain some of the variation around commonly used average trophic discrimination factors taken from the literature (Post 2002; Vanderklift & Ponsard 2003; Caut *et al*. 2009), and we speculate such variation is actually error variation due to inappropriate source tissue selection when consumers feed selectively. Given some of the observed error variation in trophic discrimination factors is likely large enough to affect subsequent ecological conclusions, consideration of source tissue selection to best represent assimilation in consumers is therefore of importance in isotopic ecology more generally. This is particularly the case for arthropods given that many consumers only feed on arthropod soft tissue components (Cohen 1995) though a majority of studies presently use whole tissues (Vander Zanden & Rasmussen 2001; Vanderklift & Ponsard 2003; Caut *et al*. 2009). We acknowledge arthropod tissue sampling may be laborious or difficult, but to improve future ecological conclusions derived from isotopic data we therefore recommend the use of arthropod soft tissues to best represent dietary sources in consumers that do not assimilate exoskeleton.

1. How consistent are differences in $\delta^{15}N$ or $\delta^{13}C$ signatures of whole, exoskeleton and soft tissue?

$\delta^{15}N$

We found marked differences in $\delta^{15}N$ between exoskeleton and soft tissues, which varied between species. Significant enrichment of aphid exoskeleton (and whole tissue) relative to soft tissue was very small and variable between food chains (mean $\delta^{15}N$ = 0.3‰), while conversely, significant depletion of hoverfly exoskeleton relative to soft tissue was large and consistent between food

chains (mean $\delta^{15}N$ = 5.2‰). Such contrast in the direction, variability and magnitude of this exoskeleton - soft tissue $\delta^{15}N$ relationship suggests species – specific tissue compositions. Though significant, small enrichment of exoskeleton over soft tissues for aphids in our study is of doubtful ecological importance, though more generally this lack of depletion in exoskeleton relative to soft tissue is uncommon in the literature (Tibbets *et al*. 2008; and see Table 3) and the mechanisms are poorly defined. Conversely, depletion of $\delta^{15}N$ in hoverfly exoskeleton (exuviae) concurs with limited available results showing depletion of 0.5‰ to 3.9‰ in insect exuviae relative to whole larvae tissue, as a consequence of high chitin content in the exoskeleton (Tibbets *et al*. 2008). Notably, the magnitude of depletion shown for hoverfly exoskeleton relative to soft tissues also concurs closely with that shown for aquatic crustaceans (Macko *et al*. 1989; Yokoyama *et al*. 2005) and is less depleted than some others ≈ 8‰ to 9‰ (Perga 2010, 2011; Table 3). Therefore whilst insect exuviae have rarely been utilised in isotopic studies elsewhere, given tissue differences identified by our review in Table 3, exuviae is hence not unrepresentative of arthropod exoskeleton more generally. Our results and those identified by our review show large differences exist in $\delta^{15}N$ between component arthropod tissues known to be differentially assimilated or avoided by consumers. We therefore recommend researchers use only source tissues assimilated by consumers, and when consumer feeding habits are unknown, urge caution on the inclusion of exoskeleton in arthropod prey.

$\delta^{13}C$

Across species we found significant $\delta^{13}C$ depletion in exoskeleton relative to soft tissue, with the magnitude of tissue differences being greater in hoverflies than aphids (mean = 2.2‰ and 0.6‰, respectively). Our results fall within a broader and less consistent arthropod literature (reviewed in Table 3). Such divergent literature results likely represent some species-level tissue differences in $\delta^{13}C$, though it is noteworthy that only Webb *et al*. (1998) records utilising lipid extraction on

samples prior to stable isotope analysis. In our study, enrichment of soft tissue $\delta^{13}C$ relative to exoskeleton was only apparent after extraction of lipids.

2. How important are tissue - specific differences in $\delta^{15}N$ or $\delta^{13}C$ in explaining differential estimations of discrimination factors for $\delta^{15}N$ or $\delta^{13}C$?

$\delta^{15}N$

We found marked variation in $\delta^{15}N$ estimates of discrimination factors across different consumer - prey tissue combinations. Such variation was more influenced by changing hoverfly tissues than aphid. Observed variation in $\delta^{15}N$ discrimination factors between common sampling practice whole tissues of 0.48‰ contrasted with correct factors based on soft tissues of 0.97‰ (soft : whole) and 2.18‰ (soft : soft). Importantly, this result demonstrates that the error inherent in using whole tissue $\delta^{15}N$ estimates, when consumers do not assimilate exoskeleton, is of notable magnitude. This strongly suggests tissue selection requires consideration in order to avoid propagating such error when utilising discrimination factors to quantify food chain length, trophic positions of consumers, or source contributions to consumer diets using mixing models, for instance. By providing empirical estimates of tissue effects on discrimination factors in this study, our findings develop the literature reviewed in Table 3 which show large $\delta^{15}N$ differences between soft and exoskeleton tissue components, but crucially do not directly compare these with whole tissues. Such a comparison between soft, exoskeleton and whole tissue as in our study, is necessary to understand if whole tissue can comprise enough exoskeleton material by mass, that exoskeleton differences from soft tissue can notably affect whole tissue $\delta^{15}N$. Tissue – specific variation in discrimination factors as shown in this study demonstrates that this can be the case.

Widely cited review studies of invertebrate $\delta^{15}N$ discrimination factors (collectively > 1400 citations on Google Scholar) are composed of > 90% arthropods, of which >95% utilise whole tissue (Vander Zanden & Rasmussen 2001; Vanderklift & Ponsard 2003; Caut *et al*. 2009). We therefore

speculate that reviewed $\delta^{15}N$ whole tissue values comprise exoskeleton tissues that, if used as discrimination factors to parameterise sources in isotopic models in subsequent studies, will constitute error when consumers do not assimilate exoskeleton. In instances where such error is significant, this will propagate and affect subsequent trophic estimates and ecological conclusions.

$\delta^{13}C$

Effects of tissue type on $\delta^{13}C$ discrimination factors were less than those of $\delta^{15}N$, with a common sampling practice whole tissue discrimination factor of -0.14‰, compared with correct soft tissue discrimination factors of -0.55‰ and -0.04‰. More generally, smaller variation in estimates of $\delta^{13}C$ than $\delta^{15}N$ discrimination factors is a consequence of lesser fractionation in $\delta^{13}C$ than $\delta^{15}N$ from prey to predator, as well established in the literature (DeNiro & Epstein 1978; 1981). This causes smaller differences in $\delta^{13}C$ than $\delta^{15}N$ both within and between aphid and hoverfly tissues and hence smaller differences in $\delta^{13}C$ than $\delta^{15}N$ discrimination factors. Smaller observed differences in $\delta^{13}C$ discrimination factors may seem of lesser consequence, but such differences must be considered relatively; in trophic systems where $\delta^{13}C$ ranges are narrow (i.e. many food chains), small tissue differences in $\delta^{13}C$ may still be important in affecting overall conclusions, for instance in discerning between sources in food chains or mixing models (Post *et al*. 2007; Tarroux *et al*. 2010). Thus we would urge caution in the use of source tissues that are known to not be consumed and advise that researchers consider their context of use.

3. How does lipid extraction differentially alter $\delta^{15}N$ or $\delta^{13}C$ signatures of whole, exoskeleton or soft tissue?

Extraction of lipids did not significantly detrimentally affect $\delta^{15}N$ of aphid or hoverfly tissues. In contrast, significant enrichments in $\delta^{13}C$ were recorded after extraction for soft, whole and exoskeleton tissues of aphids and soft tissue of hoverflies (means range 1.7% to 2.5‰). The

direction and magnitude of our results concur with other limited empirical evidence from arthropods (Bodin *et al*. 2007; Logan *et al*. 2008). The exception in our study was hoverfly exoskeleton, which showed no $\delta^{13}C$ enrichment, in contrast to aphid exoskeleton. This apparent discrepancy is likely explained by differing exoskeleton composition; for example, insect cuticles vary in their configurations of proteins (notably sclerotin) and lipid as a function of cuticle rigidity and waterproofing (Wigglesworth 1970, 1985). Many studies likely assume insect exoskeleton to be composed largely of chitin without significant lipid, and thus without a need to be subjected to lipid treatment. This is clearly not the case with aphid exoskeleton, and thus important species – specific differences in exoskeleton structure may necessitate treatment to remove significant lipid components. Notably, a significant interaction between food chain and lipid extraction showed extraction effects to be greater for aphid and hoverfly tissues on the wheat than maize food chain. We speculate such an effect is a consequence of differences in nutritional content between primary producers being propagated to consumers, as shown elsewhere (Wilson *et al*. 2011), with proportionally more lipid derived by aphids feeding on wheat plants than maize. This result infers lipid extraction effects were mediated by diet. Importantly, more generally the magnitude of effects of lipid extraction on $\delta^{13}C$ observed in our results fall within the range (>2‰) of those shown to have potentially significant consequences for deriving subsequently spurious ecological conclusions (Tarroux *et al*. 2010) suggesting that lipid in insect samples must be accounted for.

In conclusion, our results show that significant differences exist between component tissues of arthropods that are known to be selectively assimilated by consumers, and that such differences propagate notable error variation amongst discrimination factors. This will affect subsequent trophic measures and potentially ecological conclusions. Currently, tissue selection based error is not accounted for in a majority of isotopic studies that use arthropods. Implications of this study for practitioners of isotopic studies are best interpreted in the context that researchers intend to use them; in poorly defined systems where sources are isotopically similar or have larger variance, our

results indicate tissue selection within sources is important to avoid significant error, whether estimating trophic positions or dietary estimates using mixing models. When researchers are without prior knowledge, we recommend only using arthropod soft tissue components and excluding exoskeleton material. We also conclude that lipid extraction is necessary to derive accurate $\delta^{13}C$ values based on proteins for arthropod tissues. We additionally call for further research to test tissue selection effects upon derived isotopic measures and ultimate ecological conclusions, and given the laborious and difficult nature of arthropod dissection, suggest such research will be instrumental in testing potential mathematical or mass – balance corrections as a potential alternative to dissection when necessary.

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CHAPTER 3

Application of nitrogen and carbon stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) to quantify food chain length and trophic structure

3.1 Abstract

Increasingly, stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are utilised to quantify trophic structure, but few studies have tested accuracy of isotopic structural measures against known structural measures, or examined how different sources of variation in $\delta^{15}N$ and $\delta^{13}C$ affect measures of structure. Utilising species of known trophic level, we examined change and variation in $\delta^{15}N$ and $\delta^{13}C$ across 4 trophic level plant-invertebrate food chains, for both laboratory-raised and wild organisms. To test the utility of $\delta^{15}N$ and $\delta^{13}C$ to quantify structure, we subsequently derived measures of nitrogen range (NR) and carbon range (CR) which are used to quantify food chain length and breadth of trophic resources respectively, for differing food chain lengths. Our estimates of NR and CR are robust because they were calculated using Bayesian resampling procedures which propagate variance in sample means through to quantified uncertainty in final estimates.

We found $\delta^{15}N$ discrimination from sources to invertebrate consumers showed significant enrichment that was lower than literature reports for vertebrates, and that variation in enrichment was stochastic, ranged broadly (1.9‰) and importantly, propagated variation to subsequent estimates of NR. However, across differing species combinations and food chains we show NR proved robust to such variation and distinguished food chain length well, though some overlap between longer food chain lengths importantly infers a need for awareness of such limitations. $\delta^{13}C$ discrimination between source and consumer was inconsistent; generally no change or small significant enrichment was observed. Consequently, estimates of CR changed little with increasing food chain length, showing the potential utility of $\delta^{13}C$ as a tracer of energy pathways.

We therefore recommend using resampling procedures to propagate variation in source signatures and consumer discrimination into quantified uncertainty in structural measures. This study serves as a robust test of isotopic quantification of food chain structure, and a first test of terrestrial systems. Given most food chains include invertebrates, and that global estimates of aquatic food chains approximate 4 trophic levels, our use of 4 trophic level plant-invertebrate food chains makes our findings relevant for a majority of ecological systems and contexts.

3.2 Introduction

Understanding food web structure is of critical importance to a broad suite of ecological theory given that trophic dynamics between individuals, populations, species and functional guilds underpin the ecological functioning and evolution of biological communities (Doi et al. 2012, Price et al. 2012). Quantifying food web structure (trophic structure hereafter) is therefore a prerequisite to better understand how it in turn interacts with emergent properties of organisms and the environment, such as energy flux (Thompson and Townsend 2005), population dynamics (Srivastava and Bell 2009), patterns of biodiversity (Rooney and McCann 2012) and ecosystem functioning (Cardinale 2011, Thompson et al. 2012). Determination of feeding relationships between species is integral to quantifying trophic structure, and traditional methods include gut-content analysis, faecal analysis and behavioural observations. However, these methods can be laborious and may not reflect variation in digestibility and assimilation of source items, and if limited in their collection in space and time, may lead to over or under representation of source contributions (Bearhop et al. 2004). Increasingly, stable isotope ratios of nitrogen (N^{15} : N^{14}, termed $\delta^{15}N$) and carbon (C^{13} : C^{12}, termed $\delta^{13}C$) in consumer tissues are utilised to provide a temporally and spatially integrated construct of dietary niche (Bearhop et al. 2004), with $\delta^{15}N$ and $\delta^{13}C$ of consumer proteins reflecting the proteins of their food sources (DeNiro and Epstein 1978, 1981). Typically, enrichment in $\delta^{15}N$ of 2.5‰ to 3.4‰ is observed from diet to consumer (Post 2002, Vanderklift and Ponsard 2003, Caut et al. 2009), allowing determination of an organism's trophic level (Vander Zanden et al. 1997, Vander Zanden and Rasmussen 1999, Post 2002) and overall food chain length (Cabana and Rasmussen 1996, Vander Zanden and Fetzer 2007). Conversely, enrichment in $\delta^{13}C$ is much smaller between diet and consumer (Post 2002, Caut et al. 2009), and because basal sources often differ in their $\delta^{13}C$ values, $\delta^{13}C$ can be utilised to trace prey – consumer connections or food chains (Post 2002). Hence change in $\delta^{15}N$ and $\delta^{13}C$ from source to consumer as described (termed trophic discrimination factors and represented as $\Delta\delta^{15}N$ or $\Delta\delta^{13}C$), is the mechanism that crucially underpins the positioning of individuals, populations and species relative to one another in bivariate isotopic space (typically with

$\delta^{15}N$ on a y-axis and $\delta^{13}C$ on an x–axis). Importantly this subsequently allows for measures of Euclidean distances across the isotopic space occupied by populations, species or communities in order to quantify aspects of trophic structure (Layman et al. 2007, Schmidt et al. 2007, Jackson et al. 2011, Layman et al. 2011). For instance, food chain length is calculated as λ + (nitrogen range / average $\Delta\delta^{15}N$), where nitrogen range (NR) is mean difference between trophic levels of maximum and minimum $\delta^{15}N$, and λ is minimum trophic position (Vander Zanden et al. 1997, Post 2002, Layman et al. 2007). Similarly, carbon range (CR) measures breadth of trophic sources and is calculated using mean difference between trophic levels of maximum and minimum $\delta^{13}C$ (Layman et al. 2007). Observational studies have largely used such measures to quantify food chain length, typically in aquatic systems, in response to factors such as ecosystem size, disturbance and productivity (e.g. Post et al. 2000, Takimoto et al. 2008, McHugh et al. 2010). Thus the use of stable isotope ratios in an organism's tissues to provide temporally and spatially integrated dietary data is proving a very valuable methodology for trophic research.

Critically though, variation in source $\delta^{15}N$ and $\delta^{13}C$ in addition to variation in consumer $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ generates uncertainty / error in subsequent estimates of trophic structure and relationships (Vander Zanden and Rasmussen 2001, Post 2002, Matthews and Mazumder 2004, Vander Zanden and Fetzer 2007, Martinez del Rio et al. 2009). Such variation in discrimination factors ($\delta^{15}N$ -0.8‰ to 5.9‰ and $\delta^{13}C$ -2.7% to 3.4‰ excluding fluid feeders) (McCutchan et al. 2003) is well documented in the literature. This can be a consequence of multiple factors, including dietary protein quality, metabolic process and efficiency of protein assimilation and loss, fasting, growth rate, age, size, tissue type, sample size and sampling process, although there is considerable debate on which are most important (Vanderklift and Ponsard 2003, Caut et al. 2009, Martinez del Rio et al. 2009 and references therein for all). Common practice utilises mean estimates of $\delta^{15}N$, $\delta^{13}C$, $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ ignoring variability around estimates; consequently, derived estimates of trophic structure and subsequent ecological conclusions may lack accuracy.

These issues associated with variance have driven recent innovations in the analysis of

isotopic data that provide practitioners with tools to apply Bayesian inference to the calculation of trophic structure metrics (Jackson et al. 2011). These Bayesian approaches are ideally suited to testing effects of variance as they provide population and community trophic metric estimates based on resampling of variance in mean $\delta^{15}N$ and $\delta^{13}C$ estimates, effectively quantifying and propagating variation in raw data as uncertainty in subsequent metric outputs, allowing for critical examination of precision in estimates of trophic structure.

Whilst use of stable isotopes to quantify trophic structure increases, to date very few studies have examined how isotopic variance affects subsequent trophic measures, despite repeated calls for research (Martinez del Rio et al. 2009, Layman et al. 2011). Of these studies, Vander Zanden and Fetzer (2007) showed effects of variation in $\delta^{15}N$ on food chain length estimates whilst Vander Zanden and Rasmussen (2001) and Post (2002) showed variation in $\Delta\delta^{15}N$ to affect trophic level estimates, all in aquatic systems. To our knowledge, no comparable terrestrial study exists (Martinez del Rio et al. 2009). Therefore generally there exists a need to validate dynamics of $\delta^{15}N$ and $\delta^{13}C$ over multiple trophic levels and specifically, test how propagation of variation in $\delta^{15}N$, $\Delta\delta^{15}N$, $\delta^{13}C$ and $\Delta\delta^{13}C$ to final estimates of trophic structure affects their accuracy. Furthermore, variation in $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ as may affect structural measures such as NR and CR is of importance given these univariate measures underpin other bivariate ($\delta^{15}N$ with $\delta^{13}C$) measures of trophic structure in isotopic space. NR is also the most utilised isotopic metric in observational studies (Layman et al. 2011), and its function as a tool to quantify trophic level, food chain length or as a component of bivariate measures is dependent upon an assumed constant $\delta^{15}N$ enrichment with each consumer level. Variance around this assumed average enrichment constitutes unknown error in estimates of NR in observational studies. Thus, experimental validation would improve understanding of the importance of variance in $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ for affecting quantifications of trophic structure in wild systems (Layman et al. 2011), strengthening subsequently derived ecological conclusions, in addition to further catalysing the development and use of these techniques by a wider audience of ecologists.

In this study, we utilise natural plant and insect food chains raised under controlled

conditions to examine the dynamics of $\delta^{15}N$ and $\delta^{13}C$ over 4 trophic levels, and test the accuracy of the isotopic metrics NR and CR to quantify trophic structure utilising Bayesian resampling procedures. By utilising 4 trophic levels, we broaden cross – system applicability of our results to a larger repository of aquatic studies, given that global aquatic food chain lengths have been estimated at 3.5 to 4 trophic levels (Vander Zanden and Fetzer 2007). Specifically, we test 3 questions: 1) How consistent are changes in $\delta^{15}N$ and $\delta^{13}C$ across trophic levels? 2) Does NR accurately determine food chain length? 3) How does CR change with food chain length?

3.3 Methods

To test dynamics of $\delta^{15}N$ and $\delta^{13}C$ with changing trophic level, 3 replicate food chains were raised in the laboratory, with a further analogous food chain collected from the wild to allow comparison with wild systems. All food chains had 4 trophic levels consisting of: primary producer (plant) → herbivore (aphids feeding on plant phloem sap) → predator (hoverfly larvae feeding on aphids) → secondary predator (parasitoid Hymenoptera which are obligate endoparasites of hoverfly pupa, with a single parasitoid emerging from a single pupa).

For laboratory food chains, grain aphids (*Sitobion avenae*) were raised on two independent food plants; One based on a C3 photosynthetic pathway (wheat *Triticum aestivum*) and the other based on a C4 pathway (maize *Zea mays*), enabling separation of the plants on a $\delta^{13}C$ axis and thus broadening the generality of any observed patterns. Plants were raised on a common source of homogenised compost and distilled water, and introduced to aphids at 20 days (wheat) or 30 days (maize). 1st generation larvae of wild-caught hoverfly (*Syrphus vitripennis*) were fed either wheat or maize raised aphids or an approximate 50:50 ratio of both. Within each treatment, 24-48 hours after hatching, a random subset of hoverfly larvae were exposed to wild caught adult female parasitoids (*Diplazon laetorius*) to allow parasitic oviposition. All plants and insects were raised under a 16:8 light: dark cycle at 70% humidity. Plant leaves and aphids of all ages were collected at random and frozen (-20°C) prior to tissue preparation. Hoverfly larvae entered pupation 8 – 10 days after

hatching and after 72 hours pupation were frozen (-20°C) for later dissection. Prior experimentation identified 72 hour pupation as suitable to provide soft pupa tissue comparable to that likely consumed by parasitoid larvae. Parasitised hoverfly larvae were allowed to complete pupae development (19-21 days) and newly eclosed adult parasitoids were frozen (-20°C) within 12 hours without having fed.

For the wild food chain, nettle (*Urtica dioica*) leaves and nettle aphids (*Microlophium carnosum*) were collected independently and frozen (-20°C) for later preparation. Hoverfly larvae (*Syrphus vitripennis*) were collected when judged at >50% grown and laboratory raised on daily-collected wild nettle aphids until pupation, under a 16:8 light: dark cycle at 70% humidity. Pupation proceeded until either adult hoverflies or adult parasitoids (*Diplazon laetorius*) eclosed after 10-11 or 16-20 days respectively, and were frozen (-20°C) within 12 hours without having fed. Our 4 replicate food chains are hereafter termed after their plants as wheat, maize, wheat + maize (w+m) and nettle. Parasitoid hymenoptera are referred to as wasp hereafter.

Tissue Preparation & Lipid Extraction

We used tissues for sources to best represent assimilation in consumers, as shown to be important (Tibbets et al. 2008). Whilst aphids fed on plant sap, we utilised whole leaf tissue given difficulties of extracting sap and because whole leaf tissue $\delta^{15}N$ has been shown not to differ from sap (Wilson et al. 2011). For each food chain, following dissection, soft internal tissues of 60-80 aphids were pooled to produce a single sample. Hoverfly larvae soft tissue was obtained from pupae casing. Wasps represented end consumers and we utilised whole tissues. For wild hoverflies and wasps we utilised adult whole tissues. Individual hoverflies and wasps each provided single replicates. Sample sizes for plants, aphids and hoverflies were n = 15 (except nettle hoverflies, n = 7) whilst wasps were more difficult to obtain: wheat (n = 10), w+m (n = 7), nettle (n=6). For maize, no wasps were obtained due to high larval mortality. All samples were dried at 45°C for >48hrs and homogenised. Subsequently, insect samples were immersed in 2:1 chloroform : methanol solution for 50 minutes to remove free

lipid, and then left to air dry.

Stable Isotope Analysis

For all samples, 0.5mg ± 0.05 (insect) or 3mg ± 0.1 (plant) dried material was enclosed in tin capsules. Stable isotope analysis (SIA) was conducted at the Food and Environment Research Agency, York, UK. Samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ in a Fisons EA1108 elemental analyser (Carlo Erba Instruments, Milan, Italy), coupled with an Isoprime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Stable isotope ratios are reported in delta (δ) notation where $\delta^{15}N$ and $\delta^{13}C = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Isotope ratios are expressed in per mil (‰) relative to the ratio of international reference standards ($R_{standard}$) which are Atmospheric Nitrogen and Vienna PeeDee Belemnite (VPDB) for nitrogen and carbon respectively. Measures of standards placed throughout samples exhibited acceptable instrument reproducibility of < 0.09‰ (SD) for $\delta^{15}N$ and < 0.18‰ (SD) for $\delta^{13}C$ using collagen standard, insect whole tissue standard (cockroach; *Nauphoeta cinerea*), and sucrose C4 plant standard.

Data Analysis

Initial analyses utilised two-way analysis of variance (ANOVA) to test effects of the two explanatory variables *trophic level* (levels = plant, aphid, hoverfly or wasp) and *food chain type* (levels = wheat, maize, w+m or nettle) on $\delta^{15}N$ and then $\delta^{13}C$. To determine where significant differences lay between levels within treatments, subsequent one-way ANOVA for each food chain type were tested with Tukey post hoc tests, for both $\delta^{15}N$ and $\delta^{13}C$.

Within each food chain type, we then calculated mean (± SD) $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ as the difference in $\delta^{15}N$ or $\delta^{13}C$ between each source and its consumer by randomly pairing replicates (n = 6 to 15). To establish underlying sources of variation in $\Delta\delta^{15}N$, two-way ANOVA tested effects of explanatory variables *trophic link* (levels = plant-aphid, aphid-hoverfly, or hoverfly-wasp) and *food*

chain type (levels = wheat and nettle). Only nettle and wheat food chain data were used as these contained all 3 trophic links, allowing for a balanced analysis. For explanatory variables, variation in $\Delta\delta^{15}N$ was quantified using sums of squares in model outputs and was expressed as a proportion of the null model variance. All analyses were conducted in R version 2.14.1 (R Development Core Team, 2011).

Nitrogen range (NR) and carbon range (CR), calculated as the mean difference between trophic levels of maximum and minimum $\delta^{15}N$ or $\delta^{13}C$ respectively, are quantifications of trophic structure with NR representing food chain length and CR the breadth of energy sources. For all species combinations of each food chain length within each food chain type separately, NR and CR were independently calculated using Bayesian approaches to resampling of uncertainty around sample mean estimates, to provide probabilistic distributions representing 50%, 75% and 95% credible intervals of mean estimates for NR and CR, utilising the SIBER computational code (Jackson et al. 2011) in the R package SIAR (Parnell et al. 2008, 2010). Additionally for NR, for each food chain length, Bayesian resampled mean estimates across food chain types and species combinations were pooled to produce overall 95% credible intervals.

As established by preliminary experiments, we applied correction factors of – 0.7‰ for $\delta^{15}N$ and +0.4‰ for $\delta^{13}C$ to hoverflies on the wild nettle food chain (which used adult tissues) to make them directly comparable to larval hoverflies on laboratory food chains.

3.4 Results

How consistent are changes in $\delta^{15}N$ and $\delta^{13}C$ across trophic levels?

$\delta^{15}N$

Though given the same nitrogen source, $\delta^{15}N$ of wheat plants were depleted relative to maize plants by ≈2‰, whilst wild nettle plants (of independent nitrogen source) were slightly enriched (0.3‰) relative to maize. Such differences between food chain types were largely propagated to higher

trophic levels (Fig. 1a) and found to be significant; two-way ANOVA showed main effects of trophic level and food chain type significantly affected $\delta^{15}N$ ($F_{(3, 158)} = 50.54$, $p < 0.001$; and $F_{(3, 158)} = 120.64$, $p < 0.001$, respectively), with a significant interaction between these variables indicating these effects were interdependent ($F_{(6, 152)} = 11.57$, $p < 0.001$). To determine if large variances in $\delta^{15}N$ of maize and wheat plants (Fig. 1a) disproportionately affected these results, we repeated this analysis excluding these plants, but found no difference in outcome.

To ascertain patterns of $\delta^{15}N$ discrimination, Tukey post hoc tests performed on one–way ANOVA for each food chain type established where $\delta^{15}N$ differed between trophic levels. Overall, $\delta^{15}N$ discrimination factors showed significant enrichment from source to consumer (range of 1.4‰ to 3.3‰), in all but 3 instances (Table 1). Exceptional to this trend, wheat feeding aphids showed significant average depletion in $\delta^{15}N$ (-2.4‰) relative to hosts.

Given $\delta^{15}N$ enrichment was broadly consistent and larger than that observed in $\delta^{13}C$ (Table 1), for $\delta^{15}N$ we also utilised two-way ANOVA to determine sources of variation in $\Delta\delta^{15}N$ (Table 2). After disproportionate plant variation was excluded, we observed a significant interaction between trophic link and food chain type accounting for 31% of variation in $\Delta\delta^{15}N$. However, as neither variable was significant as a main effect, this significant interaction infers variation as caused by these variables was stochastic in nature.

$\delta^{13}C$

$\delta^{13}C$ values were ≈17‰ different between wheat and maize food chains, with hoverfly and wasps on the w+m food chain approximately half way between the two having integrated aphid sources from both (Fig. 1b). Two-way ANOVA showed $\delta^{13}C$ was significantly affected by an interaction between trophic level and food chain type ($F_{(6, 152)} = 19.22$, $p < 0.001$), indicating food chain effects on $\delta^{13}C$ were affected inconsistently by trophic level (Fig. 1b), with main effects of trophic level and food chain type also being significant ($F_{(3, 158)} = 57.74$, $p < 0.001$ and $F_{(3, 158)} = 6016.40$, $p < 0.001$, respectively).

Differences in $\delta^{13}C$ discrimination across trophic links and food chain types were of variable direction and magnitude (-0.7‰ to 1.9‰, excluding w+m aphid-hoverfly; Table 1). Across trophic links and food chain types, Tukey post hoc analyses showed either significant enrichment (0.6‰ to 1.9‰) or no change in $\delta^{13}C$ between trophic levels (Table 1).

Does nitrogen range accurately determine food chain length?

In most instances, estimates of nitrogen range (NR) were observed to increase with greater food chain length within each food chain (Fig. 2). Exceptional to this were low NR estimates on the wheat and w+m food chains for combinations including wheat plants.

Within a food chain, in some instances modal or credible interval values of NR varied distinctly between different species combinations of the same food chain length, but such differences were not constant across food chains suggesting a stochastic nature to such variation. Across food chains, 95% credible interval estimates of mean NR were not generally larger than 1‰ to 2‰ $\delta^{15}N$ (Fig. 2). The exception was larger estimates on the wheat food chain when wheat plants were included as a consequence of uncertainty in mean estimates (Fig. 2), propagated from large sample variation in wheat plants (Fig. 1).

Overall estimates of NR based on combining Bayesian resampled mean estimates from all 4 replicate food chains and all species combinations (for each level of food chain length), showed modal NR values to increase by between 1.2‰ and 2.7‰ with each additional trophic level (Fig. 3). Overlap in 95% credible intervals between different food chain lengths (Fig. 3a) was reduced when wheat plant combinations were excluded (Fig. 3b), and then further reduced when maize plant combinations were also excluded (Fig. 3c). Notably, there was no subsequent change in 95% credible intervals when nettle plants were additionally removed (Fig. 3d), suggesting that these wild plants did not contribute noticeably to variation in estimates of NR.

How does carbon range change with food chain length?

For estimates of carbon range (CR), we observed few consistent patterns in CR across differing food chain lengths within or among food chains (Fig. 4). Wheat and maize food chains both showed no pattern of change in CR with changing food chain length; wheat CR was <1‰ across 2, 3 and 4 trophic levels, whilst maize CR was 2‰ for combinations of both 2 and 3 trophic levels. For the w+m food chain there was marginal increase in modal CR with food chain length (2 to 4 trophic levels ≈ 0.3‰ to 2.3‰) but this was considerably less than variation in mean estimates as shown by large 95% credible intervals (>3‰), on account of two isotopically disparate $\delta^{13}C$ plant sources. Conversely, the wild nettle food chain trended towards greater modal CR with food chain length (2 to 4 trophic levels ≈ 1‰ to 3.5‰); however, overlap in 95% credible intervals between different food chain lengths was observed, whilst modal values of different species combinations of the same food chain length also differed by ≈ 1‰ to 1.5‰.

Table 1. Mean ± SD (‰) $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ across 4 trophic levels of 4 terrestrial food chains. Significant differences in $\delta^{15}N$ and $\delta^{13}C$ between source and consumer within each food chain indicated as p= <0.05*, <0.01**, <0.001***. w+m hoverfly has two aphid sources and values are given for both: (w) = wheat aphids, (m) = maize aphids, (c) = combined.

Source	**→**	**Consumer**	**Food Chain**			
$\delta^{15}N$			wheat	maize	w+m	nettle (wild)
Plant	→	Aphid	-2.40 ± 2.63***	1.12 ± 2.58		1.40 ± 0.75***
Aphid	→	Hoverfly	2.18 ± 1.25**	1.51 ± 0.69*	4.81 ± 0.87***(w) -0.92 ± 1.02***(m) 1.95 ± 3.06* (c)	3.08 ± 0.60***
Hoverfly	→	Wasp	3.31 ± 1.00***		2.49 ± 0.62***	1.38 ± 0.61**
$\delta^{13}C$						
Plant	→	Aphid	0.65 ± 0.92**	1.94 ± 0.36***		1.88 ± 0.86***
Aphid	→	Hoverfly	-0.04 ± 0.33	-0.69 ± 0.42***	9.33 ± 0.87***(w) -9.05 ± 0.79***(m) 0.14 ± 9.38 (c)	1.22 ± 0.71***
Hoverfly	→	Wasp	0.20 ± 0.30		0.39 ± 0.65	0.40 ± 0.31

Table 2. Sources of variance in $\Delta\delta^{15}N$ (‰). Variance in $\Delta\delta^{15}N$ as accounted for by either food chain or tropic link was established by expressing ANOVA model terms as a proportion of the null model variance. $\Delta\delta^{15}N$ is based upon differences in raw $\delta^{15}N$ between trophic levels, for all source-consumer links on wheat and nettle food chains only (to provide a balanced analysis). ANOVA was conducted twice: firstly for all source-consumer links and secondly excluding all links including a primary producer. Main effects of food chain and trophic link did not significantly explain variance in $\Delta\delta^{15}N$ after primary producer links were excluded. Significant model terms indicated as p= <0.05*, <0.01**, <0.001***.

	All combinations		Excludes primary producers	
Null model variance in $\Delta\delta^{15}N$	398.1		52.8	
Model Term	Proportion of null variance explained		Proportion of null variance explained	
Food Chain	0.04	**	0.02	
Trophic Link	0.42	***	< 0.01	
Food Chain * Trophic Link	0.21	***	0.32	***
Full model (all terms combined)	0.67		0.34	

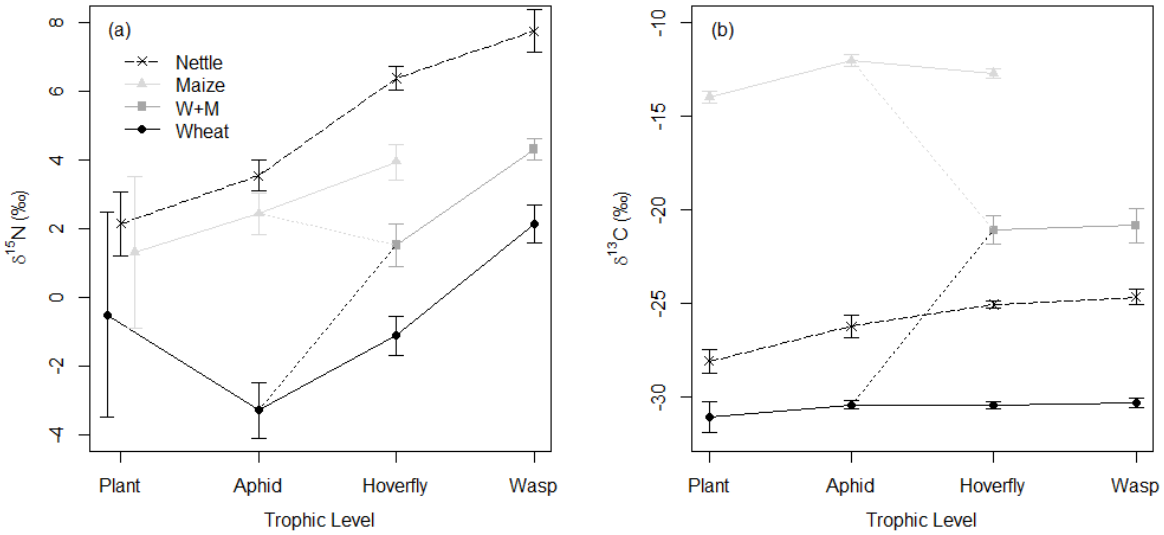

Fig. 1. (a) $\delta^{15}N$ and (b) $\delta^{13}C$ across 4 trophic levels of 4 replicate terrestrial food chains. Mean ± SD (‰) are shown. *n* = 6 to 15. For plant $\delta^{15}N$, mean ± SD are offset on x-axis for clarity. Dotted lines are trophic links between 2 aphid prey sources and their hoverfly predator.

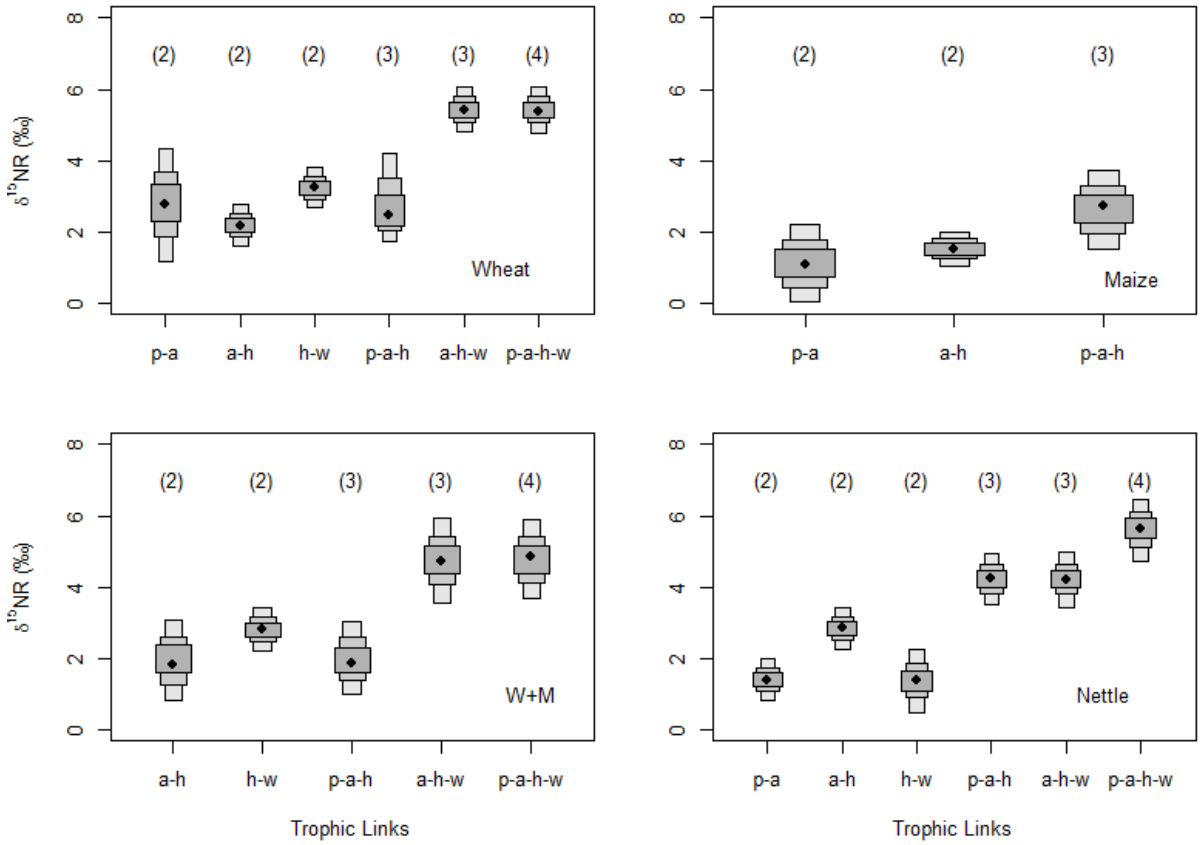

Fig. 2. Probability distributions of mean nitrogen range (NR) for different food chain lengths for each food chain. NR is difference between mean $\delta^{15}N$ (‰) of community end members, based on Bayesian resampling (*n* = 10,000) of uncertainty in sample mean estimates. Black dots represent mode (of means), while shaded boxes (dark to light) show 50%, 75% and 95% credible intervals for mean estimates. No wasps were obtained for the maize food chain. x-axis labels are species identity: p=plant, a=aphid, h=hoverfly, w=wasp. Parenthesis number shows food chain length.

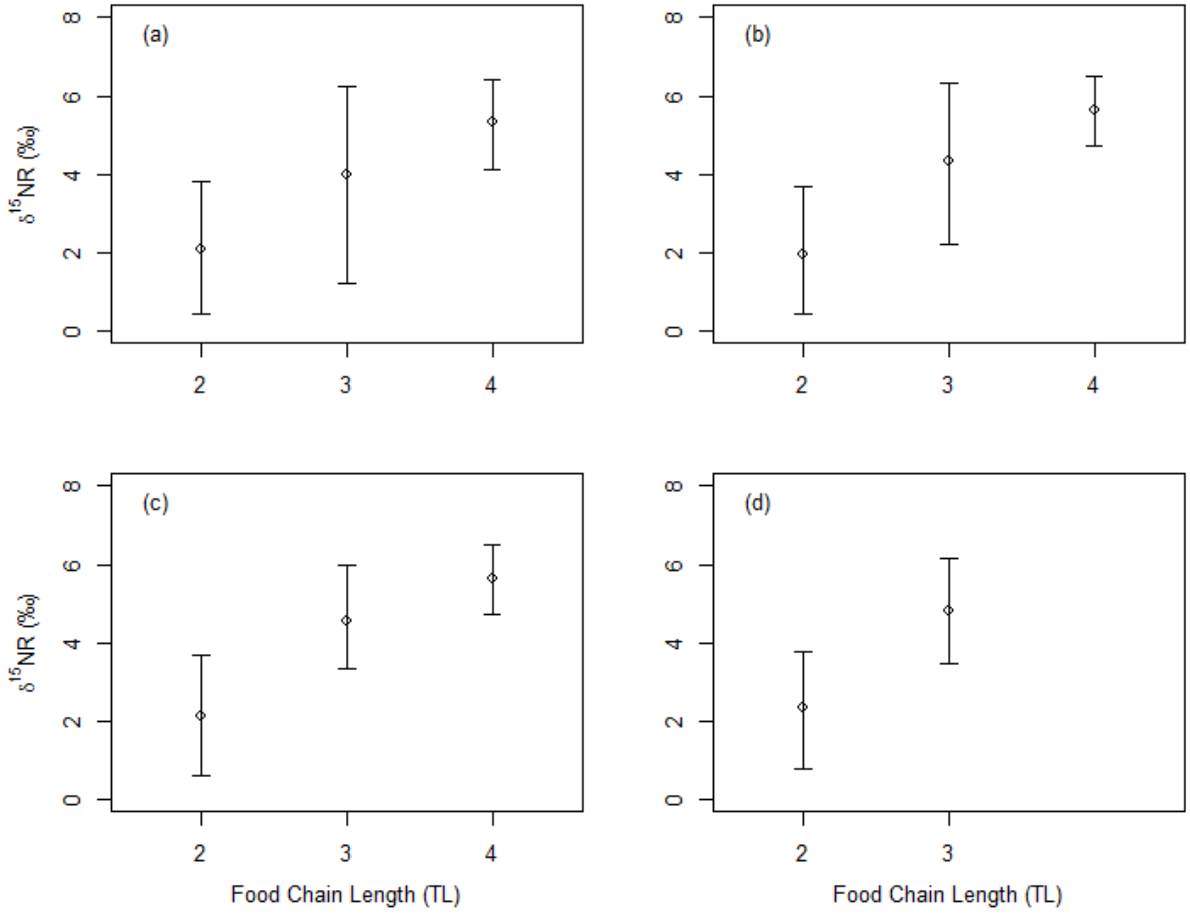

Fig. 3. Overall nitrogen range (NR) for food chains containing 2, 3 or 4 trophic levels. Values derived from combining Bayesian resampled mean values from all 4 replicate food chains. TL = trophic levels. Circles represent mode (of means) and bars 95% credible intervals for mean. a) all combinations: 2 TL n=10, 3 TL n=7, 4 TL n=3; b) excludes combinations that include wheat plants: 2 TL n=9, 3 TL n=5, 4 TL n=1; c) excludes all combinations that include wheat or maize plants: 2 TL n=8, 3 TL n=4, 4 TL n=1; d) excludes all combinations containing primary producers: 2 TL n=7, 3 TL=3.

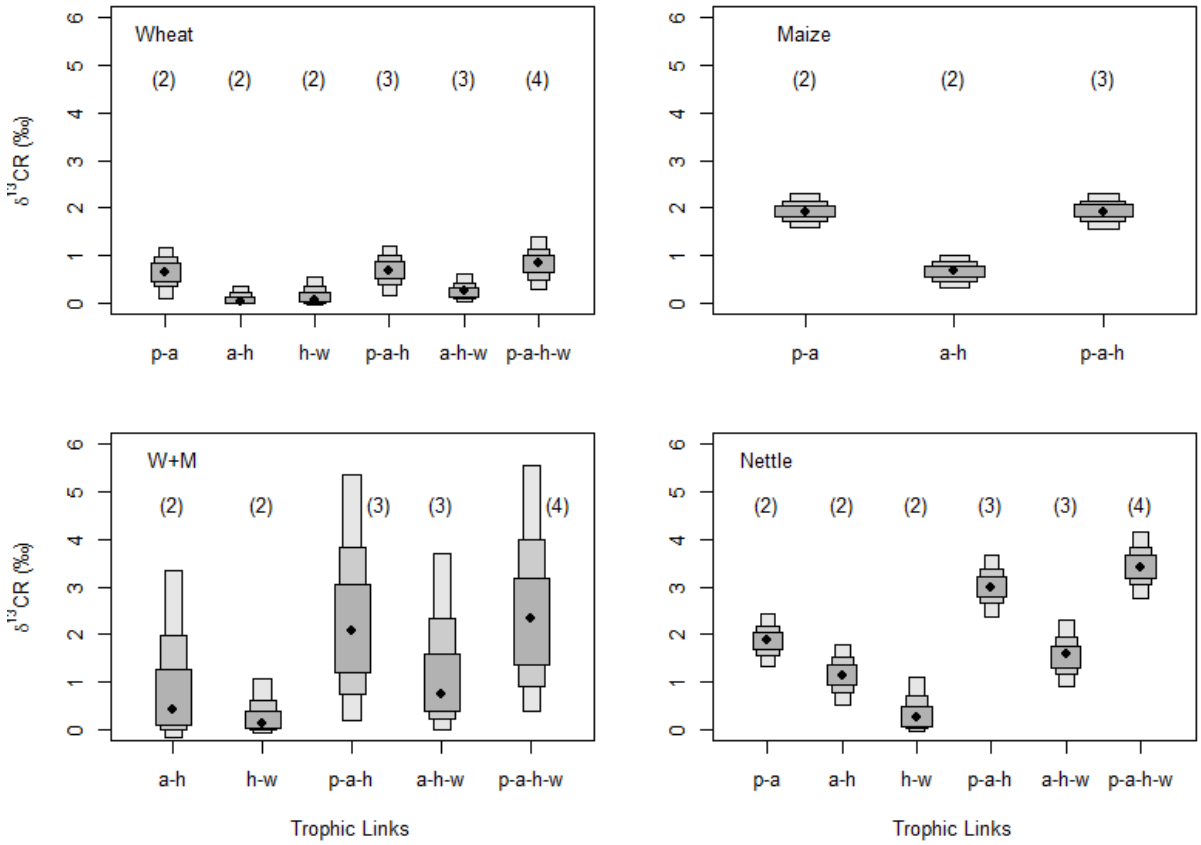

Fig. 4. Probability distributions of mean carbon range (CR) for different food chain lengths within each food chain. Distributions based on Bayesian resampling (*n* = 10,000) of uncertainty in sample mean estimates. 50%, 75% and 95% credible intervals as in Fig. 2. x-axis: p=plant, a=aphid, h=hoverfly, w=wasp. Numbers in parenthesis show food chain length.

3.5 Discussion

For the first time, we tested the accuracy of isotopic measures of trophic structure against known trophic positions using well replicated terrestrial plant and invertebrate food chains spanning 4 trophic levels. We found that despite some overlap in nitrogen range (NR) between longer food chain lengths, across a range of different species combinations and food chains NR generally quantified food chain length well, suggesting robustness to observed variation in discrimination. Additionally, we found few consistent trends in $\delta^{13}C$ discrimination with typically small (0.6‰ to 1.9‰) or no enrichment, and concurrently little and inconsistent change in CR with food chain length, emphasising the utility of $\delta^{13}C$ to trace diet – consumer pathways. We suggest our estimates of food chain trophic structure are particularly robust because they were calculated using Bayesian resampling procedures, allowing for propagation of variance in $\delta^{15}N$, $\Delta\delta^{15}N$, $\delta^{13}C$ and $\Delta\delta^{13}C$ into quantified uncertainty in final structural estimates.

How consistent are changes in $\delta^{15}N$ and $\delta^{13}C$ across trophic levels?

$\delta^{15}N$

We identified two sources variation that affect consistency of $\delta^{15}N$ observed within trophic levels; firstly variation in $\delta^{15}N$ within source species of 0.3‰ to 0.9‰ (excluding plants), and secondly variation in consumer $\Delta\delta^{15}N$ as discussed below. Within species $\delta^{15}N$ variation is well described as a consequence of (amongst others) size, diet quality, trophic position, consumer's nutritional state and mode of excretion (Caut et al. 2009, Martinez del Rio et al. 2009, Boecklen et al. 2011 and references therein). An observed (and in our context anomalous) example of this was large $\delta^{15}N$ variation in wheat and maize plants, that was not observed in wild nettle plants, suggesting that laboratory conditions affected variation. As such variation was not observed for $\delta^{13}C$ which is sourced from the atmosphere, we speculate $\delta^{15}N$ variation was explained by micronutrient pockets in soil medium through incomplete homogenisation. Alternately, stress caused by unnaturally high

and variable aphid density may have affected plant metabolism and hence nitrogen balance, as shown for other taxa (Hobson et al. 1993, Voigt and Matt 2004). As we pooled aphids to produce each aphid sample, plant $\delta^{15}N$ variation likely averaged across these multiple aphids, explaining why large $\delta^{15}N$ variation was not subsequently seen in aphids.

We found significant $\delta^{15}N$ enrichment from source to consumer in a majority of instances, varying between 1.4‰ and 3.3‰, a range concurrent with literature estimates (Post 2002, Vanderklift and Ponsard 2003, Caut et al. 2009). Averaged across food chains, such enrichment was 2.2‰ (excluding discrimination from wheat and maize plants to their aphids). This value is marginally lower than literature average estimates for invertebrates of 2.5‰ (Caut et al. 2009, Vanderklift and Ponsard 2003), and lower than the overall literature estimate of 3.4‰ (Post 2002) which is commonly employed to calculate trophic levels and food chains (e.g. Post 2002, Takimoto et al. 2008, McHugh et al. 2010). Higher trophic discrimination factors have been associated with vertebrates (Caut et al. 2009) and thus the lower enrichments noted in our insects re-emphasises the need to use taxa-specific discrimination factors when using isotopic data to calculate ecological parameters (Caut et al. 2009). Additionally, enrichment range of 1.9‰ (1.4‰ to 3.3‰) in our results was relatively large, and suggests variation in trophic discrimination factors is an important artefact in isotopic data that should be accounted for. Thus the use of averaging to produce commonly utilised trophic discrimination factors excludes variation in discrimination from final ecological estimates. Given such variation was present in our system, our subsequent use of Bayesian resampling procedures allowed us to propagate discrimination uncertainty into our estimates of food chain structure, producing estimates that we contend are more accurate and hence ecologically robust (Jackson et al. 2011).

As a single exception in source to consumer enrichment, wheat feeding aphids were on average depleted in $\delta^{15}N$ relative to hosts, concurring with other studies (Oelbermann and Scheu 2002, Wilson et al. 2011). Wilson et al. (2011) showed a negative relationship between aphid $\delta^{15}N$ discrimination and host plant total nitrogen content which is consistent with our results; aphid

enrichment was observed on maize and nettle plants that had low total nitrogen contents of 1.7% and 2.7% respectively, whilst wheat plants had higher nitrogen of 5.4%.

While examining sources of variation in $\Delta\delta^{15}N$, we found a significant interaction between trophic link and food chain type though neither variable was significant as a main effect, suggesting the interactive effects were stochastic in nature and could not be generalised. Thus in our study, discrimination variation was system and species-specific.

$\delta^{13}C$

Trophic discrimination of $\delta^{13}C$ from source to consumer was inconsistent between trophic links and food chain type, ranging from -0.7‰ to 1.9‰, and more generally observed as either showing no change or significant enrichment of between 0.6‰ to 1.9‰, concurring with discrimination reviews (Post 2002, Caut et al. 2009). Given that in our system the carbon axis was broad, such inconsistent and generally small trophic discriminations meant $\delta^{13}C$ was diagnostic of food chain type. Similarly, $\delta^{13}C$ signatures of hoverfly predators on the w+m food chain were intermediate of their two disparate aphid sources, illustrating well the usefulness of $\delta^{13}C$ data to integrate and reflect dietary sources.

Does nitrogen range accurately determine food chain length?

Using Bayesian resampling procedures to calculate nitrogen range (NR), we tested how NR changed with known food chain length within different food chain types, and then across food chains types. Excluding wheat plant–aphid combinations, we found that NR accurately determined food chain length within all replicate food chains. Only Vander Zanden et al. (1997) provides a comparable validation study of $\delta^{15}N$ with food chain length, concurrently showing a positive correlation between trophic positions of freshwater fish estimated by both $\delta^{15}N$ and traditional gut content analysis. To our knowledge, such a validation does not exist for terrestrial systems (Martinez del Rio et al. 2009).

In our study, the inclusion of wheat plant–aphid combinations depressed NR measures on the wheat and w+m food chain types. This was because aphids were depleted relative to wheat plants, such that aphids were effectively base of the $\delta^{15}N$ food chain whilst wheat constituted an additional trophic level that did not act to extend NR. This importantly shows exceptional species-specific effects may adversely affect the accuracy of isotopic measures of food chain length. As previously we had identified the wheat plant–aphid relationship as an exception to the generic enrichment in $\delta^{15}N$ from source to consumer, we feel justified in concluding that, more generally, NR predicted food chain length.

Overall, when all trophic combinations for all food chain types were combined, modal NR values increased by between 1.2‰ and 2.7‰ with each trophic level, suggesting a robustness of this technique for calculating food chain length. Excluding combinations including wheat and maize plants (on account of large variation in their $\delta^{15}N$ signatures), 95% credible intervals of NR estimates showed some overlap between food chain lengths of 3 and 4 trophic levels, inhibiting accurate estimation of food chain length at these points of overlap. Current practice uses NR to estimate food chain length and determine subsequent conclusions, but rarely has NR's use been tested, with terrestrial systems particularly understudied (Martinez del Rio et al. 2009). Based on our results, we urge caution interpreting food chain length when NR values fall in known overlap boundaries; i.e. in our study NR values of 5‰ or 6‰ could be either 3 or 4 trophic levels. Such overlap was a consequence of variation in NR values for the same food chain length across different food chain types, as caused by variation in $\delta^{15}N$ within each species (Fig. 1a) and variation in $\Delta\delta^{15}N$ between trophic links (Table 1). Of the few studies that have examined effects of variation in $\delta^{15}N$ (Vander Zanden and Fetzer 2007) and $\Delta\delta^{15}N$ (Vander Zanden and Rasmussen 2001, Post 2002) on error of trophic level or food chain length estimates, all have found error concurrent to the variation we show in estimates of NR. Importantly, our research diversifies these studies by testing empirical rather than theoretical measures of food chains, within a terrestrial context for the first time and utilising invertebrates, in addition to providing robust estimates of NR based on Bayesian resampling

of variation.

Additionally, as different food chain types in our experiment differed in their NR values for given food chain lengths as a consequence of variation in $\delta^{15}N$ and $\Delta\delta^{15}N$, so it is worth urging some caution when using NR for direct comparisons between systems of simple single-pathway food chains. It has been reasonably suggested that such variation may average out over multiple trophic levels or larger sample sizes (Martinez del Rio et al. 2009), though based on our results we would call for research to further test the importance of such variation. Pragmatically, the effect of food chain type we found was stochastic, such that sampling across multiple food chains might allow such an effect to be absorbed as 'noise' or specifically filtered out if food chain identity was categorised as a random effect. We speculate that such variation may also be averaged out when considering larger food webs containing multiple food chain pathways.

Our overall estimates of NR were based on exact trophic positions in well replicated food chains and utilised Bayesian resampling procedures that propagated uncertainty in discrimination factors to produce estimates that represented a full range of possible NR values. Given such an approach is likely to produce robust estimates of NR, and that more generally these estimates suggested that NR can accurately discriminate different food chain lengths, we conclude NR is a useful isotopic metric for quantifying food chain length.

How does carbon range change with food chain length?

Utilising Bayesian resampling procedures we calculated carbon range (CR) values for all food chain length combinations on all four food chain types. Overall, our results show that across trophic levels CR changed little (< 3.5‰) and inconsistently both within and between food chain types. Little change in CR over 4 trophic levels suggests fidelity of $\delta^{13}C$ values between primary producers and top predators, as concurrent with our earlier findings of small and inconsistent $\delta^{13}C$ discrimination (Table 1). Given global estimates of aquatic food chains approximate 3.5 to 4.0 trophic levels (Vander Zanden and Fetzer 2007) little change in CR over 4 trophic levels in our study demonstrates

more broadly the utility of $\delta^{13}C$ to trace energy pathways, be they either simple singular chains or potentially when embedded within larger trophic structures.

Studies utilising stable isotopes to quantify trophic structure and derive subsequent ecological conclusions continue to proliferate, yet few studies have specifically tested how variation in source values and consumer discrimination can affect accuracy of derived isotopic measures, with terrestrial systems and invertebrates particularly understudied. In this study we found insect $\delta^{15}N$ enrichment that was lower than literature values for vertebrates. This enrichment showed broad stochastic variation (range 1.9‰) across trophic levels within and between different food chains, propagating variation to subsequent estimates of NR. However, across a range of species combinations and food chains we show NR proved robust to such variation and distinguished food chain length well, though some overlap between longer food chain lengths importantly establishes limits in NR's precision. CR changed little with food chain length and hence $\delta^{13}C$ is potentially a useful tracer of source - consumer interactions. Having established that variation in source values and consumer discrimination affected estimates of trophic structure, we recommend the use of (Bayesian) resampling procedures to propagate variation as quantified uncertainty in final estimates of structure. Such procedures are necessary to improve accuracy and robustness of ecological conclusions in future isotopic studies. Given global estimates of aquatic food chains approximate 4 trophic levels, our use of 4-trophic level plant-invertebrate food chains makes our findings relevant to a majority of ecological systems and contexts.

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CHAPTER 4

Greater biodiversity modifies food web structure by increasing niche diversity and niche overlap

4.1 Abstract

Food web structure is known to be of underlying importance for a broad range of ecological processes. However, it remains largely untested how biodiversity *per se* affects food web structure. Nitrogen and carbon stable isotopes in tissues of organisms provide dietary axes on which to delineate species niches and overall community food web structure. In this study we utilise stable isotopes of nitrogen and carbon to quantify multiple dimensions of food web structure for grassland plant and invertebrate communities across a human induced diversity gradient, in order to test how community structure and average resource partitioning amongst community members may be affected by community taxonomic richness. We find that measures of food chain length, resource breadth, trophic diversity, and functional redundancy are all positively related to taxonomic richness, and that these effects are consistent between plant, herbivore and predator trophic guilds. These isotopic measures of community niche space provide good evidence that greater diversity increases both diversity of resources exploited and overlap in resources exploited, though we found no evidence that niches of individual taxa change as a response to diversity. We suggest these findings provide mechanistic insights to understanding coexistence and particularly ecosystem functioning, by lending support to theoretical explanations of how higher diversity may confer greater ecosystem functioning. This is achieved by more complete resource utilisation through species complementation, as shown here by both increased functional redundancy and functional diversity with increasing taxonomic richness. In naturally assembled species-rich communities we therefore demonstrate that diversity affects multiple dimensions of food web structure. Given the importance of food web structure to trophic interactions and ecosystem processes, stability and services, we suggest that human induced community structural disassembly should be acknowledged as a priority reason for stemming biodiversity loss.

4.2 Introduction

Despite many years of study, understanding causes and underlying mechanisms of food web structuring remains a fundamental agenda for future ecological research, as the consequences of structure are profound. For example, structuring of food webs can be linked to: patterns of biodiversity (Rooney & McCann 2012), how diversity regulates ecosystem functions and services (Cardinale 2011; Thompson *et al*. 2012), consequences of extinctions and invasions (Dunne *et al*. 2002; Srivastava & Bell 2009) and how communities are likely to respond to human induced habitat alteration (Melian & Bascompte 2002) and climatic change (Petchey *et al*. 1999; Hansson *et al*. 2013). Much research has therefore focused on discerning patterns of structure, typically by examining physical attributes or dimensions of food webs such as network analysis (Jorgensen & Fath 2006; Ulanowicz & Scharler 2008), trophic connectance (Vermaat *et al*. 2009; Thebault & Fontaine 2010), food chain length (Post 2002a; Vander Zanden & Fetzer 2007), niche segregation (McKane *et al*. 2002) and functional redundancy (Devoto *et al*. 2012). Whilst such quantifications of food web structure in many instances have examined structure as an explanatory variable, studies have also used structure as a response to environmental variables, such as resources (Settle *et al*. 1996; Bukovinszky *et al*. 2008), ecosystem size (Post *et al*. 2000), spatial and temporal variability (McCann & Rooney 2009), disturbance (Sabo *et al*. 2010), habitat loss (Evans *et al*. 2013) and extinction scenarios (Haddad *et al*. 2009). More recently studies have begun to link structure as both response and explanatory variable and test how the effect of biotic and abiotic environmental variables on community structure in turn influence ecosystem functions and processes (Petchey *et al*. 1999; Macfadyen *et al*. 2009; Estes *et al*. 2011). Thus food web structure is fundamentally important in affecting ecological patterns and functions, but is itself determined by a range of biotic and abiotic variables.

A critical first step in seeking to understand mechanistic causes of food web structuring is to recognise that biotic and abiotic variables affect structure and ultimately ecosystem functions and processes via modifying patterns of community diversity, for example in terms of species richness

and evenness (Petchey *et al*. 1999; Macfadyen *et al*. 2009; Estes *et al*. 2011). Thus, any given measure of food web structure will depend to some extent upon the identity and number of species in a community and their relative abundances and trophic relationships. For instance, work has shown that competition, succession, extinction and population dynamics all modify patterns of diversity and thereby indirectly affect community structure (Thebault *et al*. 2007; Schrama *et al*. 2012; Petchey *et al*. 1999; McCann & Rooney 2009, respectively). This means that in order to achieve a mechanistic understanding of the causes of food web structure we have to discern how diversity affects structure, yet to date few studies have achieved this directly (Rooney & McCann 2012).

Understanding how diversity affects structure requires the relative or average 'contributions' of species to a community's overall food web structure to be determined. An effective approach in this context is to determine the trophic niche of each species relative to one another, and consequently, dimensions of food web structure as derivatives of the sum of all member species' niche. Quantifying individual species relative effects on food web structure within communities has been utilised when investigating structure in other contexts (McKane *et al*. 2002; van Veen *et al*. 2008; Vermaat *et al*. 2009; Anderson & Sukhdeo 2011). The strength of this approach to determine diversity – structure relationships is that it allows for calculation of community trophic niche dimensions as measures of food web structure by quantifying average or cumulative species resource partitioning within each community. Subsequently, such community food web dimensions can be contrasted between different communities to mechanistically examine how species niche partitioning may change as a function of overall community diversity.

An organism's niche is often delineated by habitable ranges on *n*–number of axis representing biotic and abiotic dimensions in a theoretically quantifiable hypervolume (Hutchinson 1957). Quantifying the trophic niche for each member species within a community based on standardised axis of dietary information fits well with this conceptualisation of niche. More recently researchers have begun to use stable isotope ratios of nitrogen (N^{15} : N^{14}, termed $\delta^{15}N$) and carbon

(C^{13} : C^{12}, termed $\delta^{13}C$) in consumer tissues to provide a temporally and spatially integrated construct of trophic niche (Bearhop *et al*. 2004; Layman *et al*. 2007a; Newsome *et al*. 2007; Syvaranta & Jones 2008; Quevedo *et al*. 2009), as $\delta^{15}N$ and $\delta^{13}C$ of consumer proteins reflect the proteins of their food sources (DeNiro & Epstein 1981; 1978, respectively). $\delta^{15}N$ provides a trophic axis (Newsome *et al*. 2007) as $\delta^{15}N$ enriches from prey to predator (DeNiro & Epstein 1981: Post 2002b), whilst $\delta^{13}C$ provides a resource axis as sources often differ in $\delta^{13}C$ (Newsome *et al*. 2007). Thus $\delta^{15}N$ and $\delta^{13}C$ signatures within each species allow quantitative bivariate positioning of species relative to one another along these two dietary axis producing graphical bi-plot food webs (Layman *et al*. 2011). Subsequently, dimensional measures across this isotopic food web space allow comparisons between communities of differing diversity values (Box 1).

The way species structure themselves relative to one another along axis of resource use is crucial to understanding fundamental ecology such as coexistence and how diversity regulates ecosystem functions and processes (Salles *et al*. 2009; Cardinale 2011; Carroll *et al*. 2011). Thus improving our mechanistic understanding of how diversity affects the structure of biological communities is an ecological imperative. Relatively few studies to date have tested for such mechanisms, and to the best of our knowledge, none has sought to derive such underlying mechanisms using species-rich communities comprising natural assemblages scaling multiple trophic levels. In this study we apply novel stable isotope techniques to quantify community niche space in wild grassland communities and test the questions: How does community niche space change as a function of diversity, and what does this tell us about resource partitioning amongst taxa? We make the following non-mutually-exclusive predictions about community niche space: First, total community niche space expands with increasing community diversity; second, the niches of individual taxa contract with increasing community diversity; finally, the average niche overlap among taxa is greater with increasing community diversity. We apply tests of these hypotheses in the context of multiple facets of diversity and structure: total community taxonomic richness, taxonomic richness within trophic guilds, and functional richness within trophic guilds.

Box 1. Stable isotope approaches to quantifying food web structure

$\delta^{15}N$ and $\delta^{13}C$ are spatially and temporally integrated dietary tracers that are used to position species relative to one another in bivariate isotopic space, with distances between species approximating differences in resource use. Communities of taxa can be represented in this way to produce isotopic food webs. Euclidean distances across this isotopic space can quantify different aspects of food web structure (Layman *et al* 2007b). Briefly;

1. $\delta^{15}N$ range (NR) : Distance between species of min and max $\delta^{15}N$ providing for a measure of trophic length of the community and subsequently used to calculate food chain length;
2. $\delta^{13}C$ range (CR) : Distance between species of min and max $\delta^{13}C$ providing for an estimate of diversity of basal resources;
3. Total area (TA): Convex hull area of all species in bivariate isotopic space. Provides an estimate of total trophic niche diversity of community;
4. Mean distance to centroid (CD): Average distance of each taxa to bivariate centroid, providing a measure of average spacing of taxa and thus trophic niche diversity;
5. Mean nearest neighbour distance (MNND): Mean of distances to each taxa's nearest neighbour in bivariate space providing an estimate of density of taxa packing, and thus a measure of functional redundancy;
6. Standard deviation of nearest neighbour distance (SDNND): Provides a measure of evenness of spatial density and packing in bivariate isotopic space.

Contemporary Bayesian resampling approaches to quantifying these measures allows for the propagation of natural variation in isotopic signatures into uncertainty in final probabilistic estimates of food web structure, greatly improving robustness of estimates and conclusions (Jackson *et al*. 2011).

4.3 Methods

Field Sites

During July and August 2011 we sampled plant and invertebrate communities in two fields for each of three grassland types: extensively cattle-grazed meadows, intensively cattle-grazed pasture and golf course fairways. Grazed treatment fields were located at Rothamsted Research farm, North Wyke, Devon, UK. Golf course fairways were located approximately 12km west and thus within the same larger rural landscape. Through intensification of land management, these three grassland types represented a declining grassland sward architecture which is known to drive a decline in grassland community diversity (Woodcock *et al*. 2009). This diversity gradient provided us the opportunity to sample $\delta^{15}N$ and $\delta^{13}C$ signatures for all species within each community and test

predictions about how isotopic niche partitioning of resource use amongst community members may be affected by overall community diversity. Though qualities of management regimes differed between grasslands (e.g. sward architecture was affected by cattle grazing in the first two communities but by lawnmowers in the third) any differences in *how* they influenced species assemblage was not of concern; in the context of naturally structured communities reflective of real management, we were primarily interested in how species average resource use differed as a function of community taxonomic richness *per se*. All grassland types were mesotrophic, and with increased management intensification, were characterised by reduced sub-sets of a larger grassland species pool (UK National Vegetation Classification statuses were extensive grazing MG5, intensive grazing and golf fairways MG7; Table 1). Extensively grazed fields were characterised by low-level cattle disturbance and no fertiliser applications; intensively grazed fields by high-level cattle disturbance and twice yearly farmyard manure applications; and golf fairways by frequent mowing and inorganic fertiliser input. All fields had been maintained in their present state for >5 years and shared the same soil type.

Table 1. Characteristics of 6 grassland study sites. Grassland management intensification decreased diversity, abundance and habitat heterogeneity (measured as SD of mean sward height).

Grassland	Mean ± SD Sward Height (cm)	Plant Species Richness	Mean ± SD Plant Biomass (dry g / 0.5m^2)	Invertebrate Taxonomic Richness	Total Invertebrate Biomass (dry mg / 0.5m^2)
Extensive meadow (a)	21.02 ± 11.27	18	135.35 ± 6.82	91	738.88
Extensive meadow (b)	20.98 ± 10.51	21	159.27 ± 21.40	87	483.48
Intensive pasture (a)	11.52 ± 3.82	8	125.50 ± 15.51	68	322.52
Intensive pasture (b)	8.80 ± 4.22	8	79.11 ± 3.15	56	198.24
Golf fairway (a)	2.52 ± 1.59	6	38.45 ± 1.42	20	29.68
Golf fairway (b)	2.32 ± 1.19	8	53.60 ± 6.22	14	25.23

Field Sampling

For each field, at a distance >15m from any field margin, a 15m^2 plot was sampled to establish community plant and invertebrate diversity and biomass, and samples collected for later stable isotope analysis (SIA). Plant species richness was assessed using 3 x 1m^2 quardrats combined with 5-minute searches of the wider plot area for rarer species. All plants were identified to species *in situ* and samples collected and frozen (-20°C) within 2 hours for later SIA. Plant biomass was quantified by cutting randomly placed 5 x 1m vegetation strips of diameter 7.5cm (total area 0.375m^2), replicated twice at different locations in the plot, and weighing (g) after drying at 45°C for >5 days. Suction sampling was used to destructively sample invertebrate species richness, and was conducted on dry days between 10:00 and 16:00. 55 replicate suction samples (16 seconds each) were taken from different locations within each plot. This has been shown to be an effective methodology for sampling canopy and ground dwelling species (Brook *et al*. 2008). All invertebrates were collected in distilled water and frozen (-20°C) within 2 hours. Invertebrates were subsequently sorted in the laboratory and identified to species where possible, or genus if not. Morpho-species was used for Cicadellidae, Parasitica, Aranea and many Diptera families. Taxonomic identification and classification was consistent among communities. Given that a mixture of species, morpho-species and genus level identification was used, we hereafter refer to invertebrate diversity as invertebrate taxonomic diversity. All invertebrates were dried at 45°C for >48 hours and weighed to provide biomass (mg) per taxon prior to preparation for SIA. In each plot, sward architecture was assessed with 30 sward stick measurements taken at random to provide sward height (mean) and sward heterogeneity (standard deviation).

Sample Preparation & Lipid Extraction

All SIA plant samples were dried at 45°C for >5 days. A single leaf per plant was used for a single SIA sample. For all invertebrates, generally between 1 and 10 whole individuals were pooled to provide

enough mass for a single isotope sample, though upwards of 30 were used for a small number of taxa. Where individuals were bigger than the needed sample mass, individuals were homogenised whole, and a sub-sample taken. For snails, a section of foot was used. Though previous work has shown that within-individual tissue selection can be important for invertebrates (Perkins *et al*. 2013), samples were too numerous and often too small to accomplish dissection. However, the noise this may introduce is likely to be small when spread across such species-rich communities, and more importantly is unlikely to vary among communities which is what we are interested in comparing.

To accurately estimate $\delta^{13}C$ of proteins within individuals it is accepted practice to first remove free-lipid contained within. Lipid is naturally depleted in $\delta^{13}C$ (DeNiro & Epstein 1977) and its concentration varies between tissues, individuals and species. To account for this we conducted lipid extraction on a sub-set of samples and then applied a mathematical correction to all our samples based at either Order or Family level. For samples undergoing lipid extraction, tissue was immersed in 2:1 Chloroform: Methanol solution for 50 minutes to remove free-lipid, and then left to air dry.

Stable Isotope Analysis

For all samples, 0.35mg ± 0.05 (invertebrates) or 3mg ± 0.1 (plants) dried material was enclosed in tin capsules. Stable isotope analysis was conducted at the Food and Environment Research Agency, York, UK. Samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ in a Fisons EA1108 elemental analyser (Carlo Erba Instruments, Milan, Italy), coupled with an Isoprime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Stable isotope ratios are reported in delta (δ) notation where $\delta^{15}N$ and $\delta^{13}C = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Isotope ratios are expressed in per mil (‰) relative to the ratio of international reference standards ($R_{standard}$) which are Atmospheric Nitrogen and Vienna PeeDee Belemnite (VPDB) for nitrogen and carbon respectively. Measures of standards placed throughout invertebrate samples exhibited acceptable instrument reproducibility of < 0.10‰ (SD) for $\delta^{15}N$ and < 0.11‰ (SD) for $\delta^{13}C$ using collagen standard and insect whole tissue standard (cockroach; *Nauphoeta cinerea*); and placed amongst plant samples <

0.09‰ (SD) for $\delta^{15}N$ and < 0.11‰ (SD) for $\delta^{13}C$ using tomato (*Solanum lycopersicum*) and wheat (*Triticum aestivum*) standards.

Data Analysis

Food web metrics

Calculation of food web metrics NR, CR, TA, CD, MNND and SDNND (see Box 1 for details) were made using Bayesian resampling of variability in mean $\delta^{15}N$ and $\delta^{13}C$ of samples to provide 50%, 75% and 95% credible intervals of mean estimates for each metric, using SIBER (Jackson *et al*. 2011) in the R package SIAR (Parnell *et al*. 2008; 2010). Given we were able to obtain 1 or more replicates for approximately 70% of identified taxa within each community, we regressed modal values for each metric against metric modes based on 2 or more replicates per taxa (representing 50% of identified species) to determine if low sample size of some taxa affected overall metric accuracy. We used R^2 values of model fit to assess the effect. Estimates of community metrics as described here were used to assess predictions one and three: that with greater diversity, community niche space expands, and average niche overlap between taxa increases. All analyses described here and below were conducted using R version 2.14.1 (R Development Core Team, 2011).

Within – trophic guild taxa richness effects on community niche space

For each community, taxa were pooled into the trophic guilds *plant, herbivore, predator* and *other.* For each guild within each community, metrics were calculated as previously described and the mode of each metric extracted for analysis. For each metric separately, a General Linear Model (GLM) tested how the explanatory variables *taxa richness*, *trophic guild* and their interaction affected the metric mode. Model simplification used backwards-stepwise regression from a maximal model and ANOVA model comparisons to identify non-significant model terms for elimination. Homogeneity of variances and normality of model residuals were checked in all instances. *Post Hoc*

analyses were made when *trophic guild* was returned as significant in order to determine between which trophic guilds differences were; tests were made using ANOVA model comparisons between models in which two trophic groups had been paired and a model without pairings. When model deviance was not significantly affected by pairing guilds, these guilds were not significantly different. Analyses testing effects of within - trophic guild diversity on food web structure as described here were used to assess predictions one and three: that with greater diversity community niche space expands and average niche overlap between taxa increases.

Taxa richness effects on isotopic niche of individual taxa

To test if isotopic niches of individual taxa changed as a function of community diversity, for 17 taxa common to at least 4 communities we calculated for each taxa in each community Standard Ellipse Area corrected (SEA_c) utilising the SIBER computational code (Jackson *et al*. 2011). Samples sizes varied between taxa but were consistent for each taxa across communities (*n* = 3 to 20). Ellipse area gives a bivariate estimate of area occupied in $\delta^{15}N$ and $\delta^{13}C$ space and is robust to small sample sizes. A General Linear Mixed Model (GLMM) was used to test how the explanatory variables *taxa richness*, *trophic guild* and their interaction affected SEA_c. All 17 taxa were included in this single model with the random effect *taxa identity* used to account for inherent differences between taxa. Model simplification used backwards stepwise regression as described above. Analyses used 'lme' from the *nlme* R package (Pinheiro *et al*. 2010). Analysis as described here was used to assess prediction two: that with greater community diversity, isotopic niche space of individual taxa contract.

Functional richness effects on community niche space

Utilising only the two high diversity communities (to provide sufficient diversity) we also determined how functional richness affected TA and MNND. These metrics provide robust structural estimates through combining two resource axis in bivariate space (they inherently incorporate NR and CR) and necessarily measure different aspects of community expansion / contraction to help identify mechanisms driving any structural changes. Additionally, food web dimensions as measured by metrics analogous to MNND have been shown to be among the most important in explaining food web structure (Vermaat *et al*. 2009).

Independently for each high diversity community, we pooled taxa into functional groupings based on phylogenetic and /or feeding modes, nested within the trophic guild groupings *plant, herbivore* or *predator*. Functional groupings were: *Plant* – grass, forb, legume; *Herbivore* – sap suckers (Aphids, Cicadellidae), weevils, grazers (Lepidoptera, Gastropoda); *Predator* – spiders, coleoptera, hymenoptera. Within each community, for all functional group combinations within each trophic guild, we calculated TA and MNND as described previously. For instance, TA and MNND was calculated separately for: spiders; coleoptera; hymenoptera; spiders with coleoptera; spiders with hymenoptera; hymenoptera with coleoptera; and spiders with coleoptera with hymenoptera, within each community. This was repeated for each trophic guild. Separately for each metric within each community, we used GLMs to test how the explanatory variables *functional richness*, *trophic guild* and their interaction affected TA and then MNND. Functional richness was calculated as total number of taxa in any functional combination (e.g. spiders (15 species) + hymenoptera (8 species) = 23 species). Model simplification was employed as described above. When *trophic guild* returned as significant, *post hoc* analysis as described above was used to test which guilds were significantly different. We acknowledge that these GLMs were subject to some pseudo-replication as functional groups were used cumulatively to generate metric values for different levels of functional richness and therefore appear several times in the same analysis. Given no suitable alternatives, and that relationships were tested within single communities and not across multiple communities, we

therefore proceed with caution and compare these analytical results with graphical representations of these relationships.

To determine if effects of functional richness on MNND were caused by functional richness (i.e. addition of functional groups *within* a trophic guild) or rather, simply by cumulative addition of functional groups, we validated the use of these functional richness models through comparison of performance with null models (in which functional groups were grouped randomly *across* trophic guilds). For each trophic guild, functional richness comprised up to three functional groups (i.e. the trophic guild *plants* comprised 3 functional groups: grass, forb, legume). MNND for each two-way pairing and the three-way pairing was compared to MNND values for each of the component single functional groups and recorded as either being higher or lower. As a conservative test, if niche overlap was present between functional groups then joining groups should lower MNND relative to their component single functional group MNND values. Using a simple binomial test, we compared if the number of instances when MNND for paired or three-way functional groupings was lower than their respective single functional group's MNND was significantly higher for true functional richness groupings compared with null functional richness groupings. It was not necessary to repeat this analysis for the TA metric as addition of dissimilar functional groups across trophic guilds is likely to increase TA more than addition of similar functional groups within a trophic guild, and thus the real test of functional richness – TA relationships is naturally more conservative than a null test.

Analyses testing functional richness effects on TA and MNND as described here were used to separately assess predictions one and three: that with greater diversity, community niche space expands (TA), and average niche overlap between taxa increases (MNND).

4.4 Results

Examining three facets of diversity, we find strong evidence that changing diversity affects dimensionality of food web structure and consequently the relative partitioning of niche space amongst taxa.

Response of food web structure to diversity

Raw delta nitrogen and carbon values suggested that increasing taxa richness may have caused expansions in food web space along both resource axes and notable increase in the density of species packaging within community niche space (Fig. 1). SIBER modal estimates of mean probability distributions confirmed these trends (Fig. 2). Univariate metrics Nitrogen Range (NR) and Carbon Range (CR) both showed a trend for increasing modal estimates with increasing species richness providing evidence that greater food chain length (NR) and breadth of carbon resources (CR) were associated with more diverse communities. Concurrently with these expansions in NR and CR, Total Area (TA) recorded bivariate expansion with greater taxonomic richness, showing that higher levels of diversity occupy larger total community niche space. Interestingly, average distance to community centre (CD) did not change with taxa richness, which likely reflects contrasting effects of community niche expansion (TA) and increased density packing (mean nearest neighbour distance: MNND). Importantly, MNND decreased with greater taxonomic richness showing that on average species isotopic niches were in closer proximity when diversity was higher, suggesting greater functional redundancy in more diverse communities. Standard deviation of nearest neighbour distance (SDNND) was inconsistent and showed no clear trend with greater diversity. These described trends are based on Fig. 2 which isotopically represented >70% of all taxa identified for each community. Fig. 3 shows the same general trends as Fig.2, but was based only on taxa which had at least 2 replicates, representing approximately 50% of all taxa identified for each community. Large variance in credible intervals around each metric observed in Fig. 2 was a consequence of many taxa having only single replicates. However, such variance did not affect relative differences between metric modal values of communities of different diversity, as shown by regressions for each metric between communities represented by either 1 or more replicates per taxon (Fig. 2) or >1 replicate per taxon (Fig. 3), in which large R^2 values showed close modal fits (Table 2), with the exception of the metric CD. Thus for subsequent analyses we utilised values based on the dataset represented in Fig. 2 which

better represented true community diversity, with the exception of CD which was interpreted with caution in further analyses.

With increasing taxa richness, the expansions in food chain length, breadth of carbon resources and total niche community niche space, and the increased packing density of taxa within this space, were all found to be consistent when examined within trophic guilds for plants, herbivores and predators (Fig. 4). In each trophic guild, modal CR, NR, TA and MNND were significantly affected by taxa richness (Table 3). The trophic grouping 'other' was found to have a significantly greater effect per taxon richness than other trophic guilds on modal values of NR, TA, CD and MNND (Fig. 4, Table 3). However, the ecological meaning of this is limited as 'other' was a paratrophic grouping for the <30% taxa which could not be reliably classified into the definitive trophic guilds.

Total community niche space expands with increasing community diversity

In support of this hypothesis, community modal TA, NR and CR were observed to increase with increasing taxa richness (Fig. 2), providing strong evidence that greater diversity increases niche diversity. Interestingly, this trend was repeated consistently for taxa richness within trophic guilds, with plant, herbivore and predator taxa richness all significantly affecting TA, NR and CR (Fig. 4, Table 3). Additionally, for the two highest diversity communities (for which we had sufficient taxa richness) it was observed that TA significantly increased with greater functional richness (Fig. 5a and 5b, Table 4). More generally this TA – functional richness relationship was also found to be consistent for each trophic guild within each community (Table 4), with the single exception that plant functional richness effect on TA was less strong in the first of these communities (Fig 5a), as determined by post hoc analysis (Table 4). We acknowledge that the GLMs we use to test functional richness and trophic guild effects on TA were subject to some pseudo-replication as functional groups were used cumulatively to generate metric values for different levels of functional richness and therefore appear several times in the same analysis. However, given that overall functional richness correlates

with TA in Fig 5a and 5b, we suggest this analysis provides supporting evidence of the significance of the observed graphical relationship.

Niche of individual taxa contract with increasing community diversity

We found no evidence to support the hypothesis that isotopic niche space of individual taxa changed in response to community diversity. Using 17 taxa which were common to at least 4 of the 6 communities and for which we had sufficient replicates, we used a GLMM (random effect = taxa identity) to test how trophic area of individual taxa changed between communities as a function of overall community taxa richness. Trophic area of taxa was not significantly affected by either the interaction of community taxa richness and trophic guild (whether a taxa was plant, herbivore, predator or detritivore; $\chi^2_{(4)} = 0.98$, $p = 0.91$) or by the main effect of community taxa richness ($\chi^2_{(1)} = 0.01$, $p = 0.92$). The main effect of trophic guild is not reported as we are not interested in differences of taxa niche between trophic guilds.

Average niche overlap between taxa is greater with increasing community diversity

In support of this hypothesis we found a negative relationship between MNND and taxa richness across communities, with lowest MNND values associated with communities of greatest diversity (Fig. 2). This finding is reinforced by raw data plots (Fig. 1) showing density of species packing to increase with taxa richness. Whilst smaller MNND values indicate closer proximity of taxa in bivariate isotopic space and likely sharing of common resources, it does not by itself indicate that species are actually overlapping on either axis. However, raw data plots show clear overlap between taxa' isotopic niches, and that this increases with diversity (Fig. 1). A significant negative relationship between taxa richness and MNND was additionally found within trophic guilds for plants, herbivores and predators (Fig. 4, Table 3). Further to this, we also found this negative relationship to be significant when we grouped taxa into functional groups and tested MNND against functional

richness, within the two high diversity communities for which we had sufficient diversity to test this relationship (Fig. 5, Table 4). We validated the use of this functional richness model in the first instance through comparison of performance with a null model (in which functional groups were grouped randomly across trophic guilds). Binomial tests showed that whilst functional richness within a trophic guild significantly reduced MNND ($p = 0.04$ and $p = 0.04$ for each high diversity community respectively), functional richness based on random groupings across trophic guilds did not significantly reduce MMND ($p = 0.77$ and $p = 0.15$, respectively). Again, raw data plots suggest decreasing MNND with increasing functional richness infers both closer average proximity of differing functional groups and also greater actual overlap in isotopic niche between different functional groups (Fig. 5c and 5d). The negative relationship between functional richness and MNND was consistent for each level of trophic guild across both high diversity communities in which it was tested, with the single exception of predators in the second community which had a less strong negative relationship with MNND (Fig. 5, Table 4). As explained previously, while acknowledging pseudo-replication, the analysis in Table 4 is given in support of Fig. 5. Thus in demonstrating greater overlap of isotopic niche with greater functional richness, greater taxa richness within trophic guilds, and with greater taxa richness *per se*, these results provide evidence of the emergence of greater functional redundancy in dietary resource exploitation with increasing taxa richness.

Table 2. Taxa represented by single replicates did not cause inaccuracy in derived community metric values. Modal estimates of Bayesian resampled mean estimates were regressed for communities based on one or more replicates per taxa (Fig 2) against communities based on at least 2 replicates per taxa (Fig 3), for each measure of community structure in turn. Significant p-values are *italicised*. Importantly, R^2 values indicate a close fit for all metrics except CD and SDNND.

Metric	F	df	p	R^2
NR	4.94	1,4	0.09	0.55
CR	2.57	1,4	0.18	0.39
TA	14.2	1,4	*0.02*	0.78
CD	0.63	1,4	0.47	0.14
MNND	25.1	1,4	*<0.01*	0.86
SDNND	1.42	1,4	0.3	0.26

Table 3. Within trophic guild diversity affects multiple dimensions of food web structure. For each metric, a GLM was used to test how taxa richness per trophic guild affected metric value. Increasing diversity affected structure consistently across plant, herbivore and predator trophic guilds. When trophic guild was returned as significant, *post hoc* analysis was used to identify which trophic guilds were different. All values are F test - statistics with associated degrees of freedom in brackets. Significant terms are indicated as $p<0.05$*, $p<0.01$**, $p<0.001$***. pred = predator; herb = herbivore.

	NR	**CR**	**TA**	**CD**	**MNND**	**SDNND**
Full Model Terms						
taxa richness	$9.45_{(1,17)}$**	$89.99_{(1,20)}$***	$38.68_{(1,17)}$***	$1.83_{(1,17)}$	$24.62_{(1,17)}$***	$1.67_{(1,20)}$
trophic guild	$7.03_{(3,17)}$**	$2.04_{(3,17)}$	$7.12_{(3,17)}$ **	$19.13_{(3,18)}$***	$3.56_{(3,17)}$*	$0.70_{(3,17)}$
interaction	$0.33_{(3,14)}$	$0.88_{(3,14)}$	$2.28_{(3,14)}$	$0.36_{(3,14)}$	$0.45_{(3,14)}$	$0.45_{(3,14)}$
Post hoc tests						
plant-herb	$0.11_{(1,17)}$		$0.02_{(1,17)}$	$4.03_{(1,18)}$	$0.09_{(1,17)}$	
plant-pred	$0.35_{(1,17)}$		$0.26_{(1,17)}$	$2.84_{(1,18)}$	$0.34_{(1,17)}$	
herb-pred	$0.83_{(1,17)}$		$0.25_{(1,17)}$	$0.25_{(1,18)}$	$0.07_{(1,17)}$	
plant-other	$11.53_{(1,17)}$**		$11.62_{(1,17)}$**	$51.91_{(1,18)}$***	$8.28_{(1,17)}$**	
herb-other	$8.05_{(1,17)}$**		$10.17_{(1,17)}$**	$19.67_{(1,18)}$***	$5.67_{(1,17)}$*	
pred-other	$17.02_{(1,17)}$***		$16.40_{(1,17)}$***	$30.48_{(1,18)}$***	$5.26_{(1,17)}$*	

Table 4. Functional richness affects food web structure. Separate GLMs were used to test how the response variables TA and MNND were affected by the explanatory variables functional richness and trophic guild, for each of the two high diversity communities. When trophic guild was returned as significant, *post hoc* analysis was used to determine between which trophic guilds differences were. All values are F test - statistics with associated degrees of freedom in brackets. Significant terms are indicated as $p<0.05$*, $p<0.01$**, $p<0.001$***.

	High Diversity Community (a)		High Diversity Community (b)	
Full Model Terms	TA	MNND	TA	MNND
Functional richness	$37.14_{(1,17)}$ ***	$4.74_{(1,19)}$ *	$27.83_{(1,19)}$ ***	$10.68_{(1,17)}$ **
Trophic guild	$7.92_{(2,17)}$ **	$1.93_{(2,17)}$	$2.03_{(2,17)}$	$3.78_{(2,17)}$ *
Interaction	$1.36_{(2,15)}$	$1.18_{(2,15)}$	$0.81_{(2,15)}$	$1.31_{(2,15)}$
Post hoc tests				
plant - herbivore	$9.27_{(1,17)}$ **			$0.02_{(1,17)}$
plant - predator	$14.01_{(1,17)}$ **			$5.31_{(1,17)}$ *
herbivore - predator	$0.49_{(1,17)}$			$6.00_{(1,17)}$ *

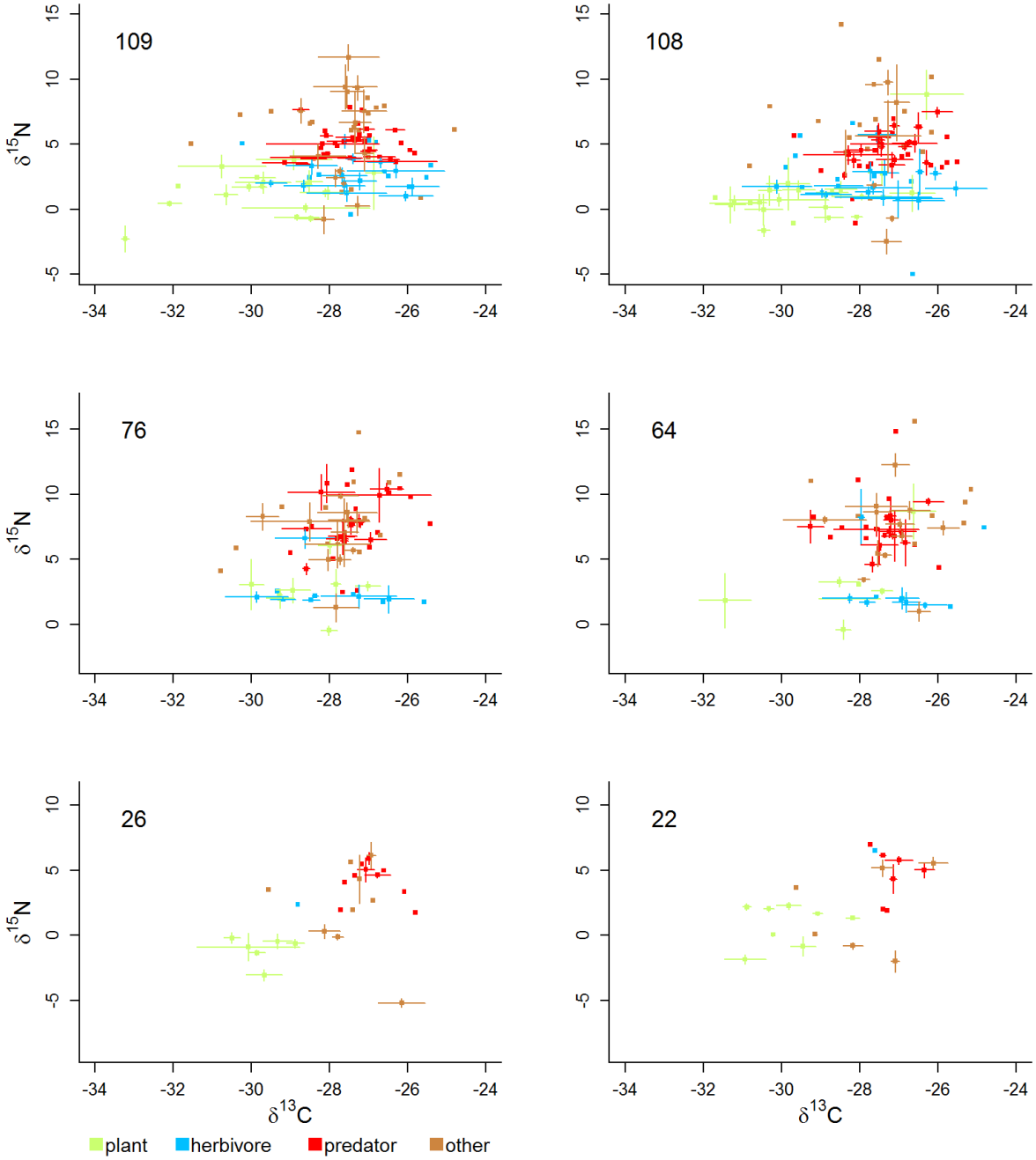

Fig 1. Raw delta (‰) stable isotope ratios of nitrogen and carbon show disassembly of community food web structure with changing taxa richness in wild grasslands subject to differing human management. Taxa richness is given as number on plot. Variance bars are standard deviation.

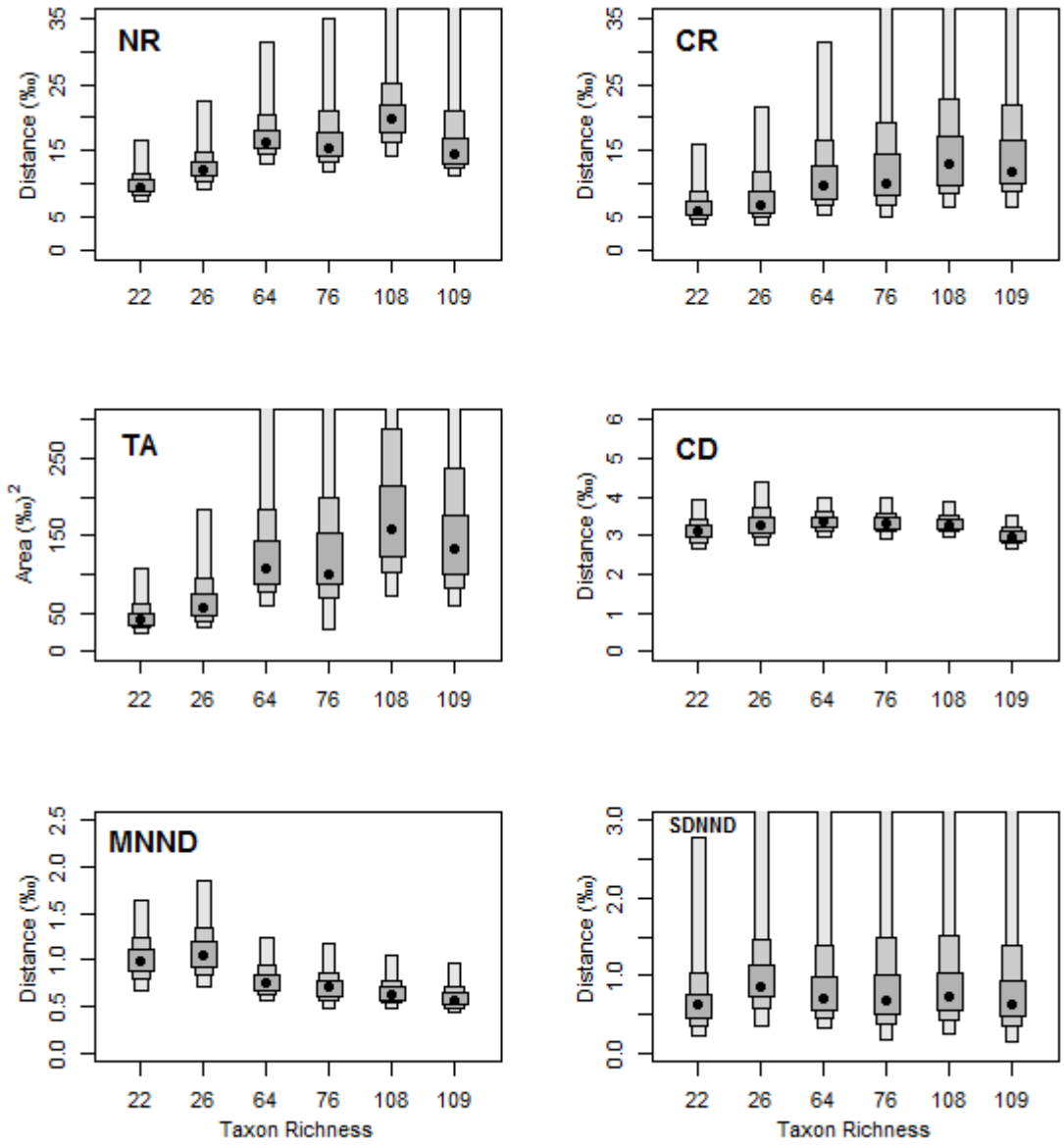

Fig 2. Biodiversity affects multiple facets of food web structure within plant and invertebrate grassland communities. Bayesian resampling of variance around mean estimates of $\delta^{15}N$ and $\delta^{13}C$ was utilised to generate estimates for each measure of structure for each community. Black dots represent mode (of mean estimates), while shaded boxes (dark to light) show 50%, 75% and 95% credible intervals for mean.

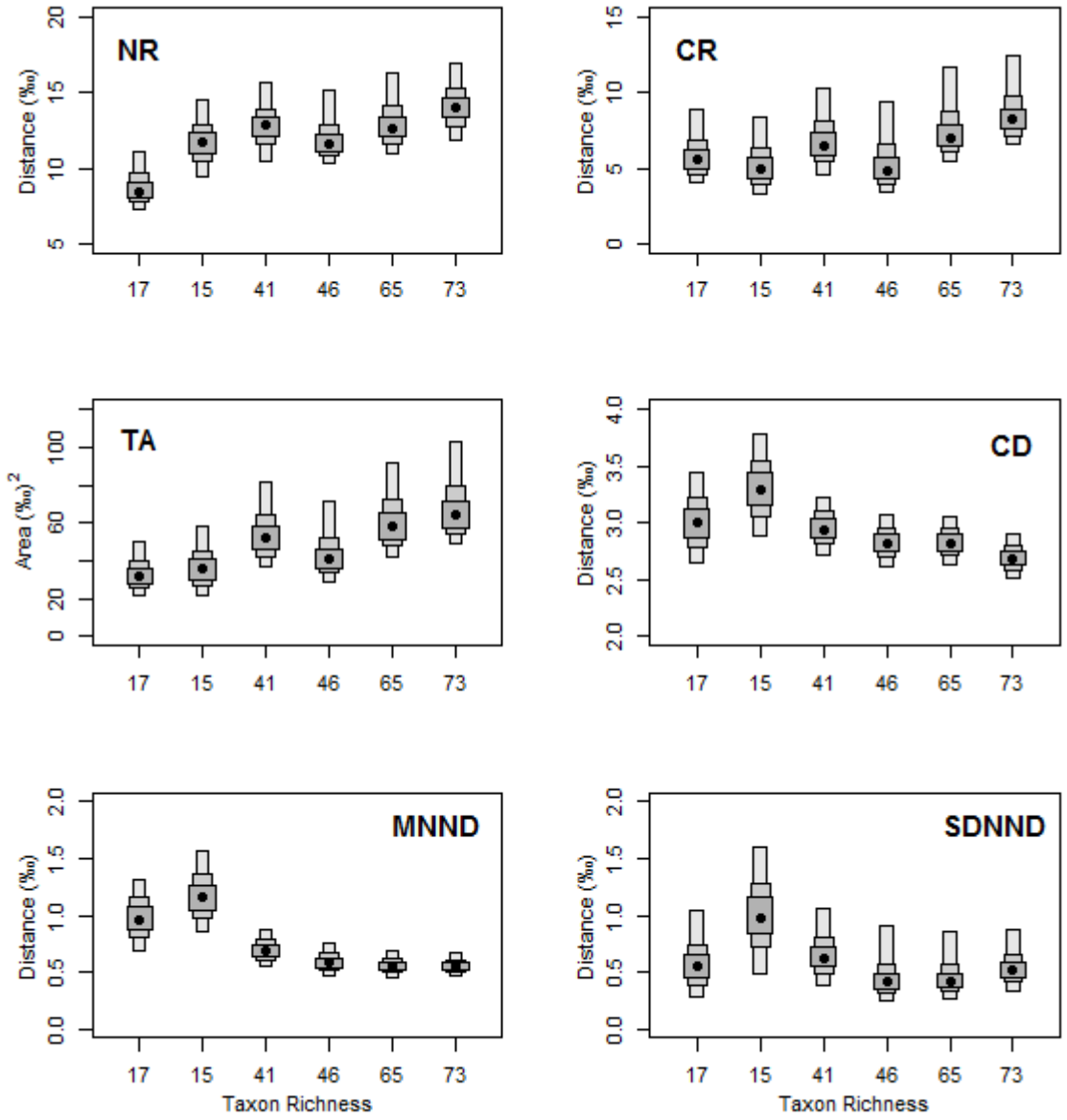

Fig 3. For subsets of ≈ 50% of taxa within each community, food web structure changes across a biodiversity gradient. Food web measures were calculated using only taxa for which at least 2 replicate $\delta^{15}N$ and $\delta^{13}C$ signatures were obtained. Modes and credible intervals calculated as in Fig 2.

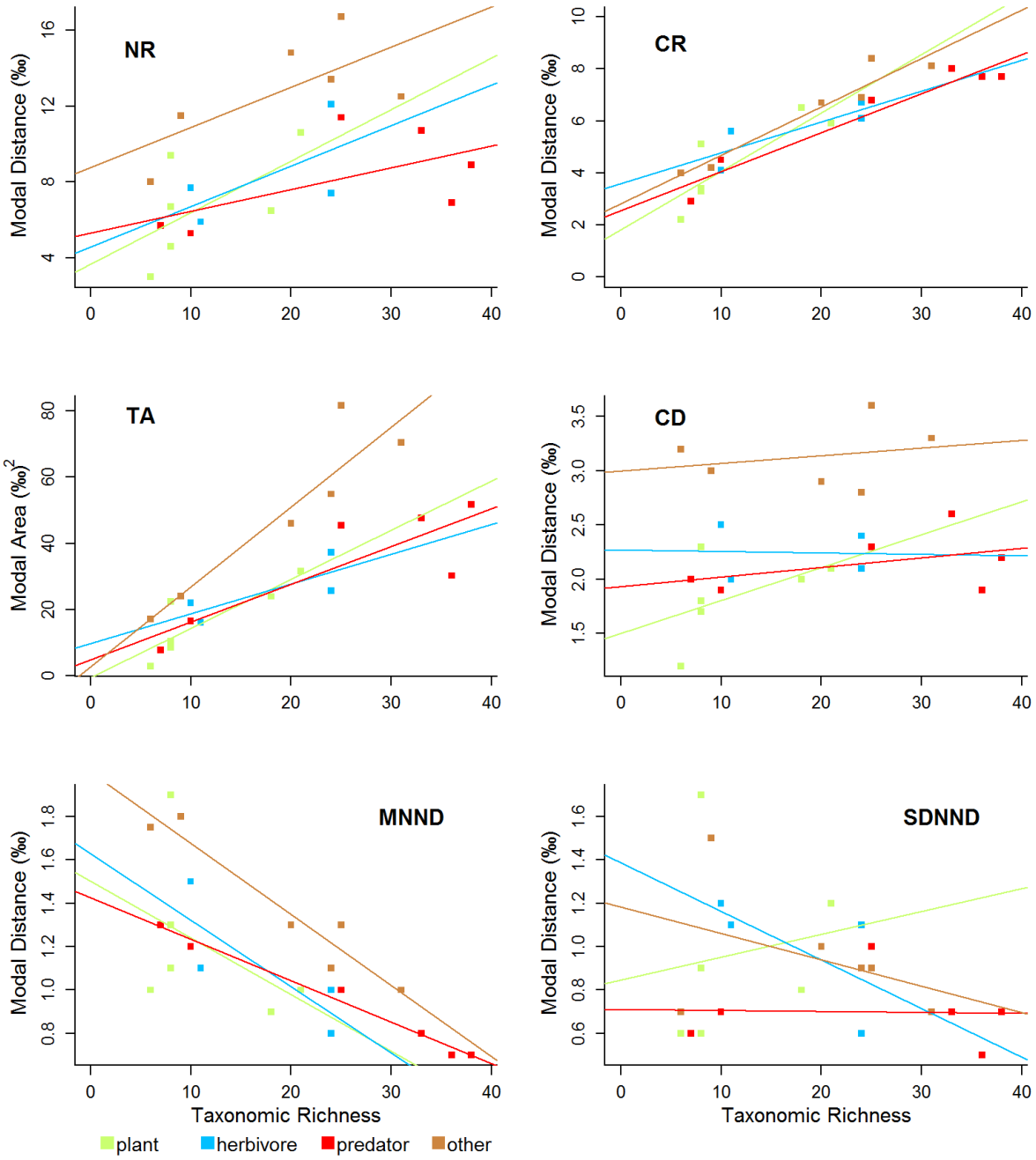

Fig 4. Changing biodiversity within trophic guilds affects multiple facets of food web structure. These effects were largely consistent between different trophic guilds. Regressions are based on modal values of Bayesian resampled mean probability estimates calculated for each measure of food web structure within each trophic guild within each community.

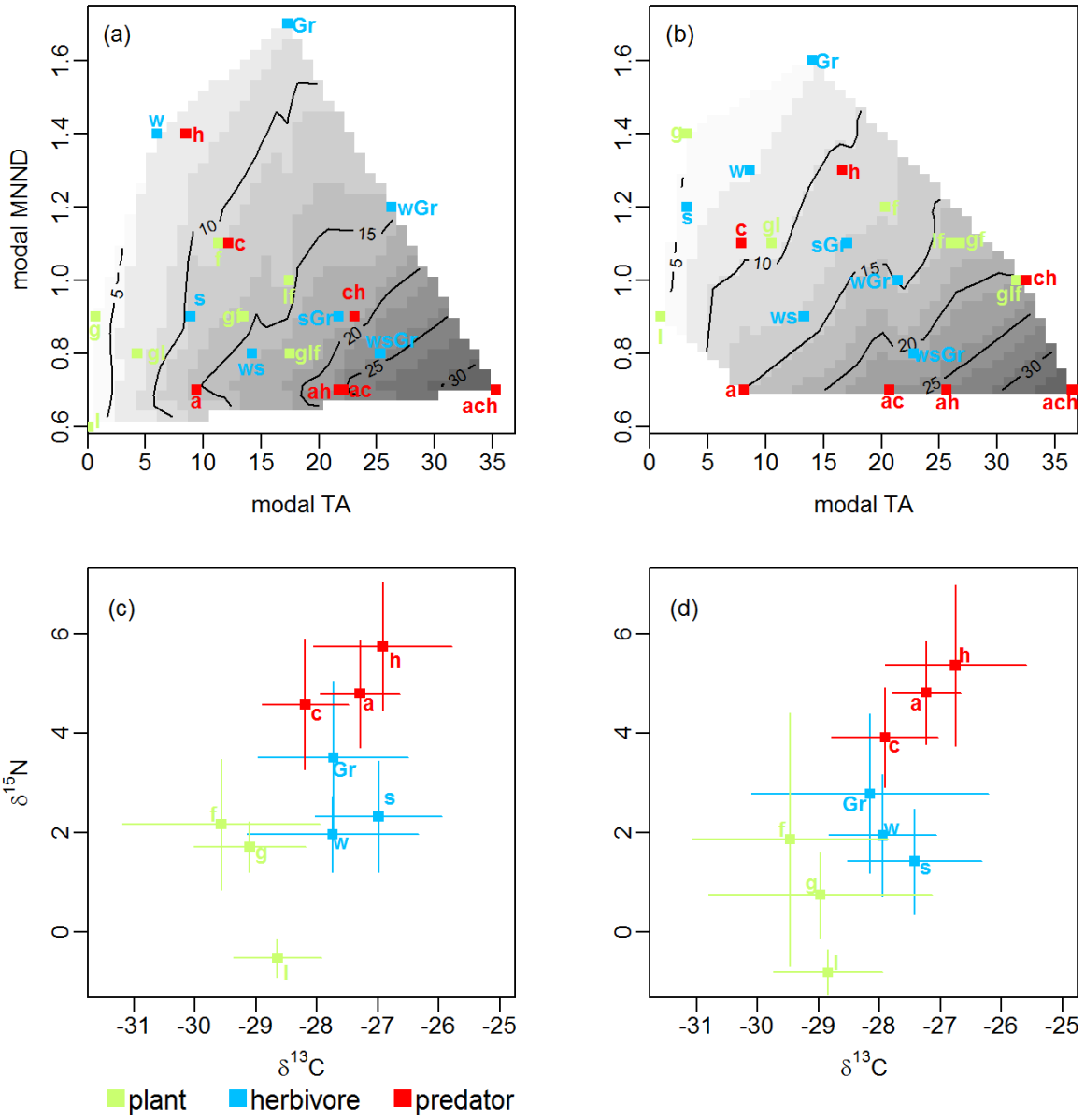

Fig 5. Functional richness affects food web structure. Increasing functional richness causes both food web expansion (increased TA) and greater functional redundancy as revealed by increased taxa niche overlap (decreased MNND). MNND and TA were calculated for functional grouping subsets of plant, herbivore and predator trophic guilds and overlaid with interpolated contours based on taxa richness values for each subset, for each of the two high diversity communities (a) and (b). Raw data (‰) are shown in (c) and (d) for each functional subset within each trophic guild for each of their above respective communities. Raw data shows overlap between functional subsets within each trophic guild, providing evidence that decreased MNND with greater functional richness in (a) and (b) infers actual overlap (not just closer proximity) between different functional groups. Plants: f=forbs, g=grass, l=legumes; Herbivores: Gr=grazers, s=sap-suckers, w=weevils; Predators: a=arachnids, c=coleopterans, h=hymenoptera.

4.5 Discussion

Utilising species-rich naturally assembled grassland communities scaling multiple trophic levels, we find strong evidence that several dimensions of food web structure change as a function of community diversity. We find that food chain length, diversity of basal resources / energy channels, overall trophic diversity and functional redundancy are all positively related to diversity, and that these effects are consistent between plant, herbivore and predator trophic guilds. In addition, we tested three predictions to identify the underlying mechanisms responsible for these diversity-structure relationships: importantly, we find good evidence that greater diversity increases both diversity of resources exploited and overlap in resources exploited, but no evidence that niches of individual taxa change as a response to diversity. We speculate that these former two mechanisms may be important in understanding how species coexist and how compensatory theory may explain greater ecosystem function with greater diversity. Crucially, we also show that across a gradient of human induced community disassembly, loss of diversity consequently affects food web structure and relative niche partitioning of resource exploitation amongst community members. We therefore suggest that understanding the effects of biodiversity loss should not be limited to ecosystem functioning but also trophic structure, and as inherently structure affects trophic interactions as well as functioning, that policy-making related to preventing habitat alteration should acknowledge that more than erosion of biodiversity is at stake.

Perhaps not surprisingly, utilising measures of taxa richness, taxa richness within trophic guilds, and functional richness within trophic guilds, we found good evidence in each instance that food web isotopic niche area increased with greater community diversity (expansions along both $\delta^{15}N$ and $\delta^{13}C$ axis and in bivariate isotopic space), showing that taxa were collectively exploiting a greater range of resources. Expansion in community niche space is likely due to greater habitat heterogeneity (as measured by increasing mean and SD of sward height; Table 1) leading to greater niche opportunities (Dimitrakopoulos & Schmid 2004; Cardinale 2011), and greater primary productivity

providing greater energy flux (as measured by dry biomass; Table 1) to consumers and thus potentially facilitating more diversity within consumer trophic levels (Borer *et al*. 2012). Expansion in $\delta^{13}C$ range (CR) with greater plant taxa richness (highlighting the utility of isotopic data to reflect resource diversity), was consistently propagated as greater CR for larger diversity values of both herbivores and predators, showing broader resource exploitation within these consumer levels. Additionally $\delta^{15}N$ range increased with community diversity, suggesting differential niche exploitation through the addition of intermediate or apex consumers (Takimoto *et al*. 2008).

We found no evidence of reduced niche for individual taxa as community taxa richness increased. This is consistent with our other findings that both community niche space and niche proximity and overlap between taxa increased. Elsewhere, species dietary niches have been shown to change as a response to levels of interspecific competition or diversity of resources. Dietary niches have been observed as restricted in the presence of interspecific competition (Bonesi *et al*. 2004) but increase in response to release from interspecific competition (Bolnick *et al*. 2010). Elsewhere, size of niche widths increased when resource diversity was greater or vice-versa (Layman *et al*. 2007a; Quevedo *et al*. 2009). However, such research has tended to focus on few populations or relatively few species; our findings take this field to the community level, with 17 taxa representing plants, herbivores and predators, covering species resource partitioning across a food web. Thus this result shows that, in grassland communities at least, increasing overlap in resource exploitation between taxa when community diversity increases does not modify isotopic niches. Given niches occupancy did not change, a potential implication of this finding is that on a gradient of resource exploitation species increasingly show resource coexistence (greater overlap) with greater community diversity. Additionally, this result raises the speculation that niche plasticity maybe dependent upon trophic position; our analysis contained many producers and herbivores, yet previous studies demonstrating niche shifts have focused on consumers (Bonesi *et al*. 2004; Layman *et al*. 2007a; Quevedo *et al*. 2009; Bolnick *et al*. 2010). It is also worth noting though, that conversely to such speculation

regarding coexistence or niche plasticity, it may be possible niche widths of taxa in our study did not change if resources were plentiful enough that many species were not in competition (DuBowy 1988). Thus in other systems or at different spatial or temporal scales niche of individual taxa may respond differently to diversity if resource thresholds for density-dependent competition are reached.

For taxa richness, taxa richness within trophic guilds and functional richness within trophic guilds, we found strong evidence in each instance that community mean nearest neighbour distance (MNND) decreased with increasing diversity, revealing that taxa increasingly exploit the same resources at higher diversity levels. Given raw data showed definite overlap between differing taxa on both $\delta^{15}N$ and $\delta^{13}C$ axis, we can be sure in this instance that increased MNND with diversity infers actual overlap and not just increased proximity in the niche of neighbouring taxa. Whilst we acknowledge that isotopic niche overlap between consumers may occur by chance due to isotopic similarity when biological resources are actually different, this is only likely to be the case in a few instances. We demonstrate a relationship between increased overlap with increased diversity using large numbers of taxa, which would be highly unlikely unless consumers were feeding on common resources in most instances.

Given that individual taxa niches did not change with diversity, this suggests such decreased proximity is the consequence of the addition of more species to already occupied niche space in less disturbed grasslands; or viewed the other way around, that loss of diversity due to human disturbance results in less overlap in resource exploitation between taxa. This is concurrent with (Laliberte *et al*. 2010) who showed plant functional redundancy was reduced by land use intensification; insights from our findings show this relationship extends across producer, herbivore and predator trophic groups. We were also able to confirm that increased niche overlap was not just the result of additional species *per se*, but was at least partly due to increased overlap between species of the same functional groupings (pre-assigned based on phylogenetic and feeding mode

similarity). Thus these findings provide empirical evidence of a potentially important mechanism for understanding how with greater diversity, the same resources may be more intensely exploited through greater functional redundancy.

Our results show how relative niche partitioning at a community level is affected by diversity, and we have good evidence that indicates two ways that niche partitioning changes, as quantified by changes in food web structure; greater exploitation of different resources (extensive) and greater exploitation of the same resources (intensive). These 'extensive and intensive' mechanisms of resource exploitation are likely to be particularly important in understanding coexistence between species, and complimentary theory in biodiversity-ecosystem function relationships (Hooper *et al*. 2005). Extensive resource exploitation obviously infers species coexist through exploiting different resources, as shown extensively elsewhere (e.g. McKane *et al*. 2002). Conversely, intensive resource exploitation infers interspecific coexistence despite resource overlap on the same dietary axis. Such coexistence may be sustained through population-limiting factors on other biotic and abiotic niche axes (Leibold 1995; Cardinale 2011). Dominant gradients affecting habitat structure and niche partitioning in our study included sward heterogeneity (Woodcock *et al*. 2009). We speculate increased habitat complexity provided additional niches for species exploiting similar resources, thus acting to structure community diversity through altering which resource gradients species or conspecifics may compete on.

In showing how niche partitioning changes at a community level as a response to diversity, our results also provide evidence of potential mechanisms underpinning biodiversity – ecosystem function relationships. Where greater diversity promotes greater functionality this may be because species effects on resource exploitation are either cumulative, such that each taxa is either exploiting different resources, or complimentary, whereby species exploit the same resources often under different circumstances, with both cumulative and complimentary effects contributing overall to more thorough / efficient physio-chemical processes (Hooper *et al*. 2005; Cardinale 2011). Rarely

though have these mechanisms been tested (Kremen 2005). Importantly our results show that greater niche diversity as quantified by more extensive resource exploitation, and greater niche overlap as quantified by more intensive resource exploitation, are both features of higher community diversity, providing for potential underlying mechanisms for cumulative and compensatory (respectively) effects to explain how diversity regulates rates of ecosystem function.

Our results also showed community diversity affected multiple dimensions of food web structure, which has important implications for ecosystem functioning (Cardinale 2011; Thompson *et al*. 2012), extinctions and invasions (Dunne *et al*. 2002; Srivastava & Bell 2009) human induced habitat alteration (Melian & Bascompte 2002) and climatic change (Petchey *et al*. 1999; Hansson *et al*. 2013), amongst others. Food chain length (NR) increased with diversity, which infers important consequences for bioaccumulation (Post 2002a), ecosystem function and top-down trophic structuring (Post 2002a; Estes *et al*. 2011). Similarly, greater resource breadth (CR) as a function of greater diversity has important implications for energy flow across trophic levels (McCann & Rooney 2009; Rooney & McCann 2012) and consequently stability of trophic structures and associated functions (Settle *et al*. 1996; Rooney & McCann 2012). As previously described, greater niche diversity and extensive resource exploitation (increased TA), and greater functional redundancy and intensive resource exploitation (decreased MNND) as caused by increasing diversity, also have important impacts on community stability and functioning (Zavaleta *et al*. 2010; Devoto *et al*. 2012). Thus a key finding of this study is that human alteration of natural food webs leads to community disassembly which significantly affects multiple dimensions of structure. As described, this is likely to have important implications for the functioning of biological communities, and therefore preservation of natural trophic structure, in addition to biodiversity, should be a priority for conservation and management of living resources.

Utilising novel application of stable isotope techniques we have shown that multiple dimensions of food web structure are significantly impacted by loss of biodiversity as caused by human habitat alteration. Importantly we have also shown how diversity affects food web structure

and community niche partitioning amongst taxa, and in doing so have empirically demonstrated that both niche diversity and niche overlap increase as a function of increasing diversity. These results are likely important for demonstrating potential mechanisms to explain compensatory theory underlying more efficient ecosystem functioning with greater biodiversity. As an aside we also advocate the continued development of stable isotope techniques for the study of food web structure and suggest the application of isotopic techniques would be likely suitable to empirically test a broad range of community ecology theory.

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CHAPTER 5

Intensification of grassland management affects food web compartmental structure and predator feeding habits

5.1 Abstract

Mesotrophic grasslands form extensive habitats throughout Europe but have suffered significant declines in plant and invertebrate biodiversity and abundance through agricultural intensification. It is currently understudied how intensification affects food web structure, particularly the differing energy pathways that comprise structure, and how this may affect predator feeding habits, which are of importance as structure - predator foraging dynamics have been linked to food web stability, function and services. We quantified above-ground plant and invertebrate species richness, abundance (biomass), and sward architecture within intensively and extensively grazed grasslands. Additionally, stable isotope ratios of nitrogen and carbon were used to estimate trophic positions and dietary composition for a suite of generalist invertebrate predators (Linyphiidae, Carabidae, Staphylinidae and Lycosoidae). Specifically, we examine how food web compartmental structure influences in what manner predators may feed on herbivore and detritivore prey. Intensification was associated with reduced sward heterogeneity, and lower plant and invertebrate diversity and biomasses. Extensive grasslands had more diverse and abundant herbivore communities, whilst intensively managed grasslands had diminished herbivore structure and lower herbivore to detritivore biomass. Relative trophic positions of predators in intensive grasslands were lower than extensive counterparts, corresponding to different prey sources. This concurred with dietary estimates for predators showing relatively more detritivore than herbivore prey for predators in intensive grasslands but relatively more herbivore than detritivore prey for predators in extensive grasslands. Using species-rich naturally assembled communities spanning multiple trophic levels we show intensification of grassland management reduced sward heterogeneity leading to trophic collapse of herbivore food web compartments. Simplification of prey base was associated with greater predator reliance on detritivore energy pathways. Importantly, the findings of this study have landscape-scale implications given that predator coupling of alternate food chain compartments is implicated in food web stability, services and functions, yet our results suggest a significant simplification of these interactions in intensively grazed pastures. We conclude that

future management strategies aimed at mitigating biodiversity losses within agricultural landscapes should include actions that increase trophic complexity as standard practice, with particular focus given to restoring food web compartmental structure and natural predator dynamics.

5.2 Introduction

Globally, intensification of agriculture is a major threat to biodiversity, with continued encroachment upon natural and semi-natural habitats (Tilman *et al*. 2001; Phalan *et al*. 2011) causing serious declines in biodiversity and species' abundances (Kleijn *et al*. 2011). Specific farming practices associated with intensification have been studied as potential drivers of declines in biodiversity, including greater pesticide application (Hodgson *et al*. 2010), intensified grazing (Kruess & Tscharntke 2002; Potts *et al*. 2009) and increased inorganic fertiliser inputs (Woodcock *et al*. 2007; Kleijn *et al*. 2009). The effects of multivariate farming practices such as these interact strongly and can therefore be considered collectively as agents of intensification, rather than as individual effects (Benton *et al*. 2003). Within north-western Europe where such intensification is particularly acute (Kleijn *et al*. 2009), stewardship schemes on farms aim to enhance biodiversity and abundance of targeted organisms such as plants (Walker *et al*. 2007), birds (Davey *et al*. 2010), beetles (Woodcock *et al*. 2007) and pollinators (Potts *et al*. 2009) through specific management strategies. Subsequently, the focus of much research has concerned both understanding how intensification modifies biodiversity and abundance, in addition to quantifying the effectiveness of such management and mitigation strategies for reversing ecological degradation (Kleijn & Sutherland 2003; Kleijn *et al*. 2011 and references therein for both). Additionally, however, loss of biodiversity through agricultural intensification acts to alter food web structure (Macfadyen *et al*. 2009; Crowder *et al*. 2010), and consequently trophic interactions (Macfadyen *et al*. 2009; Crowder *et al*. 2010), though to date much less research has focused on effects of intensification on trophic structure and dynamics. Food web structure and trophic interactions are of underlying importance in explaining patterns of diversity we observe in nature (Rooney & McCann 2012), how diversity regulates

ecosystem functions and services (Bullock *et al*. 2006; Cardinale 2011; Thompson *et al*. 2012), consequences of extinctions and invasions (Dunne *et al*. 2002; Srivastava & Bell 2009) and how communities are likely to respond to human induced habitat alteration (Schindler *et al*. 2010) and climatic change (Petchey *et al*. 1999; Hansson *et al*. 2013). The nature of trophic dynamics that underlie structure can interact to influence overall food web stability, such that food webs with greater connectance (Dunne *et al*. 2002) or functional diversity (Devoto *et al*. 2012) may have improved robustness to secondary extinctions following perturbation. In this respect, deterioration of structure itself may lead to consequential further biodiversity losses. Similarly, food web structure may influence ecosystem functions and services, for example with niche diversity of community members enhancing productivity (Zavaleta *et al*. 2010; Cardinale 2011), or via structure influencing patterns of energy flux, such as enhancement of predator populations through energy subsidy leading to greater productivity through pest control (Settle *et al*. 1996; Bell *et al*. 2008). Therefore discerning how intensified farming practices modify natural community structure and dynamics is paramount in understanding potential impacts on ecosystem resilience, function and services, and a prerequisite to developing mitigation strategies that go beyond biodiversity and abundance metrics of certain target species.

One important aspect of food web structure is the arrangement of component food chains, or compartments, that form relatively discrete energy pathways within a larger food web (Rooney & McCann 2012). For instance, detrital (brown) and autotrophic (green) energy sources have been characterised as habitat compartments within a larger food web that may each contain myriad trophic connections but relatively fewer that couple the compartments (Scheu 2001; Kardol & Wardle 2010). Characterisation of such compartments has been important in developing theoretical understanding of the potentially disproportionate effects on food web structure of species whose trophic interactions couple different compartments, mediating the transfer of energy throughout the larger food web (Scheu 2001; van Veen *et al*. 2008; McCann & Rooney, 2009). Coupling effects maybe exerted through bottom-up processes, for example green energy subsidies to brown

compartments via dead / dying organisms (Kardol & Wardle, 2010), or top-down processes, such as detritivore remobilisation of nutrients to plants (Scheu 2001), and generalist secondary consumers coupling green and brown energy pathways through feeding on prey from both (Settle *et al*. 1996; Bell *et al*. 2008). Predator mediated coupling is considered particularly important given generalist predators mobile and adaptable foraging can couple spatially and temporally distinct energy pathways (McCann & Rooney 2009; Rooney & McCann 2012). As there is an inherent asymmetry in the interaction strengths and turnover speeds of biomass between different energy pathways, such predator coupling has the important effect of dampening variability from perturbations in both the predator populations (via the opportunity to switch among alternate prey) and prey populations (predation rates fall as predators move to consuming other prey), conferring stability to the overall food web (McCann & Rooney 2009; Rooney & McCann 2012). Thus the compartmental structure of food webs and the trophic interactions of predators have important implications for the stability and functioning of communities.

Theoretical and empirical evidence from studying the effects of agricultural intensification on biodiversity and abundance suggests intensification should act to homogenise and thus simplify food web structure (Benton *et al*. 2003; McCann & Rooney 2009). However, the manner in which intensification may influence how predators might couple food web compartments remains understudied and poorly understood, particularly in wild species-rich communities (Thies *et al*. 2011). To date, manipulative experimental studies within agricultural systems (generally using small sets of species) have tested the importance of trophic coupling interactions between generalist invertebrate predators and different food web compartments, demonstrating either relatively weak coupling (Halaj & Wise 2002; von Berg *et al*. 2010), or demonstrating strong coupling driving trophic cascades that affect overall food web structure (Settle *et al*. 1996; Bell *et al*. 2008; Birkhofer *et al*. 2008) and ecosystem services (Settle *et al*. 1996; Bell *et al*. 2008). Thus these studies show the interplay of generalist predator feeding between compartments is spatially (von Berg *et al*. 2010) and temporally (Halaj & Wise 2002) variable, and likely reflects a complexity of environmental

factors (Halaj & Wise 2002; von Berg *et al*. 2009). It would therefore be beneficial to empirically determine how the relationship between food chain compartments and generalist predator feeding habits is affected by agricultural intensification in natural species-rich communities, as a necessity for developing our understanding of the likely impacts of present and future farming practices on food web dynamics.

In this study we examine how agricultural intensification may affect the trophic positions and feeding patterns of generalist invertebrate predators and link this to intensification effects on habitat heterogeneity and green and brown food chain compartments, within mesotrophic grassland communities. Grasslands form an important component of the European agricultural landscape but have suffered from significant agricultural intensification (Blackstock *et al*. 1999) and subsequent losses of biodiversity and abundances over past decades (Kleijn & Sutherland 2003 and references therein). In addition to supporting large numbers of declining species and being subject to sustained agri-conservation schemes which makes them worthy of study in their own right, relationships between agricultural intensity and grassland (sward) heterogeneity, and in turn sward heterogeneity and biodiversity, are well understood (Woodcock *et al*. 2009). Thus, grasslands provide an excellent opportunity to test the effects of intensification on food chain compartments and predator feeding habits. We use stable isotope ratios of nitrogen (N^{15} : N^{14}, termed $\delta^{15}N$) and carbon (C^{13} : C^{12}, termed $\delta^{13}C$) in consumer and prey tissues to determine the feeding habits of generalist invertebrate predators. $\delta^{15}N$ and $\delta^{13}C$ in consumer tissues can be utilised to provide a temporally and spatially integrated construct of trophic niche (Bearhop *et al*. 2004; Newsome *et al*. 2007; Layman *et al*. 2011), as $\delta^{15}N$ and $\delta^{13}C$ of consumer proteins reflect the proteins of their food sources (DeNiro & Epstein 1981; 1978, respectively). Typically, enrichment in $\delta^{15}N$ of between 2.5‰ and 3.4‰ is observed from diet to consumer (Post 2002; Vanderklift & Ponsard 2003; Caut *et al*. 2009), allowing for determination of an organism's trophic level (Vander Zanden *et al*. 1997; Vander Zanden & Rasmussen 1999; Post 2002) and overall food chain length (Cabana & Rasmussen 1996; Vander Zanden & Fetzer 2007). Conversely, typical enrichment in $\delta^{13}C$ is much smaller between diet and

consumer (Post 2002; Caut *et al*. 2009), and because basal sources often differ in their $\delta^{13}C$ values, $\delta^{13}C$ can be utilised to trace prey – consumer interactions or food chains (Post 2002). Thus the use of $\delta^{15}N$, after correction for differences in $\delta^{15}N$ baseline between communities, can be used to estimate predator trophic positions allowing comparisons between communities (Post 2002; Takimoto *et al*. 2008). Similarly, knowing prey isotopic signatures and accounting for enrichment factors allows likelihood of prey items in consumer diets to be estimated using mixing models (Inger *et al*. 2010).

We evaluate the impacts of agricultural intensification on mesotrophic grasslands through comparison of intensive and extensive cattle-grazed grassland systems. Initially we quantify biodiversity, abundance and habitat heterogeneity, as is typical for such a study. We then additionally utilise $\delta^{15}N$ and $\delta^{13}C$ to estimate trophic positions for generalist grassland invertebrate predators, and 'green' and 'brown' dietary contributions, in order to test how compartmental food web structure and subsequent predatory feeding habits are affected by intensification.

5.3 Methods

During July and August 2011 we sampled plant and invertebrate community food web structure within intensively and extensively grazed mesotrophic grasslands at Rothamsted Research farm, North Wyke, Devon, UK. For both intensive and extensive grasslands, we quantified community plant and invertebrate richness and biomass. We additionally sampled stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ in each plant and invertebrate species collected, as well as for a suite of ground-dwelling generalist invertebrate predators. This sampling was repeated in each of two fields for both grassland treatments, thus providing 4 independent communities for analysis. Intensive grasslands were representative of "conventional" beef-cattle grazing systems maximising profit and production. They were characterised by intensive grazing, twice yearly farmyard manure applications, and were classified as MG7 on the UK national vegetation classification (NVC) being dominated by the grass species *Lolium perenne*, and secondarily *Holcus lanatus* and *Phleum pratense*, with lesser amounts of legume *Trifolium repens*. Extensive grasslands were representative of higher level stewardship agri-

conservation schemes aimed at enhancing biodiversity, and were characterised by extensive grazing, no fertilizer applications, and were classed as MG5 on the NVC, having functionally diverse plant communities composed of grasses, legumes and forbs. All four fields were previously maintained as continuous grassland (no ploughing) for >10 years and were located within 0.5km of each other and shared the same soil type. The two extensive fields were smaller (both 1.2 ha) compared with the two intensive fields (5ha and 9ha). Though greater ecosystem size can positively affect diversity (Post *et al*. 2000; Takimoto *et al*. 2008), or conversely smaller field diversity can potentially be increased by greater edge effects and surrounding landscape heterogeneity (Benton *et al*. 2003), we would suggest this was not a significant problem for our study. Our within-field plots were located a minimum distance (>15m) from field boundaries which was equal between fields, and all fields were close enough together to share common landscape features and colonisation distances, such that diversity we sampled likely reflects what lives within the grasslands and not transient or source-sink dynamics of surrounding habitats.

Field Sampling

Within each field, a 15m^2 plot was established in which all sampling was conducted. Plant species richness was assessed using 3 x 1m^2 quadrats combined with 5-minute searches of the wider plot area for rarer species. All plants were identified to species *in situ* and samples collected and frozen (-20°C) within 2 hours for later stable isotope analysis (SIA). Plant biomass was quantified by cutting randomly placed 5 x 1m vegetation strips of diameter 7.5cm (total area 0.375m^2), replicated twice at different locations in the plot, and weighing (g) after drying at 45°C for >5 days. Suction sampling was used to destructively sample invertebrate species richness, and was conducted on dry days between 10:00 and 16:00. 55 replicate suction samples (16 seconds each) were taken from different locations within each plot. This has been shown to be an effective methodology for sampling canopy and ground dwelling species (Brook *et al*. 2008). All invertebrates were collected in distilled water and frozen (-20°C) within 2 hours. Invertebrates were subsequently sorted in the laboratory and

identified to species where possible, or genus if not. Morpho-species was used for Cicadellidae, Parasitica, Aranea and many Diptera families. Taxonomic identification and classification was consistent among communities. Given that a mixture of species, morpho-species and genus level identification was used, we hereafter refer to invertebrate diversity as invertebrate taxa richness. All invertebrates were dried at 45°C for >48 hours and weighed to provide biomass (mg) per taxon prior to preparation for SIA. Within each plot, we also sampled 4 ground-dwelling generalist predators, classified at a family level; Linyphiidae, Carabidae, Staphylinidae and Lycosidae. Sampling at a family level was appropriate in order to test broadly how predator guilds may feed differentially dependent upon grassland type. Linyphiidae were taken from suction samples (all identified morpho-species were pooled); Carabidae (comprising three species: *Nebria brevicollis***,** *Pterostichus melanarius***,** *Poecilus cupreus*), Staphylinidae (of the genus *Philonthus*) and Lycosidae (e.g. *Pardosa*) were collected independently using pitfall traps to ensure enough samples. Traps contained distilled water and were inspected every 2 days and trapped animals frozen (-20°C). Subsequently, predators were dried at 45°C for >48hrs prior to SIA preparation. For each plot, seeds collected from suction samples were pooled, dried at 45°C for >5 days, and weighed to provide biomass (mg) values. In each plot, sward architecture was assessed with 30 sward stick measurements taken at random to provide sward height (mean) and sward heterogeneity (standard deviation).

Sample Preparation & Lipid Extraction

All SIA plant samples were dried at 45°C for >5 days. Singular leaves per plant were used for a single SIA sample. For all invertebrates, generally between 1 and 10 whole individuals were pooled to provide enough mass for a single isotope sample, though upwards of 30 were used for a small number of taxa. Where individuals were bigger than the needed sample mass, individuals were homogenised whole, and a sub-sample taken. For snails, a section of foot was used. Though previous work has shown that within individual invertebrate tissue selection can be important (Perkins *et al*. 2013), samples were too numerous and often too small to accomplish dissection. However, the

noise this may introduce is likely to be small when spread across such species-rich communities, and more importantly is unlikely to vary among communities, which is what we were interested in comparing.

To accurately estimate $\delta^{13}C$ of proteins within individuals it is accepted practice to first remove free-lipid contained within. Lipid is naturally depleted in $\delta^{13}C$ (DeNiro & Epstein 1977) and its concentration varies between tissues, individuals and species. To account for this we conducted lipid extraction on a sub-set of samples and then applied a mathematical correction to all our samples based at either Order or Family level. For samples undergoing lipid extraction, tissue was immersed in 2:1 Chloroform: Methanol solution for 50 minutes to remove free-lipid, and then left to air dry.

Stable Isotope Analysis

For all samples, 0.35mg ± 0.05 (invertebrates) or 3mg ± 0.1 (plants) dried material was enclosed in tin capsules. Stable isotope analysis was conducted at the Food and Environment Research Agency, York, UK. Samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ in a Fisons EA1108 elemental analyser (Carlo Erba Instruments, Milan, Italy), coupled with an Isoprime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Stable isotope ratios are reported in delta (δ) notation where $\delta^{15}N$ and $\delta^{13}C = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Isotope ratios are expressed in per mil (‰) relative to the ratio of international reference standards ($R_{standard}$) which are Atmospheric Nitrogen and Vienna PeeDee Belemnite (VPDB) for nitrogen and carbon respectively. Measures of standards placed throughout invertebrate samples exhibited acceptable instrument reproducibility of < 0.10‰ (SD) for $\delta^{15}N$ and < 0.11‰ (SD) for $\delta^{13}C$ using collagen standard and insect whole tissue standard (cockroach; *Nauphoeta cinerea*); and placed amongst plant samples < 0.09‰ (SD) for $\delta^{15}N$ and < 0.11‰ (SD) for $\delta^{13}C$ using tomato (*Solanum lycopersicum*) and wheat (*Triticum aestivum*) standards.

Data Analysis

Predator trophic levels

Initially we investigated how relative trophic levels of generalist predators differed between intensive and extensive grasslands which required firstly estimating $\delta^{15}N$ for predators. For each predator sample within each community, *plant-baseline corrected* $\delta^{15}N$ was calculated by subtracting mean $\delta^{15}N$ of vegetation (based on all plant species present) from each predators raw $\delta^{15}N$. Similarly, *detritivore-baseline corrected* $\delta^{15}N$ was calculated by subtracting mean detritivore $\delta^{15}N$ from each predator sample within each community. In each community, mean detritivore $\delta^{15}N$ was calculated using equal replicates of the three Collembola orders Symphypleona, Entomobryomorpha and Poduromorpha. Initial analyses used General Linear Mixed Models (GLMM) to test how the explanatory variables *grassland type* (levels = intensive or extensive) and *predator family* (levels = Linyphiidae, Carabidae, Staphylinidae or Lycosidae) and their interaction affected either *plant-baseline corrected* $\delta^{15}N$ or *detritivore-baseline corrected* $\delta^{15}N$ of predators. To produce the simple two level factor for grassland type (intensive or extensive), predators were pooled from the 2 communities within each grassland type. The random effect *community* was used in each model to account for variance attributable to non-independence of individual replicates within and between predator families that were collected within the same communities. An analogous third GLMM compared predator $\delta^{15}N$ using *detritivore-baseline corrected* $\delta^{15}N$ for intensive grassland predators but *plant-baseline corrected* $\delta^{15}N$ for extensive grassland predators.

Community motif analysis

To determine how food web structure may differ between communities, within each community we pooled a motif of 12 species common to all communities into trophic guilds, and compared relative trophic positions of trophic guilds between communities using $\delta^{15}N$. We analysed this using a GLMM to test how the response variable $\delta^{15}N$ of motif species was affected by the interaction of the

explanatory variables *grassland type* (levels = intensive or extensive) and *trophic guild* (levels = plant, detritivore, herbivore or predator). An interaction would indicate that food web structure of trophic guilds differed between grassland types. The random effect *community* accounted for variance attributable to non-independence of motif species within the same communities, while the random effect *taxa* accounted for differences in $\delta^{15}N$ between species within trophic guilds. Motif taxa were: 3 plants (grass genus *Lolium* and *Agrostis*, the legume *Trifolium*), 3 detritivores (3 orders of Collembola: Symphypleona, Entomobryomorpha and Poduromorpha), 2 herbivores (leafhopper family Cicadellidae, and the beetle genus *Sitonia*) and 4 predators (Linyphiidae, Carabidae, Staphylinidae and Lycosidae).

Green and brown food chains

We used a simple GLM to test how relative herbivorous "green" and detritivorous "brown" biomass differed between grassland types. The response *biomass* was tested against the interaction of the explanatory variables *grassland type* (levels = intensive or extensive) and *trophic guild* (levels = herbivore or detritivore). An interaction would indicate relative differences in the green and brown potential prey availability between grassland types.

All analyses were conducted using R version 2.14.1 (R Development Core Team, 2011). For GLMMs we used 'lmer' from the *lme4* package (Bates *et al*. 2011). As shown to be important in estimating each models goodness-of-fit to the data for each GLMM we used estimates of pseudo R^2 (Nakagawa & Schielzeth 2013) using the Naglekerke statistic.

Predator diet analysis

Dietary estimates for each predator were made using stable isotope mixing models in the R package SIAR (Parnell *et al*. 2008; 2010), in which Bayesian resampling of variance in estimates of dietary

source $\delta^{15}N$ and $\delta^{13}C$ was propagated as quantified uncertainty in probability distributions of posterior estimates of mean proportional contribution of each prey item to diet. Estimates of trophic discrimination factors used in SIAR models were 2.4 ± 0.4 (SD) for $\delta^{15}N$ and 0.19 ± 0.25 (SD) for $\delta^{13}C$. These were average values based on 8 estimates for $\delta^{15}N$ and 7 for $\delta^{13}C$ reported in the literature and representing laboratory trials of terrestrial predatory arthropods (Ostrom *et al*. 1997; Oelbermann & Scheu 2002; Wise *et al*. 2006; Perkins *et al*. unpublished data).

5.4 Results

Traditional Community Assessment

As expected of intensive grassland management, intensively farmed beef pastures had lower average sward height, less sward heterogeneity, lower plant species richness and biomass, and much lower seed-set than extensive grasslands (Table 1). Invertebrate species richness and biomass were also lower in the intensive than extensive grasslands, and had fewer herbivore species (Table 1).

Predator Trophic Levels

Using a GLMM, we found *plant-baseline corrected* $\delta^{15}N$ of predators to be significantly affected by the main effects *grassland type* and *predator family* ($\chi^2_{(1)} = 12.53$, $p < 0.001$; and $\chi^2_{(3)} = 150.44$, $p < 0.001$, respectively). There was no significant interaction term ($\chi^2_{(3)} = 7.11$, $p = 0.07$). Surprisingly (because with higher diversity we predict a greater number of trophic links), within each predator family, predators in intensive grasslands had significantly higher $\delta^{15}N$ than predators in extensive grasslands, except Lycosidae which showed no difference (Fig. 1a). Significant differences in $\delta^{15}N$ among predator families within a community were consistent across grassland types (Fig. 1a), and indicated that different predator families likely fed at different trophic levels. An estimate of the model fit to the data was good: Nagelkerke = 0.50.

A second analysis where $\delta^{15}N$ of predators was corrected to a detritivore $\delta^{15}N$ baseline, showed that *detritivore-baseline corrected $\delta^{15}N$* was also significantly affected by the main effects *grassland type* and *predator family* ($\chi^2_{(1)} = 4.85$, $p < 0.03$; and $\chi^2_{(3)} = 148.77$, $p < 0.001$, respectively). Again there was no significant interaction term ($\chi^2_{(3)} = 6.20$, $p = 0.10$). Conversely to the previous green food chain result however, predator $\delta^{15}N$ was greater in extensive grasslands than intensive grasslands for each predator family (Fig. 1b), thus suggesting predators fed differentially between the grassland types. Predator $\delta^{15}N$ differed between predator families as before, though additionally Lycosidae also reflected this pattern (Fig. 1b). An estimate of the model fit to the data was also good: Nagelkerke = 0.48.

Community Motif

In order to identify why green and brown $\delta^{15}N$ baselines gave such contrasting results for predators, and determine which was the most appropriate to use, we examined the raw $\delta^{15}N$ and $\delta^{13}C$ for a motif of taxa spanning plant, detritivore, herbivore and predator trophic guilds that were common to all communities. Using a GLMM, we found $\delta^{15}N$ was affected by a significant interaction between *grassland type* and *trophic guild* ($\chi^2_{(3)} = 33.76$, $p < 0.001$) providing good evidence that $\delta^{15}N$ structure of trophic guilds differed between grassland types. An estimate of the model fit to the data was very good: Nagelkerke = 0.78. We observed good graphical evidence supporting this analysis, showing that the brown detritivore food chain had unexpectedly elevated $\delta^{15}N$ values in the intensive grasslands relative to detritivores in the extensive grasslands (Fig. 2). Notably, whilst plants and herbivores showed similar $\delta^{15}N$ values across grassland types, elevated $\delta^{15}N$ in detritivores were matched by elevated $\delta^{15}N$ for all predators within the intensive grasslands (Fig. 2). This strongly suggested that the brown food chain was an important dietary source for predators in these communities. In addition, predator $\delta^{15}N$ was >5‰ enriched relative to herbivores in intensive grasslands (Fig. 2) which, assuming typical enrichment of 2.4 ± 0.4 (SD) in $\delta^{15}N$ from prey to

predator, infers sampled herbivores could not have contributed significantly to predator diet otherwise predator $\delta^{15}N$ would have been much lower than observed. This strongly supports the result that predators feed differentially between grassland types; if we accept *detritivore-baseline corrected* $\delta^{15}N$ for the predators in the extensive communities then they have higher $\delta^{15}N$ than predators in the intensive communities (Fig. 1b). Alternately, and for the sake of being conservative, in an alternate GLMM where predators from intensive grasslands use *detritivore-baseline corrected* $\delta^{15}N$ but predators from extensive grasslands use *plant-baseline corrected* $\delta^{15}N$, the main effect of *grassland type* was not significant ($\chi^2_{(1)} = 1.49$, $p = 0.22$) which, given predators in extensive communities were modelled against a lower trophic level (plants), intrinsically suggests predators fed on different parts of the food web between grassland types. As before, the other main effect of *predator family* was significant ($\chi^2_{(3)} = 148.83$, $p < 0.001$) but the interaction was not ($\chi^2_{(3)} = 5.99$, $p = 0.11$). An estimate of the model fit to the data was good: Nagelkerke = 0.47.

Green vs Brown Food Chains

We then investigated how potential green and brown prey sources varied in abundance (biomass) across the different communities and how this related to predator isotopic signatures. Comparing only taxa of known feeding mode, initial analysis using a GLM showed *biomass* was affected by an interaction between *grassland type* and *trophic guild*, indicating that herbivores and detritivores constituted biomass differentially between grassland types. Graphical observation confirmed this result, showing herbivore biomass dominated extensive grasslands but was much reduced in intensive grasslands, with herbivore to detritivore biomass ratios greater in the extensive grasslands (Fig. 3). These findings were concurrent with raw $\delta^{15}N$ and $\delta^{13}C$ values plotted against biomass values for each taxon in each community, which showed biomass hotspots to be associated with detritivores in intensive grasslands but mixed between herbivores and detritivores in the extensive grasslands (Fig. 4). Interestingly, allowing for trophic enrichment between potential prey and generalist predators of 2.0 to 2.8 for $\delta^{15}N$ and 0 to 0.5 for $\delta^{13}C$, in both the grassland types most

generalist predators were associated with biomass hotspots. This suggests that in extensive grasslands, predators are exposed to a greater abundance of green potential prey items than in intensive grasslands. To analytically test these findings, we used SIAR to estimate dietary contribution from most abundant potential prey sources for each generalist predator within each community (Fig. 5). These outputs were in close agreement with all the above findings, broadly showing Linyphiidae, Carabidae and Staphylinidae predators in the intensive grasslands to have greater brown than green elements to their diet, whilst conversely in the extensive grasslands these predators had marginally larger green than brown elements to their diet (Fig. 5). Lycosidae dietary estimates showed no discernible differences between communities (Fig. 5). Additionally, we note that Staphylinidae had raw $\delta^{15}N$ beyond our assumed trophic enrichment estimates suggesting an intermediate food source was missing from estimates, such that we discuss dietary findings for this predator with some caution.

Table 1. Assessment of intensive farming effects on sward architecture and plant and invertebrate diversity and biomass. A comparison is made between two conventionally managed intensive beef-cattle grasslands (a and b) and two extensive beef-cattle grasslands (c and d). Sward heterogeneity is SD of mean sward. For invertebrate taxonomic richness, number of positively identified herbivore taxa is also given, in brackets.

Grassland	Mean ± SD Sward Height (cm)	Plant Species Richness	Mean ± SD Plant Biomass (dry g / 0.5m^2)	Invertebrate Taxonomic Richness (*herbivores)*	Total Invertebrate Biomass (dry mg / 0.5m^2)	Fallen Seed Biomass (dry mg / 0.5m^2)
Intensive (a)	8.80 ± 4.22	8	79.11 ± 3.15	58 (*10*)	198.24	1.14
Intensive (b)	11.52 ± 3.82	8	125.50 ± 15.51	68 (*11*)	322.52	0.46
Extensive (c)	20.98 ± 10.51	21	159.27 ± 21.40	87 (*24*)	483.48	603.44
Extensive (d)	21.02 ± 11.27	18	135.35 ± 6.82	91 (*24*)	738.88	858.48

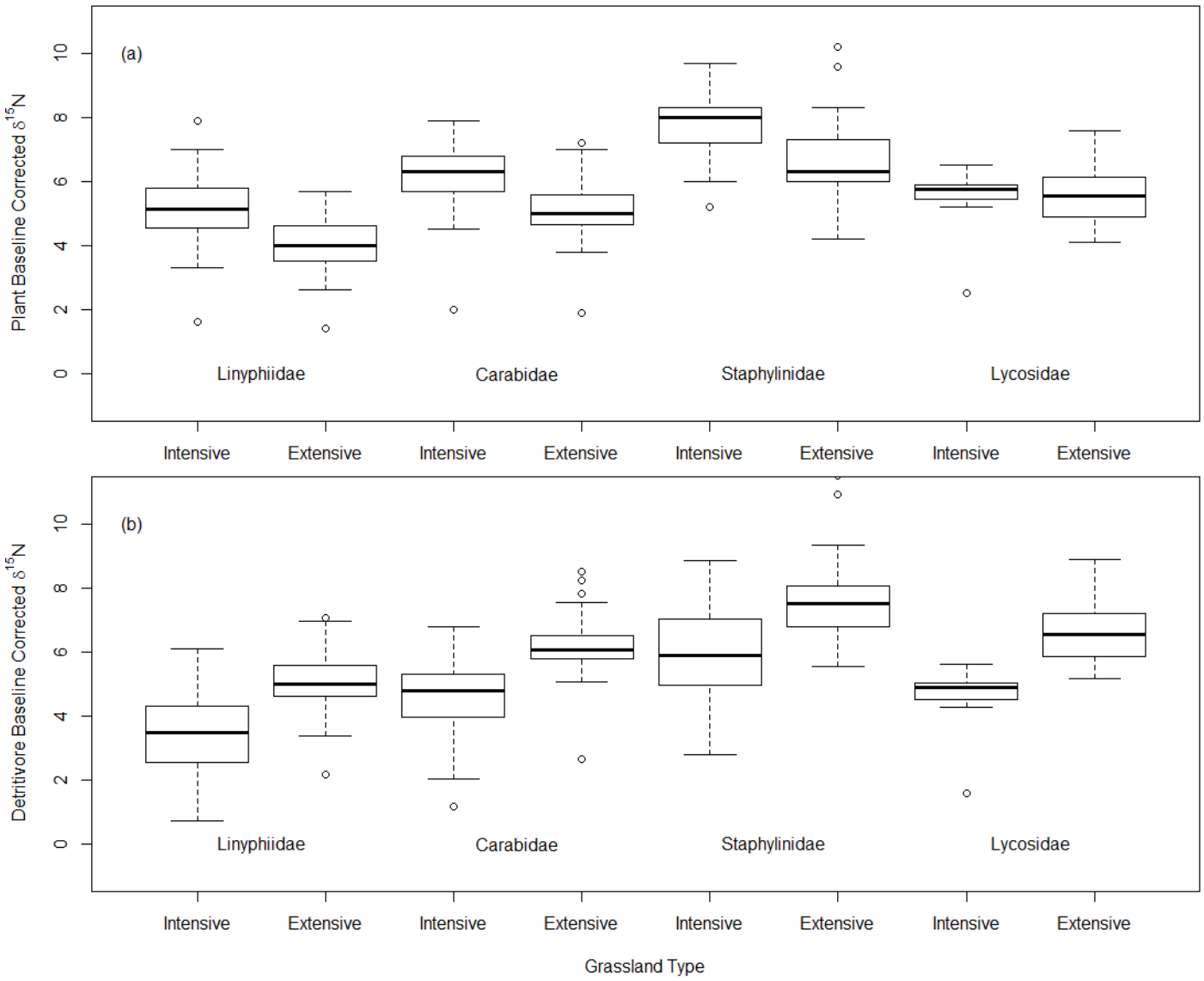

Fig 1. Baseline corrected $\delta^{15}N$ (‰) values for a suite of generalist predators differ significantly between and within intensively and extensively managed grasslands. (a) Predators in intensive grasslands have higher $\delta^{15}N$ when calculated against a plant baseline, but (b) Predators in extensive grasslands have higher $\delta^{15}N$ when a detritivore baseline is used. Significant differences in $\delta^{15}N$ between predator families are consistent across grassland types and baseline method, and show Staphylinidae feed at higher trophic levels than Lycosidae = Carabidae > Linyphiidae. Note that in this figure predators across two communities were pooled for each grassland type.

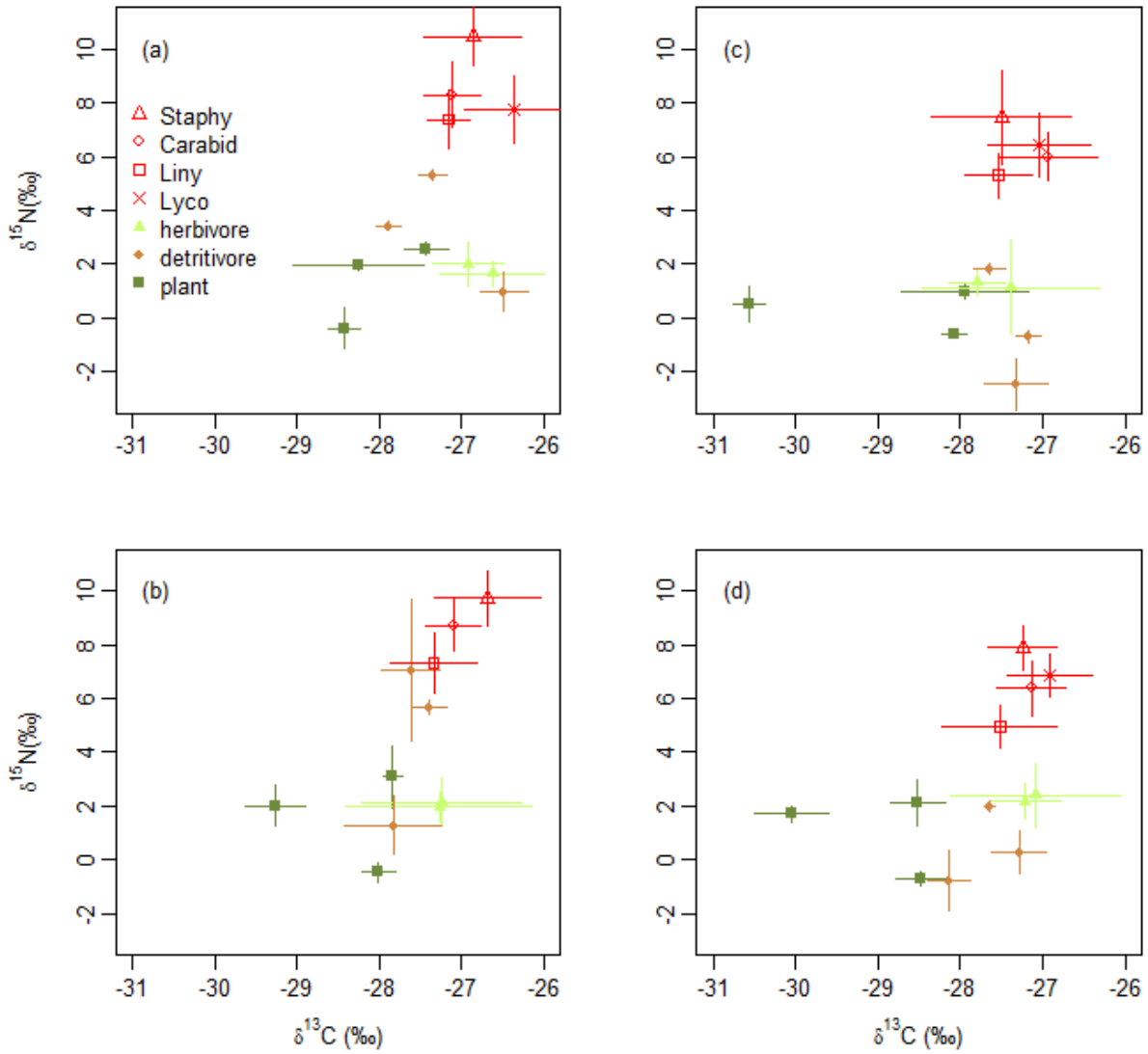

Fig 2. Raw $\delta^{15}N$ and $\delta^{13}C$ for a motif of species common to all communities. Analysis showed an interaction between trophic guild and grassland type significantly affected $\delta^{15}N$. This motif was used to identify that elevated $\delta^{15}N$ of detritivores in both intensive communities (a and b) was matched by predators in these communities, whilst plants and herbivores were similar across all four communities. In extensive communities (c and d), detritivores and predators have relatively lower $\delta^{15}N$.

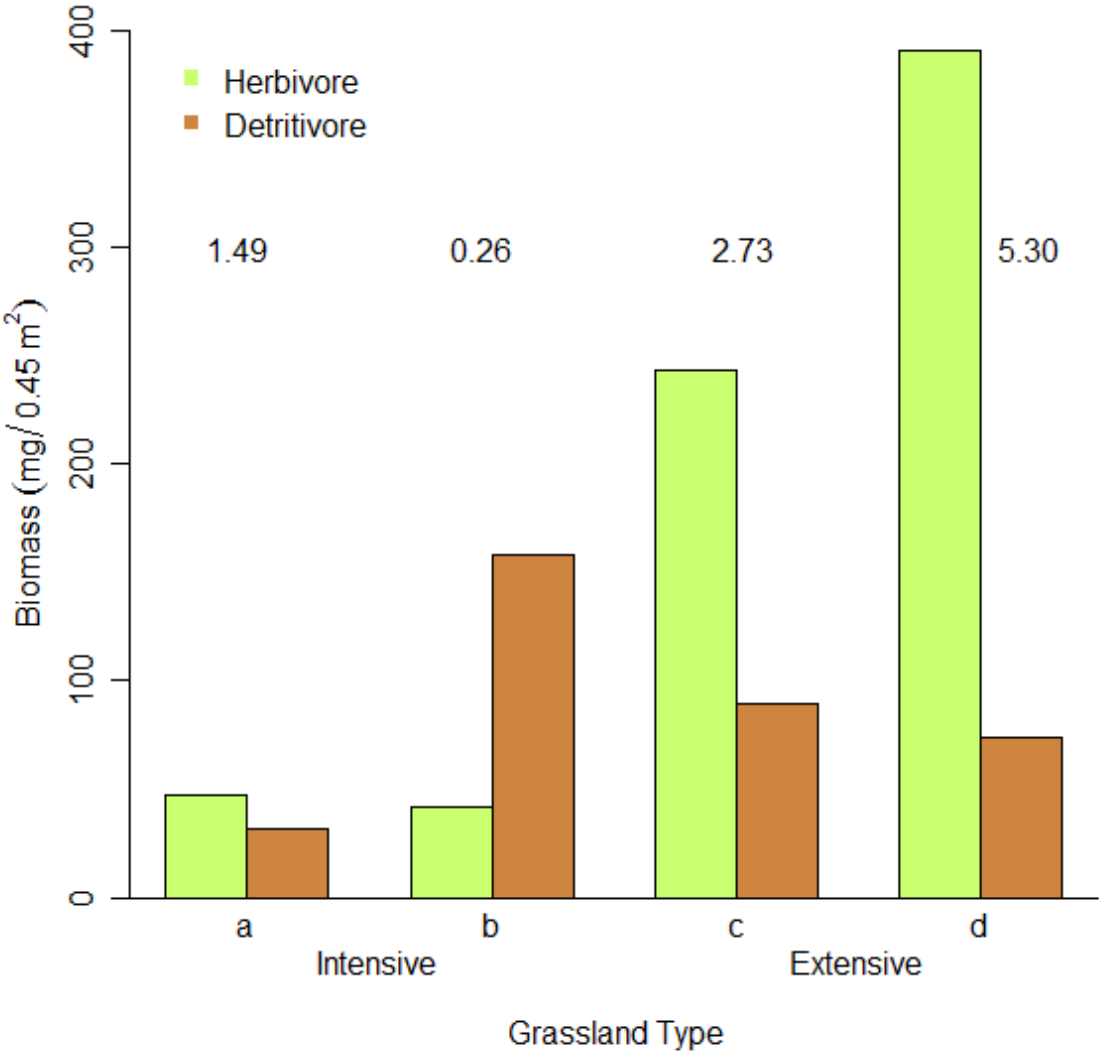

Fig 3. Green and brown food chain compartments within food webs are altered by farming practice. Total biomass (mg / 0.45m^2) of herbivorous invertebrates was less in intensive grasslands (a and b) than extensive grasslands (c and d), with the ratio of green to brown biomass being greater in the extensive grasslands. Ratios are shown as numbers above bars.

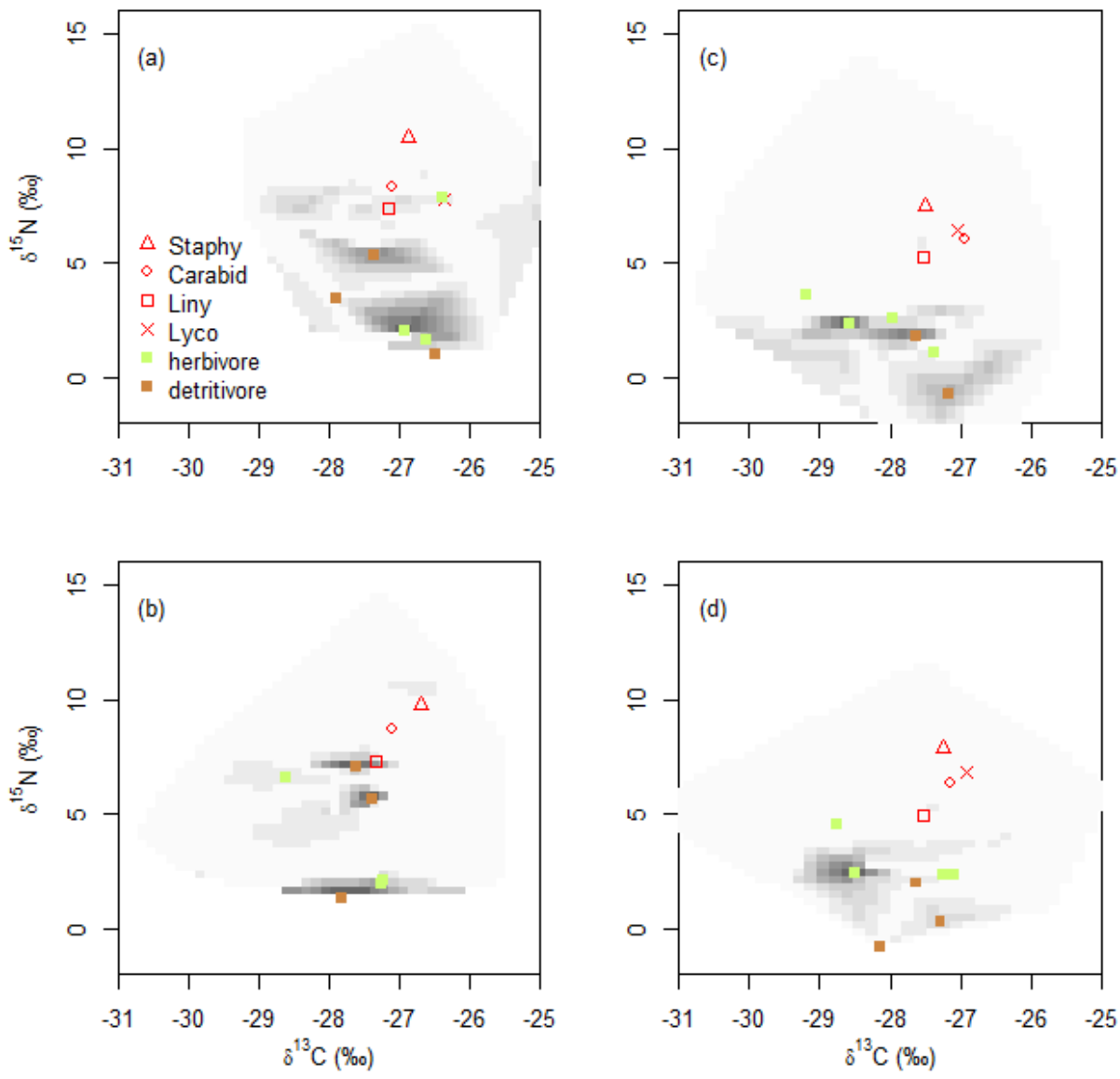

Fig 4. Intensive farming changes biomass structure of food webs. Biomass (mg) for each suction-sampled invertebrate taxa is interpolated across raw $\delta^{15}N$ and $\delta^{13}C$ of each taxa, for two intensive (a and b) and two extensive (c and d) grasslands. Invertebrate taxonomic richness is: 58(a), 68(b), 87(c), 91(d). Large differences in scale of biomass between communities meant interpolation colours were calculated independently within each community and are thus not directly comparable between communities. Potential herbivore and detritivore prey species accounting for >70% of total community herbivore or detritivore biomass are overlaid as symbols. Note that Staphylinidae, Carabidae and Lycosidae are overlaid based only on raw $\delta^{15}N$ and $\delta^{13}C$ values, not biomass, as they were sampled independently. Allowing for trophic enrichment of 2.0‰ to 2.8‰ for $\delta^{15}N$ and 0‰ to 0.5‰ for $\delta^{13}C$, most predator species (red symbols) are associated with areas of higher potential prey biomass in each community, and these areas are more dominated by detritivores in intensive grasslands.

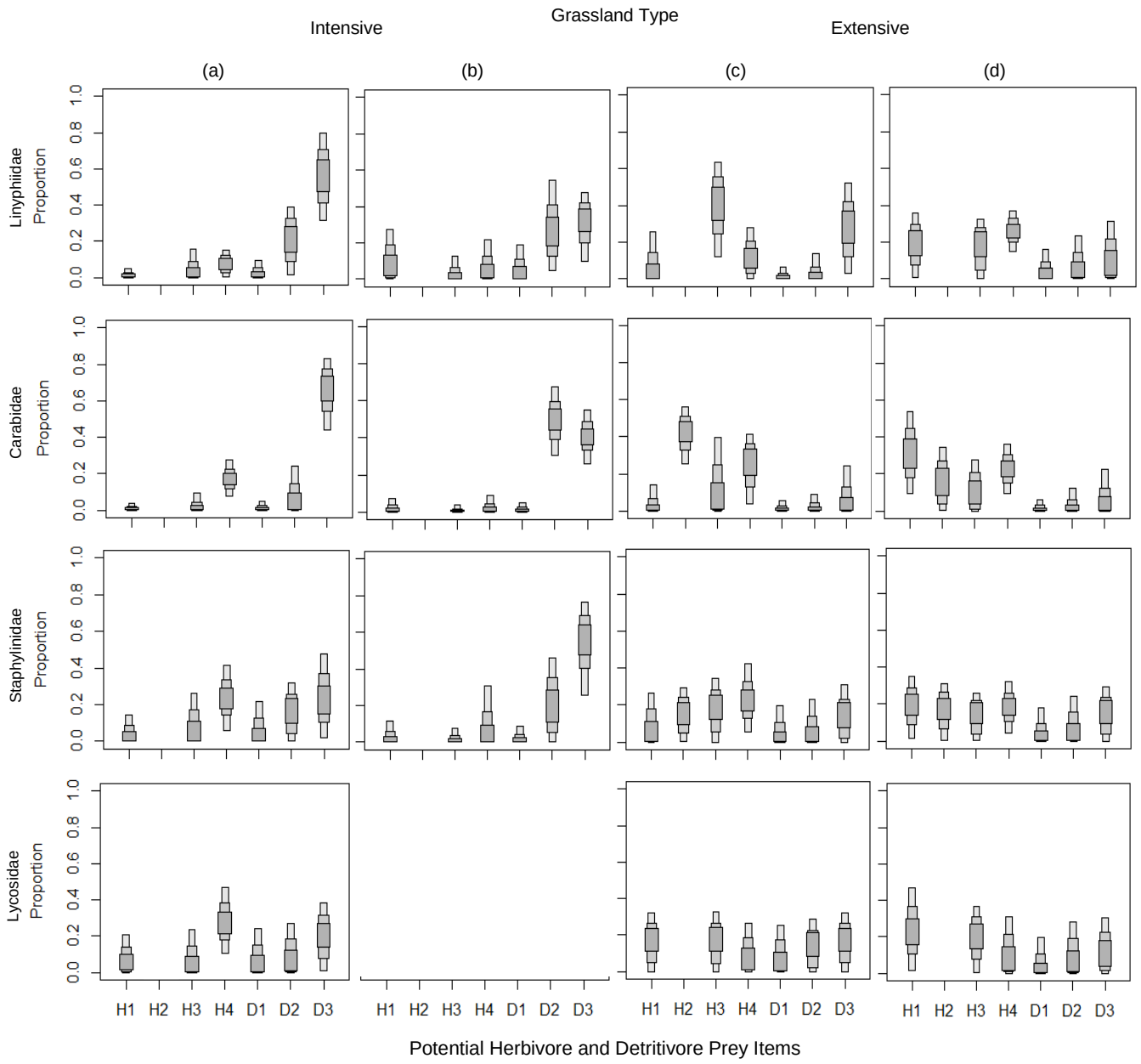

Fig 5. Dietary estimates for generalist predators have differing green and brown elements dependent upon farming practice. Bayesian resampling (*n* = 10,000) of variance in prey isotopic signatures is propagated as quantified uncertainty in final probability distributions for proportion of each dietary component. Shaded boxes (dark to light) show 50%, 75% and 95% credible intervals for mean estimates. H = herbivore, D = detritivore. H1 Cicadellidae and Aphididae; H2 Gastropoda; H3 Coleoptera: Curculionidae and Apionidae; H4 Coleoptera: Chrysomelidae; D1 Collembola: Symphypleona; D2 Collembola: Entomobryomorpha; D3 Collembola: Poduromorpha. Intensive grasslands (a and b), and extensive grasslands (c and d). No Lycosidae were obtained for community b; Gastropoda were not obtained for intensive communities, and were excluded from all Linyphiidae estimates as they were not deemed to be potential prey.

5.5 Discussion

Using species-rich naturally assembled communities, we find strong evidence that agricultural intensification of grassland management reduces habitat heterogeneity consequently altering the relative structure of green and brown food chain compartments and that this in turn can influence predator feeding habits. We show that herbivore and detritivore assemblages were abundant in extensive grasslands but that herbivore structure was diminished in intensive grasslands. Concurrent with stable isotope evidence, we additionally demonstrate predator diets contained greater brown than green elements in intensive grasslands, but more green than brown in extensive grasslands. Changes in cryptic energy flow as demonstrated in this study are likely to have important landscape-scale implications given that predator coupling of alternate food chain compartments is implicated in food web stability, services and functions, yet our results suggest a significant simplification of these interactions in intensively managed grasslands. We suggest future research should further investigate relationships between food web structural compartments, biodiversity and habitat heterogeneity in order to inform how management strategies aimed at mitigating biodiversity losses within agricultural landscapes could be effectively expanded to include actions that increase trophic complexity as standard practice, with particular focus given to restoring food web structural compartments and thus natural predator dynamics.

Traditional assessment of community structure

For measures of sward height and heterogeneity, plant diversity and biomass, and invertebrate diversity and biomass (Table 1) we found good consistency between fields within grassland type, and notable differences between grassland types. Concurrent with previous studies, we found that intensification in grassland management (stocking density, inorganic inputs) was associated with reduced sward heterogeneity - observed as reduced variation in sward height and lower plant species richness (Benton *et al*. 2003; Woodcock *et al*. 2009), and less primary resources - observed

as lower plant biomass and seed density (Vickery *et al*. 2001). Grassland intensification establishes competitive grass species through reseeding and intense grazing which has the additional effect of eliminating non-vegetative reproductive species through inhibition of life-cycle completion, reducing flower and seed resources (Vickery *et al*. 2001 and references therein). Within extensive grasslands we attribute greater invertebrate taxonomic richness and biomass to greater habitat and food resources through greater heterogeneity in sward architecture, plant species richness and plant standing biomass (Morris 2000; Vickery *et al*. 2001; Woodcock *et al*. 2007; 2010). Consequently, herbivorous invertebrate communities were both more diverse (Table 1) and abundant (Fig. 3) in the extensively managed grasslands (Morris 2000; Vickery *et al*. 2001 and references therein for both; Woodcock *et al*. 2009; 2010). For detritivores, all three Collembola orders (Symphypleona, Entomobryomorpha and Poduromorpha) were present in each community, and accounted for >70% of detritivore mass (accounting for biomass of taxa of unknown functional group that were potentially detritivores). Biomass of detritivores was variable in intensive grasslands but more consistent in extensive grasslands, though on average approximately similar between grassland types (Fig. 3). Notably therefore, these results suggest it was differences in herbivore structure that primarily determined greater ratio of herbivore to detritivore biomass in extensive grasslands (Fig. 3). Analysis using a GLM supported this finding, with a significant interaction indicating relative differences in herbivore and detritivore biomasses within communities differed between grassland types. These results are likely linked to intensification of management, which we show was associated with the loss of sward heterogeneity and plant resources, which is of direct consequence for herbivores (Woodcock *et al*. 2007; 2009; 2010). Most previous studies examining grassland intensification have focused on specific taxa, with remarkably fewer considering a larger community of species spanning multiple trophic and functional groupings. In this respect, our study makes an important contribution to our understanding of the effects of grassland intensification on food web structure. Of studies examining larger suites of taxa in the context of intensification, Woodcock *et al*. (2009) found invertebrate herbivore species richness to be enhanced by plant species richness, and

that diversity of these groups was higher in extensively managed grasslands characterised by greater habitat heterogeneity. This concurs with our findings that reductions in sward heterogeneity associated with intensive grassland management also corresponded to lower herbivore diversity and biomass. Though Woodcock *et al*. (2009) makes no measure of brown food chain compartments, their findings and the findings of this study concurrently suggest collapse of green food chain compartments as a consequence of intensive management is a key driver of structural simplification of food webs in mesotrophic grasslands. Thus, we conclude our grasslands reflect typical mesotrophic grassland management effects more generally, which is important as we now go further to discuss how this impacted predator feeding habits.

Predator Feeding Habits

We discovered that plant-corrected $\delta^{15}N$ baseline for predators produced anomalous results as subsequent motif analysis identified that detritivore-corrected $\delta^{15}N$ baseline was a more appropriate means of estimating predator trophic level, as predators were likely feeding on detritivores in intensive grasslands. Analysis using a GLMM revealed a significant interaction between grassland type and motif trophic guild providing evidence that relative difference in $\delta^{15}N$ between trophic guilds was dependent upon grassland type. Graphical observation confirmed this: whilst plant and herbivore $\delta^{15}N$ of motif species was consistent across grassland types, detritivore $\delta^{15}N$ and consequently predator $\delta^{15}N$ were enriched in intensive relative to extensive grasslands (Fig. 2). Additionally, in intensive grasslands, predators were >5‰ enriched relative to herbivores which, assuming typical enrichment of 2.4 ± 0.4 (SD) in $\delta^{15}N$ from prey to predator (Ostrom *et al*. 1997; Oelbermann & Scheu 2002; Wise *et al*. 2006), strongly suggests herbivores could not have contributed significantly to predators' diet, as predator $\delta^{15}N$ would have been much lower than was observed. It seems likely that detritivores had elevated $\delta^{15}N$ in the intensive grasslands as a consequence of either nutrient (fertiliser) inputs or within-field recycling of cow-dung providing

energy sources that entered into the detritivore food chain, as shown to alter baselines elsewhere (Bateman & Kelly 2007).

Utilising a detritivore baseline, we established that each predator family in the extensive grasslands had higher $\delta^{15}N$ than their counterparts in the intensive grasslands providing evidence of a difference in diet (Fig. 1b). Notably, this finding was supported by dietary estimates from mixing models that showed predators (Linyphiidae, Carabidae and Staphylinidae) in intensive grasslands incorporated relatively more detritivore than herbivore prey items, but that predators in extensive grasslands ate relatively more herbivore than detritivore prey items (Fig. 5). These findings are important because they provide evidence that across multiple predatory families, sources of dietary energy are derived from different energy pathways, and that this shift is associated with agricultural intensification. Our results concur with Birkhofer *et al*. (2011) examination of arable systems, where stable isotope signatures in generalist invertebrate predators showed that herbivorous prey items were consumed in larger proportions in organic (less intensive) than conventional (more intensive) fields. We build substantially on the findings of Birkhofer *et al*. (2011) by quantifying food web structural mechanisms that underpin these differences: that is agricultural intensification reduces sward heterogeneity, inducing trophic collapse of green prey food chain compartments. This is supported by lower herbivore to detritivore biomass ratios observed with greater intensification (Fig. 3), and the isotope biomass-landscapes that show predators generally associate with biomass hotspots (Fig. 4), which are more frequently comprised of herbivores in extensive grasslands but more frequently by detritivores in intensive grasslands. Importantly the predators we sampled are generalist and adaptable in their feeding habits (Bell *et al*. 2008; Birkhofer *et al*. 2008; 2011) and thus they are likely to encounter and potentially consume more abundant food sources more frequently.

We additionally observed generalist predators occupied distinct trophic niches consistently among the different grasslands, with Linyphiidae feeding at the lowest trophic level, Carabidae and Lycosidae intermediate and similar to one another, and Staphylinidae feeding highest. Staphylinidae

had high $\delta^{15}N$ values that in some instances were beyond estimated trophic enrichments from prey sources, suggesting that Staphylinidae foraging may include intraguild predation, as shown to be important in many generalist invertebrate predators (Synder and Wise 2001; Wise 2006). Alternatively there could be a missing prey source and it should be noted that suction sampling did not collect slugs (i.e. *Deroceras*) or earthworms (Lumbricina), which are potential prey items of Carabidae. However despite missing a minority of prey species, given the broad generalist diets of these predatory beetles (Keilty *et al*. 1999; Bell *et al*. 2008; Birkhofer *et al*. 2008; 2011) and the concurrence of multiple points of evidence shown across a suite of predators in this study, we would argue findings and hence conclusions of this study are likely to be robust.

In conclusion, our results show that intensification of grassland management reduced sward heterogeneity and this likely explained the observed alteration of food web compartmental structure characterised by reduced herbivore abundance and diversity. Importantly, these cascading effects were detected in the feeding habits of a suite of common predators, with a greater reliance on detrital prey items in intensive grasslands. Crucially, in seeking to improve our understanding of impacts of intensification on grassland habitats, our approach linked traditional food web assessment with predator feeding habits using novel stable isotope approaches, and in doing so, makes an important contribution to previous studies that have focused only on subsets of taxonomic diversity, or food web structure, or have not linked changes to predator dietary habits. Understanding how cascading effects are associated is critical to both understanding further ecological implications of intensification and determining mitigation strategies. Given mesotrophic grasslands are a common habitat across much of Europe and that simplification of food web structure and predator feeding habits have been linked to lower ecological stability, we recommend that future management policies aimed at mitigating biodiversity losses should be broadened to focus on restoration of food web compartmental structure, which itself will likely deliver biodiversity gains whilst also restoring natural prey-predator dynamics.

5.6 References

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CHAPTER 6

GENERAL DISCUSSION

6.1 Overview

Food web structure remains a central component of ecology due to its underlying importance in explaining patterns of diversity, ecosystem function and services (Cardinale 2011; Rooney & McCann 2012; Thompson *et al*. 2012). Additionally, understanding how food web structure influences ecosystem stability and services will likely be of increasing importance for predicting and mitigating serious detrimental impacts of human-induced habitat alteration and climate change (Schindler *et al*. 2010; Hansson *et al*. 2013). Food web structure is known to be fundamentally important in affecting ecological patterns and functions, though is itself influenced by a range of biotic and abiotic variables (Post *et al*. 2000; McCann & Rooney 2009; Sabo *et al*. 2010; Haddad *et al*. 2009). Numerous biotic and abiotic processes that affect structure do so by affecting patterns of diversity, yet biodiversity as a driver of food web structure is to date poorly understood. To improve our understanding of the wider ecological implications of food web structure it is therefore of critical importance to determine how changing diversity may alter structure, and ascertain potential mechanisms explaining these effects.

The primary aim of this thesis was to utilise novel stable isotope approaches to provide new insights into how food web structure may change in response to diversity. Given the scarcity of methodological insights into best practice when utilising stable isotope analyses, I additionally validated important aspects of their application in this respect. In the following pages I summarise key findings of this research, discuss these results within the context of food web structure and ecological functioning, and elaborate the broader implications of these findings for future research and management of natural resources.

6.2 On the use of stable isotopes for quantifying food web structure

Though the use of stable isotopes to infer ecological patterns has been practiced for some time (DeNiro and Epstein 1978; 1981) and is now widespread (e.g. Takimoto *et al*. 2008; Okuzaki *et al*. 2009; Cooper & Wissel 2012), the use of $\delta^{15}N$ and $\delta^{13}C$ to quantify trophic structure has largely been limited to investigations of food chain length (e.g. Cabana & Rasmussen 1996; McHugh *et al*. 2010) and trophic positions (Vander Zanden *et al*. 1997; Post 2002), with fewer exceptions (Layman *et al*. 2007a; 2007b; Okuzaki *et al*. 2009; Quevedo *et al*. 2009; Cooper & Wissel 2012). Given this is a growing research area, it is therefore timely that this thesis research examines certain methodological foundations on which a myriad of future ecological research is reliant to quantify food web structure and derive subsequent conclusions. Furthermore, studies of terrestrial and invertebrate systems are needed to broaden our knowledge of food web structure (Martinez del Rio *et al*. 2009; Boecklen *et al*. 2011) and through demonstration, disseminate isotopic methodological techniques to a broader audience of ecologists. Chapter 2 investigated whether tissue selection and lipid extraction were important considerations in the processing of invertebrate tissues prior to stable isotope analysis (SIA), whilst in Chapter 3 I incorporated this knowledge to undertake an accurate test of $\delta^{15}N$ and $\delta^{13}C$ dynamics over 4-tier food chains, and determine the accuracy of isotopic measures of food chain length using Bayesian resampling procedures to provide rigorous estimates. Chapter 4 extended this exploration by utilising Bayesian estimates of six community metrics to quantify food web structure of communities of differing biodiversity content.

In chapter 2 I was able to show that both tissue selection and lipid extraction were important considerations for practitioners of isotopic studies utilising invertebrates. I therefore advocate that practitioners should utilise source tissues that best represent those assimilated by consumers. Given a scarcity of previous studies examining tissue selection in isotopic studies using invertebrates (Tibbets *et al*. 2008), I suggest these results improve our knowledge of these systems as I show

evidence both for tangible effects of tissue differences on discrimination factors, and also elucidate the mechanism for this as being depletion of $\delta^{15}N$ and $\delta^{13}C$ in exoskeleton relative to soft tissues. Additionally, whilst many studies have investigated lipid extraction effects, fewer such studies have tested extraction on different tissue types (Pinnegar & Polunin 1999; Sweeting *et al*. 2006; Logan & Lutcavage 2008), particularly in invertebrates (Bodin *et al*. 2007; Mateo *et al*. 2008 and references therein). I therefore suggest that these results have additionally broadened knowledge of tissue-specific lipid extraction effects for invertebrates. Lipid extraction is currently underutilised (Mateo *et al*. 2008) likely because of the lack of understanding regarding when it is needed (Post *et al*. 2007), so I also contend that these findings are timely and provide clarity for practitioners, as I show that lipid extraction affected $\delta^{13}C$ signatures and that determination of differences between tissue-types was only possible after extraction, and thus that lipid extraction is a prerequisite to SIA of invertebrates.

Chapter 3 tested dynamics of $\delta^{15}N$ and $\delta^{13}C$ over 4-tier food chains. Using knowledge gained from chapter 2, I used lipid-extracted source tissues that best represented those assimilated in consumers. For well replicated food chains of known trophic levels, for which consumer's diets were controlled, I utilised Bayesian resampling procedures to show that the isotopic metric nitrogen range (NR) generally distinguished food chain length. Variability around estimates caused some overlap between food chain lengths of 3 and 4 trophic levels, inhibiting accurate estimation of food chain length at these points of overlap. Estimates of carbon range (CR) changed little with increasing food chain length, showing the potential utility of $\delta^{13}C$ as a tracer of energy channels. Current common practice uses NR to estimate food chain length and determine subsequent conclusions, but rarely has NR's use been tested, with terrestrial systems particularly understudied (Martinez del Rio et al. 2009). This thesis therefore provides reliable evidence that isotopic metrics can distinguish food chain structure, though I urge caution to practitioners interpreting food chain length when NR values fall in known overlap boundaries; i.e. in this study NR values of 5‰ or 6‰ could be either 3 or 4

trophic levels. I additionally ascertained the source of this overlap; variation in NR values for the same food chain length across different food chain types, as caused by variation in $\delta^{15}N$ within each species and variation in $\Delta\delta^{15}N$ between trophic links. Such variation will always be inherent in isotopic studies; being aware that error variance is present in isotopic systems is therefore the important conclusion here, as this ensures that practitioners are aware of such limitations when drawing ecological conclusions.

Chapter 4 built on these findings; Bayesian resampled community metrics based on $\delta^{15}N$ and $\delta^{13}C$ showed that community food web structure could be determined well, with NR, CR, TA and MNND all providing complimentary and insightful quantifications of different aspects of structure. Whilst TA's utility to quantify population structure has previously been demonstrated (Layman *et al*. 2007b; Quevedo *et al*. 2009) the use of metrics to quantify community structure has not been previously tested directly against changing biodiversity contents. I found CD and SDNND performed poorly in the respect that they conveyed less useful information, which may represent an inability to provide contrast unless differences in diversity are greater. Thus more generally, through a progression of discrete studies, this thesis research has identified appropriate sample preparations, tested the robustness of isotopic dynamics and measures of food chain structure, and applied isotopic quantifications of food web structure to wild communities. In doing so, I contend that this thesis furthers our understanding and experience of applying these techniques, and provides compelling evidence of the usefulness of isotopic approaches for studying food web structure.

6.3 Biodiversity effects on food web structure

To investigate how biodiversity affects food web structure, in chapter 4, I tested three non-mutually exclusive predictions by investigating three facets of diversity. I found good evidence that greater diversity increases both diversity of resources exploited and overlap in resources exploited, but no

evidence that niches of individual taxa change as a response to diversity. I showed that greater taxonomic richness led to a greater diversity of resources being exploited which I observed as expansion along bivariate resource axis and quantified as increasing measures of NR, CR and TA. This observed increased in community niche space was simultaneous with increasing species niche overlap as measured by decreasing MNND. Notably, I tested these measures across three different facets of diversity, finding that these trends were consistent for taxonomic richness across plant, herbivore and predator trophic guilds, whilst tests of TA and MNND based on functional richness in high diversity communities also reflected these patterns. Importantly therefore, in this thesis I demonstrate that diversity exerts strong effects on community structure. Elucidating these patterns however, is also of importance for providing mechanistic insights to explain coexistence and competition between species. My results showed that for greater taxonomic richness and functional richness across trophic guilds more species shared the same resource space, thus suggesting coexistence. Simultaneously greater divergence of total resource space was observed with greater diversity, showing evidence of alternative strategies that may avoid heightening competition. Fundamental ecology still seeks evidence to explain structuring processes that can account for patterns of diversity we observe in nature (Rooney & McCann 2012), including how species of near-same niche are able to coexist (Leibold & McPeek 2006). These thesis results provide good evidence that increases in niche-sharing are associated with greater diversity, providing scarce empirical insights that coexistence theory requires to build upon (Adler *et al*. 2010). Additionally, expressed differently, greater niche overlap as a consequence of greater community diversity is evidence of increased functional redundancy, providing empirical insights into mechanisms that may aid understanding of how greater diversity may determine greater ecosystem functionality. For instance, my results suggest that in diverse communities more species exploit the same resources, which provides a mechanistic basis to support complementarity theories (Kremen 2005) for how greater species richness may mediate greater efficiency in chemical and biological community processes.

In chapter 5 I additionally investigated how community structural changes were associated with predator feeding habits. In an applied context, I was able to show how human management had reduced habitat heterogeneity and how this led to a decrease in herbivorous structural compartments within grassland communities, affecting availability of predatory sources of energy and consequently modifying predator feeding habits. This study was complimentary to the previous findings in chapter 4 as it showed how diversity effects on structure were not random, but rather that diversity affected particular structural compartments of the community, such that diminishment of community structural diversity was directional. I suggest that the strength of my approach to obtain these results laid in the diversity of methods used, which combined characterisation of community biomass with $\delta^{15}N$ and $\delta^{13}C$ signatures to show how predators and either detritivore or herbivorous prey were associated, predator dietary analysis using stable isotope mixing models, and traditional biodiversity assessments. In doing so I progress from chapter 4 to demonstrate how a creative and diverse use of stable isotope data can be combined with other approaches to better determine and understand the effects of human alteration on food web structure.

6.4 The implications of biodiversity effects on food web structure and the usefulness of stable isotopic approaches to quantify them

Whilst food web structure is known to be of importance to myriad ecological processes (Rooney & McCann 2012; Thompson *et al*. 2012) and is known to be affected by abiotic and biotic factors (McCann & Rooney 2009; Sabo *et al*. 2010) few studies have examined direct effects of biodiversity *per se* on food web structure, not least in part due to the difficulty of quantifying food webs. In species-rich, wild systems subject to natural processes of community assembly, I was able to determine that diversity had important structural effects on grassland food webs. In particular, I

showed in chapter 4 that greater diversity increased both the range of resources exploited and exploitation of the same resources, which has important implications for understanding how more diverse systems may provide ecosystem-function resilience to, and efficient recovery from, natural and anthropogenic perturbations. This finding in itself is likely to be of great ecological interest given ecologists' continual search for mechanistic bases to support complementarity-theory which seeks to explain the way species exploit their environments. I therefore speculate that this piece of research will form an important basis for further uptake of isotopic approaches to quantifying food webs in order to elucidate processes of exploitation and / or coexistence, which are major themes in understanding the ordering of species in nature. In chapter 5, I was able to show that structural effects were non-random directional responses to human disturbance, and that loss of compartmental structure had consequential effects for predator feeding habits. This is of concern given that predator foraging has been implicated in mediating stability to overall food web structure and dynamics, and directly in ecosystem services such as bio-control. I suggest my work in chapters 2 and 3 may also make notable contributions to the isotopic ecological community through providing robust validatory tests of methodological approaches. As clarity and streamlining of isotopic methods for studying food webs makes their accessibility and uptake by a greater range of ecologists more likely, this in turn will also benefit ecology more broadly.

6.5 Future directions

Whilst below I make brief comments relating to future isotopic study of food webs and diversity, here I speculate on useful future directions for applications of isotopic food webs as perspectives gained from this thesis. Most strikingly, isotopic approaches allow for the quantification of food webs in a manner analogous to the mapping of landscapes using coordinates, so similarly to Geographic Information Systems (GIS), it is the overlaying of additional environmental information that can allow isotope-food webs to provide new insights to a potentially broad and exciting range of

ecology. Beyond studies within traditional community - ecology contexts applying isotopic-food web metrics to compare sub-communities across food webs or in response to environmental perturbations, other areas of ecology have not yet tested how their brand of ecology might appear at a community level. So use of isotopic-food webs might be associated within more traditional ecological spheres of community ecology, or may come from other disparate areas of ecology such as behaviour or migration. For instance, within traditional community ecology it would be insightful to overlay isotopic-food webs with abundance data, an approach I used in Chapter 5, in order to discern how energy is associated with particular trophic regions, and how this might change in relation to biotic and abiotic gradients. This may be of particular importance in understanding how energy flux influences ecosystem functioning. Food webs including quantification of where energy is located trophically are likely to provide for greater ecological insights. Incorporating other areas of ecology into community ecology through the overlying of ecological information onto an isotopic-food web may allow meta-analysis style understanding of how trophic orientation may influence life history strategies or trade-offs through imposed limitations or opportunities. For instance species positioned trophically may allow for trophic analysis of mobility, energy efficiency, life-span, reproductive strategy, transience, genetic diversity and evolutionary pace, phylogenetic age, phylogenetic relationships, extinction likelihood or importance to ecological services, functions or other human values. Such analysis may yield more detailed and surprising information than simply small things that move little, live fast, die young with high reproductive output and low parental investment occupy the bottom of the food web. Ecological applications of isotopic-food webs may be primarily limited by ecologists' creativity.

As an important point with which to conclude, I would suggest that my studies of food web structure, as made possible through the use and development of isotopic techniques, have led to findings that uphold the context in which this thesis work was undertaken - that biodiversity has important effects on food web structure. Current focus on stemming biodiversity loss has grown

from simple aesthetic objectives to recognising the importance of biodiversity for ecosystem functions and services, but given that literature shows the importance of food web structure for ecological processes, I propose that the importance of biodiversity loss to food web structure and trophic interactions should also be more widely acknowledged. Thus I contend that future research should also focus on understanding biodiversity–mediated food web structure effects on ecological processes, and that policy making should acknowledge the importance of protecting biodiversity for the purpose of protecting food web structure and trophic interactions.

6.6 References

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