Are insects key drivers of change in woodland systems under climate change?

By

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Thesis abstract

Mean concentrations of atmospheric carbon dioxide (CO_2) continue to increase globally. Whilst the impact of this on plant biochemistry, physiology and ecology has been well documented, the impact on biodiversity is less certain. Forest ecosystems are globally important habitats in terms of carbon sequestration, water cycling and housing biodiversity. Arthropods are the most diverse groups of organisms within forests and underpin key ecosystem processes such as herbivory, pollination and nutrient cycling. It remains unclear how elevated CO_2 (eCO_2) will affect forest arthropods, and what consequences this will feedback to the ecosystem. The new Birmingham Institute of Forest Research Free Air Carbon Enrichment experiment represents a unique opportunity to test the impact of eCO_2 on forest arthropods for the first time in a mature, temperate forest. Three years of sampling from forest floor to canopy has provided a characterisation of the arthropod fauna of the site. Herbivory by leaf mining Lepidoptera decreased under eCO_2, whereas the abundance of aphids increased. The flowering time of bluebells advanced by 6 days under fumigation which is likely to affect its pollinators. Whilst there were no clear effects of eCO_2 on overall arthropod abundance, longer-term monitoring may be necessary to detect trends as they develop.

‘Keep fighting the good fight’

Paul Crowley 1960-2015

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Author contributions

This thesis is presented in the 'alternative format' with chapters 2, and 5 submitted as peer reviewed research articles and chapters 3 and 4 in preparation for submission to an undecided journal. I am first author on all the papers, with SH, JS and JP providing supervision.

Chapter 2: The work presented in this chapter has been submitted to Acta Oecologica as: Crowley, L.M., Ivison, K., Enston, A., Garrett, D., Sadler, J.P., Pritchard, J. and Hayward, S.A.L., 2021 (In review). Methods for characterising spatial and temporal patterns of arthropod abundance and diversity in a mature, temperate, oak woodland. *Acta Oecologica*. LC, SH and JS conceived the study. LC and DG undertook fieldwork. LC, KI, AE and DG undertook laboratory work. LC and JS performed data analysis. LC drafted the manuscript. All authors contributed to revising the manuscript.

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Table of Contents

Thesis abstract……ii

Dedication……iii

Acknowledgements……iv

Author contributions……v

Table of contents……vi

List of figures ix

List of tables x

Data availability statement xi

1 General Introduction - Bugs, bees, carbon and trees: Effects of carbon dioxide on forest insect-plant interactions. 1

1.01 Introduction 1

1.02 Studies investigating eCO_2 to date 3

1.03 The Birmingham Institute of Forest Research FACE experiment 5

1.05 General insect response to eCO_2 10

1.06 Herbivory under eCO_2 11

1.07 Aphids and leaf miners 13

1.08 Change in community composition 17

1.09 Nutrient cycling 20

1.10 Phenology 23

1.11 Pollination 24

1.12 Summary and thesis outline 26

2 Methods for characterising spatial and temporal patterns of arthropod abundance and diversity in a mature, temperate, oak woodland. 29

2.01 Abstract 29

2.02 Introduction 30

2.03 Materials and methods 34

2.04 Results 40

2.05 Discussion 45

2.06 Conclusions 54

3 The impact of eCO_2 on leaf miner herbivory.....55
in a mature temperate woodland.....55
3.01 Abstract.....55
3.02 Introduction.....56
3.03 Methods.....60
3.04 Results.....63
3.05 Discussion.....68
3.06 Conclusions.....75
4 Go with the phloem: Evidence for increased aphid abundance.....77
in a woodland under elevated CO_2.....77
4.01 Abstract.....77
4.02 Introduction.....78
4.03 Materials and methods.....81
4.04 Results.....84
4.5 Discussion.....86
4.06 Conclusions.....88
5 Elevated CO_2 impacts on plant-pollinator interactions: a systematic map and Free Air Carbon Enrichment (FACE) field study.....90
5.01 Abstract.....90
5.02 Background.....91
5.3 Methods.....95
5.04 Results.....100
5.06 Conclusions.....111
6 Short-term CO_2 enrichment has limited impact on arthropod abundance in a mature temperate woodland.....112
6.01 Abstract.....112
6.02 Introduction.....112
6.03 Methods.....116
6.04 Results.....118
6.05 Discussion.....126
6.06 Conclusions.....132
7 General Discussion.....133
7.01 Introduction.....133
7.02 Sampling arthropods at BIFoR FACE.....134

7.03 Herbivory under eCO_2 138
7.04 Pollination under eCO_2 141
7.05 Impact of eCO_2 on arthropod abundance, diversity and phenology 143
7.06 General conclusions 146
References 149
APPENDIX I 165
APPENDIX II 174
APPENDIX III 177

List of figures

Chapter 1

Figure 1.1 *Schematic diagrams of a woodland FACE system*……4

Figure 1.2 *Aerial image and map of the BIFoR FACE facility*……6

Figure 1.3 *Image of Periphyllus acericola group*……14

Figure 1.4 *Image of a Stigmella microtheriella leaf mine*……17

Figure 1.5 *Woodland trophic structure diagram*……18

Figure 1.6 *Aphid herbivory nutrient flow schematic*……21

Chapter 2

Figure 2.1 *Map of the BIFoR FACE experimental site*……35

Figure 2.2 *Total arthropods sampled by each method*……41

Figure 2.3 *Total arthropods sampled monthly*……42

Figure 2.4 *Monthly canopy and understory beating diversity indices*……43

Figure 2.5 *Arthropods sampled by pan traps*……45

Chapter 3

Figure 3.1 *Potential impact of eCO_2 on herbivory flow diagram*……58

Figure 3.2 *Images of area measurement process*……62

Figure 3.3 *Images of oak leaf mines*……63

Figure 3.4 *Images of hazel leaf mines*……64

Figure 3.5 *Mean % of oak leaves with at least 1 mine*……65

Figure 3.6 *Mean % of hazel leaves with at least 1 mine*……66

Figure 3.7 *Mean area of oak leaf mines*……67

Figure 3.8 *Mean area of hazel leaf mines*……68

Chapter 4

Figure 4.1 *Image of Drepanosiphum platanoidis feeding on sycamore leaf*……82

Figure 4.2 *Images of the aphid clip cages*……83

Figure 4.3 *Mean number of aphids per leaf*……85

Figure 4.4 *Total number of aphids in clip cages*……86

Chapter 5

Figure 5.1 *Schematic of papers per topic from systematic review*……101
Figure 5.2 *Bluebell Flowering phenology per patch and by total number of flowers*……103
Figure 5.3 *Bluebell visitation and visitation network*……105
Figure 5.4 *Bluebell seed set*……106

Chapter 6

Figure 6.1 *Images of sampling methods*……117
Figure 6.2 *Orders sampled per sampling method*……120
Figure 6.3 *Total arthropod sampled per sampling method*……121
Figure 6.4 *Total arthropods sampled by pitfall trapping*……122
Figure 6.5 *Total arthropods sampled by pan trapping*……123
Figure 6.6 *Total arthropods sampled by Malaise trapping*……124
Figure 6.7 *Total arthropods sampled by beating*……125

Chapter 7

Figure 7.1 *Total invertebrates sampled by order*……136

List of tables

Chapter 1

Table 1.1 *Climatic variables recorded at BIFoR FACE*……7
Table 1.2 *Invertebrate sampling methods*……8

Chapter 2

Table 2.1 *Total monthly samples by array type*……36

Chapter 3

Table 3.1 *Leaf mines recorded from oak and hazel per year*……65

Table 3.2 *p-values for t-tests between number and area of leaf mines for treatment and control*……..67

Chapter 5

Table 5.1 *Bluebell patch metrics*……..97

Chapter 6

Table 6.1 *d.f. and p-values for the analysis of treatment effect on arthropod abundance using linear mixed effect models*……126

Data availability statement

All data presented within this thesis are stored and available from the Birmingham Institute of Forest Research (BIFoR) research data store (RDS). Data pertaining to this thesis are organised as follows:

- Insect sampling data – Frequency of occurrence of taxa from core sampling as set out in chapter 2.
- Leaf mine data – Leaf mine survey data and leaf mine area data, as set out in chapter 2. Leaf mine images.
- Aphid data – Aphid survey data and aphid clip cage experiment data, as set out in chapter 4. Aphid survey photos.
- Pollination under eCO_2 systematic review data – The full systematic review, including data matrix as included in appendix III.
- Bluebell data – Bluebell flowering, bluebell visitation, bluebell seed set data, as set out in chapter 5. Bluebell flowering phenology photos.

CHAPTER 1

General Introduction - Bugs, bees, carbon and trees: Effects of carbon dioxide on forest insect-plant interactions.

1.01 Introduction

Global atmospheric concentrations of carbon dioxide (CO_2) have increased by around 40% since the industrial revolution as a result of anthropogenic activity, including combustion of fossil fuels and land use change (IPCC, 2013). This global phenomenon is expected to continue throughout the next century, with concentrations projected to reach 730-1020 ppm by 2100 (Solomon, 2007). Increasing atmospheric concentrations of CO_2 are one of the key factors contributing to global climate change through its close association with increasing global mean temperatures, being responsible for around 25% of radiative forcing (Lacis *et al.,* 2010). Elevated atmospheric CO_2 (eCO_2) also has a direct impact on ecosystems through the effects on plant biochemistry, physiology and ecology due to the role of CO_2 as the fundamental reactant in photosynthesis.

Forest systems are major global biome, covering more than 42 million km^2 of the earth's terrestrial environment, which represents approximately 30% of the earth's land surface (Bonan, 2008). As such, a significant proportion of global carbon is sequestered by and stored within forest ecosystems, estimated at 2.4 ± 0.4 petagrams of carbon per year (Pg C yr–1) globally (Pan *et al*., 2011). Any impact, therefore, that eCO_2 confers upon forest ecology may have major ramifications for global carbon cycling. Furthermore, forest ecosystems may also be considered 'biodiversity hotspots', with over half of all known species associated with this habitat type (Myers, 1988).

Insects are a key component of all terrestrial ecosystems, helping to maintain ecosystem function and stability. The dominant role played by the group in ecosystem processes is largely due to high abundance and diversity as well as their disproportionately large impact on nutrient cycling in relation to their own cumulative biomass (Yang and Gratton, 2014). Forest ecosystems are no exception with insects playing a direct role in shaping woodland structure and function through their underpinning of key ecological processes. The close relationships between insects and plants is rooted in fundamental interactions such as herbivory and pollination, meaning that the fates of both groups are closely linked through mutual impacts on performance (Crawley, 1989). The scale of these interactions is so great that herbivory alone may be responsible for limiting the ability of forests to uptake carbon dioxide through a reduction of up to 70g of carbon sequestering biomass per metre squared per year (Couture *et al*., 2015). Furthermore, insects also provide an important link between above and below-ground processes via their roles in litter processing and nutrient cycling dynamics (Frost and Hunter, 2004). The huge impact insects have on forests therefore mean it is likely that previous field experiments which measure ecosystem level processes, such as Net Primary Productivity, may have underestimated these values (Gherlenda *et al*., 2016).

Insects possess characteristic life history and physiological traits, such as high abundance, short generation times and ectothermy, which make them responsive to subtle changes in climatic conditions (Cornelissen, 2011). Climate change could, therefore, conceivably have a profound effect on insect populations. The key ecological roles fulfilled by insects mean that changes in insect population level, community composition or behaviour could dramatically impact forest nutrient cycling and carbon budgets. For example, insect pest outbreaks

represent major large-scale disturbances which can switch forests from carbon sinks to carbon sources (Kurz, *et al.,* 2008; Dymond, *et al*., 2010).

1.02 Studies investigating eCO_2 to date

Attempting to understand and predict the impact of eCO_2 on forest ecosystems is difficult due to the inherent complexity of these systems. In the past, a major shortfall in our understanding in this area is how small-scale responses measured in laboratory settings translate into the open field environment.

A solution to this issue has been the development of Free Air CO_2 Enrichment (FACE) experiments, whereby CO_2 can be elevated *in situ.* This is achieved through CO_2 fumigation of unenclosed, experimental plots within a natural ecosystem (Figure 1.1), which allows the incorporation of factors such as competition, whilst negating the effect of microclimate modification imposed by chamber methods (McLeod and Long, 1999). Another advantage of this method is the ability to implement larger scale experimental plots, in which a greater range of studies may be conducted simultaneously. As of 2014, there have been 151 FACE experiments conducted in natural ecosystems worldwide since 1987 (Jones *et al*., 2014). Originally developed for crops and grassland systems, the technique was adapted for use on mature *Pinus taeda* (Loblolly pine) trees in the 1990s at DUKEFACE, North Carolina. Other 'first generation' forest FACE experiments were established in the USA in young stands of species such as Aspen (AspenFACE), and Sweetgum (ORNL FACE). Development of this technology has led to increases in performance whereby achievement of target elevated atmospheric CO_2 concentrations is similar to comparable closed-chamber systems (McLeod

and Long 1999), with one-minute averages of CO_2 concentration within 20% of the target for >95% of the time (Zavala *et al*., 2009).

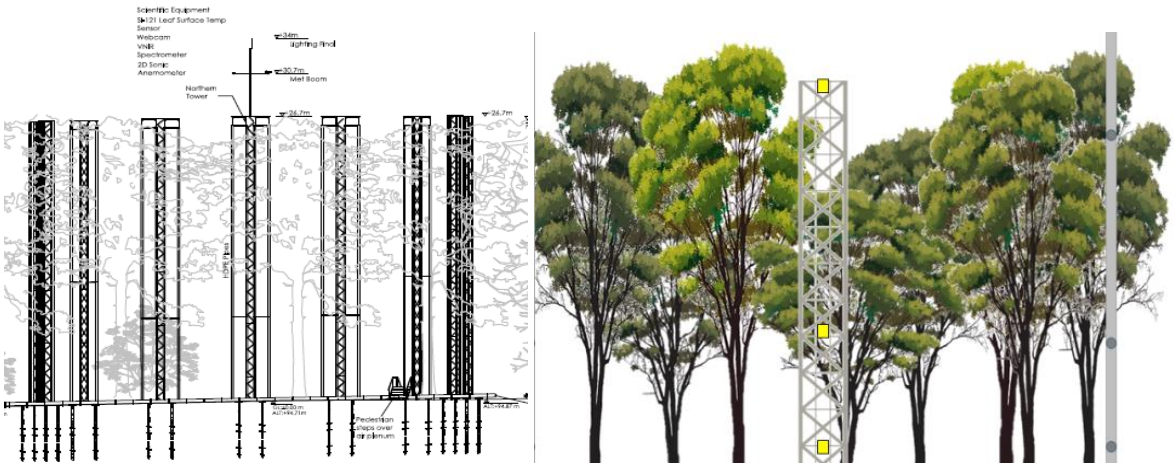

Figure 1.1 - Schematic diagrams of a woodland FACE system showing side on view of towers which support CO_2 delivery pipes. Towers are installed in a circular arrangement around the experimental plot, typically with a diameter of ~30 m. Tower height is to the top of canopy, which for most woodland FACE sites is 20-30 m. Image credit: BIFoR.

FACE arrays deployed in forests therefore represent one of the most reliable methods of testing how these forest systems will respond to future atmospheric CO_2 conditions, providing valuable insight on the system-wide impacts under natural, unenclosed conditions (Ainsworth and Long, 2005; Norby *et al*., 2016).

The first generation of forest-based FACE experiments began to explore how forest invertebrate communities respond to eCO_2 and the potential consequences of this to the system (Knepp *et al*., 2005; Stiling & Cornelissen, 2007; Hillstrom *et al*., 2014). This area of research, however, has mostly been undertaken in relatively few sites in the USA (North Carolina, Wisconsin and Florida) and is not globally representative given the immense diversity of both forest ecosystems and the insects within them.

1.03 The Birmingham Institute of Forest Research FACE experiment

The 'first generation' of forest FACE experiments were largely limited to young, managed, homogenous systems (Facey *et al*., 2016). The responses of mature, heterogenous, forest ecosystems to CO_2 fumigation has not been empirically tested at the ecosystem scale and may differ from the systems previously examined. The limited implementation of FACE experiments in old-growth forests represents a deficiency in our understanding, especially given the significant role of these biomes as global carbon sinks (Jones *et al.,* 2014). A suite of 'second generation' forest FACE experiments are currently being established to address this key knowledge gap. The first of these was the 'EucFACE' experiment, which began operation in 2012 in Mediterranean-type sclerophyll forest in Australia (Drake *et al*., 2016).

The Birmingham Institute of Forest Research (BIFoR) FACE experiment is the second such experimental facility, and to date, the only other which is operational. At BIFoR FACE the 'ten-year response of a mature, temperate, deciduous forest ecosystem to a 150-ppmv step-change in atmospheric CO_2' concentration will be studied in detail (MacKenzie et al., 2016). This level of CO_2 elevation represents predicted atmospheric concentrations in 2050 under current rates of anthropogenic emissions (Prather *et al.* 2001).

The BIFoR FACE facility has nine 30m diameter experimental arrays which consist of three infrastructure treatment arrays receiving CO_2 fumigation, three infrastructure control arrays receiving ambient air fumigation and three non-infrastructure control arrays (Hart *et* al., 2019) (Figure 1.2).

Figure 1.2 – Aerial image of the BIFoR FACE facility showing infrastructure (left) and a schematic plan of the facility detailing array locations (right). Image credit: BIFoR.

The facility is located in Staffordshire, UK (52°47'58"N, 2°18'15"W) in a 21-hectare mature, semi-natural broadleaved woodland (>200 years continuous tree cover) characterised with English oak, *Quercus robur L.*, 'standard' primary trees and a common hazel, *Corylus avellana* L. understory layer. There are several other species of tree dispersed across the woodland including sycamore, *Acer pseudoplatanus* L., hawthorn, *Cretaegus monogyna* Jacq. and Ash, *Fraxinus excelsior* L. This diversity means that BIFoR FACE has the most complex canopy structure of all forest FACE experiments to date (Hart *et al.,* 2019).

As a long-term, multidisciplinary study, the site is very well instrumented. A wide range of biological and environmental factors are routinely recorded (Table 1.1). Such extensive monitoring will allow valuable system-wide insights into the relationship between the biotic factors measured and the environmental covariables which influence them, for example herbivory, photosynthetic rate and temperature/humidity.

Table 1.1 – Details of some of the key variables which are continuously recorded across the BIFoR FACE facility.

Variable	Details
Air temperature	Across all arrays at 2 and 25 m elevation.
Ambient CO_2 concentration	6 x 32 multiport sampling system.
Barometric pressure	At 1.2 m, 20 m, 30 m & 40 m on the flux tower.
Humidity	Across all arrays at 2 and 25 m elevation.
PAR	At 1.2 m, 20 m, 30 m & 40 m on the flux tower.
Sap flow	2 trees in each array.
Soil moisture	Depths: Soil surface, 10 cm and 25 cm.
Soil respiration	9 soil collars per array.
Soil temperature	Depths: Soil surface, 10 cm and 25 cm.
Throughfall precipitation	2 rain gauges per array.
Total radiation	At 1.2 m, 20 m, 30 m & 40 m on the flux tower.
Wind direction	Across all arrays at 25 m elevation.
Wind speed	Across all arrays at 25 m elevation.

As a second-generation FACE experiment, BIFoR FACE represents a unique opportunity to measure the response of insects to eCO_2 in a temperate heterogenous woodland system. Results from this study site can be combined with the findings from other second-generation FACE experiments including the existing EucFACE (within Australian Cumberland Plain forest) and the potential Amazon FACE (Brazilian Amazon basin tropical rainforest) to build a truly global picture of forest insect responses to eCO_2.

1.04 Insect sampling in forest ecosystems

The traits which confer the ecological dominance of insects, such as high abundance, varied life cycles and breadth of habitat specialisations, also make it difficult to accurately and completely measure their population and spatiotemporal distribution. Whilst methods such as insecticidal canopy fogging may allow a fairly comprehensive assessment of invertebrate diversity (Blanton, 1990), such methods would oversample the system and have direct, lasting

impacts. The answer, therefore, is to collect a small, representative number of individuals using a variety of sampling methods within the different structural components of the forest system (e.g. soil, shrub and canopy layers) as well as across seasonal timelines (critical in temperate systems). Sample data can then be extrapolated up to provide an indication of absolute total values. Various sampling techniques are employed by entomologists in sampling programmes to collect data on insect populations (Leather and Watt, 2005). These techniques usually involve a method for catching individuals, either temporarily or permanently, so that they can be recorded. In certain situations, direct observations of individuals can be made from a sampling unit, for example the number of individuals per leaf for a numerous pest species. More often, sampling involves some form of trapping using specifically designed equipment. Some sampling techniques are considered 'active' meaning they positively attract individuals by taking advantage of a certain behaviour (Leather and Watt, 2005). Alternatively, sampling which relies on chance for an individual to be recorded is 'passive'. Given the various nuances of different sampling methods, different techniques possess inherent bias and will disproportionately favour certain taxa (Table 1.2).

Table 1.2 - Different invertebrate sampling methods with details of key taxa sampled by each method. Adapted from Grootaert et al., 2010.

Method	Collection Type	Key groups
Portable suction devices	Active	Hemiptera, Araneae.
Beating	Active	Coleoptera, Araneae, Hemiptera.
Sweep net	Active	Hemiptera, Araneae.
Visual observation	Active	All, particularly larger, non-cryptic species.
Fogging	Active	All, except endogenous species.
Coloured pan traps	Passive	Diptera, Hymenoptera, Aphididae.
Emergence traps	Passive	Diptera, Saproxylic species.

Light traps	Passive	Heterocera, Nematocera.
Malaise traps	Passive	Flying insects, particularly Diptera & Hymenoptera.
Sticky traps	Passive	Diptera, Hymenoptera, Aphididae.
Suction traps	Passive	Diptera, Aphididae.
Pitfall traps	Passive	Epigeal invertebrates, particularly Carabidae, Staphylinidae, Araneae, Opiliones, Formicidae and Diplopoda.

Forests are particularly complex ecosystems and inherently more difficult to sample due to their high structural diversity. Furthermore, a large proportion of forest biodiversity occurs in components of the system which are difficult to access, such as below-ground or many metres up in the canopy (Speight, 2005). A number of important ecosystem processes occur predominantly in these difficult to reach parts of the ecosystem, for example the majority of photosynthesis which occurs in a forest, does so in the upper strata of the canopy (Holbrook and Lund, 2004). This has led to a disparity between the functional importance of these habitats and how well represented they are in the majority of ecological sampling.

In order to accurately determine the interaction between insects and forest ecosystems under environmental change, it is vital to have a representative and comprehensive sampling programme. Some sampling methods are demonstrably more suitable for accomplishing this within forest systems, whilst others need to be adapted to negate specific difficulties associated with the forest environment. Where they can be accommodated, it can be advantageous to use 'standard' ecological techniques, for example, pitfall trapping, which can be successfully utilised in a wide range of situations, including forests (Woodcock, 2005). This can allow for comparisons to be more easily made between different systems/studies.

1.05 General insect response to eCO_2

The magnitude of CO_2 concentration increases predicted to be experienced under global climate change over the next 50-100 years (+50ppm – +500ppm), and thus those used in FACE experiments, is unlikely to directly impact insect physiology. Although there are many examples of the direct effects of CO_2 on insects, such as in host finding by sanguivorous flies or rhizophagous larvae (Nicolas and Sillans, 1989), these are in relation to relatively large concentration gradients. These are frequently multiple orders of magnitude greater than the 150ppm typically used in FACE experiments. I am unaware of any examples of small increases in atmospheric CO_2, such as is the case in global atmospheric increases, having a direct impact on insects. In fact, many insect species regularly inhabit environments where CO_2 concentrations are routinely many times greater, e.g. under bark, in snow-covered soil or within dung (Nicolas and Sillans, 1989). Any direct effects of eCO_2, would instead likely act through altering behaviours such as host finding or oviposition (Stange, 1999).

Insects are instead expected to respond to small increases in CO_2 via indirect, plant-mediated mechanisms due to the close interdependency of the two groups. eCO_2 has been shown to directly impact plant physiology, growth and biochemistry (Curtis and Wang, 1998; Pringle, 2016). In particular, greater concentrations of atmospheric CO_2 are associated with increased C:N in plant tissue and/or alterations of plant defence mechanisms (see section 1.06). These changes will in turn have an associated impact on herbivorous insects and their immediate predators and parasitoids. It is through these trophic cascades that insects as a group often exhibit rapid and easily detectable changes to even small perturbations to environmental conditions.

When the impacts of eCO_2 on forest insects has been investigated, it was generally found that there was a decrease in abundance (Facey *et al*., 2016) and diversity (Altermatt, 2003). These responses are, however, inconsistent and tend to be species specific (Sanders *et al*., 2004; Hillstrom *et al*., 2014). Given the functional diversity of insects as a taxonomic group, a high degree of variation in responses of insect abundance and diversity would be expected as different groups are able to exploit or suffer from changes in their environment. Changing environmental conditions may also be associated with local extinctions, changes in endangered species status or altered pest status of species (Coviella and Trumble, 1999).

1.06 Herbivory under eCO_2

A guild is a group of species which utilise the same resources, such as parasitoids or folivorous herbivores. Herbivorous insects often have a particularly close relationship with their host plant and, therefore, will experience any plant-mediated effects of eCO_2 more than other invertebrate guilds. This sensitivity means that insect herbivores are expected to be amongst the first groups of organisms in the system to exhibit detectable responses to CO_2 fumigation (Cornelissen, 2011). Insect herbivore performance may be impacted by changes in leaf nitrogen content, water content, carbohydrate content and secondary plant compounds associated with herbivore defence (Bezemer and Jones, 1998).

eCO_2 is associated with increased C:N ratios in the tissues of woodland plant species (Gifford *et al*., 2000; Nowak *et al*., 2004) as a result of increased carbon uptake (Leakey *et al.,* 2009). Nitrogen is a limiting factor for most herbivores (Mattson, 1980), therefore, a decrease in N availability in plant tissues represents a decrease in nutritional value for insect herbivores. This decline in host plant quality is associated with increased larval developmental time,

decreased pupal weight and decreased survival in a number of herbivore species, particularly across the Lepidoptera (Stiling and Cornelissen, 2007). Other species respond to this decline in palatability by exhibiting behavioural changes such as compensatory feeding (Robinson *et al*., 2012). By increasing feeding rate, these herbivores may be able to mitigate the detrimental effects of lower quality food. Increased feeding may, however, lead to increased exposure to defensive compounds, particularly in plants species that alter allocation of secondary compounds, negatively affecting the growth and survival of herbivores (Coviella *et al.,* 2002). Furthermore, longer developmental times and slower growth rates also increase a herbivores exposure to natural enemies such as parasitoids and predators e.g. the 'slow-growth–high-mortality hypothesis' (Benrey and Denno, 1997).

eCO_2 is also directly associated with an increase in defensive compounds (Ryan *et al*., 2010) and defence efficiency (Fu *et al*., 2010) via increased production of photosynthates allowing increased allocation to secondary metabolites. Such increases in plant defence can have a significant detrimental impact on insect herbivore performance (Landosky and Karowe, 2014). In other instances, eCO_2 has also been shown to have the opposite effect through the disruption of the regulation of plant defence gene expression, making the plant more vulnerable to herbivores or influencing herbivore behaviour (Zavala *et al.*, 2009). Disruption of plant defences in this way has been associated with increased feeding rate, for example a dilution of phenolic compounds leading to increased herbivory by a Chrysomelid beetle on *Salix myrsinifolia* (Veteli *et al*., 2002).

The changes in the magnitude or direction of herbivory under eCO_2 are, therefore, highly species specific (Hillstrom *et al.,* 2014) and are often unique to each insect-plant pair (Coviella

and Trumble, 1999). Research has shown that eCO_2 can lead to either: (i) reduced herbivore diversity, richness and abundance (Altermatt, 2003; Cornelissen, 2011), with an associated decline in herbivory (Stiling *et al*., 2002; Hamilton *et al*., 2004) or (ii) an increase in canopy herbivory damage, due to compensatory feeding, which may significantly affect the capacity of a forest ecosystems to act as carbon sink (Couture *et al*., 2015). To date, amongst the studies examining changing patterns of herbivory in response to eCO_2 there is a bias towards leaf chewing defoliators (Cornelissen, 2011). The overall response of an ecosystem, therefore, is likely to vary depending on the species composition (both plant and insect) and abiotic conditions. Regarding the latter point, the impact of an increasing frequency of extreme events, such as drought or flooding – as is predicted under climate change, on these relationships remains largely unknown. There is some evidence that eCO_2 alters plant responses to extreme events, e.g. by leading to increased senescence (Warren *et al*., 2011). It is less clear, however, how this relates to herbivory, as a mass herbivory event has never coincided with a forest FACE experiment throughout the combined >100 years they have been operational.

1.07 Aphids and leaf miners

The feeding method and plant tissues fed upon by herbivorous insects varies greatly. Species which feed in similar ways can be grouped into feeding guilds such as 'leaf-chewers', 'phloem-feeders', 'root-feeders', 'leaf miners' etc. Variation between feeding guilds may partly explain the differential responses exhibited by herbivores in general to changing environmental conditions such as eCO_2. Aphids (Hemiptera: Aphididae) and leaf miners (Endophagous insect

larvae) represent two groups of insect herbivores from different feeding guilds that are abundant and ecologically important in deciduous woodland ecosystems.

Many studies that have examined the potential impacts of climate change on insect herbivores have used aphids as a model group due their characteristic life history, ease of use within the laboratory and economic relevance as major pests. Aphids are true bugs (Hemiptera) in the Aphididae family which feed on plant sap by piercing the phloem with specially adapted mouthparts. Aphids reproduce asexually for much of their life cycle and are frequently very abundant in the field, , meaning they play an important role in ecosystem processes such as herbivory, litter inputs and food webs (Throop and Lerdau, 2004).

Figure 1.3 – A group of Periphyllus acericola alates with nymphs on the underside of a sycamore, Acer pseudoplatanus, leaf. A fairly common aphid species in UK deciduous broadleaf woodland.

As phloem feeders, aphids will exhibit changes in feeding behaviour in close association with any changes in host plant physiology (Auclair, 1963). This trait suggests that aphids are expected to be able to easily exhibit compensatory feeding in the face of changing plant biochemistry, meaning they fall into the only herbivore feeding guild which is expected to show an overall positive response to eCO_2 (Sun *et al.,* 2016). This ability may be limited by changes in the host plant's resources allocation, resulting in no overall changes in aphid population (Awmack and Harrington, 2000). The responses of different aphid species, however, are known to vary, even when feeding on the same plant species. For example, *Myzus persicae* and *Brevicoryne brassicae* are two species of aphid which showed either increased or decreased offspring production respectively when reared on *Brassica oleracea* under eCO_2 (Bezemer *et al.*, 1999). In order to understand, therefore, how specific species of aphid within a particular system may respond, it is necessary to empirically test the responses of these host-aphid species combinations. Furthermore, the presence of natural enemy communities will also have a bearing on aphid responses at the population level (Awmack *et al*., 2004).

The majority of studies which have examined the impact of eCO_2 on insect herbivores have focused on free-feeding species (Cornelissen, 2011). Leaf mining larvae are a relatively under-studied feeding guild yet represent a useful indicator group due to their sessile nature and exceptionally close association with the host plant. A 'leaf miner' can be defined as an endogenous feeding insect herbivore larva. This group mainly consists of Lepidopterans, particularly Nepticulidae, but also includes some Coleoptera (Curculionidae), Hymenoptera (Tenthredinidae) and Diptera (mostly Agromyzidae). In some systems, leaf miners can be the most frequently encountered herbivore and provide a good opportunity to measure feeding

history and mortality via examination of the leaf 'mine' feeding trace (Stiling *et al*., 1999). The limited mobility of larvae, spending their entire larval stage within a single leaf, has several advantages. It means that comparing the effects of eCO_2 vs. control sites is much easier, as the insect will not move between treatments. Accordingly, any negative consequences experienced by leaf miners will be more pronounced than those of equivalent free-living species, as the ability to switch food source is removed (Johns and Hughes, 2002). The decline in quality of plant tissue as a food source which is associated with eCO_2 has been shown to decrease leaf miner density in a scrub oak community (Stiling and Cornelissen, 2007). Evidence for whether leaf miners are able to mitigate the negative impacts through compensatory feeding is mixed. Development rate, survival, pupal mass and adult mass were all found to be reduced with no evidence of increased feeding in *Dialectica scalariella* and *Chromatomyia syngenesiae* (Smith and Jones, 1998; Johns and Hughes, 2002), whereas for *Pegomya nigritarsis* eCO_2 was associated with increases in mine size and, therefore, level of feeding, as well as no change in pupal mass suggesting successful compensatory feeding (Salt *et al*., 1995).

Figure 1.4 – The serpentine mine of Stigmella microtheriella in a common hazel, Corylus avellana, leaf.

1.08 Change in community composition

In a broad sense, the responses of plants and their insect herbivores to eCO_2 varies between different functional groups, with different herbivore feeding guilds exhibiting opposing responses (Hillstrom and Lindroth, 2008). For example, leaf-chewers, such as leaf mining Lepidoptera, have been shown to increase feeding by 30% in an attempt to mitigate the impact of declining food quality, whilst populations of phloem feeders, such as aphids, increased and with development time decreasing by 17% (Bezemer and Jones, 1998). Varying responses between groups suggests that under eCO_2 there may be winners and losers amongst the feeding guilds, potentially leading to significant changes in community

composition without an effect on overall insect abundance (Hamilton *et al.,* 2012). The ramifications for such shifts in guild structure are not clear, but, given the key role played by these groups, could be important at the ecosystem level. Furthermore, forests represent one habitat type within a greater landscape, meaning the effect of eCO_2 on woodland species may have implications for the surrounding environment, such as agricultural ecosystems.

Responses will also vary within guilds and are often weak and species specific (Coviella and Trumble, 1999; Hillstrom *et al*., 2014). The high degree of variation within both phloem-feeding aphids and leaf mining Lepidoptera, however, mean guild-wide generalisations cannot be made (Salt *et al*., 1995; Smith and Jones, 1998; Hullé *et al*., 2010). It can be expected that key species within each guild will drive major processes in a particular ecosystem through bottom-up regulation, and therefore, elucidating the unique responses of these species is vital to gain an understanding of system-wide change.

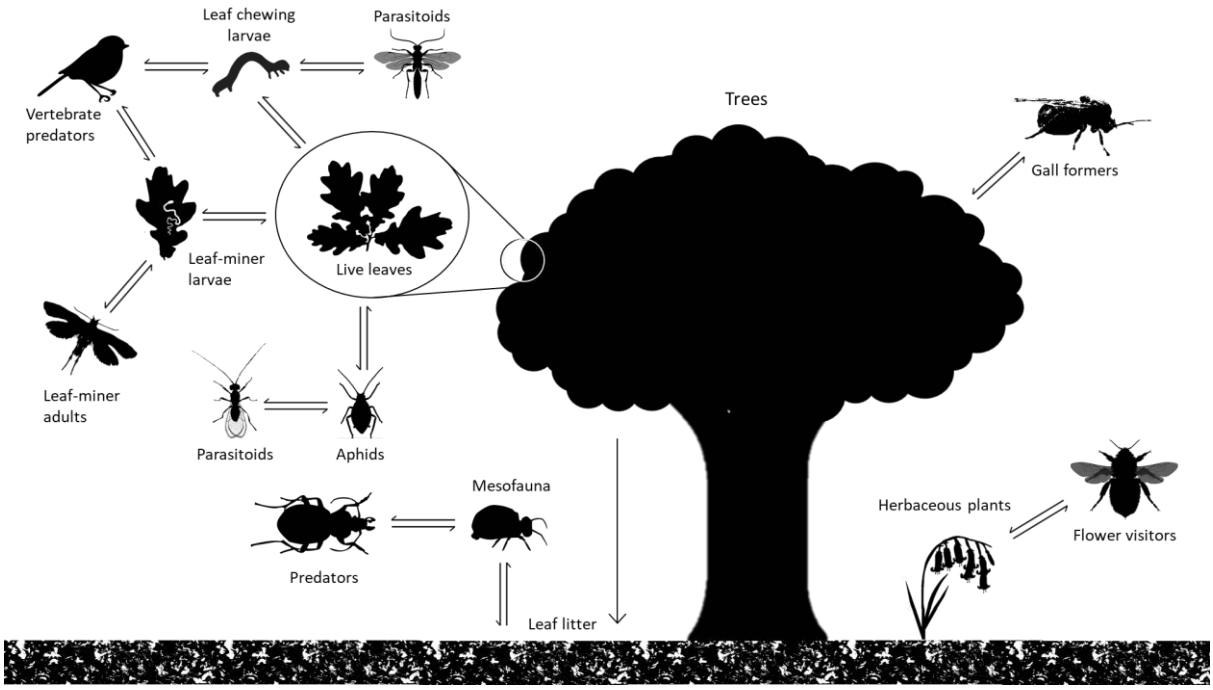

Figure 1.5 – A diagram detailing trophic structure of some key ecological processes within a forest ecosystem. The dominant tree species forms a 'hub' around which these processes operate, such as herbivory, pollination and nutrient cycling.

The responses at higher trophic levels will also govern shifts in guild structure. Top-down pressure from predators and parasites is an important regulator of insect herbivore populations (Rosenheim, 1998). Changes in population, performance or behaviour of herbivores will have knock-on effects for higher trophic levels, which in turn alters the level of control exhibited by these groups. For example, under eCO_2 increases in aphid populations have been allayed by increased suppression by harlequin ladybird beetles, *Harmonia axyridis* (Coccinellidae) (Hentley *et al*., 2014).

Increased mortality associated with some herbivore groups under eCO_2 may also result from an increased rate of parasitism (Stiling *et al*., 1999). Aphids are known to be more vulnerable to natural enemies under eCO_2 (Awmack *et al*., 1997). There are several routes by which this mechanism could act, either by influencing parasitoid performance, host defence efficacy or both. For example, eCO_2 was shown to diminish aphid alarm pheromone responses in *Chaitophorus stevensis* (Mondor *et al.,* 2004).

Development time and the proportion of time spent feeding both increase as a result of declining food quality, which can further increase the risk of predation or parasitism (Price *et al.,* 1980). Alternatively, the impact of eCO_2 may be more pronounced on early instars (Bezemer and Jones, 1998), which, along with decreased growth rates, may affect the average size of hosts available for parasitism. Host size effects are known to have significant impact on parasitoid fitness (Opp and Luck 1986) which may further impact on top-down control of herbivore populations.

The direction of responses of tri-trophic interactions to environmental perturbances are difficult to predict due to their inherent complexity. Gaining a better understanding of the

impact of changing climate on complex trophic interactions is necessary to understand how key ecosystem processes may be affected (Facey *et al.,* 2014). In order to detect these affects a comprehensive, representative sampling programme is required to begin to build an understanding of the relationship between environmental changes and invertebrate communities.

1.09 Nutrient cycling

Insect herbivores, particularly leaf chewers, produced a significant volume of waste (frass, honey dew, greenfall, etc.) during the action of feeding. This material is nutrient rich (Gherlada *et al*., 2016), with high concentrations of N and P, and often in a more labile form than leaf litter (Lovett *et al*., 2002; Madrich *et al*., 2007). The production of frass, therefore, represents a significant link in the cycling of nutrients back into the soil, increasing the rate at which nutrients, such as N, move through the system (Frost and Hunter, 2007). In this way herbivory is a key component linking above- and below-ground processes (Bardgett and Wardle, 2003). Changing patterns of herbivory under eCO_2 will impact upon frass production which will directly affect nutrient cycling (Frost and Hunter, 2004; Kagata and Ohgushi, 2012). For example, compensatory feeding may lead to an increase in frass production, thus increasing the rate of N flow into the soil.

In addition to altering the quantity of frass produced, changing leaf chemistry may also affect the biochemical constitution of frass. The impact of eCO_2 on the biochemistry of frass produced remains relatively untested and may contribute to a more complete understanding of the impacts of changes in atmospheric C on nutrient cycling.

Different feeding guilds may also have differing impacts on nutrient cycling. Phloem feeders such as aphids produce sugar rich honeydew rather than frass during feeding, in which the C and N is highly labile (Stadler *et al.*, 1998). Given the large numbers of aphids during population booms associated with specific stages of the life cycle and the associated quantities of honeydew produced, this has been shown to affect C and N dynamics at the ecosystem level (Grier and Vogt, 1990). Honeydew production by individual aphids can increase under eCO_2, in association with increasing feeding rates in response to altered phloem C:N (Sun *et al.*, 2009). Whilst the amino acids in honeydew have not been found to alter under eCO_2 (Sun and Ge, 2011), it is also possible that the chemical composition of honeydew may change in other ways, such as the C:N ratio. Changes in composition or quantity of honeydew may, therefore, confer a significant effect on overall nutrient dynamics of the system. Honeydew is also involved in interactions with other insects, such as being used in host finding by parasitic hymenopterans (Bouchard and Cloutier, 1984), or as a nectar-substitute by certain pollinators including *Bombus spp.* (Moller and Tilley, 1989). Changing chemical composition of honeydew is likely to have a significant effect on these interspecific interactions.

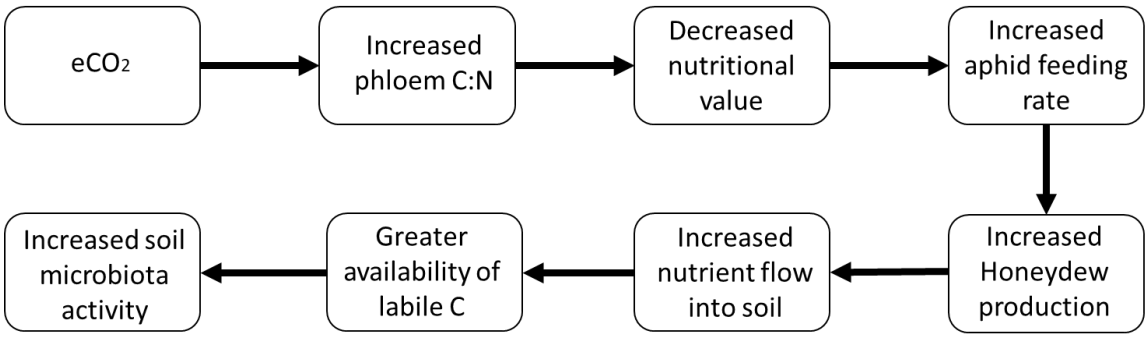

Figure 1.6 – Schematic detailing a potential nutrient flow cascade in relation to aphid herbivory under elevated CO_2.

Many species of insect herbivores periodically undergo rapid increases in population during 'outbreak events'. These disturbance events occur as a result of the interplay between multiple different top-down and bottom-up regulatory factors, for example predators and pathogens (Dwyer *et al.,* 2004). For example, winter moth, *Operophtera brumata* (Linnaeus, 1758), is a common species of moth which undergoes intermittent outbreaks, the intensity of which is thought to be regulated by the host plant, parasitoids and temperature (Buse and Good, 1996; Kerslake *et al.,*1996). Changing environmental conditions are associated with increase in frequency and intensity of these insect outbreaks (Volney and Fleming, 2000), although they remain difficult to predict, and the specific impact of eCO_2 on these outbreaks remains unclear. When outbreak events do occur, they can greatly impact both plant performance and nutrient flow dynamics, e.g. decreasing sap flow and increasing rate of organic C and N deposition into soil (Stadler *et al*., 2001; Kristensen *et* al*.,* 2019). This will also affect soil bacteria abundance and composition, which in turn will impact other processes such as soil respiration or leaf litter decomposition.

Herbivory has also been shown to affect leaf litter quality by impacting leaf decomposition rate (Findlay *et al*., 1996). The association between eCO_2 and reduced soil microarthropod species richness may be partly explained by these impacts on leaf litter (Hansen *et al*., 2001). Frass production and leaf litter decomposition are the two key processes in the recycling of nutrients from the canopy to the soil, with insects and other related arthropods the main drivers of both of these processes. To understand how these processes may be affected by eCO_2, it is necessary to examine the responses of the organisms driving them. Sampling epigeal invertebrates, such as by pitfall trapping, will help to identify these responses.

1.10 Phenology

Phenology is the measure of the timing of an organism's life history events (e.g. emergence from an over-wintering diapause), which are often highly responsive to environmental stimuli. Increasing evidence suggests that climate change is altering the relative phenology of many species, affecting events such as bud burst, flowering, and leaf senescence which have advanced on average by 2.5 days per decade since 1971 in temperate latitudes (Körner and Basler, 2010). Some tree species have been shown to experience earlier canopy bud burst in spring, leading to increased ground shading earlier in the year and could therefore impact on ground flora development (Roberts *et al.*, 2015). In this example flowering time of herbaceous woodland plants may be delayed as a consequence, which would have major implications for a range of insect-plant interactions.

The phenology of a species is influenced by a range of environmental variables, chiefly amongst them is temperature. This interaction is, however, highly nuanced, rather than a simple correlation between current thermal conditions and the induction of physiological processes. In reality, phenology is often determined through various interactions between an organism's thermal history, exposure to other biotic and abiotic variables and individual phenotype. Furthermore, a species' phenology is influenced by and influences that of other species, for example in a tritrophic system where species interaction strength is maintained through phenological synchronisation (Buse *et al.*, 1999).

CO_2 concentration is amongst the environmental variables known to impact plant phenology (Springer and Ward, 2007), although it remains unclear what impact eCO_2 will have on

temperate forest plants as these effects are variable and often species specific (Asshoff *et al*., 2006).

1.11 Pollination

A 'pollinator' is an agent which moves the pollen from the anther of an Angiosperm flower to the receptive stigma of a conspecific, thus achieving 'pollination' by facilitating fertilisation of the ovules (Faegri and Van der Pijl, 2013). This is often conflated with the term 'flower-visitor' which refers to an animal, usually an insect, which comes into contact with a flower, typically in order to feed on or collect floral resources such as nectar and pollen. The majority of flower visits will not result in successful pollination, often due to the flower visitor not immediately visiting a second, receptive flower of the same species, or failing to transport enough pollen to ensure likely transmission to the stigma. Many studies mistakenly report on 'pollinators', failing to distinguish these from 'flower-visitors' which is a more general term for anthophilous species (Kevan and Baker, 1983).

Pollination is an important ecological process for which many species of plant rely on insect pollinators. For example, 35% of global food production is reliant on insect pollination services (Klein *et al*., 2007). The relative success of pollination has implications for plant reproductive success, which could, in turn, impact plant species turnover and even plant community composition (Wilcock and Neiland, 2002).

Both plants and their insect pollinators exist in a spectrum of specialisation vs generality. Some species are pollinated by a diverse assemblage of flower-visiting insects whereas other interactions are performed by a highly specialised single species pair (Motten *et al.,* 1981). The majority of plant-pollinator assemblages are, in fact, generalised, with most plants being

served by several pollinators and most pollinators visiting several species of plants (Herrera, 1996; Waser *et al*., 1996). The relative complexity of a plant-pollinator interaction network is, therefore, variable and will influence the resilience of the pollination interactions under environmental change.

eCO_2 is one of a number of changing environmental factors which impact flower phenology, which could influence plant-pollinator interactions. For example, it has been widely shown that, alongside temperature, eCO_2 directly effects flowering time in many species of both cultivated and wild plants (Springer and Ward, 2007). Many pollinators are also experiencing phenological shifts under climate change, often experiencing earlier emergence times in response to increasing mean temperatures (Bale and Hayward, 2010). It is likely, therefore, that eCO_2 will impact on the timing of plant-pollinator interactions, potentially exacerbating the impact of temperature on phenology. This may lead to a loss of synchrony due to temporal mismatch following independent shifts in the phenology of some plant pollinator pairs (Forrest, 2015). Such changes could have significant effect on the system due to the important role played by plant-pollinator interactions in woodland ecosystems, including species composition and carbon-cycling (Pringle, 2016).

As well as flower phenology, eCO_2 may also affect the production and composition of nectar and pollen, which act as the currency of plant-pollinator interactions. Currently, the literature regarding this is relatively sparse, mostly focussing on relatively few economically important crop species. These differ from wild species, typically allocating more mass to reproduction with no change in C:N allocation under eCO_2, whereas wild plants typically allocate more C to structural and defensive tissues under these conditions (Jablonski *et al.,* 2002). Whilst there is

clear evidence that eCO_2 affects plant tissue C:N in a wide range of species (Gifford *et al.*, 2000), this has generally not yet been extended to floral resources. Subtle changes in the physiology and growth of plants, such as altered composition of nectar or pollen, could eventually lead to changes in ecosystem assemblages through impacts on 'higher order biotic interactions and lifetime fitness' (Bradley and Pregitzer, 2007). Overall, most studies have found that plants tend to increase their reproductive output under eCO_2 (Ward and Strain, 1999).

1.12 Summary and thesis outline

The majority of studies investigating the effect of eCO_2 on insects have been undertaken in controlled laboratory conditions, with few assessments of the impact of eCO_2 on insects under field conditions, such as within FACE experiments. Furthermore, these have been performed almost exclusively in relatively simple ecosystems such as crop systems or young, homogenous stands of trees (e.g. Stiling *et al.*, 2002; Hamilton *et al.*, 2004; Sanders *et al.*, 2004; Hillstom *et al.*, 2014). Forest age and diversity influences insect community structure and composition, with more mature and heterogenous woodland systems often supporting more diverse insect assemblages (Tews *et al.*, 2004; Jeffries *et al.*, 2006). The greater number of interactions associated with this additional complexity is likely to influence insect responses to eCO_2 as well as the feedback of these responses on the ecosystem. For example, the greater species diversity associated with mature forests may confer an additional degree of resilience, meaning that eCO_2 does not impact the arthropod community as much as it might in a simpler system.

The maturity of the ecosystem may also influence the response to eCO_2 due to the differential physiology of older trees. It remains unclear whether eCO_2 will have similar effects on mature trees compared with young, growing trees upon which the majority of studies to date have focussed (Hunter, 2001). The magnitude of the response of mature trees is expected to be small and in a complex system such as this, the effects are likely to be dynamic and species-specific (Asshoff *et al.,* 2006). Furthermore, the effect of eCO_2 on many groups of insects remains unassessed in any meaningful way (Coviella and Trumble, 1999). Measuring the responses of insects within this system will, therefore, act as a useful indication of how the system as whole may change. Examining responses across broader ecological scales is required to develop knowledge beyond the simple pairwise interactions which most research previously has focused on (Facey *et al.,* 2014). Furthermore, some aspects cannot be accurately replicated at smaller scales, such as the impacts of changing phenological patterns on interspecific interactions or multi-trophic interactions. Only ecosystem scale experiments can provide the robust, relevant empirical data required. This evidence would have implications for commercial forestry, in terms of pests, invasive species and productivity as well as wider environmental science due to the role of forests in carbon sequestration and provision of ecosystem services.

Against this background, this thesis seeks to take advantage of the unique opportunity presented by BIFoR FACE to determine the impact of eCO_2 on insects for the first time in a mature, temperate woodland at the ecosystem scale. This will be achieved by addressing the following hypotheses:

1) A characterisation of the arthropod community within a mature woodland ecosystem and an assessment of sampling method efficacy.

2) eCO_2 leads to a decrease in the abundance and increase in compensatory feeding of leaf miners.

3) eCO_2 leads to an increase in aphid abundance, growth rate and fecundity.

4) Bluebell flowering phenology is delayed under eCO_2, with an associated decrease in flower visitation and seeds production.

5) eCO_2 leads to an increase in abundance of certain orders and a decreased abundance of alternative orders over the course of 3 years of fumigation.

The work addressing these objectives is presented in the following chapters as a series of research articles centred around the BIFoR FACE experiment which have, or shall be, submitted for publication. There is, therefore, necessarily some degree of repetition between chapters, for example within the methods sections.

CHAPTER 2

Methods for characterising spatial and temporal patterns of arthropod abundance and diversity in a mature, temperate, oak woodland.

The work presented in this chapter has been submitted for publication as: Crowley L M, Ivison K, Enston A, Garrett D, Sadler J P, Pritchard J and Hayward S A L, (In review). Methods for characterising spatial and temporal patterns of arthropod abundance and diversity in a mature, temperate, oak woodland. *Acta Oecologica*.

2.01 Abstract

Arthropods underpin fundamental ecological processes such as herbivory, pollination and nutrient cycling, and are often responsive to subtle changes in environmental conditions. Thus, changes in their abundance and phenology may be crucial indicators of system-wide responses to climate change.

The new Birmingham Institute for Forest Research (BIFoR) Free Air Carbon Dioxide Enrichment (FACE) facility provides a unique opportunity to undertake a comprehensive assessment of spatial and temporal patterns of arthropod biodiversity and abundance in a mature forest. This is an essential first step before attempting to measure the potential impacts of eCO_2 on arthropod populations. Two fundamental criteria are: i) sufficiency of sampling to determine differences between structural layers of the woodland system, e.g. ground, shrub, sub-canopy and canopy layers, ii) a temporal resolution that can identify seasonal patterns of change (phenology). This paper sets out the methodological approaches employed to achieve these objectives.

A total of 22,568 invertebrates from 108 families were sampled using a range of techniques across all major strata. Diptera were the most abundant order sampled and had the greatest number of families represented (45). Yellow pan traps collected more arthropods than white or blue traps. Upper canopy diversity was greater than that in the understory samples.

Phenology patterns generally followed the anticipated seasonal cycle, with increasing abundance and diversity from spring to summer. Temperature was the best predictor of Malaise and pitfall trap collections. Precipitation was not correlated with any phenology patterns/trap data.

Patterns of abundance, diversity and phenology were consistent across this heterogeneous site, and eCO_2 treatments did not significantly alter these patterns during year 1. These data provide an important baseline from which to assess the impacts of eCO_2 over the 10-year BIFoR FACE experiment, and highlight the importance of employing diverse sampling methods, temporal replication and measuring environmental factors over appropriate timescales.

2.02 Introduction

Accurate and detailed assessments of biodiversity are key to our understanding of how complex ecosystems may respond to changing environmental conditions. Arthropods play an integral role in both terrestrial and freshwater ecosystem function and stability due to their high abundance, diversity and disproportionate impact on nutrient cycling compared with their cumulative biomass (Yang and Gratton, 2014). Many groups of arthropods are currently experiencing significant global declines (Biesmeijer *et* al., 2006; Conrad *et al.,* 2006; Brooks *et al.,* 2012; Hallmann *et al*., 2017; Lister and Garcia, 2018; Sánchez-Bayo and Wyckhuys, 2019),

which has been linked to several factors including habitat loss and degradation, climate change, pollution, pesticides, invasive species and introduced pathogens (Wagner, 2020). Long term experiments are needed in order to build an accurate picture of how arthropods are responding to climate change, including how processes such as herbivory, pollination and nutrient turnover may be affected and the associated impact this has on the ecosystem. Furthermore, due to their small size, high abundance, short life cycles and ectothermic physiology, arthropods are typically highly responsive to subtle changes in environmental conditions (Cornelissen, 2011). This sensitivity means that they are expected to be amongst the first group of organisms to respond to climate change and thus represent a useful indicator group (Ferris and Humphrey, 1999).

In order to accurately measure these potential changes, it is essential that we gain an accurate understanding of current spatial and temporal patterns of arthropod abundance and diversity in different systems as a baseline. Furthermore, quantification of these components may be strongly influenced by acute environmental conditions and sampling methodology.

Around 30% of the earths land surface, >42 million km^2, is covered by forests (Bonan, 2008). Forest ecosystems are, therefore, of global importance in terms of housing biodiversity, regulation of water cycling and carbon sequestration (Jenkins, 2002). Understanding the implications of eCO_2 on ecosystem processes within forests is vital to elucidate how this fundamental change may affect their performance in the near future, and Free Air Carbon Dioxide Enrichment (FACE) experiments are an important tool to investigate community-level impacts of near future climate scenarios *in situ* (McLeod and Long, 1999). The Birmingham Forest Research (BIFoR) FACE facility represents the only mature temperate woodland FACE

experiment currently running in the northern hemisphere. While previous forest FACE experiments have studied invertebrates, these first-generation experiments typically only examined young, plantation/monoculture forests (Knepp *et al*., 2005; Stiling & Cornelissen, 2007; Hillstrom *et al*., 2014). Consequently, it remains unclear how invertebrates will respond to eCO_2 in a mature, heterogeneous woodland systems, and if the associated feedbacks will operate in an analogous way. Mature forests also have a high degree of structural diversity, particularly across their vertical profile, which is comprised of several layers including soil/ground, leaf litter, field/shrub layer, understory and canopy. This structural diversity not only contributes to an overall increase in biodiversity, but also the complexity of spatial and temporal (phenological) species distribution patterns (Schowalter and Ganio, 1998). Characterising this complexity, therefore, necessitates the use multiple invertebrate sampling techniques over entire seasonal timescales. This level of detail has been lacking from many previous FACE experiments, and whilst they have been employed within more recent FACE studies, e.g. EucFACE (Facey *et al.,* 2016), the Eucalyptus forest systems are not as diverse nor experience such extensive seasonal/phenological changes in insects as encountered in temperate deciduous woodland.

Various techniques are employed for sampling invertebrates, each with their own advantages and limitations. Different techniques disproportionately favour certain taxa depending on life history, abundance and behaviour, and will therefore produce a different 'sample profile'. For example, pitfall trapping is an extensively utilised sampling method to capture epigeal beetles, spiders and ants, but tends to under-represent Hymenoptera and Diptera (Southwood and Henderson, 2009). The sample profile will also vary in different habitats, across habitat layers, seasonally and under different environmental conditions (Yi *et al*.,

2012). In order to build a more complete overall profile of the biodiversity, therefore, it is often necessary to employ several techniques simultaneously (Kitching *et al*., 2001; Leather and Watt, 2005). Comparisons of catches under standardised conditions also allows a more accurate assessment of sampling method efficacy and reliable interpretation of results from ecological studies using these techniques.

The complexity of mature woodlands means it is challenging to perform an accurate, detailed and representative assessment of the arthropod assemblages. In particular, the canopy is infrequently sampled due to practical difficulties of access and is often under-represented in biodiversity sampling, despite the functional importance of arboreal invertebrates (Schowalter, 1995). Against this background, the current study assessed different sampling methods to characterise the arthropod fauna of the Birmingham Institute for Forest Research Free-Air CO_2 Enrichment facility across the full vertical profile of the woodland system. The purpose is to provide a baseline against which any changes in abundance, diversity or phenology can be compared throughout the duration of this unique 10-year experiment. The impact of eCO_2 is assessed using this first year's data to determine if there were any immediate impacts on distribution patterns, for example, CO_2 is a known attractant for certain Dipterans. I also interpret invertebrate trap data in light of seasonal patterns of temperature and precipitation to identify other potential climatic drivers of arthropod abundance, diversity or phenology. Finally, outputs from different sampling methods are also compared to provide an assessment of their efficacy in characterising the site, and if there is any sampling redundancy.

2.03 Materials and methods

Experimental site

The study was conducted at the Birmingham Institute for Forest Research Free-Air CO_2 Enrichment ('BIFoR FACE') experimental facility, located in Staffordshire, UK (52°47'58"N, 2°18'15"W) as described in Hart *et al*. (2019). The site comprises 21 hectares of mature, semi-natural broadleaved woodland (>200 years continuous tree cover), characterised by >150-year-old 'standard' English oaks, *Quercus robur*, and a previously coppiced common hazel, *Corylus avellana*, understory. There are several other species of tree dispersed across the woodland including sycamore, *Acer psuedoplatanus*, hawthorn, *Cretaegus monogyna*, and ash, *Fraxinus excelsior.*

There are 9 experimental arrays across the site, comprising 6 infrastructure arrays of which 3 are CO_2 fumigated treatment arrays and 3 are non-fumigated control arrays. The remaining 3 arrays are non-infrastructure controls (Figure 2.1). CO_2 enrichment commenced in April 2017 and will continue throughout the 10-year duration of the FACE experiment. Treatment arrays receive CO_2 fumigation to elevate the average concentration across the array to 150ppm (~550ppm total) above ambient (~400ppm), measured in real time (Norby *et al*., 2016).

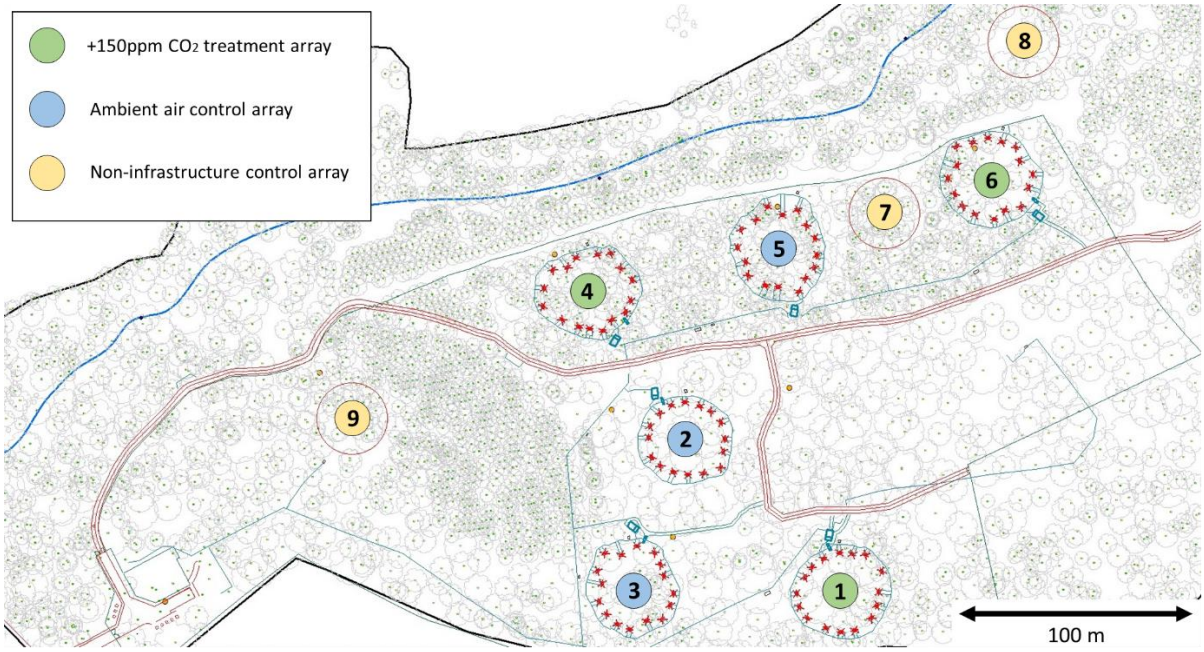

Figure 2.1 – The BIFoR FACE experimental site in Staffordshire, showing array locations and numbering. 1, 4 and 6 are infrastructure treatment arrays, receiving +150ppm CO_2 above ambient. 2, 3 and 5 are infrastructure control arrays, receiving ambient air via the same infrastructure. Arrays 7, 8 and 9 are non-infrastructure controls. Red marks within infrastructure arrays denote CO_2 delivery pipe support towers. Adapted from an image from BIFoR.

Invertebrate sampling

Five sampling methods were selected in order to maximise sampling efficacy whilst minimising physical impacts on the site and in an effort to avoid oversampling. Both active and passive sampling methods were employed for one full year from March 2017 to February 2018. This sampling period commenced one month before eCO_2 'switch on' in order to allow detection of any immediate, acute effects of fumigation on insects within the woodland. Sampling was conducted across four layers (ground, field/shrub, understory and canopy) in the last week of each calendar month. The location of traps within the arrays was generated randomly before they were installed.

Table 2.1 – Total number of samples derived from each sampling method per month by array type.

Sampling method	Treatment arrays	Control arrays	Non-infrastructure control arrays	Total per month
Pitfall traps	2 x 3	2 x 3	2 x 3	18
Malaise traps	1 x 3	1 x 3		6
Pan traps	3 x 3	3 x 3	3 x 3	27
Understory beating	1 x 3	1 x 3		6
Canopy beating	1 x 3	1 x 3		6
Total	24	24	15	63

Pitfall trapping. To sample ground layer invertebrates, two pitfall traps (passive) were installed in each experimental array (18 in total) which is consistent with other forest FACE experiments (Sanders *et al*., 2004; Facey *et al*., 2016). The traps consisted of a 570ml plastic cup, (8cm diameter and 10cm depth), positioned so the rim was level with the soil surface. Pitfall traps were left for a two-week bedding in period before any sampling took place to allow for any increased catch rates derived from 'digging in' effects to subside (Greenslade, 1973). When operational, traps were filled to about 1/3 with water with a drop of scentless detergent to break the surface tension, and covered by a tile held on metal legs to prevent rain and debris from falling in. When not operational, between trapping periods, pitfalls were closed with a lid to prevent unwanted by-catch. The sampling period was 7 days, with traps collected in at approximately the same time of day as they were deployed.

Malaise trapping. A Malaise trap (passive) (Watkins and Doncaster, UK) was deployed in each of the infrastructure treatment and control arrays (total 6) to sample flying insects. Malaise traps (main screen 180cm x 160cm) were operational over a 24-hour sampling period from approximately 10:00am to 10:00am during which the collection bottle was attached, filled to 1/3 with water plus a drop of scentless detergent. During 'non-operational' intervals the collection bottle was removed, and the trap left open.

Pan-trapping. Flower-visiting insects were sampled using coloured pan traps (active), which have previously been effectively used in a FACE experiment (Hillstrom & Lindroth, 2008). A single post supporting three different coloured pan traps mounted on a crossbar approximately 1m off the ground was installed in each experimental array (total 9). The pan traps consisted of a plastic bowl of 20cm diameter and 10cm depth, spray painted yellow (~580nm), blue (~475nm) or white. The coloured pans were operational over a 24-hour sampling period from approximately 9:00am to 9:00 am, during which they were half-filled with water plus a drop of scentless detergent (as above).

Understory and Canopy beating. Understory (common hazel) and canopy (oak) vegetation were each sampled by beating (active) at a single location in each experimental array once a month (6 understory and 6 canopy). Insecticidal approaches, such as 'fogging', were not viable as these would have a large, lasting impact and affect subsequent sampling. To avoid damage to vegetation the foliage was agitated, instead of being struck with a stick, as conducted by Altermatt (2003) to sample canopy arthropods in a forest FACE experiment. Due to the logistical limitations of sampling in the canopy, a large plastic funnel was used instead of a traditional full-sized beating tray. An area of approximately 1 square metre was systematically agitated over the course of 30 seconds above a plastic funnel of 25cm diameter, connected to a collecting pot. *Quercus robur,* English oak, was selected as the dominant canopy species and was sampled at a height of between 25m and 30m, at a point which was within reach from the central tower. *Corylus avellane,* Common hazel*,* was selected as the dominant understory species and was beaten from ground level, directly below the point in the canopy where oak beating occurred. Beating occurred during every month that the trees had photosynthetically active leaves (April-October).

Processing and identification of samples. Samples were collected and placed into 70% ethanol for long term storage before identification. During identification, all arthropods in each sample were counted and identified to order under a stereomicroscope (SMZ140; Motic, Spain). Initial counting and identification took approximately 15 minutes on average per sample, equating to 174 hours in total. All pan trap and beating samples were identified to family. Coleoptera from pitfall samples were acknowledged as a key group and also identified to family.

Diversity indices. Simpsons and Shannon-Wiener diversity indices were calculated at family level for understory and canopy beating samples.

Meteorological data

Temperature. Mean air temperature was calculated from hourly means measured by a Campbell Scientific 107 Thermistor and recorded on a Campbell Scientific CR300 series datalogger fitted to one of the towers of each FACE array at a height of the upper canopy (approximately 22m). Mean temperature was then calculated for the time windows during which each sampling method occurred. The time window for meteorological measurements related to beating sampling was set at twenty-four hours from 00:00 to 23:59 of the day the sampling took place. The time windows for meteorological measurements related to Malaise and pan trapping were set at forty-eight hours from 00:00 the day the traps were deployed to 23:59 the day samples were collected. The time windows for meteorological measurements related to pitfall trapping was set at 168 hours (= 7 days) from 00:00 the day the traps were deployed to 23:59 the day samples were collected.

Precipitation. Mean throughfall precipitation was calculated for the site from measurements taken from 2 ARG100 tipping bucket Rain gauges in each array and recorded on a Campbell Scientific CR300 series datalogger. Total throughfall was calculated for the same time windows as mean temperature.

Statistical analyses

All statistical analyses were performed in R, version 3.5.2 (R Core Team, 2015). The two pitfall trap samples taken from the same experimental array simultaneously were pooled to negate pseudo replication.

eCO_2 analysis. The analysis of treatment effect on arthropod abundance was initially performed using a Generalised linear mixed model (GLMM) with Poisson error distribution. Sampling method and month were included in the model as fixed effects and array as a random effect. The model was refitted with negative binomial error distribution in response to overdispersion and validated by inspection of residual plots (Brooks *et al.,* 2017).

Meteorological analysis. The analyses of mean temperature, maximum temperature, minimum temperature and throughfall precipitation on arthropod abundance for each sampling method during the respective time windows were performed using a Generalised Linear Model with quasi-Poisson errors.

Pan trap colour analysis. The variance of arthropod abundance across the three coloured pan traps was heterogeneous, and the distribution was non-normal. Consequently, these data were analysed using a Kruskal Wallis test, followed by a Dunn post-hoc test for multiple comparisons.

Canopy vs. understory comparison. The variance of arthropod abundance within canopy and understory samples was homogenous, and the distribution normal. Simpson's and Shannon-Wiener diversity indices of canopy and understory arthropods were also shown to have homogenous variance and a normal distribution. Therefore, the analyses of abundance, Simpson's diversity and Shannon-Wiener diversity of canopy vs understory were performed using paired t-tests. Species richness has been shown to strongly correlate with both genus and family numbers (Báldi, 2003). As a result, family can successfully be used as a surrogate for species diversity for taxa difficult to identify past family level (Derraik *et al*., 2002).

2.04 Results

Spatial patterns of abundancy and biodiversity: comparison of trapping methods.

Over the 12-month sampling period a total of 22,568 arthropods were collected and identified, comprising 24 orders within the Phylum: Arthropoda. Of these orders, 12 were within the Class Insecta, 4 within Arachnida, 3 within Entognatha and 7 from other Classes. Diptera were the most abundant order overall, with 10,869 individuals, and the most frequently sampled in the pitfall (Ground layer), Malaise and pan traps (field/shrub layer) (Figure 2.2a, b, c). Coleoptera and Hymenoptera were the second and third most sampled orders, with 2,795 and 2,643 individuals collected respectively. Of the individuals identified to family, 65% belonged to just 6 families, specifically Staphylinidae (32.9%), Leoididae (4.5%) and Carabidae (8.1%) for the Coleoptera; Sciomyzidae (13.1%) and Chironomidae (3.0%) for the Diptera; and Platygastridae (3.4%) for the Hymenoptera. Araneae were the most frequently sampled group by canopy and understory beating (34.2%, Figure 2.2d).

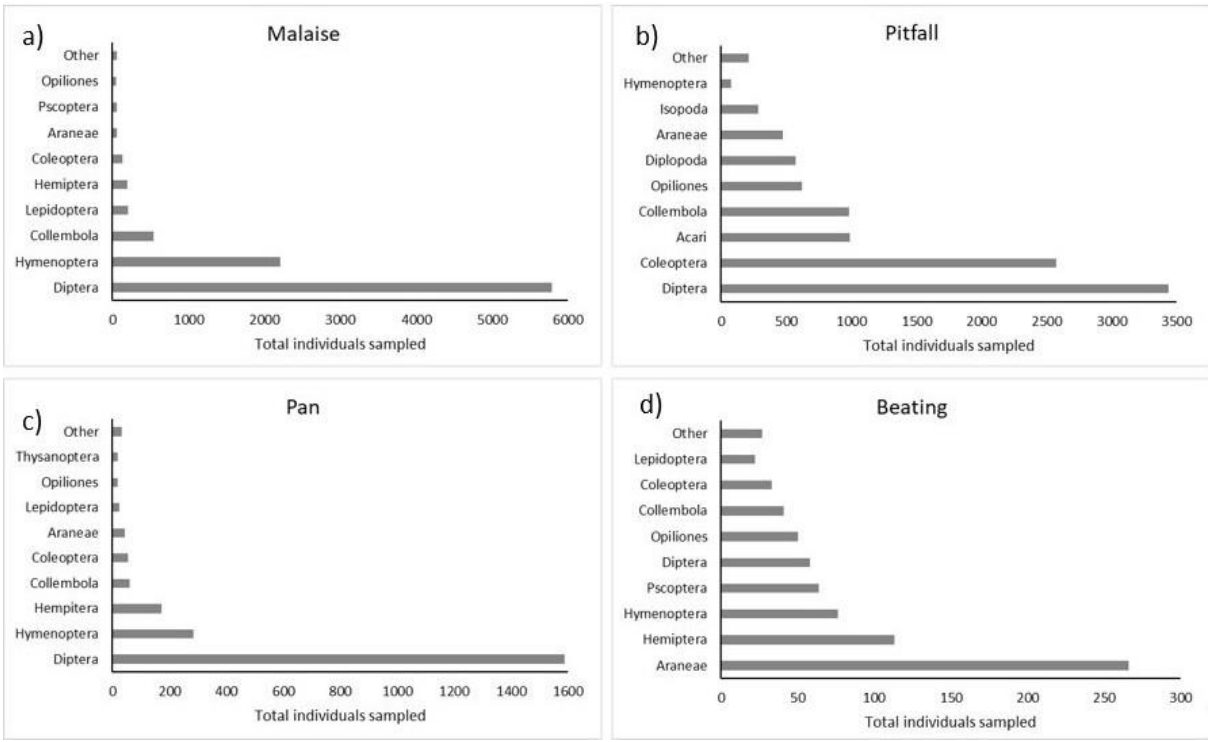

Figure 2.2: Total number of arthropods from the 9 most frequently sampled orders from Malaise (a), pitfall (b), pan trapping (c) and combined canopy and understory beating (d). Totals are cumulative across the complete 12-month sampling period (March 2017-February 2018).

A total of 10,230 arthropods were sampled from the ground layer using pitfall traps (Figure 2.3a), 9,289 from Malaise traps (field/shrub layer), 2,299 from pan traps (field/shrub layer) (Figure 2.3b), 471 from canopy beating and 279 from understory beating (Figure 2.3c). 108 families were identified from the pitfall traps (Coleoptera), pan traps (all Insecta) and beating (all Insecta). eCO_2 had no significant effect on the overall abundance of arthropods sampled ($p>0.1$), whereas sampling method, and therefore spatial distribution did have a significant effect ($p<0.1$).

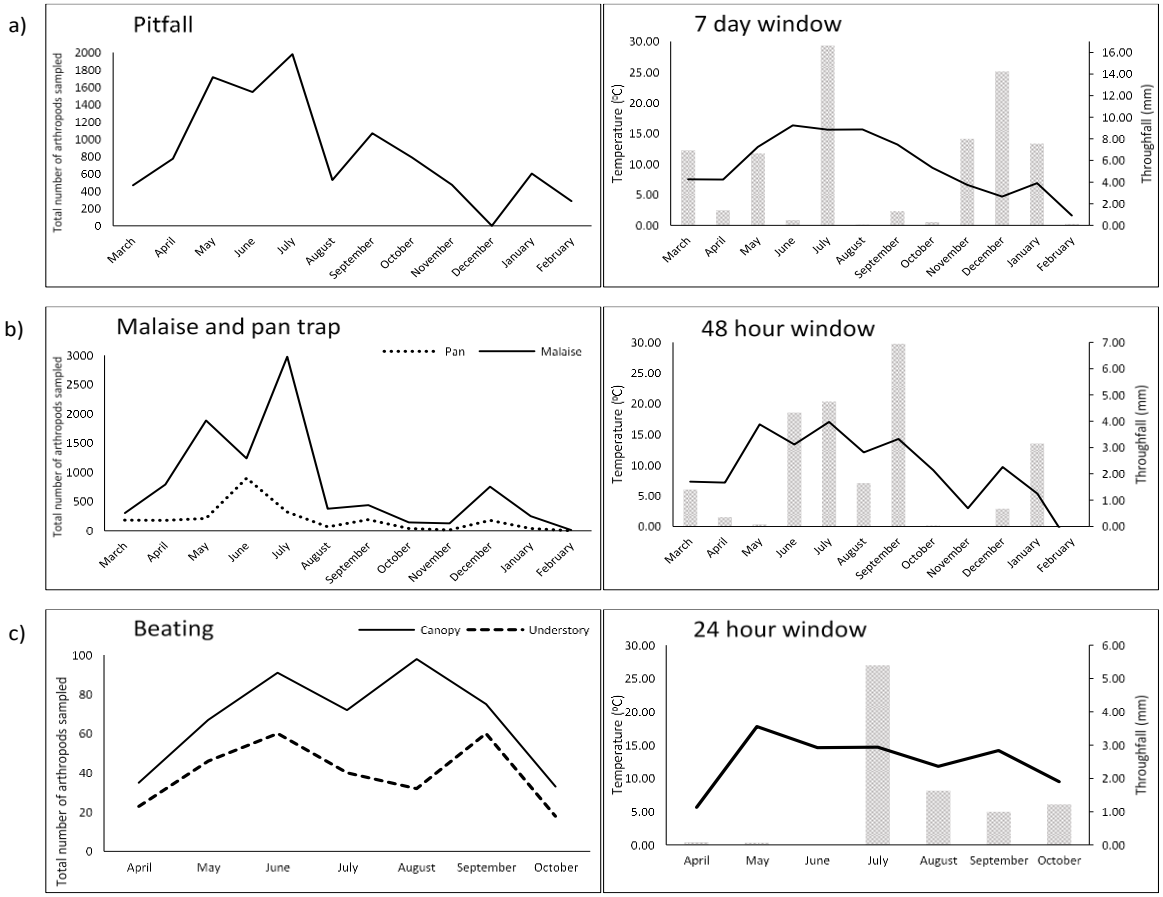

Figure 2.3 – Total number of arthropods sampled monthly by pitfall (a), pan and Malaise trapping (b) and canopy and understory beating (c) over the 12-month sampling period (March 2017-February 2018). Mean temperature and total throughfall precipitation during the associated time window is displayed next to each sampling method, 7 days for pitfall (a), 48 hours for pan and Malaise trapping (b) and 24 hours for beating (c).

Arthropod abundance was significantly higher in the canopy compared to the understory across the 12-month period (Figure 2.3c; $t = 3.87$, $p < 0.01$). This difference was greatest in August 2017 and driven mainly by Araneae and Braconidae. Family level diversity in the canopy was also greater than in the understory for all months, except September, for both Simpson's and Shannon-Wiener indices (Figure 2.4a and 2.4b respectively). Overall mean Simpsons and Shannon-Wiener diversity index scores of the canopy were both significantly greater than understory ($t = 2.557$, $p = 0.04$ and $t = -2.8707$, $p = 0.0284$).

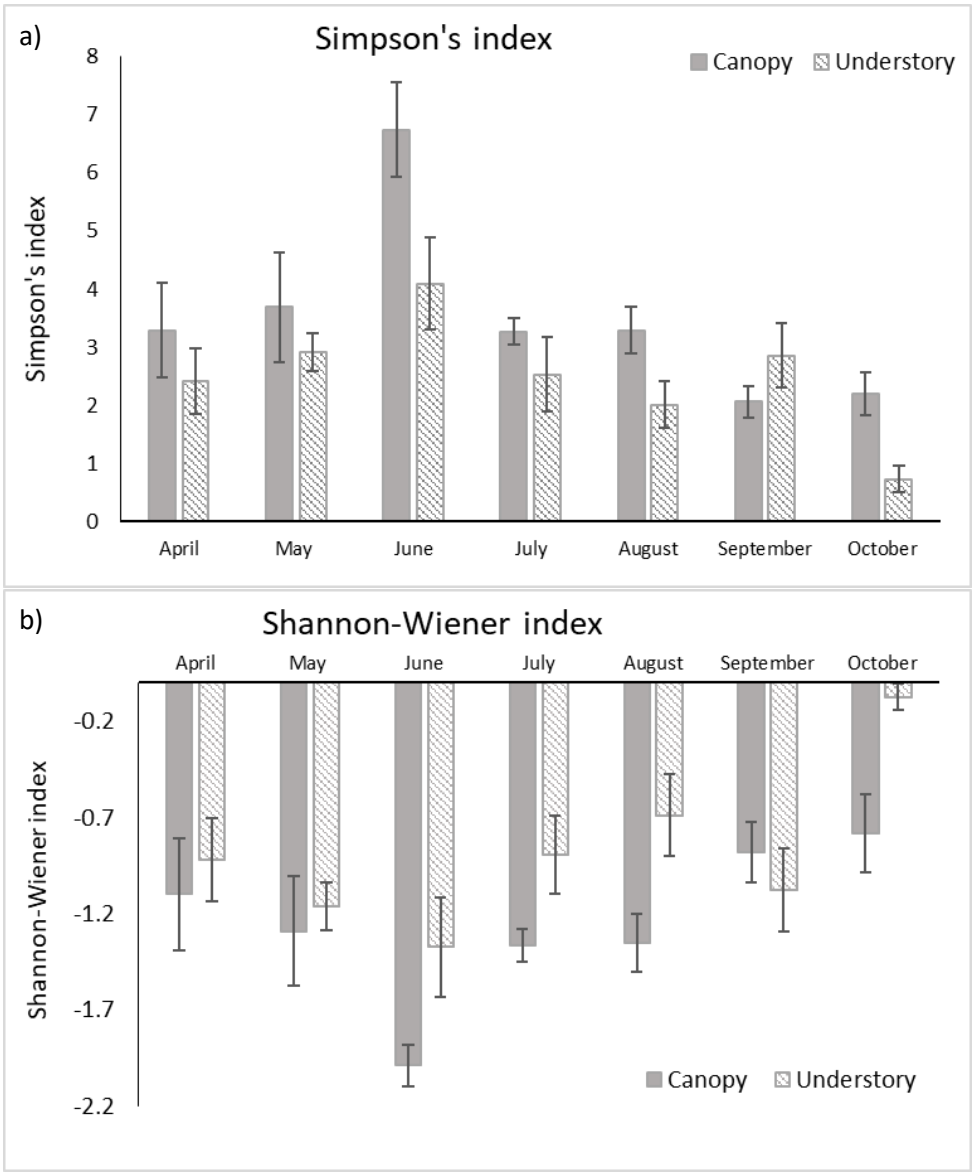

Figure 2.4 - Mean (+/- S. E.) Simpson's diversity index (a) and Shannon-Wiener diversity index (b) for canopy vs understory beating samples across the 7-month period (April to October 2017).

Temporal patterns of abundance and diversity: phenology and climate profiles.

There was a clear phenological pattern of arthropod abundance, with total numbers (collected across all trapping methods) increasing each month from March 2017 to a peak of 5,387 individuals in July 2017 (Figure 2.3). Sampling month had a significant effect on the overall abundance of arthropods sampled ($p<0.01$). There was a significant decrease in the total number caught in August using all sampling methods except canopy beating, which experienced its highest overall catch during this month. Total abundance rebounded slightly

in September followed by a consistent decline in abundance until November 2017. Pitfall traps collected more arthropods than any other method for every month between August 2017 to February 2018 except December 2017, when both Malaise and pan trap collections increased slightly (Figure 3a vs. Figure 3b). Mean December temperatures for the 7-day pitfall collection period were low (4.70°C), compared with Nov (6.61°C) and January (6.89°C), while mean 48-hour temperatures for Malaise and pan trapping periods were high (9.67°C) compared to November (2.96°C) and January (5.34°C) (Figure 2.3a & 2.3b).

Mean temperatures calculated for each 48-hour Malaise trap collection period had a significant correlation with arthropod abundance using this method (Figure 2.3b; $p<0.001$). Mean temperatures for each 7-day pitfall trap collection period also had a significant correlation with numbers collected (Figure 2.3a; $p<0.01$). No correlations were found between temperature and abundance for pan traps or beating methods, and throughfall precipitation did not correlate with arthropod abundance from any sampling method.

Pan trap colour

Yellow pan traps consistently collected significantly more arthropods than either the blue or white traps for all months, with overall means of 14.6, 2.92 and 3.77 individuals respectively (Figure 2.5a; Kruskal-Wallis $\chi^2 = 6.3765$, $p = 0.04$). Numbers collected in white and blue traps were not significantly different from each other across the first year overall ($p = 0.3$). Sciomyzidae were the most abundant family sampled by pan traps (33.4%), of which 99.6% were caught in yellow traps (Figure 2.5b).

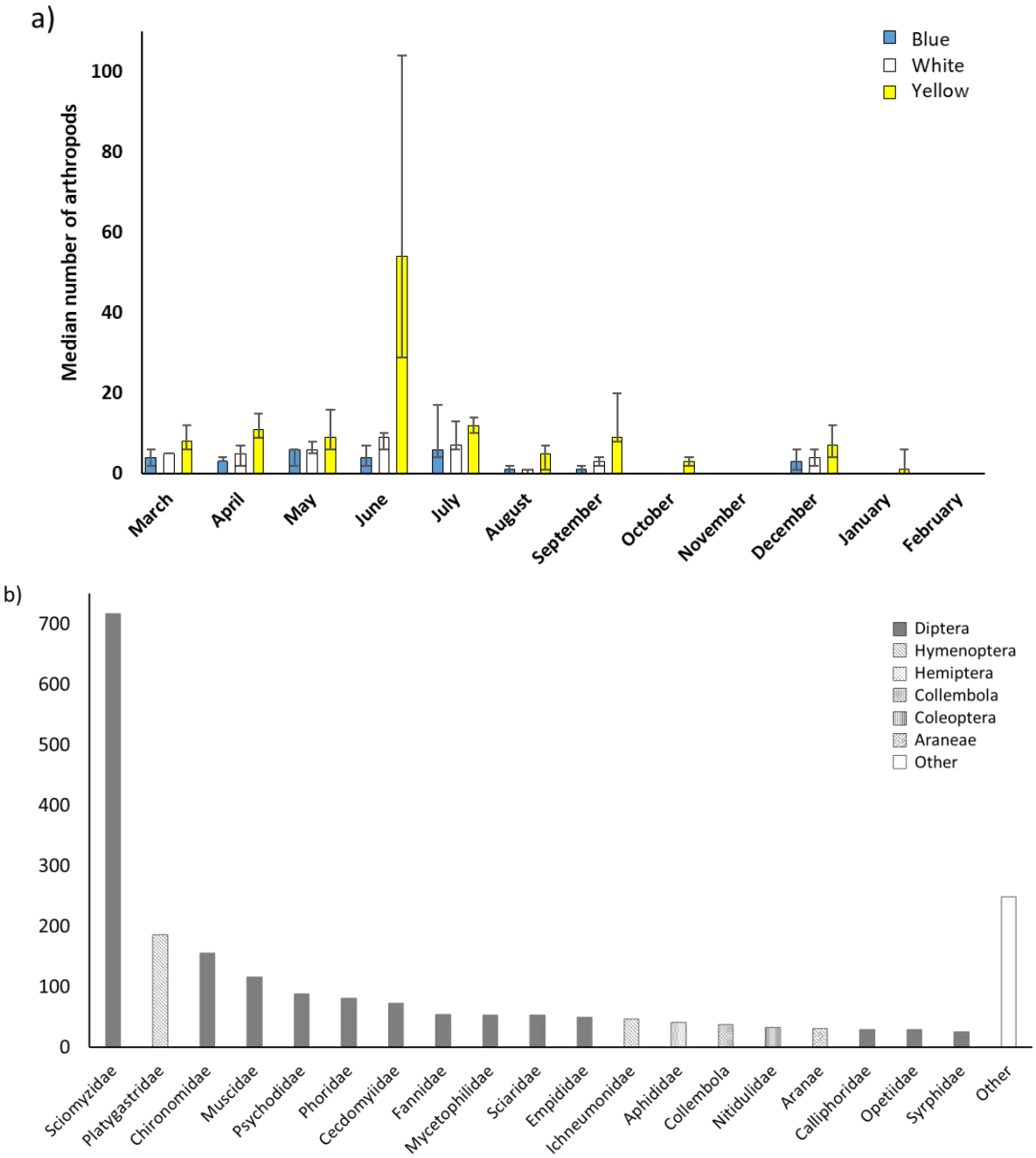

Figure 2.5: (a) Median (+/- Interquartile range) number of arthropods sampled by blue, white and yellow pan traps each month across the 12-month sampling period (March 2017-February 2018). (b) Total number of arthropods from the 19 most frequently collected families in all pan trap samples. Totals are cumulative across all 3 colours and the complete 12-month sampling period (March 2017-Febraury 2018).

2.05 Discussion

This study provides an important spatial and temporal characterisation of arthropod abundance and diversity across a mature oak woodland, and importantly a site that will

experience 10 years of +150ppm CO_2 as part of the BIFoR FACE experiment. It also allows us to evaluate the efficacy of different sampling methods which will be fundamental for future studies determining mid- to long-term impacts of eCO_2 on arthropods in temperate forest ecosystems.

General arthropod abundance and diversity

The sample composition across all trapping methods was dominated by just three orders, with over 72% of the individuals belonging to Diptera, Coleoptera and Hymenoptera. Whilst this high abundance suggests that these groups may be key drivers of ecological processes, these taxa are also highly diverse in both species and functional groups. Other forest FACE experiments also found similar sample composition at order level, suggesting that the sample obtained is both representative and comprehensive (Altermatt, 2003; Hillstrom & Lindroth, 2008; Stiling *et al*., 2010; Facey *et al.,* 2016). Diptera were the most abundant order sampled, comprising 48% of the total. This is unsurprising given that this order has been found to dominate several other ecosystems, e.g. an Arctic ecosystem (Schmidt *et al.,* 2017).

Similarly, at the family level it was found that a relatively small number of families drove the overall abundance patterns observed, with 65% of individual belonging to just 6 families (Staphylinidae, Sciomyzidae, Carabidae, Leoididae, Platygastridae and Chironomidae).

Abundance does not necessarily correlate with biomass, which may be a more useful response variable in relation to ecological functionality (Saint-Germain *et al.,* 2007). Previous studies have, however, found that whilst significant changes in abundances can occur across a wide range of woodland arthropod groups, this does not always correlate with significant changes in biomass (Facey *et al.,* 2016).

An alternative approach to address these issues is DNA metabarcoding (Taberlet *et al.,* 2012). Whilst this technique provides a 'next-generation' approach towards assessing biodiversity, it also has limitations. Metabarcoding can provide rapid determination of the number of species from environmental samples (Coissac *et al*., 2012), but requires a reference database to be effective and with current methods sampling is destructive. Furthermore, whilst DNA barcoding is an excellent tool for describing sample diversity, it is less useful for measuring abundance and biomass (Elbrecht & Leese, 2015), which are important metrics in studies which seek to determine the effect of environmental factors on arthropod populations and ecological processes.

Spatial distribution

The methods used to sample each layer in this study varied considerably, for example sampling period duration or 'active' vs 'passive'. Whilst this allows efficient sampling of each individual layer to build a picture of the arthropod community composition, it is not possible to compare layers based on numbers of individuals produced alone.

The ground layer was dominated by Nematoceran Diptera and epigeal Coleoptera. Almost all of the Diptera sampled from this layer were adult Chironomidae and several other fly families which possess larvae which feed in leaf litter. Whereas the larvae themselves were rarely sampled, undoubtably due to their limited motility, adults were sampled in large numbers. This is most likely due to their greater motility as they walk and fly throughout this layer in search of either mates or oviposition sites. The most abundant beetle families were Staphylinidae and Carabidae. Whilst both these families are large and functionally diverse, the majority of species identified from the samples in this study were predatory.

The samples from the field/shrub layer consisted chiefly of Diptera and Hymenoptera. The two most abundant Dipteran families were Sciomyzidae, whose larvae are predators/parasites of Gastropoda, and Chironomidae whose larvae are abundant in tree holes, rotting vegetation and soil, playing an important role in detritus processing and trophic cycles (Armitage *et al.,* 2012). The majority of the Hymenoptera sampled belonged to the family Platygastridae, which are typically egg parasitoids of Diptera, Coleoptera and Hemiptera.

Araneae were by far the most abundant order from the canopy. These are exclusively predatory, although even within the canopy layer there were species sampled from a range of different hunting guilds. The next most abundant families were Aphididae and Braconidae. Aphididae are phloem feeding herbivores which are often very abundant due to large, rapid population increases during certain stages of their lifecycle. Braconid wasps are the second most diverse family of parasitoid wasps, with an equally diverse host range, which, for a number of common species, includes aphids.

Importance of the canopy

A large proportion of the biomass and biodiversity of a mature temperate woodland occurs within the canopy layer (Halle, 1995) and this layer is particularly important for a large number of arthropods (Ulyshen, 2011). In order to accurately sample the habitat, it is, therefore, vital to include all layers across the vertical profile, including the canopy. Despite this, many studies of woodland biodiversity omit or have limited representation of the canopy layer due to inherent difficulties associated with sampling many metres off the ground. This is

particularly prevalent for temperate forests, for which typically less attention has been paid to canopy arthropods than for tropical forests (Ulyshen, 2011).

Studies have found mixed results in regard to patterns of abundance and diversity of arthropods across the vertical layers of temperate forests, with the canopy supporting higher diversity (e.g. Sobek *et al*., 2009), equal diversity (e.g. Stork & Grimbacher, 2006), or lower diversity (e.g. Hirao *et al*., 2009) in different situations. The overall number of individuals sampled by canopy beating was considerably smaller than the numbers in the pitfall, Malaise and pan trap samples, however, this reflects differences in sampling method rather than an abundance gradient. Direct comparisons of abundance can only be made between samples taken in the same manner e.g. active vs passive or sampling period. In this study I found that overall abundance and diversity of arthropods in the canopy was consistently greater than the understory when sampled in the same way, i.e. beating. This is an important result in the characterisations of the particular patterns of diversity within this specific ecosystem. There are a number of variables which may be driving this difference, including height, structural differences, tree species, phenology and microclimate. Furthermore, the extent of interconnectivity between the canopy and other layers remains unclear. A high degree of connectivity could influence the sample profile due to movement of arthropods into and out of the canopy, meaning that the timing and conditions of sampling is particularly important.

Phenology and climate data

Unsurprisingly, given the temperate location of the BIFoR FACE site, there was a clear, strong seasonal phenology in both abundance and diversity of arthropods within the woodland. These seasonal patterns highlight the importance of characterising phenology in two ways.

First, temporal replication can ensure results are more representative of the system as a whole and not skewed by stochastic events (Southwood & Henderson, 2009). For example, during August the abundance of individuals sampled decreased by 80% relative to the previous month, before increasing again in September. This event would have provided a false characterisation of the site if sampling had occurred only in August. Second, the sampling interval of phenology sampling is crucial, and if not frequent enough can miss key phenological events (Southwood & Henderson, 2009). Optimisation of sampling intervals is, therefore, again a trade-off between precision and practicality.

Our study also highlights the importance of interrogating climate data within time periods relevant to the sampling methods employed, and not just using, for example, monthly or yearly means (e.g. Lister & Garcia 2018). There are several instances where mean temperatures for the 48-hour Malaise or pan trapping periods give a very different picture of climate conditions for a particular month than when looking at the 7-day mean temperatures for the pitfall trapping periods, e.g. December 2017 (Figure 2.3). This is an important finding as it highlights that the duration of sampling period as well as the duration of environmental monitoring influence whether or how the relationship between then is interpreted.

The lack of a correlation between arthropod abundance, particularly flying insects which form the majority of arthropods sampled in this study, and precipitation is an interesting and unexpected result. This suggest that woodland systems may be more buffered against the effects of precipitation, perhaps due to the structural component of the trees/canopy. The next step is to characterise microclimate conditions relevant to the locations of these trapping methods, e.g. soil temperature and moisture availability for ground layer, shrub layer

air temperature and above canopy precipitation. This resolution of microclimate data was not available for the first year of sampling at the FACE site but is now in place for the remainder of the experiment.

The different sampling methods also reveal variation in phenological patterns across vertical layers within the woodland system, both in magnitude and direction. For example, in August the total number of arthropods sampled from the canopy increased from the preceding month whereas understory decreased (Figure 2.3c). This temporal variation may be driven by actual shifts in arthropod abundance, climatic factors or seasonal variation in sampling method efficacy. For example, movement behaviours such as flight, often vary seasonally in relation to life history and voltinism, which would affect sample frequencies for flight interception traps (Basset, 1991). This is well characterised by fluctuations in the number of Aphididae sampled, which exhibited low overall abundance but experienced two large peaks in May and September. These peaks were driven by an influx of alate aphids, which likely corresponds to the phenology of host alternation or dispersal flights (Dixon, 1977). Climate can also directly influence trap performance, for example if temperatures drop below insect thermal activity thresholds then the frequency and duration of movement is curtailed (Coleman *et al.,* 2015). Equally, extended periods of precipitation will restrict flying insect movement in particular. Continuous sampling throughout the entire year is therefore important in order to allow subtle temporal changes in arthropod abundance and diversity to be measured in relation to seasonal climate patterns, whilst also allowing detection of shifts in phenology between years.

Pan trap colour

Pan traps are an effective method for sampling flower visiting insects within forested ecosystems, particularly when a range of colours are used (Campbell & Hanula, 2007). The dominance of Diptera and Hymenoptera in the pan trap samples highlights the relative importance of these groups as potential pollinators within the woodland ecosystem. In the present study this dominance was largely driven by flies in the Sciomyzidae, Chironomidae and Muscidae families and wasps in the Platygastridae family. These three Dipteran families are mostly comprised of saprophages whereas the Platygastridae are parasitoids. This is consistent with other studies which have also found that these feeding guilds dominate pan trap samples in temperate forests (Hillstrom & Lindroth, 2008).

The data from this study corroborates the evidence that colour of pan traps is important in determining efficacy, with yellow pans consistently sampling the greatest number of individuals in this system. This is consistent with previous studies which demonstrate that high reflectance colours are more effective (Vrdoljak & Samways, 2012). As well as the physical properties of different colours, their effectiveness may also be influenced by the relative abundance of flowers of the same colour in the surrounding landscape. It has even been suggested that catch sizes might be inversely proportional to the availability of flowers of the same colour in bloom in the vicinity (Cane *et al.,* 2000), representing a 'dilution effect'. There was a high abundance of blue and white flowers in bloom throughout the flowering period at this site, such as common hogweed, *Heracleum sphondylium* L.*,* and common bluebell, *Hyacinthoides non-scripta* (L.) Chouard ex Rothm., and the relative paucity of yellow

flowers. This, coupled with the consistent greater number of arthropods samples by yellow pan traps, potentially provides support to the dilution effect hypothesis.

There was a low overlap in the taxa caught by different pan trap colours. In some instances, entire families were sampled almost exclusively by one colour, for example Panorpidae in blue or Sciomyzidae in yellow pan traps. The different sample profiles produced by each colour means that in order to produce an extensive and representative sample, using a combination of colours is the most effective method (Campbell & Hanula, 2007).

Future impacts of eCO_2

No immediate response in arthropod distribution was detected in response to commencement of fumigation. For example, a small, temporary, positive response to higher CO_2 concentrations may have been expected to be observed immediately after switch-on in groups such as Nematoceran Diptera which are effectively trapped with CO_2 enrichment (Petrić *et al.,* 1999). The likely reason that this was not found to be the case is that the concentration gradients experienced were relatively modest at the scale of individual insects. This study also found no significant effect of eCO_2 on the abundance, diversity or phenology of arthropods over the course of the first 12 months of this 10-year experiment. This result is unsurprising as it is expected that a highly complex, mature system such as this would take longer than 12 months to respond in a way that would be detectable in broad scale changes to arthropod abundance and diversity. Despite this, the characterisation of the fauna provides an important baseline to allow the detection of future changes in response the eCO_2. Long-term monitoring of the experiment is on-going, and the impact of eCO_2 can only be fairly assessed after multiple years of treatment.

2.06 Conclusions

It is perhaps unsurprising that no meaningful changes in arthropod diversity or abundance were observed during the first year of CO_2 fumigation. The characterisation of the arthropod fauna during the first 12 months of the BIFoR FACE experiment has demonstrated that a combination of sampling methods is required to produce a comprehensive and representative sample due to the differences in sample profile produced by each method. There is no indication of sampling redundancy, as omission of any sampling method would result in the absence of one or more important functional groups. The data also demonstrate that experimental arrays at the BIFoR FACE site provide a good representation of a heterogeneous woodland across the eCO_2 and control treatments. Sampling of such complex systems must involve sufficient temporal replication to detect phenological patterns, which may be a key part of the ecosystem response to changing environmental conditions. Finally, the different layers of the woodland have been shown to produce significantly different samples, therefore a complete sampling programme across vertical layers of this woodland are is required to adequately sample this structural diversity. In particular, this study suggests that the canopy is a key layer within a mature forest ecosystem which may exhibit different patterns of faunal abundance and diversity.

CHAPTER 3:

The impact of eCO_2 on leaf miner herbivory in a mature temperate woodland.

The work presented in this chapter is being prepared for submission for publication as: Crowley, L.M., Sadler, J.P., Pritchard, J. and Hayward, S.A.L., (In prep). Elevated atmospheric CO_2 causes a decline in leaf miner herbivory in mature oaks via changes to leaf chemistry.

3.01 Abstract

Insect herbivory is an important ecosystem process affecting nutrient flow through all terrestrial ecosystems. It is unclear, however, how plant-herbivore relationships will respond to increasing concentrations of atmospheric carbon dioxide (eCO_2), and how feedback from these responses will in turn affect the wider ecosystem. The new Birmingham Institute of Forest Research (BIFoR) Free Aire Carbon Enrichment (FACE) facility provides a unique opportunity to investigate these responses in a mature, temperate woodland. Leaf mining insect larvae are a particularly useful feeding guild to study these responses, as their entire larval life history takes place within a single leaf and produces an easily quantifiable trace of their feeding history. eCO_2 is known to increase C:N ratios in many plant species, reducing their nutritional value, which could result in compensatory herbivore feeding. However, eCO_2 also influences the production of secondary metabolic (defensive) compounds in plants, which could negatively impact on leaf miner performance. Eight key species of Lepidopteran leaf miners feeding within oak and hazel leaves at BIFoR FACE were identified. The abundance and mine area of these species were measured in 2017 and 2018. Approximately 43% of oak leaves and 22% of hazel leaves surveyed contained at least 1 mine, which is considerably higher than the number found in previous studies. There was no difference in the overall

abundance of leaf mines between treatment and control on either tree species in both years. Equally, there was no difference in the mean mine area under eCO_2 and control conditions in 2017. Mean mine area was, however, significantly smaller for *Stigmella sp*. on oak under eCO_2 in 2018. Given the lack of any evidence of compensatory feeding in any species and the inconsistent response between oak and hazel, it is likely that the response is driven by an increase in defensive compounds. The decrease in mine area on oak represents a decline in leaf area consumed from 1.19% of the total leaf area per tree to 0.85%. This represents an important decrease in herbivory, with an associated decrease in C flow which potentially has implications for the ability of the forest to act as a carbon sink.

3.02 Introduction

Increasing global concentrations of atmospheric CO_2 are having a profound effect on plant biochemistry, physiology and ecology (Wang *et al.,* 2012). For example, elevated CO_2 (eCO_2) is associated with enhanced photosynthesis and an increase in C:N in plant tissue (Nowak *et al*., 2004). Many species of insects have a close relationship with plants, either as a shelter, a food source or as a food source for their prey. Changing environmental factors which influence plants are, therefore, also expected to indirectly impact the insects associated with them. Insect herbivores are particularly sensitive to subtle changes in host plant biology, perhaps more so than any other guild.

Insect herbivory is a key factor influencing ecosystem processes such as nutrient cycling. For example, it has been demonstrated that in a temperate forest insect herbivory was responsible for the removal of up to 70g of carbon sequestering biomass per square metre per year (Couture *et al*., 2015). Any impact of eCO_2 on insect herbivores via host plant

responses has, therefore, implications for the wider ecosystem via feedback loops. Insect herbivores are also abundant throughout most terrestrial ecosystems, including forests (Chapter 2), therefore processes which impact them are likely to also affect overall abundance and biodiversity of insects more widely. This has implications for processes which are underpinned by insects, such as food webs.

Previous studies have found mixed responses of herbivores to eCO_2 when tested empirically. These responses vary amongst different systems and are often dependent on feeding guild (Bezemer and Jones, 1998). Even within feeding guilds, sometimes responses may be idiosyncratic and species specific (Hillstrom *et al.*, 2014). In general, the responses of herbivores to eCO_2 typically fall into one of two outcomes: reduced performance or compensatory feeding (Cornelissen, 2011). A reduction in herbivore performance would be characterised by slower growth, reduced development and/or increased mortality, with an associated decrease in herbivory. This may be driven by declining host plant quality, increased plant defence or a combination of both (Figure 3.1). Alternatively, compensatory feeding is a behavioural response to lower nutritional value of host plant tissue whereby feeding rate increases. This would be associated with an increase in overall herbivory, assuming no significant changes in plant defence.

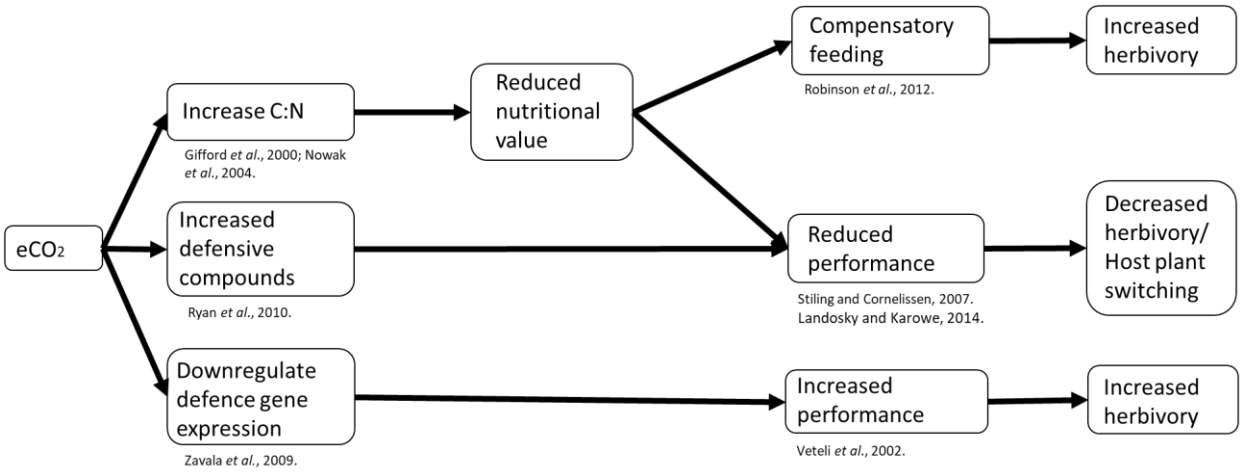

Figure 3.1 – Theoretical potential impact of eCO_2 on insect herbivory leading to the observed response.

Forests are a globally important ecosystem in terms of carbon storage, water cycling and housing biodiversity (Ozanne *et al.,* 2003). Our understanding of the relationship between eCO_2 and mature, complex forest systems, however, remains incomplete. Characterising the effects of eCO_2 on such forests via the modification of herbivory is an important step to develop a more complete understanding on the potential impacts of climate change. Free Air Carbon Dioxide Enrichment (FACE) experiments are an example of an *in situ*, long-term method which can be employed to redress this knowledge gap. The Birmingham Institute of Forest Research (BIFoR) FACE facility represents a unique opportunity to investigate the effect of eCO_2 on herbivory within a mature, temperate forest ecosystem. Whilst there were no clear changes in overall insect abundance in the first 12 months of the experiment (Chapter 2), there may be significant changes in the abundance of specific functional groups, such as insect herbivores, which become apparent when the group is studied more closely.

Leaf miners are a guild of herbivores defined as endogenously feeding insect larvae. This guild represents an excellent model system in which to study the responses of herbivores to eCO_2 as they are sessile (so can be linked to an individual plant), endophagous (so are strongly influenced by host plant biology), abundant (any impacts are ecologically relevant at an

ecosystem level) and the entire larval life history is often captured in the mine/feeding trace (so life history metrics can be quickly and easily quantified). Being sessile within a single leaf/plant makes leaf miners particularly suited to study the impact of eCO_2 on herbivory compared to more mobile, free living species which may move between plants or even between experimental arrays. The majority of leaf miner species belong to the Lepidoptera, which mainly feed on trees, and Diptera, which mainly feed on forbs, with a varying number of leaf miner species associated with any given host plant species.

It has been demonstrated that density of leaf mines is correlated with total nitrogen content of the leaf (Faeth *et al.,* 1981), which could mean that under eCO_2 the abundance of leaf miners is reduced due to increasing leaf C:N ratios. Alternatively, rather than population level responses in terms of decreased abundance, leaf miners may respond at the level of the individual. For example, individual behavioural shifts such as compensatory feeding could lead to greater leaf mine area. The ability to compensate for reduced N availability is known to vary within the leaf miner guild (Bezemer and Jones, 1998), therefore it follows that the response of leaf miners to eCO_2 will similarly vary depending on species and context. Indeed, investigations into leaf miner performance under eCO_2 to date have not only varied in the magnitude of response, but also in direction. Kampichler *et al.* (2008) reported a small increase of 0.1% to 0.5% of oaks leaves with mines under eCO_2 compared to controls after 1 year of fumigation, but not in subsequent years. Stiling and Cornellisen (2007), on the other hand, found that densities of all species of leaf miners were lower under eCO_2 for all 8 years of a long-term experiment. Whilst previous studies, such as these, have investigated leaf miners under eCO_2, this has never been investigated in a mature, heterogenous, woodland system across multiple tree species with a large diversity of leaf miners. It remains unclear

how leaf miners at BIFoR FACE will respond to eCO_2, both over the short, and the long term, with potential for either increased or decreased associated herbivory depending on the mechanism (Figure 3.1).

Aims of the study

Against this background, this study aims to:

a) Characterise the key species of leaf miners feeding in the dominant tree species, *Quercus robur* and the main understory species, *Corylus avellana,* at the BIFoR FACE facility. The most abundant 4 leaf miner species feeding on each of these tree species are identified based on mine architecture.
b) Asses the hypothesis: The abundance of leaf miners is reduced under eCO_2 and this effect is greater in the second year following fumigation.
c) Assess the hypothesis: Mean mine area is greater under eCO_2 and this effect is greater in the second year following fumigation.

3.03 Methods

Study site and tree species

The study was conducted at the site of the Birmingham Institute for Forest Research Free-Air CO_2 Enrichment ('BIFoR FACE') experimental facility (see Chapter 2). That canopy is principally comprised of 150-year-old English oaks, *Quercus robur,* with a common hazel, *Corylus avellana*, understory, which were selected as the study species.

The FACE facility operates by fumigation of 3 experimental treatment arrays with CO_2 to elevate average CO_2 concentration to 150pp above ambient (~550ppm), with simultaneous

fumigation of 3 control arrays with ambient air (~400ppm). The facility commenced fumigation on 03rd April 2017 which continued throughout the 2017 and 2018 growing seasons.

Leaf mine surveys

On the 3rd November in 2017 and the 27th of September in 2018, leaf mine surveys were conducted in each experimental array (6 arrays in total). Two hundred freshly fallen oak leaves were sampled from the litter layer within 10m of the centre of each array. Leaves were selected haphazardly with the assumption that they fell from an oak within each array. Common hazel, *Corylus avellana,* leaves were surveyed directly from trees within 10m of the centre of each array at a height of between 0 and 3m (N = 200 leaves).

Each leaf was visually inspected, and the number of mines recorded. The percentage of leaves which contained at least 1 mine was calculated for each array.

Leaf mine area measurements

On the 28th September 2017 and the 20th of September in 2018, 20 leaves containing at least 1 mine were collected directly from the canopy of a single oak at a height of between 25m and 30m in the centre of each treatment and control array (6 arrays in total). Twenty leaves containing at least 1 mine were also collected from hazel trees in each array. These leaves were taken back to the laboratory and photographed (Nikon d60) against a scaled background (Figure 3.2). The area of each leaf and each mine was measured using ImageJ software (Schneider *et al.,* 2012).

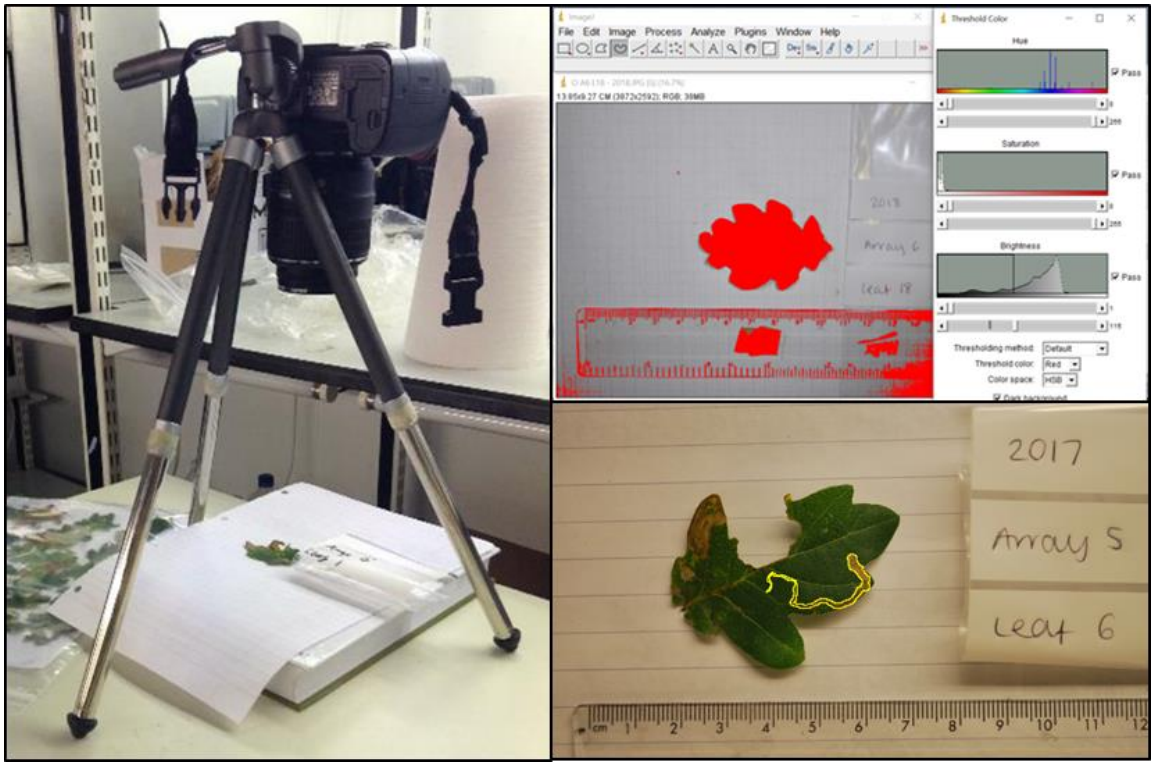

Figure 3.2 – Left: The leaf imaging laboratory set-up with leaves on scaled background. Top right: Screenshot of leaf area measurement process using colour thresholding in imageJ. Bottom right: Screenshot of leaf mine area measurement process in imageJ with Stigmella sp. mine selected.

Statistical analysis

All statistical analyses were performed in R, version 3.5.2 (R Core Team, 2015). A t-test was performed to compare mean percentage of leaves with at least one mine between treatment and control for each tree, each year. In order to test percentage of leaves with at least one mine between years, a paired t-test was performed for each tree species.

Mean mine area (cm^2) was compared between treatment and control for each leaf miner species, for each year and for each tree species were compared using paired t-tests and Wilcoxon rank-sum test when distributed normally and non-normally respectively.

3.04 Results

Key leaf miner species

The most abundant leaf mines in the oak leaves were made by *Stigmella spp*. Schrank, 1802*, Phyllonorycter quercifoliella* (Zeller, 1839), *Ectoedemia albifasciella* (Heinemann, 1871) *and Tischeria ekebladella* (Bjerkander, 1795) (Figure 3.3). Mines of *Stigmella* could not be reliably identified to species level from mine architecture alone without larvae or dissection of adults.

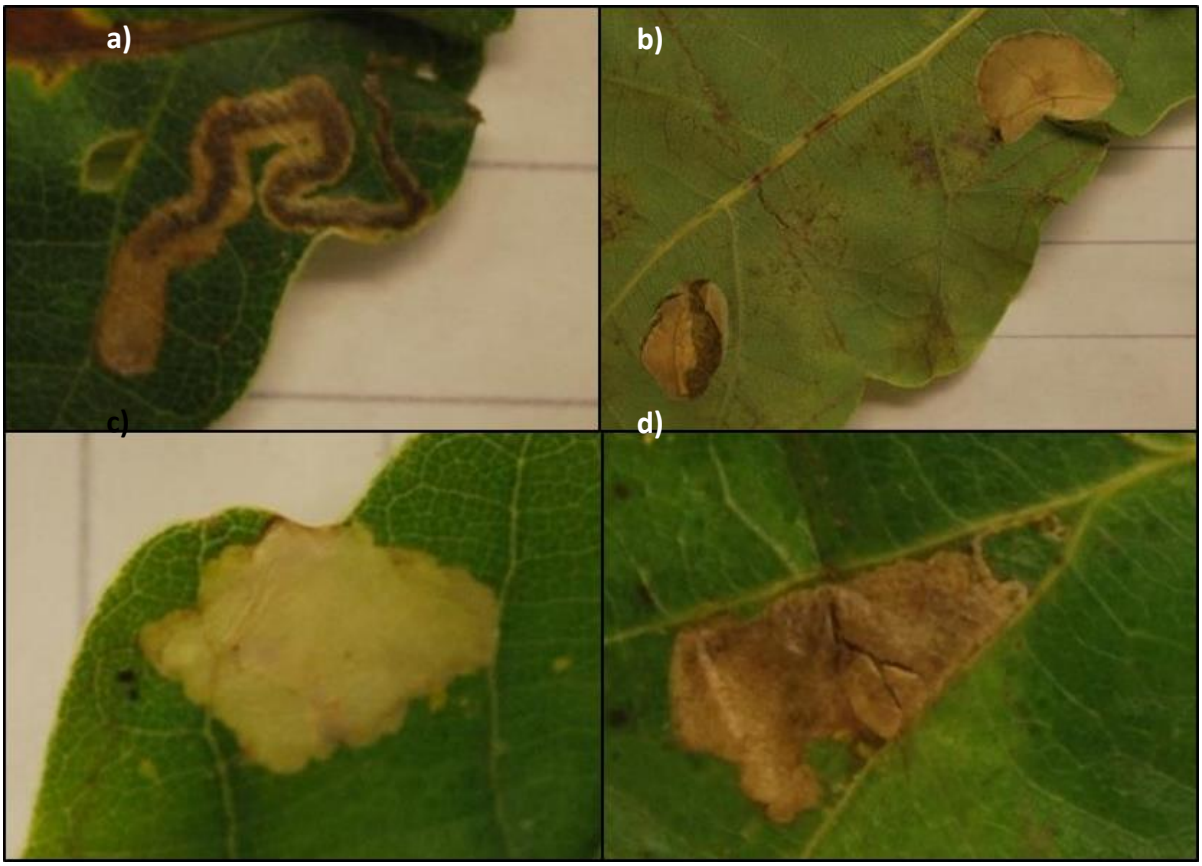

Figure 3.3 – Typical mines of a) Stigmella sp. b) Phyllonorycter quercifoliella c) Tischeria ekebladella d) Ectoedemia albifasciella in oak, leaves.

The most abundant leaf mines in the hazel leaves were made by *Stigmella microtheriella* (Stainton, 1854), *Phyllonorycter coryli* (Nicelli, 1851), *Phyllonorycter nicellii* (Stainton, 1851) and *Parornix devoniella* (Stainton, 1850) (Figure 3.4).

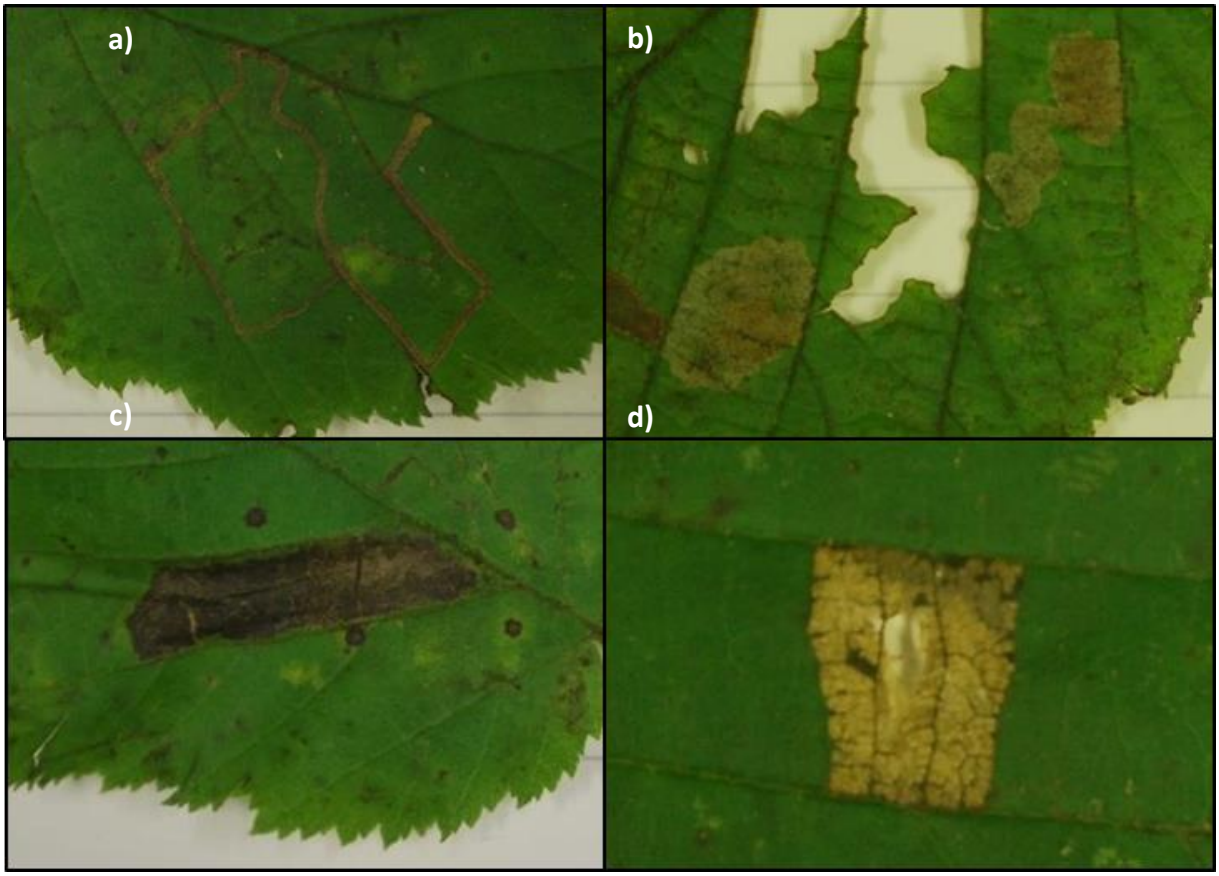

Figure 3.4 – Typical mines of a) Stigmella microtheriella b) Phyllonorycter coryli c) Phyllonorycter nicellii d) Parornix devoniella in hazel leaves.

Leaf miner abundance

Oak

A total of 1500 mines of all miner species were recorded from the 2,400 oak leaves surveyed across 2017 and 2018 (Table 3.1), with no significant difference in the number of mines between years ($p = 0.3418$).

There was no significant difference in the mean number of mines in oak leaves between eCO_2 treatment and control for either year ($p = 0.8345$ and $p = 0.090$ - Figure 3.5).

Hazel

The 2,400 hazel leaves surveyed across 2017 and 2018 contained a total of 633 mines of all leaf miner species (Table 3.1). There was no significant difference in the percentage of leaves with at least 1 mine between years (p = 0.2178).

There was no significant difference in the mean number of mines in hazel leaves between eCO_2 treatment and control plots for either year (p = 0.8918 and p = 0.7596 - Figure 3.6).

Table 3.1 – Summary of: the total number of mines recorded from 1500 leaves surveys each year for each tree species; the percentage of leaves surveyed with at least 1 mine; p-value of t-test of number of leaves with at least 1 mine between eCO_2 treatment and control.

		2017	2018
Oak	Total mines	743	757
	% leaves with at least 1 mine	41	44.4
	p-value treatment vs control	0.8345	0.909
Hazel	Total mines	322	311
	% leaves with at least 1 mine	23.2	20.75
	p-value treatment vs control	0.8918	0.7596

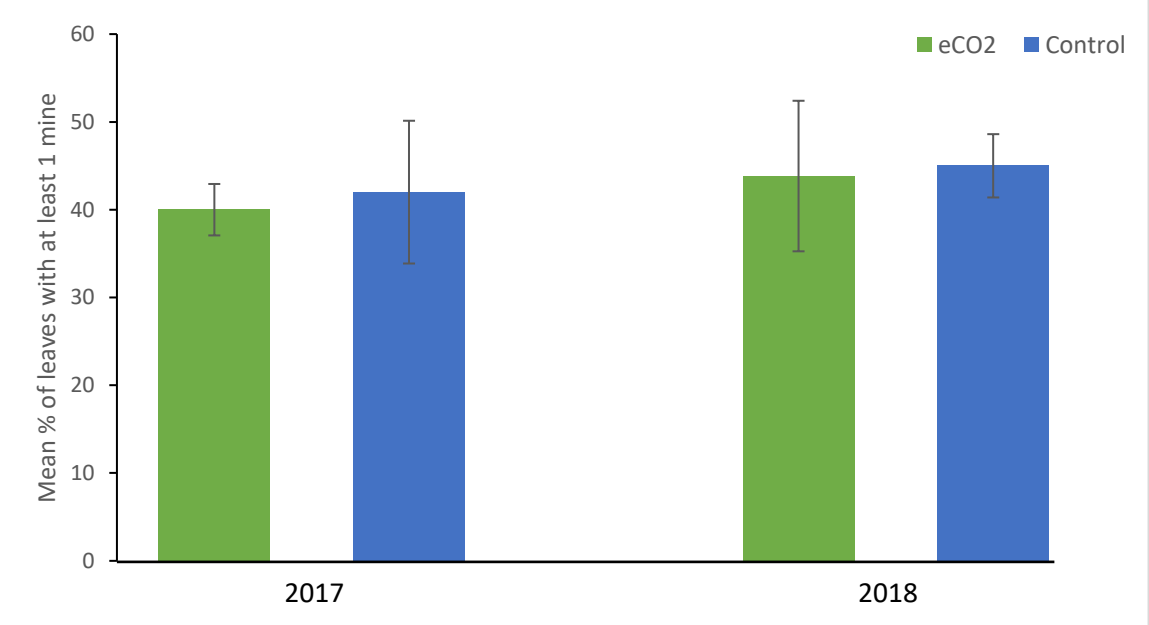

Figure 3.5 – The mean percentage of oak leaves with at least 1 leaf mine eCO_2 from treatment and control conditions in 2017 and 2018. Error bars denote +- standard error.

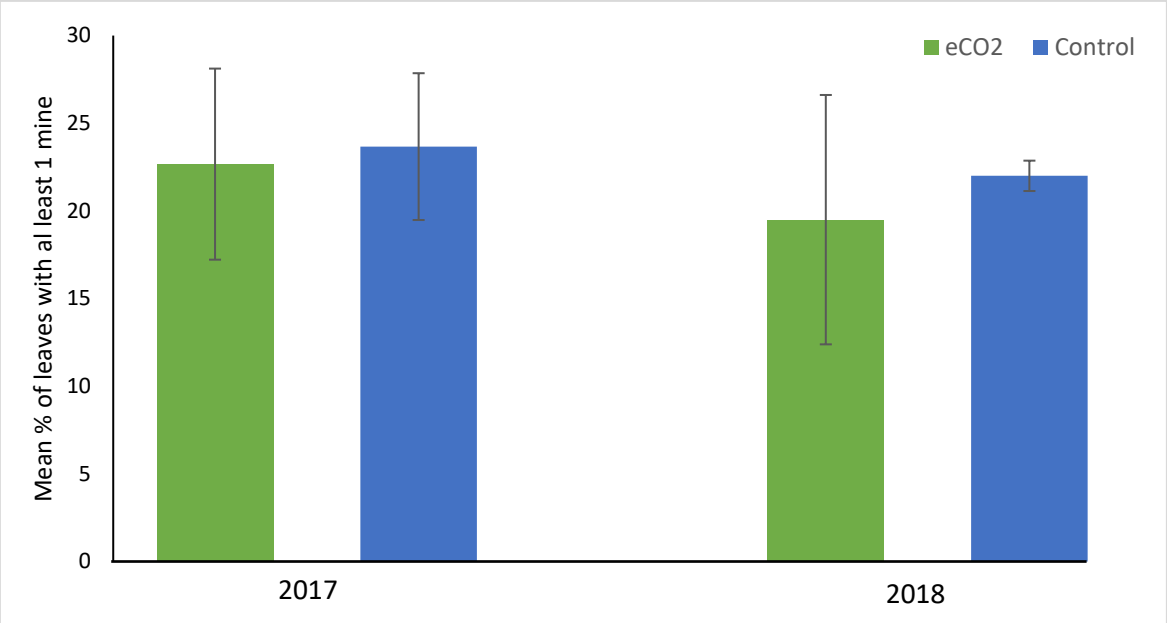

Figure 3.6 – The mean percentage of hazel leaves with at least 1 leaf mine from eCO_2 treatment and control conditions in 2017 and 2018. Error bars denote +- standard error.

Leaf miner herbivory

Oak

The area of 163 leaf mines were measured from the 120 oak leaves sampled in 2017 and 240 mines in 2018 (Figure 3.7). Overall, 31.8% of these mines were made by *Stigmella sp*. with a mean area of 0.313cm^2, 31.8% by *Phyllonorycter quercifoliella* with a mean area of 0.448cm^2, 5.7% by *Tischeria ekebladella* with a mean area of *0.325*cm^2 and 30.8% by *Ectoedemia albifasciella* with a mean area of 0.199cm^2.

There was no significant difference in the mean area of mines between treatment and control arrays for any species of leaf miner in 2017 (Table 3.2). In 2018 the mean area of *Stigmella* spp. mines in oak leaves was significantly lower under eCO_2 ($p = 0.0163$). The mean area of *P. quercifoliella* and *E. albifasciella* were also lower under the treatment, although this was not significant ($p = 0.1089$, $p = 0.4$ respectively). The mean area of *T. ekebladella* mines was also not significantly different under the treatment in 2018 ($p = 0.3939$).

*Table 3.2 – p-values for t-tests of mean mine area between eCO_2 treatment and control for each leaf miner species, each year for oak and hazel. *denotes significant values.*

Tree species	Leaf miner species	2017	2018
Oak	*Stigmella sp.*	0.2159	**0.0163***
	P. quercifoliella	0.9485	0.1089
	T. ekebladella	0.6428	0.3939
	E. albifasciella	0.3074	0.4
Hazel	*S. microtheriella*	0.6976	0.07128
	P. nicellii	0.999	0.3651
	P. coryli	0.6819	0.8979
	P. devoniella	0.2257	0.6428

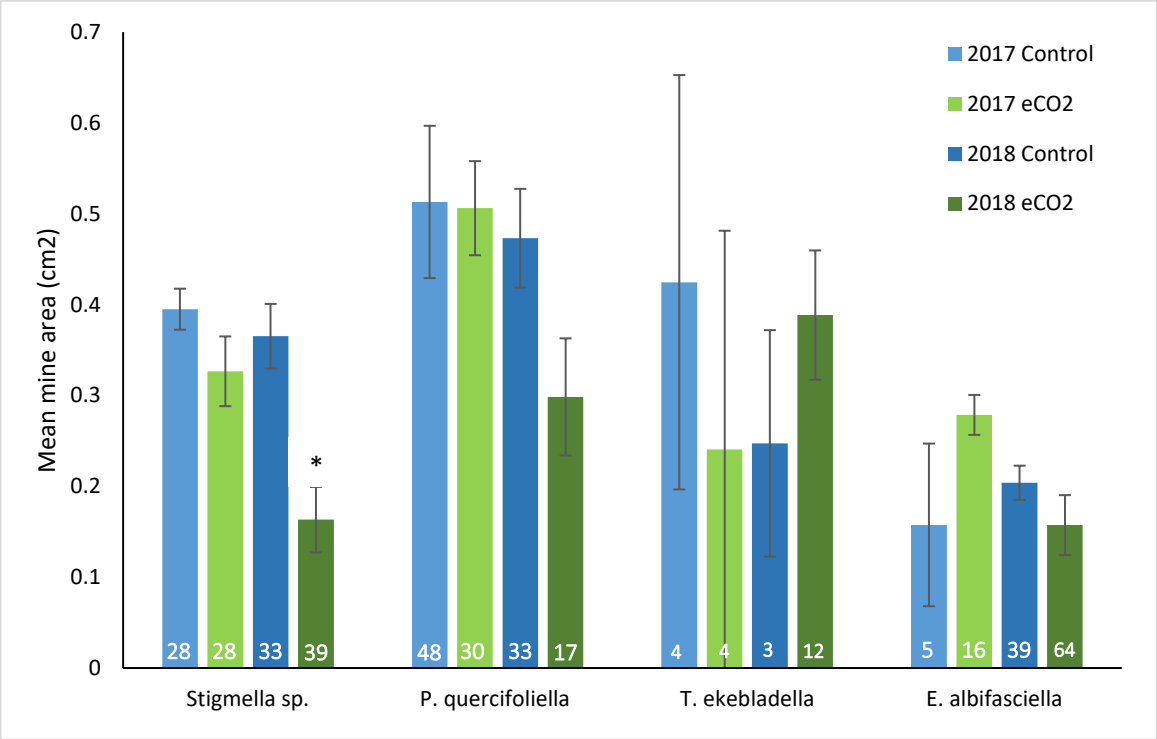

*Figure 3.7 – Mean area (cm^2, +-SE mean) of mines of Stigmella sp., P. quercifoliella, T. ekebladella and E. albifasciella in oak leaves from treament and control arrays for 2017 and 2018. The number at the base of bars denote sample size. *Denotes statistical significance of the difference in mean mine area between treatment and control for a species in a particuar year.*

Hazel

The area of 212 leaf mines was measured from the 120 hazel leaves sampled in 2017 and 192 mines in 2018 (Figure 3.8). Overall 60.9% of these mines were made by *Stigmella microtheriella* with a mean area of 0.542cm^2, 16.3% by *Phyllonorycter nicellii* with a mean

area of 1.416cm^2, *16.1%* by *Phyllonorycter coryli* with a mean area of 1.474cm^2 and *6.7%* by *Parornix devoniella* with a mean area of 0.912cm^2.

There was no significant difference in the mean area of mines in hazel leaves between treatment and control for any species in 2017, nor 2018 (Table 3.2).

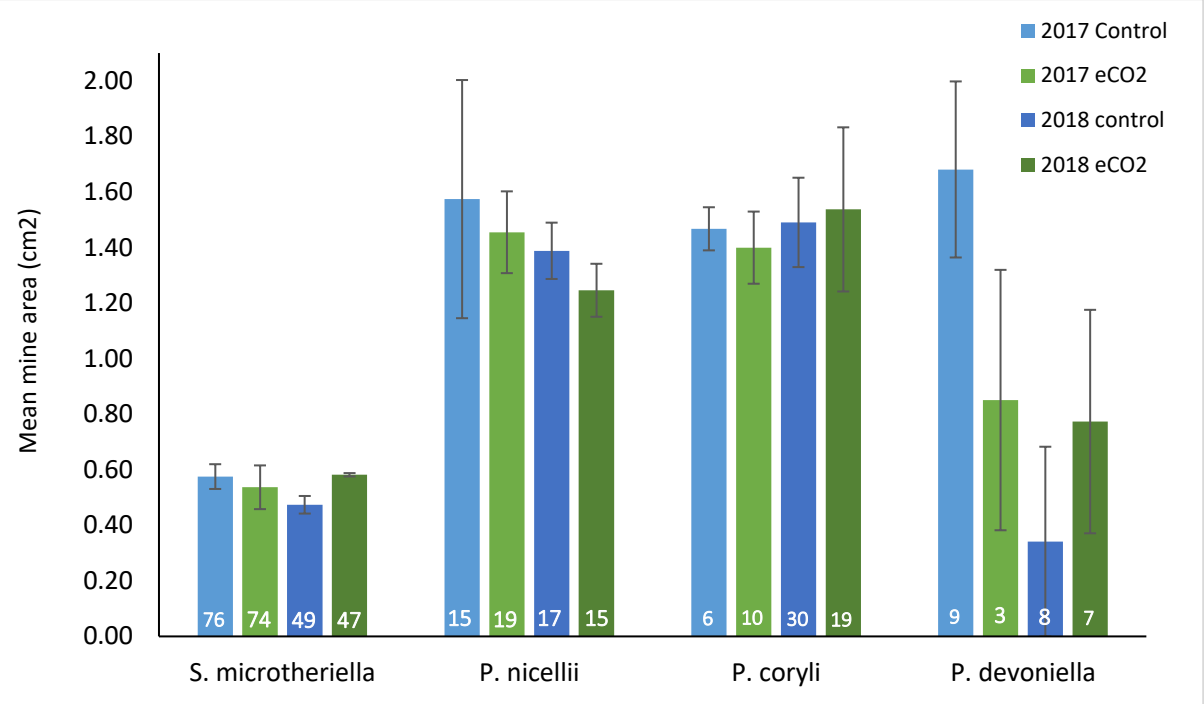

Figure 3.8 – Mean area (cm^2, +-SE mean) of mines of S. microtheriella, P. nicellii, P. coryli and P. devoniella in hazel leaves from treament and control arrays for 2017 and 2018. The number at the base of bars denote sample size.

3.05 Discussion

Dominant Leaf miner species

Oak trees support a huge diversity of insects including a large number of leaf mining species. Lepidoptera represent 36 of the 40 species of leaf miner species recorded from *Q. robur* in the UK (Pitkin and Plant, Unpublished), particularly from within the Gracillariidae and Nepticulidae families. *Phyllonorycter quercifoliella* was the most abundant leaf miner species in oak leaves in this study and was also the species with the greatest mean mine area on oak.

This suggests that *P. quercifoliella* consumed the greatest amount of leaf tissue of any oak leaf miner species (based on abundance by mean mine size), thus is a key leaf herbivore of this tree species.

In the UK 21 species of leaf miners have been recorded from *Corylus avellana*. Whilst hazel supports fewer species of leaf miner than oak (almost half as many), a similar proportion are Lepidoptera. Only 3 species are recorded from both tree species (*Orchestes signifier, Coleophora currucipennella* and *Gypsonoma dealbana*). *Stigmella microtheriella* was the most abundant species on hazel in this study, with nearly four times as many mines as other species. *Phyllonorycter nicellii and P. coryli* mines had the greatest mean mine area of all species identified in this study (1.416cm^2 and 1.474cm^2). Despite having the smallest mean mine area of the 4 most abundant hazel leaf mines, the much greater abundance of *S. microtheriella* mines suggests that this species consumed the greatest amount of leaf tissue and is, therefore, a key hazel leaf herbivore.

Leaf miner abundance

The overall number of oak leaves with at least 1 mine found in this study (41%) was much higher than found in previous studies. Relatively few studies in the published literature describe leaf mine abundance on oak trees, but in various other species of *Quercus* overall mean leaf mine densities are reported at 5.6% (Faeth *et al*., 1981), 5.1% (Rickman and Connor, 2003), 5.7% (Bultman and Faeth, 1986) and 2.8% (Aguilar and Boecklen, 1992). Some studies describe periodic increases in abundance in certain years (e.g. Faeth *et al.,* 1981), but these are temporary increases followed by a return to more typical densities in subsequent years. The consistent abundance of leaf mines in successive years in the present study

suggest that this is not part of a single year population boom, either in response to environmental conditions or long-term population cycles. The abundance of leaf mines on this species of oak, within this particular ecosystem (a mature temperate forest), has not been previously characterised and it is likely that it simply routinely experiences much higher densities of leaf mines. Crucially, the previously studied *Quercus* species are evergreen, unlike *Q. robur*. Thus, the seasonal accumulation of leaf mines is likely to be quite different to that of deciduous species.

Even fewer studies characterise leaf mine abundance on *C. avellana*. Similar to the finding for *Q. robur*, the overall mean density of leaf mines on hazel in the present study (23.2%) is markedly greater to those previously reported (3.8%) (Péré *et al.,* 2010). The paucity of published characterisations of leaf miner abundance on hazel, however, means it is not possible to draw meaningful conclusions on the relative abundance observed in this study compared to other ecosystems.

For both tree species, the abundance of leaf mines did not differ spatially (between arrays) or temporally (between years), suggesting that populations of leaf miners were relatively stable. The abundance data also fails to demonstrate a significant change in leaf miner distribution in response to eCO_2. This somewhat matches the trends in overall insect abundance explored in chapter 2. Any impacts eCO_2 may incur on leaf miners are likely to, therefore, instead operate via changes to leaf miner performance, such as feeding rate. Such changes may, however, confer small impacts on fecundity which are not detectable in the short-term, but may accumulate over multiple generations to eventually impact abundance in the mid- to long-term future.

Leaf mine area

The area of a leaf mine provides a direct measure of larval feeding and, thus an indication of individual performance. The lack of a significant increase in mine area under eCO_2 for any species suggests compensatory feeding is not occurring in these species. In fact, the reverse was found in this study, with a significant decrease in mine area in oak leaves under eCO_2 in the second year for *Stigmella sp*. This decrease in mean mine area in 2018 is also consistent, although not significant, for *P. quercifoliella* and *E. albifasciella.* Whilst this pattern is not detected in *T. ekebladella* (and *E. albifasciella* in the 2017 control), these means are derived from a much smaller number of observations (≤12) with an associated large variance, therefore it is not possible to draw meaningful conclusions for this species.

Although it was not possible to determine mines of *Stigmella sp.* beyond a genus level identification, it is likely that across the site there are at most 3 closely related species (*S. atricapitella, S. roborella and S. ruficapitella*) and the individuals sampled were mostly all the same species. Due to the lack of definitive identifications, however, it must be acknowledged that it is possible that interspecies variation influenced the results.

The lack of any observed change in mine area in 2017 may be explained by the timing of feeding in relation to commencement of fumigation. As 2017 was the first year of experimental fumigation, oak trees had only experienced a high CO_2 environment for a few months at most. Given that oak is a long-lived species, it is expected that it will take longer to respond to changing environmental conditions such as this. The deciduous nature of *Q. robur* further compounds this effect, as the leaves being mined in 2017 would have been flushed with nutrients and carbon absorbed and assimilated in years prior to CO_2 enrichment.

In contrast to the clear decrease in mean mine area for species mining oak, there were no significant differences or consistent patterns in the mean area of mines on hazel. This indicates that the observed differences between hazel and oak were potentially driven by mechanisms such as differing physiological responses of the tree to eCO_2. For example, the two tree species may differ in the extent to which carbon allocation is altered in a CO_2 enriched environment. Should one species increase allocation to herbivore defence whereas the other increase specific leaf area, the consequence for leaf miners would be markedly different. Overall mean mine area in hazel was 3 times greater than for oak (1.09cm^2 vs 0.32cm^2 respectively), whilst mean typical forewing length of the adult leaf miners is almost identical (7.125mm vs 7.25mm respectively). This difference in mine area between the two host may be causes by several factors, such as variation in nutritional value whereby a greater amount of leaf tissue needs to be consumed in order to complete development. This highlights the difference in the environments in which leaf miners develop between different host species.

The decrease in herbivory on oak could be due to a decrease in the nutritional value of leaves, derived from increased C:N, resulting in reduced performance, such as slower developmental rate, smaller achieved pupal mass or even increased mortality. Should this have been the case, however, it would be reasonable to expect evidence of compensatory feeding in at least some of the species (Couture *et al*., 2015). Furthermore, a similar increased C:N would be expected in hazel, therefore parallel declines in mine area (and thus extent of feeding) in hazel miners should be observed. Neither compensatory feeding nor parallel decreases in mean mine area on hazel was observed, suggesting that an alternative mechanism drove these results.

One such potential mechanism that may explain the different results for the two tree species are differences in the production of defensive compounds. Oaks produce a range of secondary metabolic compounds, such as tannins and phenols, to defends against herbivores (Imaji and Seiwa, 2010), whereas hazel does not. Many plants are known to increase carbon allocation to these compounds in response to eCO_2 (Stiling and Cornelissen, 2007; Cavagnaro *et al.,* 2011), indeed eCO_2 has been found to lead to temporary increase in phenolic compounds in oak (Dury *et al*., 1998). It is unclear how eCO_2 may impact defensive allocation in hazel. This hypothesis potentially also explains the consistent response observed in all three species of oak leaf miner (for which there were sufficient observations), the delay in the response and the lack of a similar response in hazel.

It may be possible to determine whether this hypothesis is correct via biochemical analysis of both oak and hazel leaves to measure C:N and concentration of defensive compounds. Indeed, this is the objective on ongoing work, which was unable to be complete and included within this thesis due to delays associated with the impact of the ongoing global pandemic on laboratory operations. A temporal series of measurements could also be employed, which would allow the tracking of seasonal fluctuations in these components. Simultaneous measurements of mine area may also allow direct correlation between these values and miner feeding and developmental rates. Finally, miner fate could also be determined in order to provide data on survival, which would be expected to decrease under increased production of defensive compounds.

Both decreased nutritional value and an increase defensive compounds represent bottom-up impacts on leaf miners, but top-down regulation should not be ruled out as a potential driver.

Declining foliar quality can lead to greater mortality in leaf miners due to increased parasitism (Stiling *et al*., 1999). Slower growth rates prolong the time period larvae are vulnerable to parasitoid attack whilst decreased nutritional value of food source may compromise their ability to defend against it. Increased parasitism could offset any increases in leaf miner abundance, for example the lack of differences in leaf miner abundance observed in this study may be the result of otherwise increasing populations experiencing greater regulation by parasitoids. In another leaf miner species, however, plant quality was found to be a stronger determiner of survivorship and fecundity than top-down pressure (Lill and Marquis, 2001).

The importance of leaf miners at the ecosystem level.

The high abundance of leaf miners indicates that this feeding guild may confer significant impacts to ecosystem functioning. Allometric equations published in the literature along with the data presented by this study allow the calculation of approximate values for the extent of photosynthetically active material lost to leaf miner herbivory for oak. Based on the allometric equation:

$$\log(\text{estimated leaf number}) = 0.92 + 2.55 \log(\text{GBH})$$

from Gripenberg *et al.* (2008), the number of leaves of an individual oak tree can be estimated using the diameter at breast height (GBH). The mean GBH of the oak trees in this study was 70cm which gives:

$$\log(\text{estimated leaf number}) = 0.92+2.55\log(70)$$

$$\text{estimated leaf number} = e^{(0.92+2.55*4.249)}$$

$$\text{estimated leaf number} = 127{,}218$$

The mean area of oak leaves was approximately 18cm^2, which suggests a total leaf area of around 2,289,924cm^2, or 229m^2 per tree. The survey data showed that there were ~750 mines per 1200 oak leaves, or 62.5 per 100 leaves, meaning that by the end of the season each tree contained an approximate mean of 78,125 mines. The overall mean mine area within the control arrays was 0.35cm^2 which would give a total mined leaf area of 27,343.75 cm^2, or 2.73m^2 per tree. This would represent around 1.19% of total leaf area.

Under eCO_2 in 2018 overall mean mine area decreased to 0.25cm^2. Following the same approximations, this would equal a total mined area of 19,531.25cm^2, or 1.95m^2, representing around 0.85% of total leaf area.

Similar equations do not exist for hazel, so it is not possible to repeat this calculation for this species. Based on similar measurements of leaf area and mine area, however, an approximation of percentage lost to miner herbivory per leaf and the relative effect of eCO_2 on this can be made:

Mean hazel leaf area was approximately 35cm^2, whilst overall mean mine area under control conditions was 0.92cm^2. This represents approximately 2.63% of total leaf area lost to leaf miner herbivory. Under eCO_2 overall mean mine area was similar at 1.03cm^2, equating to 2.94% of leaf area lost to leaf miner herbivory.

3.06 Conclusions

This study identifies key species of leaf mining herbivores on English oak and common hazel in a typical temperate, mature woodland. *Phyllonorycter spp. and Stigmella spp.* were consistently the most abundant leaf mining species as well as those which tended to construct the largest mines.

Importantly, this study also provides an important characterisation of the responses of these species to eCO_2 within this setting. Leaf miner abundance did not change in response to eCO_2 although mean mine area was consistently reduced on oak after the 2nd year of fumigation. An increase in defensive compounds is a more likely mechanism driving this response than decreased nutritional value alone, although a combination of the two or other factors such as changes in top-down regulation could also be implicated. This decrease in mine area represents a decrease in herbivory which, given the abundance of leaf mining larvae, may have significant implications at the ecosystem scale, for example the fate of carbon in the system. Furthermore, if these responses are sustained moving into the mid-long term, the associated decrease in leaf miner performance (developmental rate, achieved pupal mass, mortality) may begin to influence abundance. Differing responses amongst tree species and leaf miners also indicate that there will be both winners and losers under future climate scenarios. The decreased herbivory exhibited on oak suggest that it may be better equipped to deal with herbivory under eCO_2, possibly via increased allocation of resources to defence. The high abundance of leaf mines and the total leaf area lost to this herbivory on oak suggest this is a significant pressure, and a reduction in herbivory could be an important advantage. eCO_2 may provide the conditions that allow oak trees to mitigate some degree of herbivory, therefore allowing this species to by a winner compared to species such as hazel. The decrease in herbivory also represents a decrease in the rate at which carbon flows from the leaves to the ground. Herbivores process carbon from plant tissue into frass, which is a much more labile form of C, thereby acting as an important vector in the carbon cycle. Any decrease in herbivory, therefore, interrupts this process, increasing the tree, and wider forests ability to act as a carbon sink.

CHAPTER 4:

Go with the phloem: Evidence for increased aphid abundance in a woodland under elevated CO_2.

The work presented in this chapter is being prepared for submission for publication as: Crowley L M, Enston A, Money J, Sadler J P, Pritchard J and Hayward S A L, (In prep). Go with the phloem: Evidence for increased aphid abundance in a woodland under elevated CO_2.

4.01 Abstract

Aphids are one of the most destructive agricultural pests globally, as well as being abundant in forest/woodland habitats. They are predicted to be amongst the few insect herbivore 'winners' under future elevated CO_2 (eCO_2) scenarios. More field- experiments are needed, however, in order to determine species specific responses, including impacts on fecundity and mortality at the population level. I studied aphid population responses to eCO_2 on common sycamore, *Acer pseudoplatanus,* within a temperate woodland, at the Birmingham Institute for Forest Research (BIFoR) Free-Air CO_2 Enrichment (FACE) facility. A survey of the aphid species, *Drepanosiphum platanoidis*, *Periphyllus testudinaceus* and *P. acericola*, showed an increase in the abundance and population density of all three species under eCO_2 (~550ppm), although differences were only significant for *D. platanoidis*. The number of nymphs produced by individual *D. platanoidis* alates isolated in clip cages was not significantly affected by the eCO_2. These results suggest that *D. platanoidis* could be amongst the species of aphids that could increase in abundance under eCO_2, but that population level responses are not driven by improved individual performance.

4.02 Introduction

Increasing global concentrations of atmospheric CO_2 indirectly impact insect herbivores via changes to host plant quality – most notably changes in C:N ratios (Hillstrom *et al.,* 2014). Responses to elevated CO_2 (eCO_2) are known to differ between insect species (Bezemer and Jones 1998; Sanders *et al.,* 2004), with both 'winners' and 'losers' emerging within different plant-insect systems studied to date. Feeding guild is a key trait which may influence an insect herbivores response to eCO_2, with performance varying depending on plant tissue consumed and the feeding mechanism. Based on current data, phloem feeders are the only feeding guild expected to experience improved performance under eCO_2 (Sun *et al.,*2016).

Aphids (Hemmiptera: Aphididae) are an abundant group of insect herbivores which feed principally on plant phloem. They are one of the most destructive insect pests globally, but particularly in temperate regions, where many tree species also serve as hosts. The nutritional quality of the host plant phloem for aphids is defined principally by the quantity and quality of amino acids (McNeil and Southwood, 1978), and this is a key driver of their life history, with declining food quality triggering a switch from parthenogenesis to sexual reproduction (Douglas 2003). Because of their role in transmitting plant pathogens, as well as direct feeding damage, aphids have been an important model group for studying the effects of climate change on insect performance. Current evidence suggests that aphids will generally do better under eCO_2, for example with increased growth rate, fecundity, survival and abundance (Sun and Ge, 2011; Robinson et al, 2012). One potential mechanism driving this increase in performance is believed to be plasticity in behavioural traits, such as increased phloem ingestion rate to compensate for lower concentrations of amino acids (Sun & Ge 2011). By

exhibiting compensatory feeding as a plastic response, aphids may be able to mitigate the decrease in nutritional value of phloem as plant C:N ratios increase in response to eCO_2.

The response of different aphid species to eCO_2 is inconsistent, however, and often varies depending on the aphid-host pairing (Bezemer *et al.*, 1999). The fate of aphid populations under eCO_2 will also be governed not just by changes in C:N ratios, but also by plant defence mechanisms. These are also known to differ between plant species under eCO_2 in association with varying increases in the concentration of defensive compounds in plant tissue (Ryan *et al.,* 2010). Furthermore, shifts in reproductive allocation by host plants under eCO_2 may also reduce the carrying capacity of the plant with an associated with decrease in aphid abundance (Awmack and Harrington, 2000).

Sycamore, *Acer pseudoplatanus* L.*,* is a common, deciduous, broad-leaved tree native to Eurasia and naturalised in the UK (Peterken, 2001). Twelve species of aphid are known to feed on sycamore (Blackman and Eastop, 2008), of which 8 occur in the UK (Baker, 2020). The three species which are most commonly found feeding on sycamore leaves are *Drepanosiphum platanoidis* (Schrank, 1801), *Periphyllus testudinaceus* (Fernie, 1852) and *Periphyllus acericola* (Walker, 1848). These species, and *D. platanoidis* in particular, are often abundant in late spring, feeding on the underside of young leaves. Following this period of rapid population growth, these species enter a period of aestivation of up to 8 weeks throughout the summer when conditions, such as rainfall, are less favourable (Wellings *et al., 1985*).

The abundance of these species at any given time of year is governed by seasonal cycles in response to changing environmental factors. For example, it has been shown that reproductive activity of *D. platanoidis* is shaped by seasonal changes in quantities of amino acids within sycamore leaves and intraspecific competition (Dixon *et al*., 1993). Temperature is also known to affect reproductive rate (Wellings, 1981). Given the variation in responses of different aphid species to eCO_2, it is likely that the responses of different aphid species on sycamore will vary. A previous study by Docherty *et al*. (1997), found that relative growth rate of *D. platanoidis* and *P. testudinaceus* on one-year old saplings was not altered under 600pm CO_2. It remains unclear, however, how eCO_2 will affect these species at a larger, ecological scale within mature, complex systems with a greater number of interacting species. For example, changes to top-down pressure from natural enemies may limit or reverse responses to changing host quality.

Drepanosiphum platanoidis is known to aggregate on specific leaves in a uniformly spaced distribution, the density of which depends on number of aphids and associated level of self-induced competition (Dixon and Logan, 1972). Whilst it has been shown that larger leaves are more likely to have a greater number and higher density of aphids (Dixon and Mackay, 1970), it is unknown how increasing concentrations of eCO_2 may impact this behaviour.

This paper examines the effect of eCO_2 on the abundance and distribution of the three most common UK aphid species on sycamore leaves in an open, ecosystem scale experiment. This is achieved by assessing the following hypotheses:

1) The abundance and density of *Drepanosiphum platanoidis*, *Periphyllus testudinaceus* and *Periphyllus acericola* feeding on Sycamore leaves is greater under eCO_2.
2) The growth rate and fecundity of individual *Drepanosiphum platanoidis* feeding on Sycamore leaves is greater under eCO_2.

4.03 Materials and methods

Experimental site

The study was conducted at the Birmingham Institute for Forest Research Free-Air CO_2 Enrichment ('BIFoR FACE') experimental facility, located in Staffordshire, UK (52°47'58"N, 2°18'15"W) as described in Hart *et al*. (2019). The site comprises 21 hectares of mature, semi-natural broadleaved woodland (>200 years continuous tree cover), characterised by >150-year-old 'standard' English oaks, *Quercus robur*, and a previously coppiced common hazel, *Corylus avellana*, understory. There are several other species of tree dispersed across the woodland including sycamore, *Acer psuedoplatanus*, hawthorn, *Cretaegus monogyna*, and ash, *Fraxinus excelsior.*

There are 3 experimental treatment arrays at the site, which fumigate a 30m diameter area of forest with +150ppm CO_2 above ambient (~550ppm total) measured in real time (Norby *et al*., 2016). There are a further 3 control arrays which fumigate a similar area with ambient air (~400ppm). CO_2 enrichment commenced in April 2017 and will continue throughout a minimum 10-year duration.

The two largest and most mature sycamore trees in each array were selected as experimental trees (12 trees in total). These trees are approximately 20-40 years old following a compartment thinning of the site in the 1980s.

Aphid survey

On the 06/06/2019, five leaves from each of the experimental sycamore trees were surveyed. Leaves were selected haphazardly from those within reach (<3m height). Digital photographs of the underside of these leaves were taken against a white background with an appropriate scale, whilst minimising disturbance to the leaf (Figure 4.1). Images were then analysed to determine the number, identity and developmental stage (nymph vs imago) of all aphids present. Leaf area was calculated using ImageJ software (Schneider *et al.,* 2012).

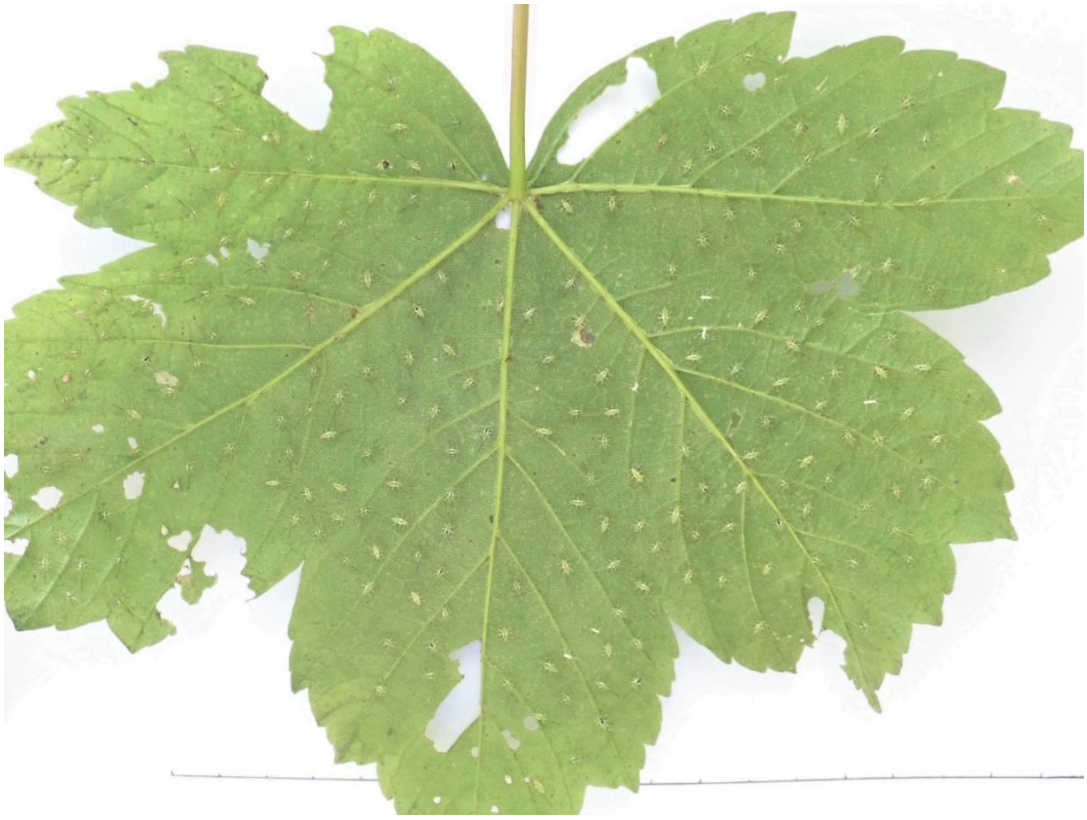

Figure 4.1 – Image of Drepanosiphum platanoidis alates feeding on a sycamore, Acer pseudoplatanus, leaf with characteristic spaced out distribution.

Clip-cage experiment

On 14/05/2019, two leaves with 4th instar *Drepanosiphum platanoidis* nymphs were located for each of the two experimental sycamore trees within each array. A single individual of these nymphs was enclosed within a clip cage (c.f. MacGillivray and Anderson, 1957) giving a total of 4 cages per array. Only 4th instar nymphs were selected in order to synchronise the age of aphid samples (to aid growth monitoring) and to maximise the likelihood that the individuals being studied had developed on their given experimental tree for at least 1 generation. Clip cages were comprised of two pieces of plastic tube (25mm diameter) with fine gauze over one end and a ring of foam over the other, held together with a sprung metal clip (Figure 4.2). Each aphid was isolated on a 4.91cm^2 area of the underside of a sycamore leaf for 4 weeks. The time taken to moult to the alate stage was recorded, as well as weekly number of offspring produced and mortality until 11/06/2019. This was when reproduction plateaued before individuals entered the summer aestivation period. The number of ultimately surviving nymphs which moulted each week was used to indicate developmental rate (% moulted per week).

Figure 4.2 – The clip cages used to isolate sycamore aphids on sycamore leaves in situ.

Climate data

Hourly mean air temperature was measured by a Campbell Scientific 107 Thermistor and recorded on a Campbell Scientific CR300 series datalogger fitted to one of the towers of each FACE array at a height of the upper canopy (approximately 21m). From this, weekly mean air temperature was calculated throughout the experimental period for each array for the 7 days preceding the measurement.

Statistical analyses

All statistical analyses were performed in R, version 3.5.2 (R Core Team, 2015). The impact of the treatment effect on the surveyed abundance of *D. platanoidis* was analysed using a negative binomial, mixed effects model with tree nested within array. Abundance data for *Periphyllus testudinaceus* and *P. acericola* were linearised using a Tukey transformation, and impacts of the treatment analysed with an ANOVA (with tree nested within array). The effect of treatment on the population density of each aphid species was tested using an ANOVA (with tree nested within array). The number of nymphs produced by *D. platanoidis* within clip cages under eCO_2 and control conditions was assessed with a Wilcoxon rank-sum test. The developmental rate, measured as the number of nymphs which had moulted at each time point, under treatment and control was tested with a paired t-test.

4.04 Results

Aphid survey

A total of 1,979 aphids across all 3 species were recorded from the 60 leaves surveyed. This comprised 460 *D. platanoidis*, 85 *P. testudinaceus* and 1434 *P. acericola*. The majority of

individuals recorded (71.8%) were *P. acericola* dimorphs aestivating in dense clusters of up to 346 individuals.

Drepanosiphum platanoidis had a significantly greater abundance and density under eCO_2 compared with control arrays ($p = 6.03x10^{-5}$ and $p = 2.88x10^{-4}$ respectively; Figure 4.3). *Periphyllus testudinaceus* and *P. acericola* also had greater abundance and population density under eCO_2, but neither were significant (abundance p = 0.449, p = 0.395 respectively; density p = 0.223 and p = 0.692 respectively).

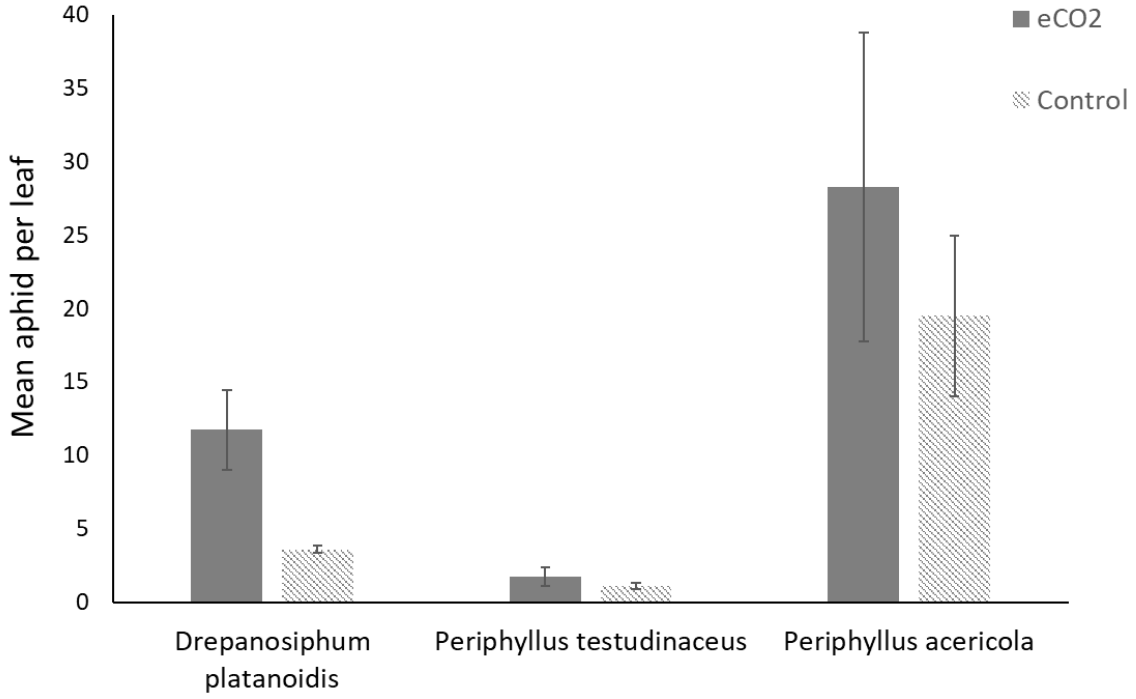

Figure 4.3 – Mean number of each aphid species per leaf from 30 leaves under eCO_2 and control conditions (+-SE).

Clip-cage experiment

There was no difference in mean weekly air temperature between any of the arrays, suggesting this would not be the cause of any differences in developmental rate or fecundity observed. A total of 82 progeny were produced over the 4-week observation period by the

original 24 aphids in clip-cages, with a mean of 3.42 nymphs per aphid (figure 4.4). By 21/05/2020, 60% of the 4th instar nymphs had undergone their final moults into alates, and 100% of surviving nymphs had done so by 04/06/2020. Whilst there were a greater number of nymphs produced by aphids under eCO_2, this was not significantly different from control arrays (W = 62, p = 0.572). The developmental rate of nymphs was not significantly different under eCO_2 (df = 4, p-value = 0.242, Figure 4.4).

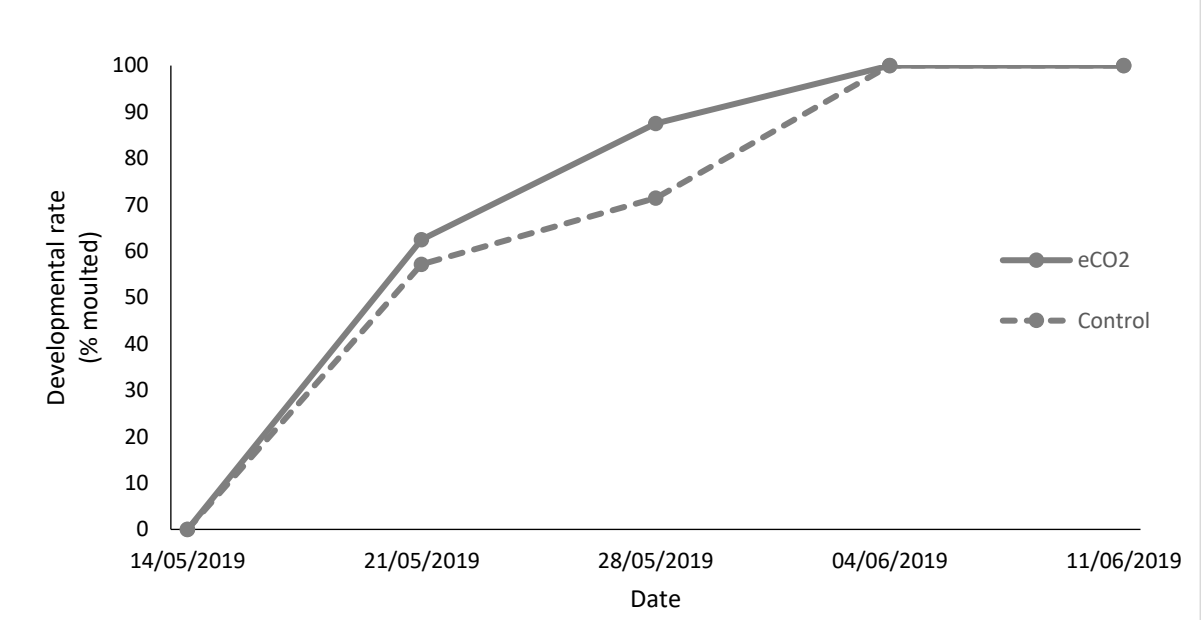

Figure 4.4 – Weekly developmental rate of D. platanoidis (represented as the percentage of ultimately surviving nymphs which have moulted) in all 12 clip cages under eCO_2 and all 12 clip cages in the ambient air control.

4.5 Discussion

The increased abundance and density of all aphid species under the treatment supports the hypothesis that aphid performance, in general, could improve under eCO_2. The differing magnitude of this response between *D. platanoidis* and the two *Periphyllus* species, however, suggests that this response may be idiosyncratic and influenced by several different factors. For example, aphid population increase under eCO_2 may be influenced by soil nitrogen levels

(Newman *et al.,* 2003), therefore varying sensitivities of aphid species to this effect may lead to a divergence in the response to eCO_2. These species also each possess differing relative phenologies and ranges of host species, which may also influence their sensitivity to CO_2. This is consistent with the findings of previous studies that suggest the magnitude of responses of aphids to eCO_2 will be species specific (Newman *et al.,* 2003; Sun and Ge, 2011).

An acute deterioration in host plant quality triggers diapause and/or dispersal in aphids as well as many other insects (Hunter and McNeil, 1997). It remains unclear, however, how sustained changes in plant host quality will affect insect herbivore populations. Small effects conferred on herbivore fecundity may accumulate over multiple generations and thus drive larger shifts in population structure. The timing of this study (in late spring) means that aphids were in a period of feeding and population growth, shortly before a period of aestivation. As such, the aphids in this study are likely to be the second generation of the year, which were deposited onto the maturing leaves by fundatrices earlier in the spring. Whilst these species do undertake limited dispersal (Dixon, 1969), the majority of individuals are expected to have remained on the same host tree as parental generations. Whilst movement into eCO_2 arrays cannot be ruled out as a causal factor, it is unlikely that this is the primary driver of the observed effects.

Whilst the number of nymphs produced by *D. platanoidis* was not significantly greater under eCO_2, it is expected that individual performance of aphids under eCO_2 may not explain population responses (Awmack *et al*., 2004). One possible explanation of the discrepancy between individual performance (no significant differnce in number of nymphs produced or developmental rate) and population trends (increased abundance and density) may be either

a decrease in the strength of intra-specific competition, or increased tolerance to it. This species clusters on suitable leaves, likely in response to microenvironment (Dixon and Mackay, 1970), thereby exposing itself to competition. Whilst competition is not desirable, the benefits of aggregation must outweigh the imposed costs. Any modification of this cost or benefit as an indirect consequence of eCO_2 could lead to the pattern observed.

Alternatively, increased aphid abundance may be a result of decreased predation by predators such as Coccinellids or parasitoids such as Braconids. Several factors influence the degree of this top-down regulation including phenology, natural enemy abundance, and the ability of natural enemies to locate aphids (Hentley *et al.*, 2014). The impact of eCO_2 on one or several of these factors could reduce regulation by natural enemies resulting in the increased abundance observed. Current sampling data from across the site over the three years proceeding this experiment do not indicate a significant difference in aphid natural enemy abundance under the treatment (see Chapter 2). Coarse level analysis of guild abundance may be insufficient to detect subtle difference in top-down pressure exerted on aphid population by natural enemies. This level of detail is difficult to replicate in smaller experiments and highlights current gaps in our knowledge regarding how mechanisms explored at small scale translate to field scale.

4.06 Conclusions

This study found increased abundance of the three most common UK sycamore aphid species under eCO_2 (~550 ppm) in a mature oak woodland ecosystem, however, these increases were only statistically significant for *D. platanoidis*. There was no impact of eCO_2 on developmental rate, fecundity or mortality in *D. platanoidis*, suggesting other, population-level, factors might

be influencing changes in this species abundance. This may be driven by changes in intraspecific competition, although numbers of all species were reasonably comparable across eCO_2 vs. control. Alternatively, abundance/population density may be regulated differentially by natural enemies. Current sampling data fails to indicate any significant difference in insect community structure between eCO_2 and control arrays, although direct assessments of parasitism/predation may be required to adequately explore this hypothesis.

CHAPTER 5

Elevated CO_2 impacts on plant-pollinator interactions: a systematic map and Free Air Carbon Enrichment (FACE) field study.

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5.01 Abstract

The impact of elevated CO_2 (eCO_2) on plant-pollinator interactions is poorly understood. This study provides the first systematic review of this topic and identifies important knowledge gaps. In addition, I present field data assessing the impact of eCO_2 (150 ppm above ambient) on Bluebell (*Hyacinthoides non-scripta*)-pollinator interactions within a mature, deciduous woodland system. Since 1956, only 71 primary papers have investigated eCO_2 effects on flowering time, floral traits and pollination, with a mere 3 studies measuring the impact on pollination interactions. My field experiment documented flowering phenology, flower visitation and seed production, as well as the abundance and phenology of dominant insect pollinators. I show that first and mid-point flowering occurred 6 days earlier under eCO_2, but with no change in flowering duration. Syrphid flies and bumblebees were the dominant flower visitors, with peak activity recorded during mid- and late-flowering periods. Whilst no significant difference was recorded in total visitation or seed set between eCO_2 and ambient treatments, there were clear patterns of earlier flowering under eCO_2 accompanied by lower pollinator activity during this period. This has implications for potential loss of synchrony in

pollination systems under future climate scenarios, with associated long-term impacts on abundance and diversity.

5.02 Background

Insect mediated pollination is required by the majority of angiosperm species in order to achieve sexual reproduction (Ollerton *et al*., 2011). This ecosystem service, therefore, has a direct impact on plant reproduction and turnover in many ecosystems, and critically underpins our food security. Phenological synchrony with plant flowering times is crucial for many insect species utilising floral resources as a food source, as well as for organisms at higher trophic levels feeding on (or parasitizing) these insect pollinators (e.g. Mortensen *et al*., 2016). There is now clear evidence that climate change is increasing global mean temperatures and changing patterns of precipitation, which has affected the phenology and physiology of plants and their pollinators (Hegland *et al*., 2009; Bale and Hayward, 2010; Owen *et al*., 2013; Phillips *et al*., 2018). This, in turn, has led to adverse impacts such as a phenological mismatch between plants and their pollinators (Memmott *et al*., 2007; Schenk *et al*., 2018). Such impacts could result in realignment of interaction networks, changes in populations and even local extinctions, and may be a significant contributing factor of observed declines amongst many pollinator species (Powney *et al*., 2019).

Elevated concentrations of atmospheric Carbon Dioxide (eCO_2) are also hypothesized to influence plant-pollinator interactions, e.g. through impacts on plant growth, biochemistry, physiology, phenology etc. (Curtis and Wang, 1998; Pringle, 2016). However, assessments of the potential impact of eCO_2 on pollination interactions are limited. This is an important gap in our knowledge because any negative impacts eCO_2 confers on these interactions could

adversely impact populations of both the plants and their pollinators. This would represent yet another stressor potentially contributing to declining pollinator populations in combination with other factors such as habitat loss and fragmentation, agrochemicals, pathogens and alien species (Potts *et al*., 2010).

A key mechanism by which eCO_2 is expected to impact pollination is through changes in plant flowering phenology. The term 'flowering phenology' comprises several constituent events including floral bud burst, maturation and release of fertile pollen, production of nectar, the stigma becoming receptive to pollen and floral senescence. A review by Springer and Ward (2007) of studies across a range of cultivated and wild plants found varied responses of flowering time to eCO_2. Light (photoperiod and illuminance), temperature (soil and air), nutrient availability (N, P, etc.) and water availability (precipitation and soil moisture) are also all known to affect flowering time (Simpson *et al*., 1999; Lesica and Kittelson, 2010), further complicating the picture. Many of the studies examining the direct effect of eCO_2 on flowering phenology have used propagated plants in controlled environments, but this approach misses potential interacting effects of other variables such as local environmental/microclimate conditions and species interactions. Given that the scale and direction of the response to eCO_2 varies depending on species and context, further empirical studies are necessary in order to explore these responses in different species and systems, particularly in situ ecosystem (field) scale experiments.

Another mechanism through which eCO_2 could affect pollination is by altering the amount and/or biochemical composition of floral resources. Pollen and nectar are the primary currency in plant-pollinator interactions, and so any changes in the quantity or quality of this

resource could have significant impacts on flower-visiting insects. Pollen is an important protein and lipid source for many insect species, including hoverflies (e.g. Haslett, 1989), and is vital for obligate palynivores such as bees. Whilst there has been more focus on the impact of eCO_2 on pollen rather than nectar, studies are still scarce. There is evidence that eCO_2 leads to a decrease in pollen quantity in some horticultural species, such as *Lycopersicon lycopersicum* and *Cucurbita pepo* (López-Cubillos and Hughes, 2016), and a decline in pollen quality (protein content) in *Solidago spp*. (Ziska *et al*., 2016). In other species, however, the reverse was noted, with increased pollen production under eCO_2 in species such as ragweed, *Ambrosia artemisiifolia* (Ziska and Caulfield, 2000) and Loblolly Pine, *Pinus taeda* (LaDeau and Clark, 2006). Nectar can be a rich source of both amino acids and sugars (Gardener and Gillman, 2002). There is evidence that the volume, sugar concentration, and sugar composition of nectar are all influenced by temperature and water availability (Pacini *et al*., 2003), yet data on the direct effects of eCO_2 on nectar production or composition are very limited. I found only 10 studies assessing the effect of eCO_2 on nectar, again with varying responses. For example, Lakes and Hughes (1999) reported a reduced nectar volume, whilst López-Cubillos and Hughes (2016) noted an increase in nectar production. eCO_2 is known to increase C:N ratios and alter the nutritional value of plant tissue such as leaves (Bezemer and Jones, 1998; Gifford *et al*., 2000), but it remains untested whether similar changes occur in pollen or nectar biochemistry. What is clear, is that changes in nutritional quality or quantity of floral resources can have significant consequences for pollinator development and reproductive success (Vaudo *et al*., 2018), as well as immune/disease responses and overall health (Dolezal and Toth, 2018). Thus, examining the role of eCO_2 within the context of

pollinator nutritional ecology will be a key part of understanding plant–pollinator interactions, coevolution, and the restoration of declining pollinator populations under climate change.

Beside the impact on phenology and floral resources, it is also possible that eCO_2 may affect pollination via other pathways, such as interfering with the production or detection of floral volatiles and thus disrupting plant-pollinator communication (Jamieson *et al*., 2017). As pollination is a complex, multispecies, ecological interaction, it is inherently difficult to detect, disentangle and predict how it is impacted by shifting environmental factors such as eCO_2. Empirical data from *in situ*, ecosystem scale experiments is required, therefore, in order to answer these complex questions.

Exploring the consequences of eCO_2 on ecosystem processes such as pollination is particularly difficult in complex ecosystems such as forests. This is due to the difficulty of manipulating CO_2 concentrations at an appropriate spatial scale. Free Air Carbon Dioxide Enrichment (FACE) experiments are an invaluable tool in this context, where unenclosed forest/woodland plots are fumigated in situ and ecosystem responses measured to provide vital real-world data (Norby *et al*., 2016). There are currently only two large scale forest FACE experiments running globally. In the southern hemisphere, EucFACE (Australia) has been fumigating a eucalyptus forest with CO_2 since 2012 (Drake *et al*., 2016), but this facility has yet to publish any studies on pollinator systems. In the northern hemisphere, the 'Birmingham Institute of Forest Research' (BIFoR) FACE facility (UK), has been fumigating a mature oak woodland system with CO_2 since 2017, and provides the perfect opportunity to examine the impact of eCO_2 on plant-pollinator interactions in this important temperate ecosystem.

Against this background, the current study had the following objectives: 1) To undertake the first systematic mapping of the literature investigating the effect of eCO_2 on floral traits and pollination in order to highlight key knowledge gaps for future study, and 2) To assess the impact of eCO_2 plant-pollinator interactions within a complex, mature deciduous woodland against the following hypotheses:

1) The flowering phenology of common Bluebell (*Hyacinhoides non-scripta*) is delayed under eCO_2.
2) Insect visitation to bluebell flowers is reduced under eCO_2.
3) The mean seed count per fruit of bluebells is reduced under eCO_2.

5.3 Methods

Systematic review

A systematic review was preformed to provide a transparent, comprehensive and objective overview of the quantity and quality of evidence related to pollination under eCO_2, following published guidelines (James *et al*., 2016). A comprehensive search of the literature was performed in January 2020 and repeated in December 2020 using the online database Web of Knowledge (WoK v5. 3), in English language only. A scoping process was performed to optimise the search terms so that the search was as comprehensive as possible whilst reducing the volume of irrelevant material. The final search terms used were: Title = ((CO_2 OR "carbon dioxide") AND (('flower* time' OR 'flower* phenology') OR ((pollinat* OR nectar OR pollen)))). The search was also performed using the same search terms in the online search engine Google Scholar, and the first 80 results, sorted by relevance, were included. The results of the search were assessed against the inclusion criteria by examination of the

abstract, and further exploration of the text where this was unclear. The inclusion criteria were set as: 1) The article must report the results of a primary empirical study, 2) the explanatory variables must include eCO_2, 3) the response variables must include either flowering phenology, floral resources or pollination. Studies reporting effects on reproductive allocation, fruit production or seed production were not included. Any article which did not pass all 3 inclusions criteria, or was a duplicate, was excluded.

Review papers included in search results, which passed all other inclusion criteria, were then further examined to identify any additional primary research articles. The final set of articles that had passed the inclusion criteria were read in full and entered into the database by extraction of the relevant data (Appendix III).

Field experiment

Location: The field experiment was conducted at the Birmingham Institute for Forest Research Free-Air CO_2 Enrichment ('BIFoR FACE') experimental facility, located in Staffordshire, UK (52°47'58"N, 2°18'15"W) as described in Hart *et al*. (2020). The facility is located within a semi-natural, mature, temperate woodland with English oaks, *Quercus robur*, as the dominant tree species and an understory comprised mainly of common hazel, *Corylus avellana*. In brief, 3 experimental arrays fumigate 30 m diameter plots with 150 ppm above ambient CO_2, with 3 control arrays which fumigate with ambient air. Fumigation commenced on 03rd April 2017, thus the woodland system had been exposed to eCO_2 for a period of 2 years prior to the experiment.

Plant study system: The common bluebell (*Hyacinthoides non-scripta*, Asparagaceae) is a widespread spring-flowering bulbous perennial which occurs throughout Atlantic Western

Europe. It is an ideal model species for studying how field-layer flowering plants within temperate woodlands might respond to eCO_2 due to its abundance, floral composition, flowering phenology and insect mediated pollination. The species is locally abundant throughout the experimental site in both eCO_2 and ambient arrays. Typically, 7-20 flowers are produced on a raceme which open in an acropetal sequence, each lasting 2-3 weeks. This species reproduces vegetatively by budding and sexually by seed. Insect mediated cross-pollination is required to produce a full seed set, with self-pollinated flowers producing fewer fruits and seeds, conferring a degree of 'effective self-incompatibility' (Corbet, 1998). Each array contained a single patch of bluebells with a mean area of 3.5 m^2 (SE = +-1.3) in the ambient arrays and 8.2 m^2 (SE = +-3.8) in the treatment arrays (patch sizes ranged from 0.7 to 12.6 m^2, Table 1).

Table 5.1 - Bluebell patch metrics for each experimental array.

Array	Treatment	Patch size (m^2)	Total number of racemes	Total number of flowers	Total number of fruits
1	eCO_2	0.71	60	257	167
2	Ambient	1.60	88	581	405
3	Ambient	6.03	150	933	613
4	eCO_2	11.31	230	1495	985
5	Ambient	2.90	96	536	285
6	eCO_2	12.57	250	1879	1279

Environmental data: To determine whether other environmental variables differed significantly between experimental arrays, soil temperature (°C), soil moisture (m3/m3) and patch-level illuminance (LUX) were recorded. Soil moisture was measured using CS655 probes (Campbell Scientific, Utah, USA) and recorded on a Campbell Scientific CR300 series datalogger. Mean monthly soil moisture and temperature were calculated for the three years preceding this study. Mean daily soil moisture and temperature were also recorded

throughout the duration of the flowering period. Illuminance was recorded for each patch throughout the flowering period using a smartphone light meter application (Lux Meter, My mobile tools dev, Android).

Flowering phenology: Trail cameras (SAS-DVRODR05, Konig, Edmonton, Canada) were used to monitor the flowering phenology of the bluebells throughout the 2019 flowering period. Cameras were installed facing each experimental patch at a height of ~50 cm and took photographs twice a day. From these photographs, the date of specific flowering stages (first flowering date, mid-flowering date and flowering duration) was determined. based on the 6 flowering stages defined by Corbet (1999). Total flowers in bloom in each patch were counted weekly.

Insect visitation surveys: A 30-minute flower visitation survey was conducted at each patch every two weeks throughout the bluebell flowering period (3 time points). The flowering period was subsequently divided into three time windows around these survey points ('early', 'mid' and 'late') to facilitate analysis. Surveys of all patches were performed in succession on the same day between 11:00-14:00, in a random order. Surveys were conducted on days when air temperature, wind, precipitation and cloud cover were as similar as possible. During each survey, every visit made by an insect to a bluebell flower within the patch was recorded and the insect identified to species level, or genus level for taxa where this is not possible from field identification (or family level for Ichneumonidae). A 'visit' was defined as each individual event when an insect entered/landed on a flower, potentially coming into contact with the floral reproductive organs (cf. Faegri and Van Der Pijl, 2013).

Seed counts: After all flowering was completed, 60 racemes were collected from each patch. The number of flowers produced and fruits that developed were recorded. The total number of seeds developing within 3 fruits from each raceme were then counted. One early, one mid and one late fruit were selected. This was determined by their position on the raceme, which corresponds to the period in which they flowered.

Statistical analyses: The impact of the treatment on first flowering date, mid-flowering date and flowering duration was assessed by ANOVA. Pearson's product-moment correlations were performed between each of the flowering date measures (day of year first flowering, day of year mid-point flowering and flowering duration), and each environmental variable during the flowering period (light intensity, soil moisture and soil temperature). Comparisons of mean monthly soil moisture, soil temperature and mean light intensity during flowering period between treatment and ambient arrays were performed using Wilcoxon rank-sum tests.

The relationship between number of visits to a patch and patch size was tested with a linear regression. To analyse the effect of eCO_2 treatment on number of visits per unit area of each patch, a Wilcoxon rank-sum test was used. The effect of time period on number of visits was tested using a Kruskal-Wallis rank sum test. The effect of both treatment and time period on seed set were tested by fitting Generalized least squares model and applying the *varIdent* weights term to control for the heterogeneity in the sample period using nmle package version 3.1-144 (Pinheiro *et al*., 2020). The analysis of the interaction between mean number of seeds per fruit and mean number of flower visits was performed using a generalised linear model with gaussian errors distribution. All statistical analyses were performed in R, version

3.5.2 (R Core Team, 2015). All the analyses were validated by examining model residuals (where appropriate) using model fits and inspection of model covariates residual spreads (Zuur and Ieno, 2016).

5.04 Results

Systematic review

The search process returned a total of 189 articles, 74 articles from Web of Knowledge, 80 from Google Scholar and 35 from examination of reference lists within review articles. Of these, 73 articles passed the inclusion criteria, with publication years ranging from 1956 to 2020 (Appendix III).

The mean treatment concentration of CO_2 for these 73 studies was 730 ppm, with a mean control concentration of 360 ppm. More than 118 plant species from 32 families were investigated with 146 individual species level responses reported. There is a strong bias in the geographic location of the studies, with over 72% performed in North America (53%) or Europe (19%). There were 2 or less studies from Africa, Central America or the Middle East, and none from South America.

Fifty-five articles investigated the impact on eCO_2 on flowering phenology (Figure 1, Appendix III). Flowering time varied from -60 to +10.8 days under eCO_2 compared to controls, with a mean response of -3.73 days. The greatest mean advance in flowering date under eCO_2 was exhibited by Ericaceae (-60), Solanaceae (-11.67) and Euphorbiaceae (-9), whereas the greatest mean delay in flowering was by Geraniaceae (+1.88), Amaranthaceae (+2.03) and Cucurbitaceae (+10.8).

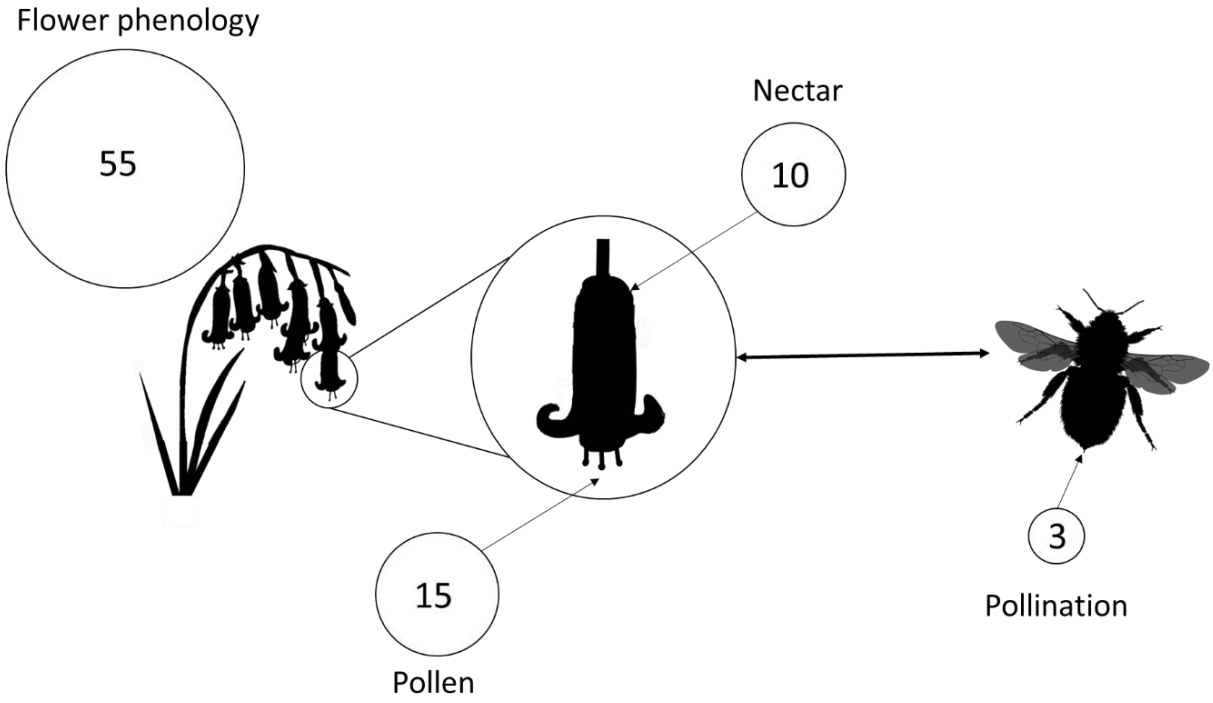

Figure 5.1 - Components of common bluebell, Hyacinthoides non-scripta, pollination interaction and the number of published studies (on any species) considering the impact of eCO_2 on each component. The area of the circle is proportional to the number of studies.

Fifteen studies included eCO_2 effects on pollen (Figure 1, Appendix III), with 6 reporting an increase in pollen production and 4 showing an associated decrease in quality through reduced protein content or increased metabolites. There were mixed results of the effect of eCO_2 on nectar, with 3 articles showing increased production, 2 decreased and 3 with no change. Similarly, the response of nectar sugar content varied with 2 and 1 studies reporting increasing and decreasing concentration respectively. Three studies directly measured the impact of eCO_2 on more than just floral traits (Figure 1, Appendix III), of which 2 looked at a single crop species *ex situ*. These investigations found either increased visitation or decreased pollinator longevity, but neither were significant.

BIFOR FACE Field experiment

Environmental parameters: Over the course of the proceeding three years mean soil temperature did not differ significantly between treatment and control arrays with an overall mean of 9.7°C (+-0.65) and 9.5°C (+-0.64) respectively (p = 0.7613, Appendix III). Mean soil moisture was also not significantly different over the same period with an overall mean of 16.27 m3/m3 (+-1.02) in eCO_2 arrays and 14.86 m3/m3 (+-1.26) in ambient arrays (p = 0.3928, Appendix III). During the flowering period mean light intensity in eCO_2 arrays was 2852 lx (+-314) and 3420 lx (+-491) in ambient arrays and as such also not significantly different (p = 0.361).

Bluebell flowering phenology: Under eCO_2 the mean date of first flower opening advanced by 6 days relative to the ambient control and the mean mid-point, between first flower opening and final flower senescing, also advanced by 6 days (Figure 2a). The advance of mid-flowering date under eCO_2 was statistically significantly (F = 12.893, p = 0.02295). The duration of flowering was not significantly different under eCO_2 (F = 0.0091, p = 0.9286) with a mean of 46 days under eCO_2 and 45 days for the ambient patches. Mean peak flowering occurred in the late period for ambient patches, whereas mean peak flowering shifted to during the mid-flowering period in eCO_2 patches (Figure 2b).

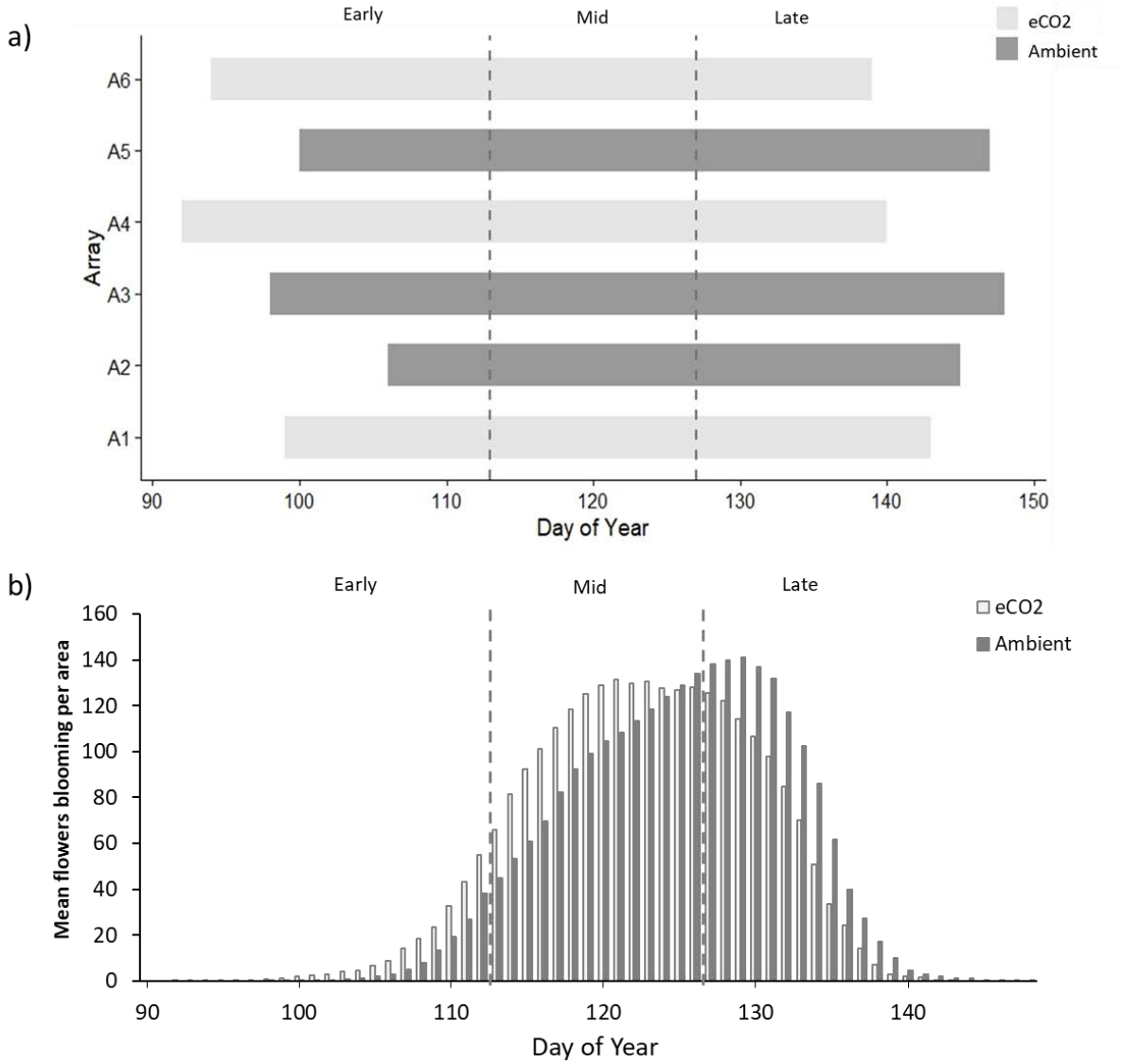

Figure 5.2 - a) Patch-wide flowering period of the common bluebell, Hyacinthoides non-scripta, per FACE array. Bars commence on the day of year when the first flower opened and end when last flower senesced. Dashed lines denote the boundaries for 'early', 'mid' and 'late' time windows within flowering period. b) Daily mean total number of flowers blooming per patch area (m^2) for eCO_2 and ambient patches. Totals were based on weekly counts and interpolation for missing values, cross-referenced against daily phenology photographs.

Pearson's correlations of light intensity, soil temperature and soil moisture were non-significant between first flowering date ($p = 0.1411$, $p = 0.4806$, $p = 0.1127$), mid flowering date ($p = 0.2226$, $p = 0.6628$, $p = 0.2215$) and flowering duration ($p = 0.3167$, $p = 0.3255$, $p = 0.219$).

Insect visitation and seed production: Insect visitation of bluebells commenced as soon as the first flowers opened, but at low rates with a mean of 1 visit/patch during the early flowering period (30 min observation periods). Visitation was significantly lower in the early flowering period compared to the later flowering periods ($p = 0.0436$), with the mean number of visits per patch rising to 5.8 and 4.8 in the 'mid' and 'late' flowering periods respectively (Figure 3a).

The overall number of visits under eCO_2 were much higher than the overall number of visits under ambient CO_2, however visits were significantly correlated with patch size ($p = 0.0029$, $R^2 = 0.8914$). There were no significant differences in visitation per area between treatment and control arrays ($p = 0.6866$).

A total of 18 species/species groups visited bluebell flowers during the surveys (Appendix III), of which 10 made repeated visits (Figure 3b). Hoverflies (Diptera: Syrphidae) were the most frequent visitor during all three flowering periods, contributing 55.7% of total visits (Figure 3a). The hoverflies *Platycheirus spp*. made the greatest number of visits, peaking in the mid-flowering period. *Rhingia campestris* made the second highest number of visits of any hoverfly species, with 88% of these occurring in the late flowering period. Bumblebees (Apidae: Bombus) represented 22.9% of total flower visits, with *B. pratorum* workers the most common bumblebee observed. For all other *Bombus* species, visits were made exclusively by queens.

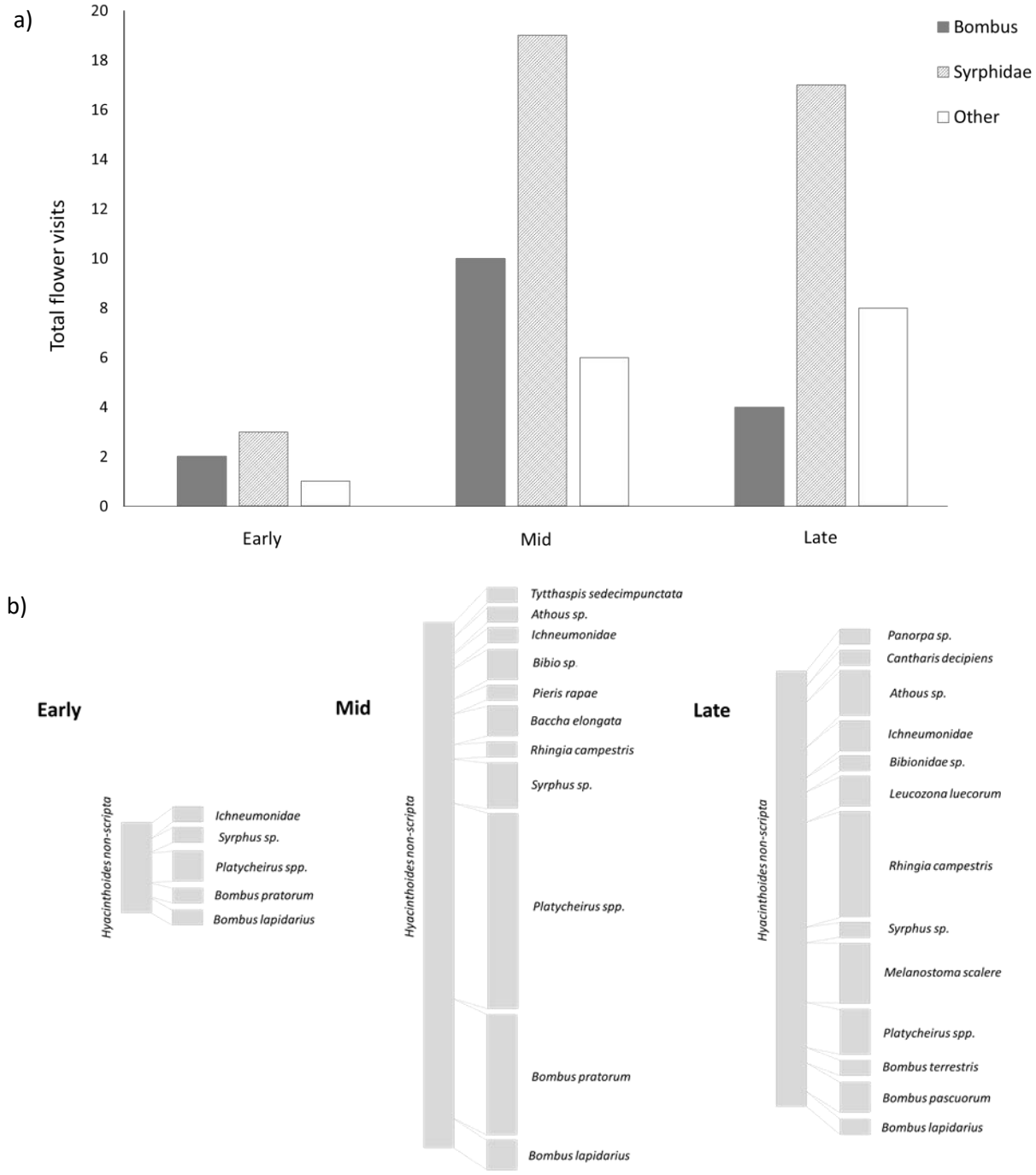

Figure 5.3 - a) Total number of flower visits made by bumblebees (Bombus), hoverflies (Syrphidae) and all other flower visitors during each time period. Data is based on 30 minute observations at each array for each time period during flowering. b) Sankey diagram of the visitation network during each flowering time period. Size of bars are proportional to total number of visits by each taxon during each time period.

Seed set followed a similar temporal pattern (Figure 4), with an initial mean of 4.91 seeds per fruit from early-flowering fruits. This increased to 7.48 mean seeds per fruit for mid-flowering

period, and 6.30 mean seeds per fruit during late-flowering period (p = 0.0526). There was a significant correlation between total number of flower visits recorded and mean number of seeds per fruit produced from flowers which bloomed during the corresponding period (p = 0.0348).

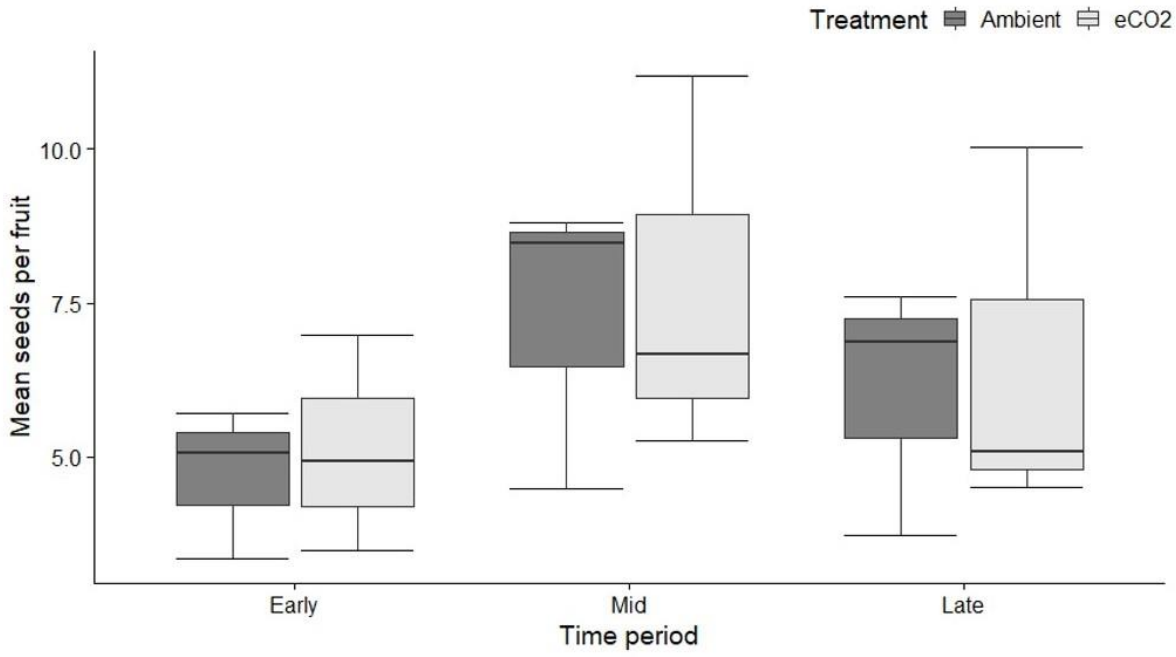

Figure 5.4 Mean seeds per fruit for eCO_2 vs ambient for each time period during flowering. N = 60 fruits per patch (total 360) at each time point.

5.5 Discussion

Systematic review

Compared with the number of studies which examine the effect of other climate change variables, such as temperature and precipitation patterns (Potts *et al.*, 2010), there is a paucity of peer reviewed literature which investigates the impact of eCO_2 on pollination. The systematic review of the literature revealed here, indicates that the majority of publications examining pollination under eCO_2 reported on the impacts on flowering time (75%), which is

likely due to this variable being easier to measure from direct observation. This covered a wide range of flowering species from a reasonable phylogenetic spread of families, although the importance of insect pollination to their pollination ecology varied considerably. For example, the large proportion of studies reporting the effect on Poaceae (10), which are largely wind pollinated (Culley *et al*., 2002).

A key finding of this review is the net advance in flowering phenology under eCO_2 by 3.73 days. This conforms with the findings of previous studies assessing large numbers of species (e.g. Springer and Ward, 2007) and adds further evidence to the conclusions that increasing atmospheric CO_2 will disrupt flowering phenology, leading to an advance in flowering for many species. This may be due to related increases in growth rates in response increasing photosynthetic rates (He *et al*., 2005). The phylogenetic spread of phenological responses to eCO_2 across various plant families is also potentially interesting, however, in this review the strongest mean responses, in either direction, are underpinned by the findings of a smaller number of studies. For example, five of the six families with the strongest mean response, in either direction, are all derived from the results of a single study. Therefore, whilst the findings of this review suggest there is a pattern in the response across the plant phylogeny, more studies would be needed to provide a robust assessment and with multiple assessments of individual plant families.

There were substantially fewer studies examining the impact of eCO_2 on floral resources, which is likely due to the additional methodological steps required to sample and measure these properties. Where this was measured the results are equally mixed, i.e. no consistent response across species. Direct measurements of floral traits such as flowering time and floral

resources do not provide a direct quantification of pollination, however, and therefore can only be used to infer the impact of eCO_2 on this interaction.

Insect mediated pollination is a complex interaction between multiple species, which is perhaps why so few studies to date have directly measured the impact of eCO_2 upon it. Only 4% of the studies found by this review directly measured an aspect of the effect of eCO_2 on pollination, revealing an important knowledge gap. Furthermore, none of these studies were performed *in situ*, therefore potentially missing the effects of important interactions which cannot be replicated in controlled environments. Empirical studies addressing this specific area are urgently needed to improve our predictions of plant-pollinator interaction changes under climate change.

4.2 BIFoR FACE Field experiment

To our knowledge, this is the first study to empirically measure the impact of eCO_2 on pollination directly *in situ* and the first assessment of a pollination interaction in a FACE experiment. Flowering phenology of common bluebell was found to advance by a mean of 6 days under eCO_2 (Figure 2). This is consistent with the overall mean effects of eCO_2 on flowering time reported in the articles included within the systematic review. Importantly, other variables such as soil moisture, soil temperature and light intensity, which are all known to influence phenology (Schemske *et al*., 1978), did not vary significantly between eCO_2 and ambient arrays within the BIFoR FACE facility for the 3 years preceding this study, meaning they are unlikely to help explain any observed differences in bluebell flowering between patches. This allows us to focus particular attention on the contribution of eCO_2 on bluebell flowering traits, as well as plant-pollinator interactions. eCO_2 is associated with increased

growth in many plant species (Wang *et al*., 2012), which may lead to increased and/or earlier flower production. Whilst there were a larger number of flowers in the eCO_2 arrays, this was directly related to patch size (Table 5.1) which was a pre-existing condition of the distribution of bluebell across the site. Neither flowers per area, nor flowers per raceme varied significantly between treatment and control arrays therefore there is no evidence of an increased reproductive allocation under eCO_2, although this cannot be fully assessed without further historical data on flower production per patch prior to fumigation.

The temporal patterns of insect visitation, and the associated consequences for seed set (Figures 5.3 and 5.4), suggest that early flowers are less successful for *Hyacinthoides non-scripta.* Increased resource allocation to flowering early in the flowering period would, therefore, be less efficient. I found that the early flowers also had a greater duration which is consistent with the theory that earlier spring flowers experience lower visitation. Bluebell flowers are generally long lived, compared to many other woodland flowers (Corbet, 1999), suggesting an overall low frequency of visits, which is consistent with the findings of this study.

Many of the dominant pollinator species recorded visiting bluebells in this study, such as *Bombus pratorum*, *B. lapidarius*, *B. pascuorum*, *B. terrestris*, *Rhingia campestris* and *Platycheirus spp*., have been previously recorded visiting bluebells (Corbet and Tiley, 1999). This adds to the evidence that they are the key species in the bluebell pollination interaction networks in Britain. Many of these hoverflies and bumblebees were also observed to be covered in bluebell pollen whilst moving between racemes and are, therefore, highly likely to be facilitating pollination. This is not true of all insect visitors, however, with numerous

interactions failing to transfer pollen to a receptive stigma of a conspecific flower. Thus, visitation does not always equal pollination. In order to avoid conflation of 'pollinators' and 'flower visitors', studies which attempt to measure pollination must also measure pollination success, e.g. by measuring seed set. Corbet (1998) found insect pollination to be directly related to seed set in bluebells. My results support this, further suggesting that seed set may be a useful indicator of pollination success for this species.

Over 90% of all insect visits to bluebells occurred during the mid- and late-flowering periods, with many important (based on number of visits) species (e.g. *Rhingia*) only active in the late period. The advance in flowering phenology driven by eCO_2 observed in this study, therefore, is potentially shifting peak flowering (and thus seed production) away from the peak flight period of key pollinators. Other insect species may of course 'step in' to provide a pollination service, but recent evidence from alpine environments suggests altering pollinator communities can have significant negative effects on plant reproductive success (Richman *et al*., 2020) and there is often less redundancy in pollination service provision for species that emerge early in the year. Negative impacts on important pollinator species could also be significant, for example it is worth noting that for many of the *Bombus* species observed, only queens were recorded visiting bluebells. Thus, this flower likely represents an important resource for queens emerging, somewhat nutrient deprived, from their overwintering diapause. Any reduction in the availability of this resource, e.g. resulting from a phenological mismatch, could reduce the success of queens subsequently establishing a colony.

The phenological relationships between plants and insect pollinators are, of course, influenced by many factors other than eCO_2, and while neither temperature nor precipitation

had a significant effect in the current study, their influence across longer time scales is clearly evident. Both factors are known to affect flowering phenology (Rafferty and Ives, 2011), and temperature (importantly not just during spring) seems to be the dominant factor influencing insect emergence following winter diapause (Bale and Hayward, 2010). Indeed, global shifts in the synchrony of multiple species interactions, based on historic data, appear to be driven by temperature (Kharouba *et al*., 2018, but predictive models are now also needed in order to more effectively plan conservation and food security strategies. The current study indicates that models for any plant-insect interactions would be wise to include eCO_2 as a parameter, to determine if it will either exacerbate or reduce temperature-driven changes in phenological synchrony.

5.06 Conclusions

eCO_2 is likely to directly impact plant-pollinator interactions in addition to other climate change variables, yet few studies have directly measured these impacts. My results showed a consistent advancement of bluebell flowering under eCO_2 in a deciduous woodland, which is also consistent with the mean effect established across studies of other plant species (in both lab and field settings). If this pattern continues under future eCO_2 scenarios, then greater phenological mismatches may occur than predicted by temperature-based models alone, with the main flowering period of several plant species potentially losing synchrony with the peak flight period of key pollinators. This could lead to a shift in the plant-pollinator network resulting in a declining forage resource for certain pollinators, as well as a decrease in in plant seed set. Importantly, this impact is likely to be greater for plant species with short flowering periods and/or very specialised plant-pollinator relationships.

CHAPTER 6

Short-term CO_2 enrichment has limited impact on arthropod abundance in a mature temperate woodland.

6.01 Abstract

Forest ecosystems house a high degree of biodiversity, with a large proportion of this species richness comprising arthropods, which also underpin many important ecosystem processes. Elevated CO_2 (eCO_2) has the potential to impact woodland arthropod populations, predominantly via plant-mediated effects leading to trophic cascades throughout the ecosystem that can potentially alter ecosystem function with associated implications for processes such a nutrient flow and carbon sequestration. Arthropods were monitored over the course of 118 weeks at the Birmingham Institute of Forest Research (BIFoR) Free-Air Carbon Enrichment (FACE) facility using an extensive sampling programme from forest floor to canopy. Monthly sampling by pitfall, pan and Malaise trapping plus canopy and understory beating yielded 58,413 individual invertebrates which were identified to order. There were no-significant differences in the total abundance of invertebrates sampled between eCO_2 and control arrays either for any sampling method cumulatively or for any individual order, with the exception of grouped 'other orders' for understory beating. Overall, variation between years exceeded variation driven by eCO_2, although it is not possible to rule out eCO_2 effects over longer timescales or finer taxonomic resolution.

6.02 Introduction

Increasing concentrations of CO_2 have the potential to profoundly alter ecosystem function via its impacts on processes such as carbon cycling, plant growth, and productivity (Ainsworth and Long, 2005). It remains less clear how elevated CO_2 (eCO_2) will impact biodiversity,

especially given the potential for these impacts to act synergistically with other stressors such as increasing temperature, changing patterns of precipitation and increased frequency of extreme weather events (Wang *et al.*, 2012).

Forests house a significant proportion of global terrestrial biodiversity, with over half of all known species associated with this habitat leading to the characterisation of many forest ecosystems as 'biodiversity hotspots' (Myers, 1988). Arthropods are the most abundant and diverse organisms in these systems and important drivers of ecological processes. Despite the key role played by arthropods in processes such as herbivory, pollination and nutrient flow, it remains unclear how forest arthropods will respond to climate change, in particular eCO_2. Whilst direct impacts of eCO_2 on arthropod species are unlikely, plant-mediated indirect impacts are expected (Grodzinski *et al.*, 1999).

Previous studies have found that the response of forest arthropod to eCO_2 is subtle and context specific (Hillstrom *et al.*, 2014). In general, eCO_2 in forests has been associated with decreased insect diversity (Altermatt, 2003) and abundance (Facey *et al.*, 2016). Responses are, however, variable and often guild specific (Sanders *et al.*, 2004). For example, in a first-generation forest FACE experiment on within a young pine plantation, predatory feeding guilds such as Araneae and parasitic Hymenoptera increased under eCO_2, whilst herbivorous species such as phytophagous Lepidoptera and Coleoptera decreased (Hamilton *et al.*, 2012).

Forest ecosystems also play a major role in the sequestration and long-term storage of carbon. The impact of eCO_2 on forest biodiversity is likely, therefore, to indirectly effect ecosystem functioning in terms of carbon flow. For example, increases in the abundance of a particular herbivore feeding guild under eCO_2 may lead to an increase in herbivory with an

associated increase in the consumption of photosynthetically active plant biomass. This would have consequences for the rate of biochemical processes such as photosynthesis and thus potentially decrease the ecosystems ability to offset further increases in atmospheric CO_2 via the carbon fertilisation effect. This is an example of how insect herbivory may in fact be capable of turning a forest system from a carbon sink into a carbon source (Chen *et al*., 2016).

Long-term, ecosystem scale experiments across a range of different forest habitats are, therefore, required to build an accurate and complete assessments of the impact of eCO_2 on arthropod populations and the feedback this confers on ecosystem function (Facey *et al*., 2016). The BIFoR FACE experiment thus represents a unique opportunity to study these impacts for the first time in a mature, temperate woodland.

Chapter 2 set out and explored how arthropod abundance and diversity varied spatially and temporally at the BIFoR FACE experiment, as well as the influence of sampling method on sample composition. Whilst there were no significant responses to eCO_2 detected in the first 12 months, such impacts are likely to only be detected after multiple years of exposure, especially given the maturity, complexity and seasonality of the ecosystem. The characterisation of the most abundant families sampled in Chapter 2 also provides an indication of likely order level responses for families which share specific traits. For example, more than 80% of Hemiptera sampled were Aphididae, which were found to be more abundant under eCO_2 in Chapter 4, so it may be expected that the abundance of Hemiptera increases under eCO_2 across the longer time scales. Alternatively, Lepidoptera possess leaf-chewing larvae, such as the leaf mining species explored in Chapter 3, which may be expected to decrease in abundance under eCO_2 in response to decreased nutritional value of host

tissue or increased plant defences. Collembola were the 4th and Acari 5th most sampled taxa in Chapter 2, both of which mostly predominately soil mesofauna which are expected to decrease in abundance under eCO_2 due to declining litter quality or plant-derived changes to soil (Hansen *et al*., 2001). Over 90% of Hymenoptera sampled in the first 12 months belong to families of parasitoids such as Ichneumonidae and Platygastridae, which have been found to decrease in abundance under eCO_2 (Facey *et al*., 2016), potentially mirroring declines in their hosts such as Lepidoptera larvae. If pollination interactions are disrupted, as was suggested may occur under eCO_2 in Chapter 5, flower visiting insects may decrease in abundance, which could be detected using methods such as pan trapping. Order level responses such as these, however, may not necessarily be detectable at broad taxonomic, restricted spatial or limited temporal scales. Responses may be masked by interspecific variation, high individual mobility and longer lead times respectively.

This chapter continues the line of investigation established in Chapter 2 over multiple years, with a focus on how eCO_2 impacts arthropod abundance across a comprehensive range of orders. Furthermore, the initial study characterised the sample profile of different sampling techniques, highlighting the guilds and taxa favoured by each method. This allows more targeted investigations of the responses of different arthropod groups to eCO_2, as well as an assessment of whether sample profiles are consistent over a longer time-period.

Aims of the study

This study aims to build on the findings of the study in chapter 2 which investigated the impact of CO_2 fumigation on arthropods within a mature temperate forest over the first 12

months. This study will focus in on the potential impacts on arthropod abundance over multiple years of fumigation. This will be achieved by addressing the following hypotheses:

a) The most abundant orders sampled overall and by each sampling method after 3 years is the same as after 1 year.
b) Overall arthropod abundance is reduced after 3 years of eCO_2. The strength of this effect increases with time via a time x treatment interaction.
c) The abundance of epigeal arthropods sampled by pitfall trapping is reduced after 3 years of eCO_2. The strength of this interaction increases with time.
d) The abundance of arthropods sampled by pan trapping is reduced after 3 years of eCO_2. The strength of this interaction increases with time.
e) The abundance of aerial arthropods moving within the field layer is reduced after 3 years of eCO_2. The strength of this interaction increases with time.
f) The abundance of arboreal arthropod with the canopy and understory layers is reduced after 3 years of eCO_2. The strength of this interaction increases with time.

6.03 Methods

Experimental site

The study was conducted in a mature, temperate, broadleaved woodland within the BIFoR FACE facility in Staffordshire, UK (see chapter 2). The FACE facility consists of nine 30m diameter experimental arrays throughout the site, of which 3 fumigate with CO_2 to +150ppm above ambient, 3 fumigate with ambient air and 3 are non-infrastructure controls, as described in Hart *et al.* (2019). CO_2 fumigation commenced on 03rd April 2017, operating during daylight hours throughout the summer growing period.

Invertebrate sampling

Invertebrates were sampled using the five methods outlined in chapter 2 (Figure 6.1; pitfall trapping, pan-tapping, Malaise trapping, canopy beating and understory beating). Sampling commenced in March 2017 and continued every 4-6 weeks until June 2019 for a total of 27 time points over 118 weeks. Beating occurred only during the growing season when oak and hazel trees had leaves (15 time points). All samples were collected into 70% ethanol for long term storage before identification. During identification, all arthropods in each sample were counted and identified to order using a stereomicroscope.

Figure 6.1 – The sampling methods used in the core sampling programme. Top left – pitfall trapping, top right – pan trapping, bottom left – Malaise trapping, bottom right – beating.

Statistical analysis

All statistical analyses were performed in R, version 3.5.2 (R Core Team, 2015). The two pitfall samples and the 3 different colour pan trap samples taken in each array were pooled. The total abundance of all arthropods collected by each sampling method was analysed with a linear mixed effect model, with treatment (eCO_2, ambient control and non-infrastructure control), time (week since start of sampling) and the treatment x time interaction as fixed effects and array as a random effect. The abundance of the 5 most abundant orders collected by each sampling method (4 most abundant orders plus all remaining orders grouped as 'other' for pitfall trap, pan trap and Malaise trap; 2 most abundant order plus all remaining orders grouped as 'other' for canopy and understory beating) were also analysed with linear mixed effect models, with treatment, time and the treatment x time interaction as fixed effects (plus trap colour for pan trap samples) and array as a random effect (plus replicate for pitfall samples).

6.04 Results

Most abundant orders

A total of 22 orders were sampled across all sampling methods. Malaise traps sampled the most different orders (19), and canopy beating the least (14). Diptera were by far the most sampled order overall with 23,446 individuals sampled (40.14% of all invertebrates sampled) and were also the most abundant order sampled by all sampling methods with the exception of beating. The next most sampled orders overall were Coleoptera (8,058), Collembola (7,264), Hymenoptera (5,914) and Acari (3,187) (Figure 6.2). Araneae were the most abundant order sampled by both canopy and understory beating (36.1% and 36.8%

respectively), with Hemiptera the second most abundant by both (20.8% and 13.7% respectively).

The relative proportions of each order sampled overall and within each sampling method did not differ between the first 12 months and the overall total (Figure 6.2). The ranking of the most abundant order for each sampling method was similarly unchanged, with only minor switches for Hymenoptera and Collembola overall, Collembola and Acari for pitfall and Coleoptera and Collembola for pan trapping. The most abundant two orders across all sampling method and for each sampling method separately were consistent between the first 12 months and the overall total.

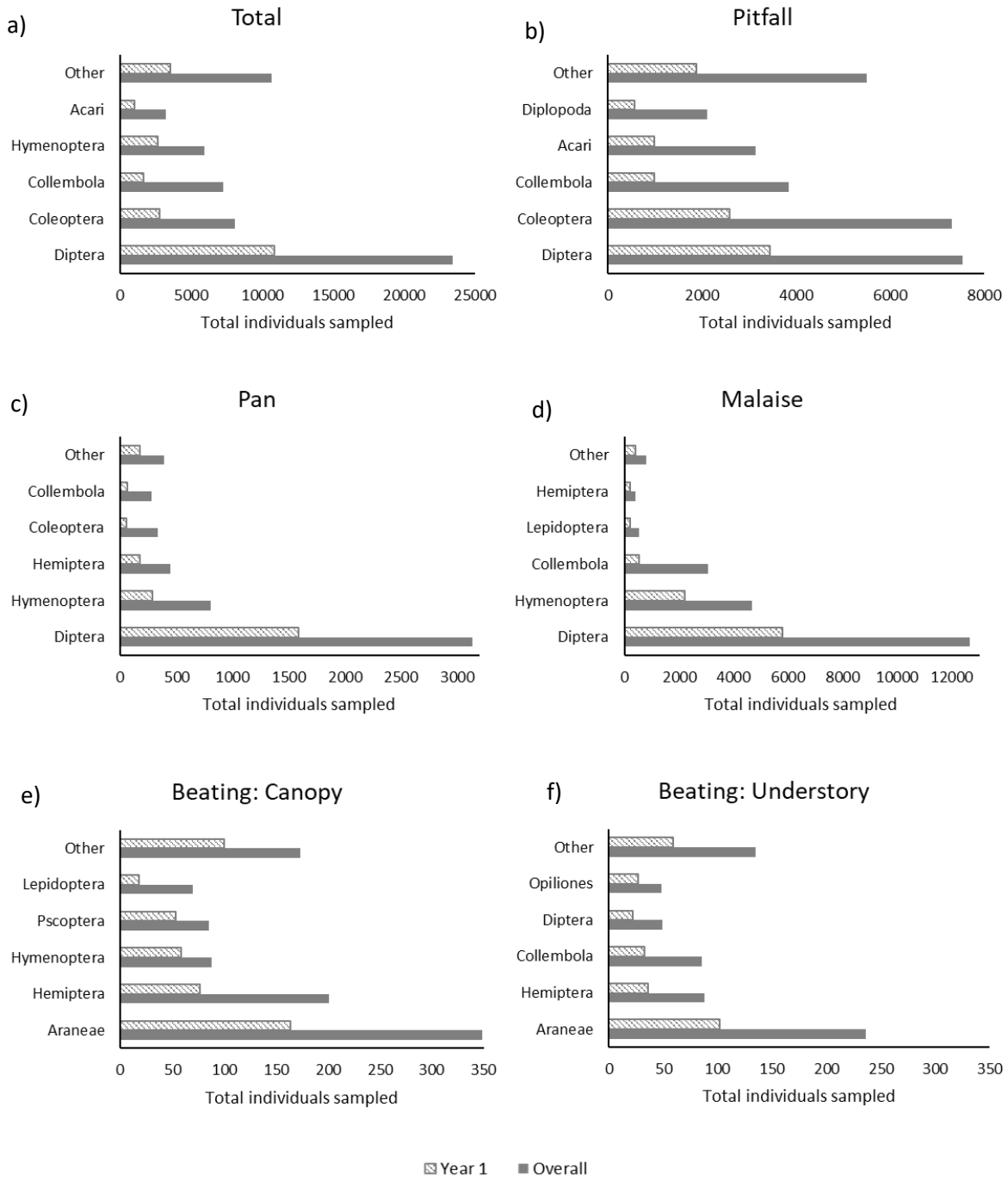

Figure 6.2 – Total individuals sampled from the 5 most abundant orders for a) all sampling methods, b) pitfall trapping, c) pan trapping, d) Malaise trapping, e) canopy beating and f) understory beating. Solid bars represent overall total and hatched bars represent the total from the first 12 months.

Overall arthropod abundance

A total of 58,413 individual invertebrates were sampled over 118 weeks. 29,383 invertebrates were sampled by pitfall traps, 22,028 by Malaise traps, 5,395 by pan traps, 966 by canopy beating and 641 by understory beating (Figure 6.3). Neither treatment (Table 6.1), time, nor

the treat x time interaction had a significant effect on total arthropod abundance for any trap type, with the exception of time for pan trap samples which had a significant decreased in abundance with increasing time ($p = 0.0377$). Trap colour also had a significant effect on pan trap sample abundance ($p < 0.0001$).

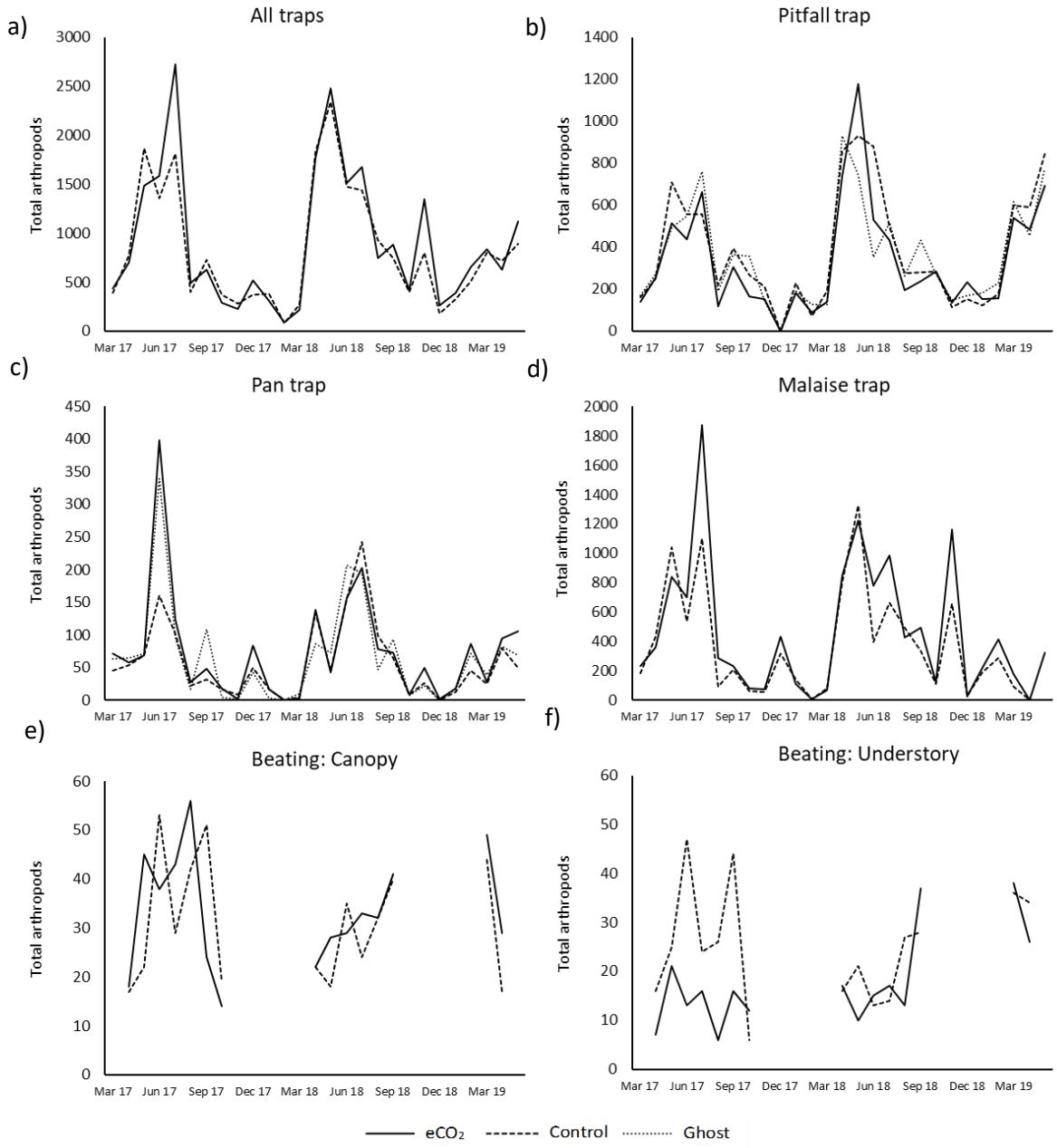

Figure 6.3 – Total arthropods sampled monthly from March 2017 to June 2019 for eCO_2, control and non-infrastructure control ('Ghost') arrays; for a) all sampling methods, b) pitfall trapping, c) pan trapping, d) Malaise trapping, e) canopy beating and f) understory beating.

Epigeal arthropod abundance

The treatment had no significant effect on the abundance of Diptera, Coleoptera, Collembola, Acari, Diplopoda or other orders sampled by pitfall trapping (Table 6.1). Time did have a significant effect on the abundance of Collembola, Diplopoda and other orders (p = 0.0017, p = 0.0065 and p = 0.0058). The most frequent Dipteran families sampled were Chironomidae (non-biting midges) and Phoridae (scuttle flies). Staphylinidae (rove beetles), Carabidae (ground beetles) and Leoididae (round fungus beetles) were the most frequent families of Coleoptera sampled.

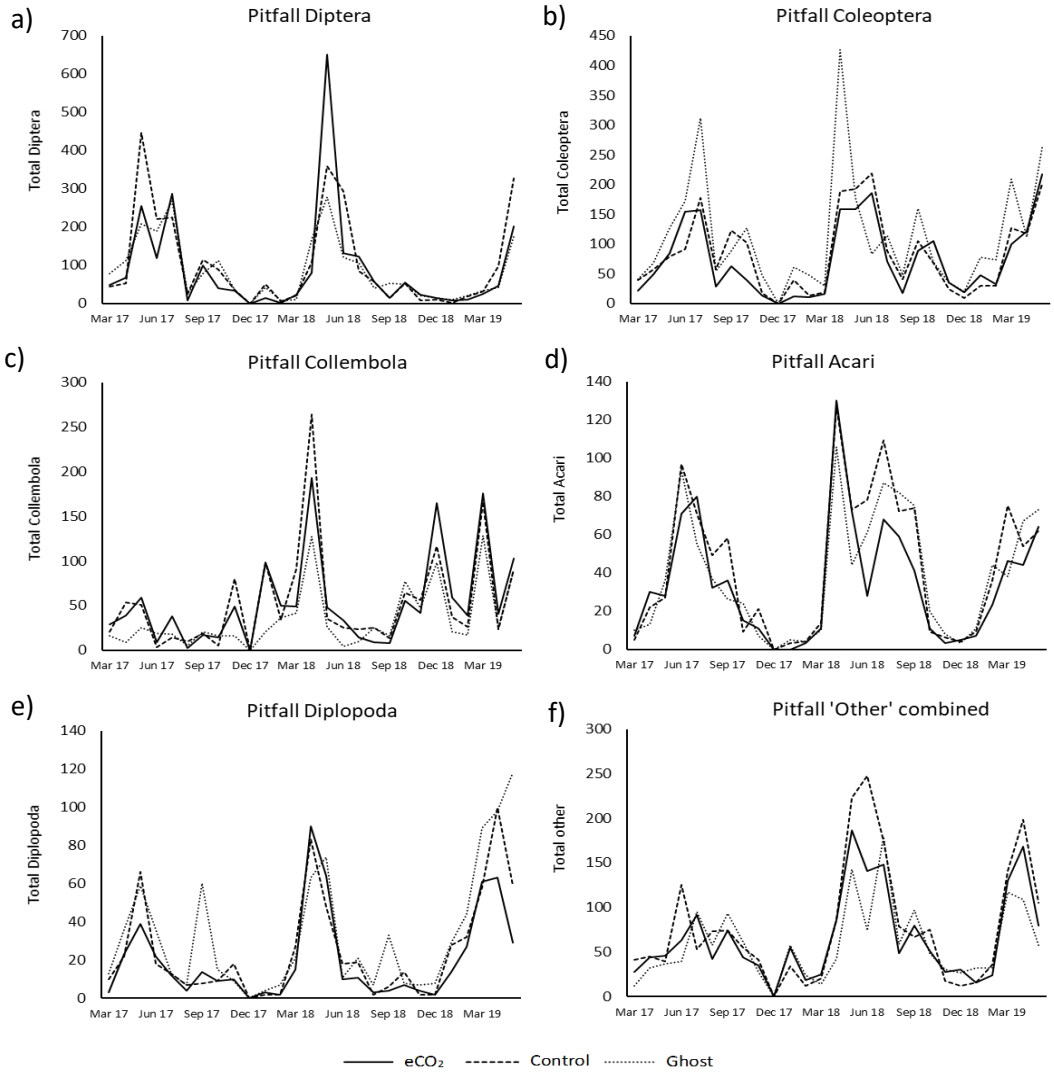

Figure 6.4 – Total individuals sampled by monthly pitfall sampling from March 2017 to June 2019 for eCO_2, control and non-infrastructure control ('Ghost') arrays for the top 5 most sampled orders; a) Diptera, b) Coleoptera, c) Collembola, d) Acari, e) Diplopoda and f) all other orders.

Flower-visiting arthropod abundance

The treatment, time and treatment x time interaction has no significant effect on Diptera, Hymenoptera, Hemiptera, Coleoptera, Collembola or other orders for pan trapping (Figure 6.5, Table 6.1). Pan trap colour had a significant effect on all but Collembola and other orders, with yellow traps sampling a greater abundance of Diptera, Hymenoptera and Hemiptera ($p = 0.0001$, $p < 0.0001$ and $p < 0.0001$) and white traps sampling a greater abundance of Coleoptera ($p = 0.0005$). The most abundant families sampled by pan traps were Sciomyzidae (snail-killing flies) and Muscidae (house flies) for Diptera, Platygastridae (gall midge wasps) and Ichneumonidae (ichneumon wasps) for Hymenoptera and Aphididae (aphids) for Hemiptera.

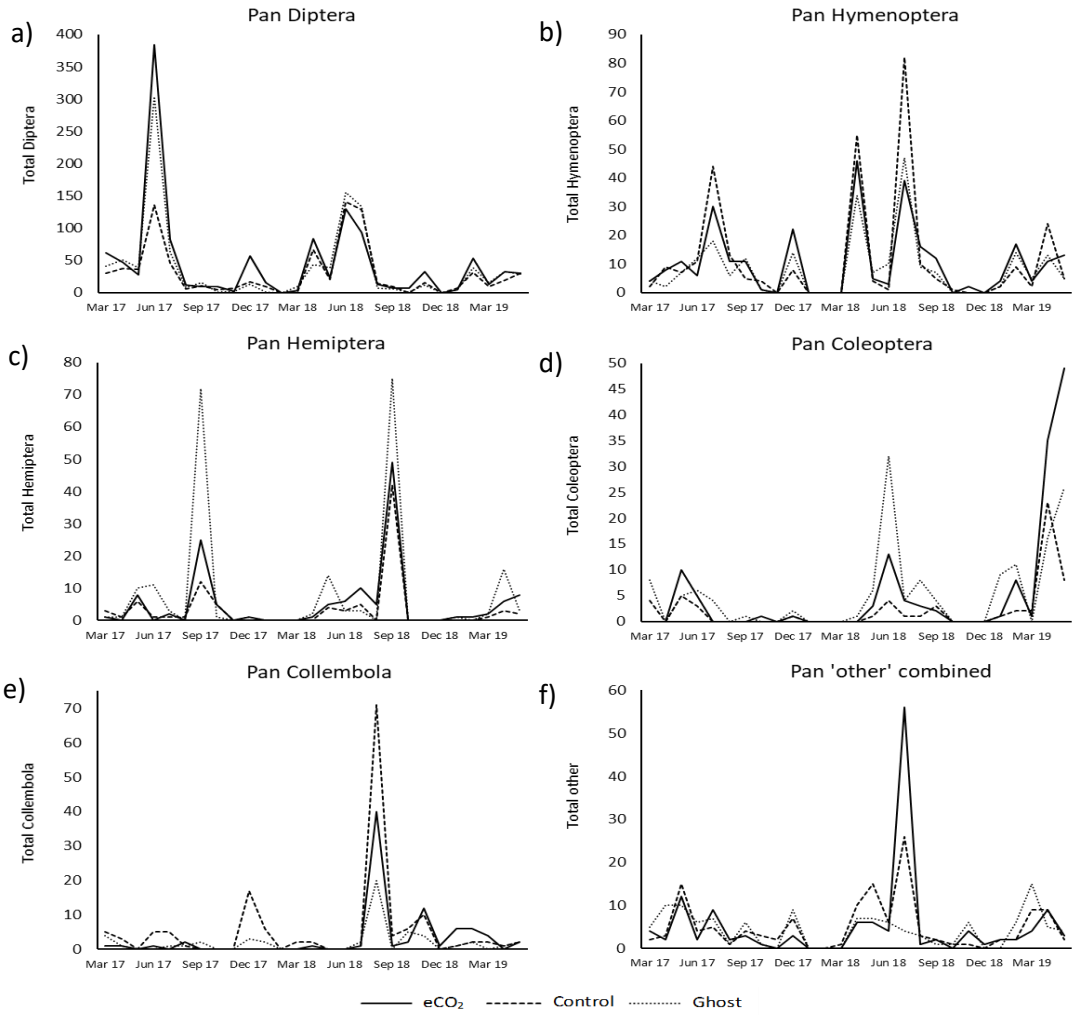

Figure 6.5 – Total individuals sampled by monthly pan-trap sampling from March 2017 to June 2019 for eCO_2, control and non-infrastructure control ('Ghost') arrays for the top 5 most sampled orders; a) Diptera, b) Hymenoptera, c) Hemiptera, d) Coleoptera, e) Collembola and f) all other orders.

Flying arthropod abundance

CO_2 treatment, time and treatment x time interaction had no significant effect on the abundance of Diptera, Hymenoptera, Collembola, Lepidoptera, Hemiptera or other orders sampled by Malaise trapping (Figure 6.6, Table 6.1). In the Malaise samples the most abundant: Diptera families were Chironomidae, Cecdomyiidae (gall midges), Fannidae (lesser house flies), Mycetophilidae (fungus gnats) and Sciaridae (dark-winged fungus gnats); Hymenoptera families were Platygastridae, Ichneumonidae and Braconidae (braconid wasps); Lepdioptera family was Geometridae (geometer moths); and Hemiptera family was Cicadellidae (leafhoppers).

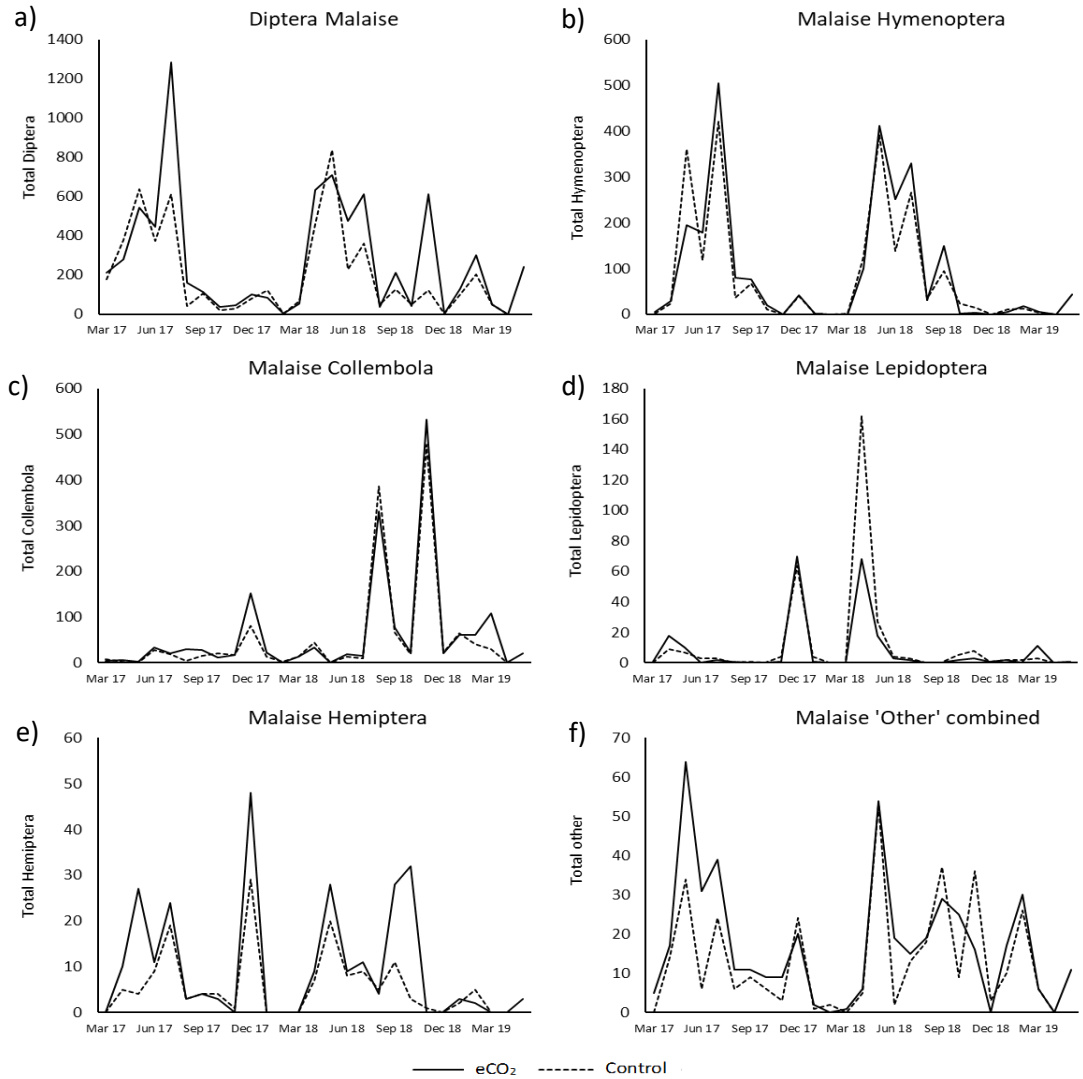

Figure 6.6 – Total individuals sampled by monthly Malaise sampling from March 2017 to June 2019 for eCO_2 and control arrays for the top 5 most sampled orders; a) Diptera, b) Hymenoptera, c) Collembola, d) Lepidoptera, e) Hemiptera and f) all other orders.

Arboreal arthropod abundance

The abundance of Araneae, Hemiptera and other orders sampled by both canopy and understory beating were not significantly impacted by the treatment, time of treatment x time interaction (Figure 6.7, Table 6.1). The most abundant Araneae families sampled by beating were Theridiidae (comb-footed spiders), Linyphiidae (money spiders) and Tetragnathidae (long-jawed orbweb spiders) whilst the most abundant Hemiptera family was Miridae (plant bugs).

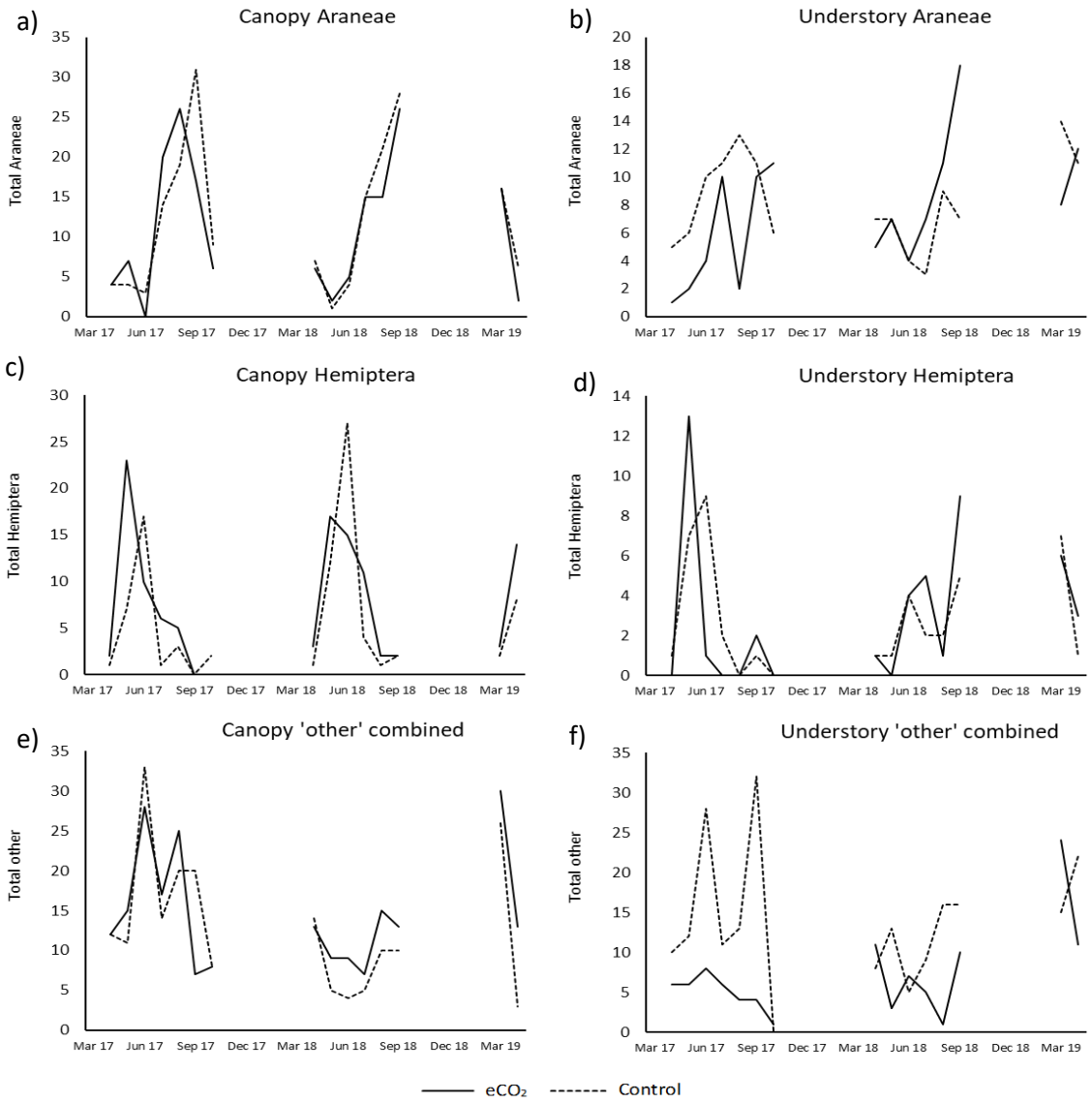

Figure 6.7 – Total individuals sampled by canopy and understory beating from April 2017 to May 2019 for eCO_2 and control arrays for the top 2 most sampled orders. a) Canopy Araneae, b) Understory Araneae, c) Canopy Hemiptera, d) Understory Hemiptera, e) Canopy all other orders and f) Understory all other orders.

Table 6.1 – Table of d.f. / p-values for treatment effect on abundance from linear mixed effect modelsfor overall invertebrate numbers sampled by each sampling method and each of the 5 most abundant orders sampled by each.

	Pitfall	Pan	Malaise	Canopy	Understory
Overall	6 / 0.4406	6 / 0.3855	4 / 0.7512	4 / 0.6574	4 / 0.1552
Diptera	6 / 0.6491	6 / 0.1492	4 / 0.7263	-	-
Coleoptera	6 / 0.4540	6 / 0.3663	-	-	-
Collembola	6 / 0.5905	6 / 0.2618	4 / 0.8725	-	-
Acari	6 / 0.7848	-	-	-	-
Diplopoda	6 / 0.9640	-	-	-	-
Hymenoptera	-	6 / 0.7527	4 / 0.9621	-	-
Hemiptera	-	6 / 0.9135	4 / 0.3962	4 / 0.5519	4 / 0.7126
Lepidoptera	-	-	4 / 0.8725	-	-
Araneae	-	-	-	4 / 0.9479	4 / 0.2970
Other	6 / 0.6798	6 / 0.8537	4 / 0.1628	4 / 0.6754	4 / 0.1125

6.05 Discussion

This study provides an assessment of the impact of eCO_2 on the abundance of forest arthropods after the first 3 years of fumigation at the BIFoR FACE facility. A clear temporal pattern was seen in the overall abundance of arthropods sampled, which peaked in late spring/early summer before dropping off rapidly over the course of the summer and into the autumn. For many species this seasonal pattern is predominantly driven by the phenology of the host tree species, for example the invertebrate community on oak trees (Southwood *et al.*, 2004). When examined at order level, seasonal abundance varies strongly between years, demonstrating the high temporal variation which is commonplace in complex ecosystems such as mature forests. For example, seasonal peaks in Collembola abundance are much greater in 2018 than 2017, possibly underpinning the significant relationship between Collembola sampled via pitfall trapping and time. Whilst there were similar significant effects

of time on the abundances of Diplopoda and 'other orders' sampled by pitfall traps, and overall pan trap abundance, this link was not significant for most sampling methods and specific orders. This is most likely due to the majority of the variance in abundance being driven by seasonal cycles and the dataset being limited to 118 weeks. It may be possible that other time effects may be significant if sampling operated over several years. Perhaps more interestingly, such time effects could potentially drive a time x treatment interaction, with potential impacts of eCO_2 on the system accumulating over time. There were no significant time x treatment interaction effects on arthropod abundance in this study, but similarly to the time effects, these may operate over longer timescales.

Despite the lack of widespread or consistent shifts in arthropod abundance driven by the treatment or time, it is possible to pick out more specific increases. For example, in 2018 a peak in abundance can be seen across several groups sampled by pitfall trapping. A possible explanation of this phenomena is the mass defoliation event that occurred in late spring 2018, when there was a highly synchronous emergence of defoliating Lepidoptera larvae feeding in the canopy. This 'outbreak event' resulted in almost complete defoliation of the oak trees, with an associated influx of nutrients into the litter layer via frass and greenfall, which is known to affect soil processes and nutrient levels (Hillstrom *et al.*, 2010). This influx of nutrients may have driven the observed increase in the abundance of Collembola and detritivorous Diptera (Chironomidae and Phoridae), and subsequently their predators (Carabidae). A key species implicated in the herbivory event was the winter moth, *Operophtera brumata,* which is a key oak herbivore and has highly synchronous larval hatching in response to host tree phenology (Tikkanen and Julkunen-Tiitto, 2003). This species is well known to have cyclical population increases every 9-10 years (Tenow, 1972), with the

large increase in abundance reflecting a peak in this cycle. A prelude to this event can be seen in the large number of *O. brumata* males (females are apterous) sampled in December 2017. This maybe in response to warmer than average temperatures in the days preceding the sampling period, triggering mass eclosion of dormant pupae, as temperature is known to influence the timing and success of eclosion (Peterson and Nilssen, 1998). This increase in abundance followed through to the following spring when the offspring of this generation hatched to become major contributors to the defoliation event, which is reflected in the peak in Lepidoptera larvae sampled in June 2018. Localised fluctuations in abundance are, therefore, are likely to be influenced by fine scale (both spatially and temporally) weather conditions during crucial points in the lifecycle. Climate variables may also influence the effects of eCO_2 (Robinson *et al*., 2012). For example, Zvereva and Kozlov (2006), show that the effects of eCO_2 and elevated temperature tend to counteract each other. In order to determine more fully the impact of eCO_2 on the relationship between other climate variables and arthropod populations, longer term monitoring within eCO_2 experiments is needed to increase the range of weather events observed.

The peak in Hemiptera in September 2017 and 2018 was due to an influx of alate aphids. This peak in sampling activity of this group was due to dispersal flights undertaken by aphids at the end of summer (Dixon, 1977). Other 'spikes' in abundance of orders can similarly be attributed to likely events, particularly for pan trap samples, and are often underpinned by a single family. For example, the peak in Diptera in June 2017 and Coleoptera in June 2019 were driven by large increases in the abundance of Sciomyzidae and Nitidulidae (pollen beetles) respectively. Pollen beetles are also known to undertake dispersal events during summer and are attracted to white and yellow surfaces (Kirk-Spriggs, 1996), leading to the

short-term peak in the number sampled by white and yellow pan traps. The cause of the temporary increase in Sciomyzidae is less clear, but due to the larvae being parasitoids of gastropods, likely reflects trends in abundance in the host populations, which may be driven by precipitations patterns.

The only consistent significant effect on arthropod abundance were differences driven by pan trap colour. This is consistent with the finding in Chapter 2, with yellow pan traps consistently sampling a greater abundance of arthropods. As well as trap colour having a significant effect on overall pan trap abundance, this relationship was also significant for Diptera, Hymenoptera, Coleoptera and Hemiptera. These taxa are well known to be attracted to specific colours (Campbell and Hanula, 2007), therefore this result was expected. The lack of a significant effect of trap colour on the fifth most sampled taxon by pan traps, Collembola, suggests that springtails were not actively attracted to any particular colour trap. Instead, unlike the other groups which are attracted to the wavelengths of the colours used, springtails are likely to have simply fallen into the traps serendipitously from the canopy above.

The composition of orders and families sampled is fairly typical of a broadleaved deciduous woodland (Hillstrom and Lindroth 2008), although fewer Anthomyiidae (root-maggot flies) were sampled than expected. The predominance of Diptera in the sampling meant that trends in this order often match overall trends across all sampling methods. The most abundant families are predominantly detritivores associated with leaf-litter (Chironomidae, Phoridae, Mycetophilidae, Leoididae, Collembola, Acari) and their predators (Staphylinidae, Carabidae, Empididae, Acari). Herbivores (Aphididae, Geometridae, Cicadellidae,

Cecdomyiidae) and their parasitoids (Sciomyzidae, Platygastridae, Ichneumonidae, Braconidae) comprise the majority of the rest of the most abundant groups. The presence of Diptera in each of these guilds further highlights the importance of this order in ecosystem functioning.

Many of the arthropods sampled are highly mobile, with winged adults capable of flying considerable distances in search of resources (e.g. pollinators searching for flowers) or during dispersal (e.g. aphid host switching). The limited spatial extent of the study means that for certain groups it may be difficult to attribute observed responses as a treatment effect as individuals may move between experimental arrays. The different sample profiles generated by each method mean that sampling techniques vary in their susceptibility to this issue. For example, arthropods sampled by pitfall trapping are typically wingless or disinclined to fly often or very far (e.g. Carabidae, Phoridae, Collembola and Acari) and therefore tend to be far less mobile than those sampled by flight interception traps such as Malaise traps (larger Diptera and Hymenoptera). Similarly, the majority of arthropods sampled from the canopy and understory were not strong flyers (e.g. Araneae and Miridae) and are unlikely to move between trees often. Methods such as pitfall trapping and beating may, therefore, be better able to detect the impact of eCO_2 due to the less mobile nature of the species most effectively sampled by these methods.

Arthropod orders are highly diverse in both species and their life histories. Samples were mostly identified to order due to practical and logistical constraints, however, it is possible that trends in response to eCO_2 were present at finer taxonomic resolution. Despite the functional diversity of some orders sampled (e.g. Diptera), several other orders, or at least the

main families sampled, are not as functionally diverse. For example, Collembola (omnivourous grazers), Hemiptera (mostly phloem feeding aphids) and Hymenoptera (mostly parasitoids) can be grouped into single functional groups. One potential solution to overcome taxonomic uncertainty with bulk sampling may be the use of techniques such as metabarcoding. Ross *et al*. (2020) use this technique to assess the impact of eCO_2 on the assemblage of orbatid mites in the soil. This is an example of how metabarcoding can be an effective tool to rapidly assess diversity, however it is less effective at determining abundance or biomass, for example due to primer bias (Elbrecht & Leese, 2015). Furthermore, without complete DNA reference libraries, traditional morphological techniques are still required to move beyond diversity analysis on observable taxonomic units.

The lack of a significant effect of eCO_2 on the abundance of arthropods sampled suggests that major shifts in community assemblies were not present after just under 3 years of fumigation. This does not necessarily mean that arthropods are not responding to the step-change in atmospheric conditions. There are two main potential pathways by which profound changes could still be operating but remain undetected. The first is that responses to eCO_2 are occurring at a finer taxonomic scale than order. Family, genus or even species-specific responses may become masked or lost in the noise of coarser level analysis. The relatively small number of families sampled from most orders, however, suggests that should this be the case, any impacts would need to be small in order for the net change to remain indistinguishable from zero at the scale of the analysis performed. The other mechanism by which changes have thus far remained undetected is temporal. Given the maturity of the system and the speed at which the dominant tree, oak, responds to environmental conditions, it may be possible that more time is required for small changes in arthropod

assemblage accumulate sufficiently enough to be detected. Small impacts on individual arthropod fitness, for example by changes in host plant nutritional value, may take several years to develop into population level responses detectable via broad measurements of arthropod abundance.

6.06 Conclusions

There were no short-term significant changes in arthropod abundance when comparing eCO_2 with control or ghost arrays. Clear seasonal patterns were found in the abundance of arthropod orders, and the dominant families from each order were identified. Sampling method had a strong influence on the sample profile, with the abundance of different orders varying across sampling methods. Variation in abundance between years was also greater than variation caused by eCO_2, highlighting the importance of environmental parameters in governing arthropod abundance. It may be possible to detect the impacts of eCO_2 on arthropod community assemblages either through long-term monitoring and/or analysis at finer scale taxonomic resolution.

CHAPTER 7

General Discussion.

7.01 Introduction

In the face of dramatic changes in global climate and unprecedented biodiversity loss, it is becoming increasingly important that we gain an understanding of the drivers of ecosystem change. As with any global trend, the contributing factors are vast, numerous and nuanced, therefore, in order to attempt to mitigate the negative consequences of these global changes, we need much more than just a detailed understanding of the impacts of each factor, and instead an understanding of how factors interact with each other. Current research has focussed disproportionately on aspects such as temperature and precipitation at the expense of others, such as increased concentrations of gaseous pollutants (e.g. CO_2 and NOx).

Increasing global concentrations of atmospheric CO_2 represent a potentially significant driver of change in terrestrial ecosystems, yet the extent of this remains unclear. Large scale, open, *in situ* experiments are vital tools to address current knowledge gaps in relation to how eCO_2 will impact ecosystem processes. FACE experiments are an example of such an experimental tool, which have been successfully deployed in a range of different ecosystems, highlighting the differences in ecosystem response, depending on factors such as species composition, nutrient availability and environmental parameters (Ainsworth and Long, 2005). Until the establishment of the BIFoR FACE facility, a FACE experiment had never been undertaken in a mature, temperate forest. This facility, therefore, provides a unique opportunity to address this knowledge gap and measure the response of mature woodland ecosystems to

atmospheric CO_2 conditions which we are almost certain to experience in the next 50 years, i.e. 550 ppm (IPCC, 2000).

The response of arthropods to eCO_2, in particular via their interaction with plants, is likely to have a significant bearing on ecosystem function in the future. Characterising the diversity of arthropods within this ecosystem, and understanding their responses to eCO_2 represented the core focus of this thesis, and was addressed by tested the following hypotheses:

1) A characterisation of the arthropod community within a mature woodland ecosystem and an assessment of sampling method efficacy.

2) eCO_2 leads to a decrease in the abundance and increase in compensatory feeding of leaf miners.

3) eCO_2 leads to an increase in aphid abundance, growth rate and fecundity.

4) Bluebell flowering phenology is delayed under eCO_2, with an associated decrease in flower visitation and seeds production.

5) eCO_2 leads to an increase in abundance of certain orders and a decreased abundance of alternative orders over the course of 3 years of fumigation.

7.02 Sampling arthropods at BIFoR FACE

Accurate, comprehensive and representative biodiversity monitoring is fundamental to understanding how climate change is currently, and will in the future, impact upon ecosystem functioning. In order to begin cataloguing the responses of arthropod communities to eCO_2, it is necessary to monitor both spatial and temporal patterns of abundance, diversity and phenology, as well as any environmental factors that might influence these patterns. Chapter

2 provides details of the core sampling programme which was established at BIFoR FACE to monitor arthropod populations, and which will continue over the 10-year timespan of the experiment. It is important to remember that, in isolation, each sampling method is an imperfect reflection of the true community present due to bias in the likelihood of different taxa to be sampled in that way. Collectively, however, these methods appear to provide a comprehensive representation of biodiversity one might expect to find in a mature oak woodland (Southwood *et al*., 2005).

In total, 68,399 individual invertebrates were sampled or recorded across all experiments at BIFoR FACE between February 2017 and July 2019 (Figure 7.1). This comprised 32 different orders (Coleoptera, Dermaptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Mecoptera, Megaloptera, Neuroptera, Odonata, Orthoptera, Pscoptera, Siphonaptera, Thysanoptera, Trichoptera, Entomobryomorpha, Poduromorpha, Symphypleona, Araneae, Opiliones, Pseudoscorpiones, Ixodida, Sarcoptiformes, Trombidiformes, Geophilomorpha, Lithobiomorpha, Glomerida, Julida, Polydesmida, Isopoda, Gastropoda, and Haplotaxida).

The majority of these invertebrates were sampled from the continuous core sampling programme outlined in Chapter 2, and as such were identified to order and placed into long-term storage in the BIFoR archive. The predominance of Diptera across the majority of sampling/survey methods reinforces the ecological importance of this order within this ecosystem. Flies play an important role in nutrient cycling, decomposition, pollination and food webs, yet are often neglected in research in favour of more charismatic groups (e.g.Bazzanti *et al*., 2008; Orford *et al*., 2015).

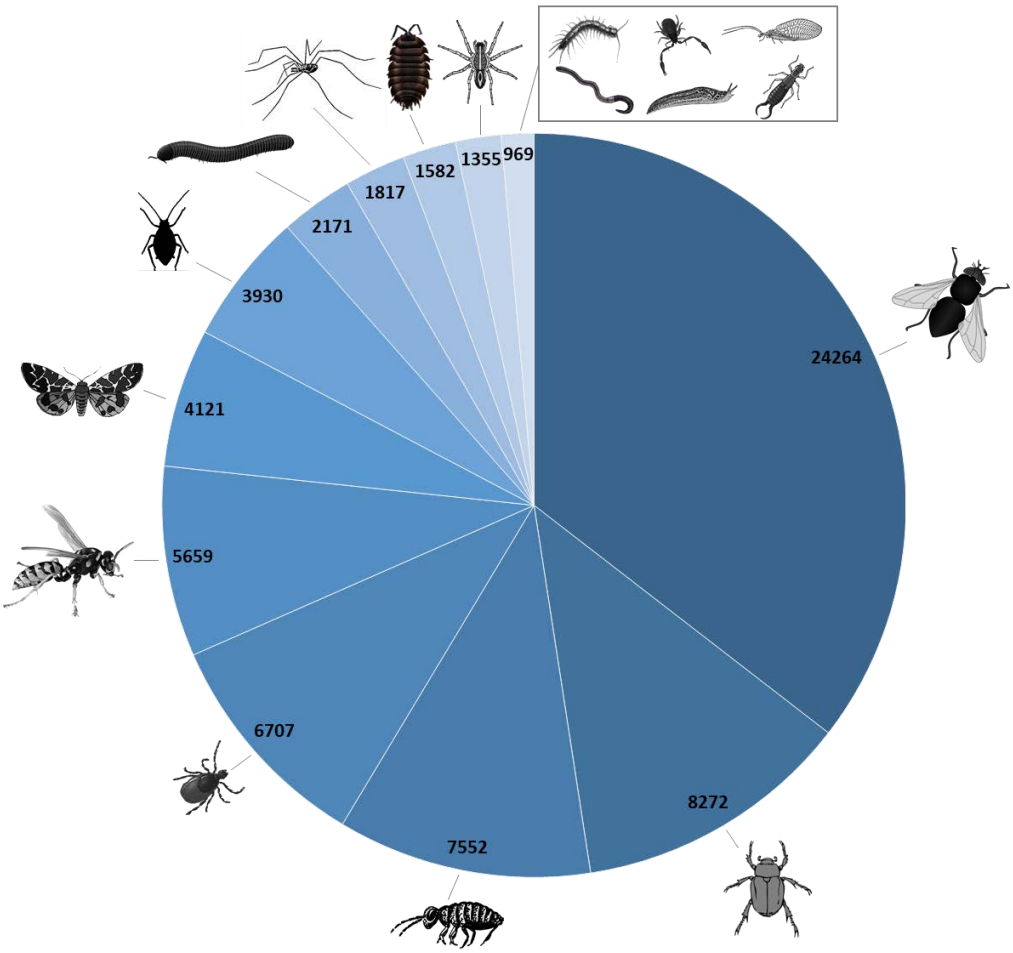

Figure 7.1 – The total number of invertebrates sampled, counted or otherwise recorded across all experiments, by order. The final portion represents all other invertebrate orders cumulatively.

Over the course of the various experiments outlined in this thesis, 375 invertebrate species were identified (Appendix I). The bulk of these species level identifications were of Lepidoptera (173). This is undoubtedly due to the relative ease of which adult Lepidoptera can be identified based on wing patterning, and the regular operation of a Skinner light trap (approximately quarterly). Species level identification was not a major component of most of the experiments, with either a small number of abundant, readily identifiable species used as models (e.g. the sycamore aphid, *Drepanosiphum platanoidis*, in Chapter 4) or mass sampling with identification to a broader taxonomic level (e.g. identification to order/family in the core sampling programme in Chapters 2 and 6). Identification was limited to order/family level

within the large-scale sampling programme due to significant practical and logistical undertaking that would be required to identify tens of thousands of individual invertebrates to finer taxonomic resolution. A comparable level of order or family scale identification was performed by similar forest FACE experiments (e.g. Altermatt, 2003; Hamilton *et al*., 2012; Facey *et al*., 2016). An alternative approach is to identify individuals into functional groups (e.g. Sanders *et al*., 2004; Facey *et al*., 2016), which can allow for easier interpretation of guild responses, although a degree of detail is lost by grouping all individuals into one of just 5-7 groupings. Emerging, molecular methods, such as DNA metabarcoding and eDNA, present exciting new possibilities for large scale, accurate monitoring, however, currently traditional techniques are still required alongside. For example, barcode reference libraries remain incomplete for large numbers of species, particularly more obscure groups, whilst limitations arising from variations in biology, such as mitochondrial introgression, are becoming apparent (e.g. Cong *et al*., 2017). It is likely that eventually modern technologies will overcome these limitations, but until such a time an integrated approach remains optimum.

The number of species level determinations made during the course of the experiments within this thesis barely scratches the surface of the true invertebrate species richness at the site. Yet, even this modest species list vastly eclipses that of vertebrates (57 species), or indeed any other group, recorded from the site, highlighting the relative dominance of invertebrates in underpinning the species richness of terrestrial ecosystems. Despite this dominance, invertebrates, including arthropods, still receive less research focus and conservation effort, representing a taxonomic bias towards larger, more charismatic vertebrate fauna (Troudet *et al*., 2017).

Regular, intensive sampling of a site using a range of techniques also allows the potential discovery of species which may otherwise be missed with less rigorous, short-term surveying. This proved to be true with the sampling at BIFoR, which produced the discovery of the rarely recorded wasp, *Embolemus ruddii* (Appendix II). A single female of this small, apterous, aculeate wasp was caught in a pitfall trap in November 2017. It is the only species from the family Embolemidae in the UK (Edwards, 1997), and is very rarely seen, with only 20 previous UK records, the last of which from Kent in 1997. The paucity of observations of this species means very little is known about its biology, but it is believed to parasitise tree-root feeding Cixiidae (Varrone and Olmi, 2010).

7.03 Herbivory under eCO_2

Herbivory is a key ecological process which influences nutrient flow, energy flow and population dynamics of species within terrestrial ecosystems such as temperate forests. Furthermore, it is a direct interaction between plants as hosts and herbivores as consumers and can influence the surface area of plant biomass available for photosynthesis, thus potentially impacting whether a plant, and wider ecosystem, acts as a net carbon source or sink. Herbivory is, therefore, expected to be particularly sensitive to eCO_2 due to the direct impacts it confers on plant growth, physiology and biochemistry (Pringle, 2016). Chapters 3 and 4 investigated the responses of insect herbivores belonging to two different feeding guilds, leaf-miners and phloem feeders respectively. Leaf-mining Lepidoptera were chosen due to their sessile nature and close association with the host plant; and aphids due to their abundance, short generation time and global significance in food security.

It was expected that leaf-miners would exhibit a compensatory feeding response to eCO_2, as has been found in other CO_2 enrichment experiments (e.g. Stiling and Cornelissen, 2007). This response would mitigate the decrease in nutritional value of the host plant due to the increased C:N of leaf tissue. Somewhat surprisingly, the reverse was found to be the case, with a consistent decrease in mine area, and therefore feeding extent, found on oak and no significant change in amount of feeding on hazel. One possible cause of this disparity in the responses of leaf miners on oak and hazel is that the tree species differ in the extent of their physiological response to eCO_2. oak has a greater background level of defensive compound accumulation in its leaves, suggesting a higher level of nutrient allocation to defence, which is indicative of a strong antagonistic interaction between the tree and its herbivores. Many plant species have been shown to increase carbon allocation to defence under eCO_2 (Jablonski *et al.*, 2002). Against this background, the oak trees may have increased production of defensive compounds such as phenolics under eCO_2 (Dury *et al.*, 1998) which may have tipped the balance of the interaction against the leaf mining herbivores. Meanwhile, it is unclear whether hazel exhibits similar increases in defensive compounds under eCO_2, or whether C allocation is increased elsewhere (e.g. root growth).

In contrast, aphids are among the few herbivore groups which are expected to do well under eCO_2 (Sun *et al.,* 2016). As phloem feeders they are already specialised to deal with low nitrogen content of the tissue they feed on through possession of intracellular symbionts that provide amino acids (Sandström and Moran, 1999). They are, therefore, potentially already equipped to deal with further relative decrease in N concentration in host plant tissue, as is the case under eCO_2. Aphids may also be able to easily increase feeding rate in response to decreased relative Nitrogen content of host plant tissue without increasing exposure to

defensive compounds. This is because secondary metabolites do not accumulate in phloem in the same way as leaves, so aphids may not be impacted by this in the same way that leaf-chewers might.

The results presented in Chapter 4 aligned with this hypothesis. Aphids were more abundant under eCO_2, with greater densities feeding on sycamore leaves. The results of the clip-cage experiment isolating individuals showed no difference in nymphs produced or growth rate, suggesting that positive population level responses were not driven by individual scale increases in fecundity or growth rate, which is also consistent with previous findings (Awmack *et al*., 2004). The exact mechanism driving the population level increase in aphid abundance remains unclear but is likely either bottom-up release from competition or top-down decrease in regulation by parasites/predators.

In order to further explore the impact of eCO_2 on insect herbivory, it may be useful to test the hypothesis generated by these experiments that responses are driven by increases, or relative lack of, in defensive compounds in plant tissue of different species. This could be addressed by biochemical analysis of tissue to measure concentrations of secondary metabolites under eCO_2 across different seasonal timepoints. The C:N ratio could also be simultaneously analysed to test its relationship with herbivore abundance, feeding rate and growth rate. It would also be beneficial to better quantify the impact of top-down regulation by natural enemies, and how the interaction between herbivore and predator/parasitoid is modified under eCO_2. In order to achieve this, it may be necessary to identify key model tritrophic interactions which can be monitored in the field, such as oak-aphid-parasitoid.

7.04 Pollination under eCO_2

Chapter 5 considered another important ecosystem process: pollination. This topic has garnered increased interest over recent years, with several studies exploring the impacts of climate change on pollinator diversity as well as pollination ecology (e.g. Hegland *et al*., 2009; Phillips *et al*., 2018). These studies tend to focus on factors such as mean air temperature or precipitation patterns, but very few examined the potential effects of eCO_2. Whilst there is limited evidence within the literature that eCO_2 impacts flowering time (Springer and Ward, 2007) and floral resources (Ziska *et al*., 2016), the impacts on plant-pollinator interactions had not previously been systematically reviewed. The systematic literature mapping undertaken, therefore, was the first detailed review of pollination under eCO_2 (Appendix III). It clearly highlighted the lack of quantified evidence of the impact of eCO_2 on pollination interactions directly and indicated the need for field experiments investigating the effect of eCO_2 on pollination interactions. FACE experiments are a useful tool to achieve this *in situ* at the ecosystem scale. The bluebell experiment was subsequently designed to address this knowledge gap. The review and primary research experiment are presented together in a hybrid study, in similar style to previously published research articles (e.g. Stiling and Cornelissen, 2007).

Bluebells are an ideal model pollination system as they are locally abundant, provide an important floral resource, have a long, discrete flowering period and seed set is determined by insect pollination (Corbet, 1998; Corbet, 1999). This was further confirmed by the close link between extent of flower visitation and seed set revealed in this study. The advance in the timing of first flowering by 6 days under eCO_2 is consistent with the mean change in flowering period found by studies on other plant species in the systematic review.

Furthermore, this change in flowering phenology could not be explained by variation in soil temperature, soil moisture or light intensity (none of which differed significantly across arrays), and so is likely to be a true eCO_2 treatment effect. Syrphid flies and bumblebees were the dominant flower visitors, corroborating existing studies (Corbet and Tiley, 1999), and highlighting their importance in bluebell pollination networks. Given that both of these taxa are both widespread and seasonally abundant, it suggests that they may also play an important role in pollination networks of other spring-flowering angiosperms.

The lack of a significant effect of eCO_2 on flower visitation or seed set found in my study does not necessarily indicate that it will not impact pollination systems in the future. The effects of advances in flowering may accumulate over multiple years, gradually disrupting pollination networks in tandem with other stressors such as increasing temperature. Furthermore, the highly mobile nature of flower visiting insects means that any influence of eCO_2 on visitation was dampened over the spatial scale of the experiment. Earlier flowering is associated with a lower number of pollinating species (Petanidou *et al*., 2014), therefore the trend of advancing flowering time under future CO_2 increases could eventually cause widespread disruptive shifts in pollination network dynamics. The low level of both flower visitation and seed set early in the flowering period suggests that advances in flowering may lead to an increase in the number of receptive flowers outside of peak pollinator flight period, resulting in a decline in the effective pollination delivered. Furthermore, phenological shifts in pollinator networks may reduce floral resource availability and decrease the diet breadth of pollinators, which modelling has indicated could affect 17–50% of all pollinator species (Memmott, *et al*., 2007), potentially leading to species loss. A loss of synchrony in plant-pollinator interactions under future scenarios may, therefore, have long-term impacts on abundance and diversity of both

plants and the insects which visit their flowers. Whilst the relatively long flowering period of bluebells makes it a good model flower species for this study, many species have much shorter flowering periods, therefore may be even more sensitive to such changes if flowering times are similarly affected by eCO_2. Similarly, pollinators differ in the length of their flight period, meaning those with shorter active periods may be more susceptible to changing phenology of the flowers they interact with. This may be further compounded by extreme weather events, which typically adversely affect pollinators, particularly those with short or early flight periods (Nicholson and Egan, 2020). Previous studies have suggested that changes in phenology driven by temperature alone may be equally tracked by both plants and their pollinators (Bartomeus *et al*., 2011). The results in this chapter, however, suggest that the impacts of eCO_2 should also be factored in, which potentially has profound implications for the modelling of pollination under future climate scenarios.

It remains unclear whether these responses will increase in magnitude cumulatively across multiple years or remain proportionally constant in relation to the increase in eCO_2. For example, will flowering be advanced further under the same regime of CO_2 fumigation the following spring or remain at a mean of 6 days earlier than under ambient conditions? This uncertainty can be addressed by repeating the experiment over multiple subsequent years and comparing the results. Environmental conditions, such as temperature, would need to be carefully measured and factored into this analysis due to the high degree of annual variation.

7.05 Impact of eCO_2 on arthropod abundance, diversity and phenology

Whilst Chapter 2 focussed on arthropod abundance, diversity and phenology across the first 12 months of the experiment in greater detail, a longer-term look at the abundance of orders

was undertaken in Chapter 6. In general, no significant differences in abundance, diversity or phenology were detected under eCO_2 across arthropods as a whole, or for any specific order, at least at the spatial, temporal and taxonomic levels tested.

The lack of a clear signal indicating order level responses to eCO_2 over the first 12 months was unsurprising. Given that any impact of eCO_2 on arthropod populations are expected to be plant mediated, it would take time for these responses to feed through the system, particularly for slow growing species such as oak. Furthermore, the dominant plant species in the ecosystem are deciduous, potentially further delaying any impacts due to a large reduction in photosynthetic activity, and thus the impact of eCO_2 on plants, for the months between leaf fall and bud burst. Over multiple years, the variation in abundance of arthropods between years was greater than the variation between eCO_2 and control arrays. This kind of annual variation was expected before sampling occurred and is typical in complex ecosystems such as this, yet represents 'noise' in the data which may make it difficult to discern potentially more subtle responses to eCO_2. This variation across years also highlights the impact and importance of extreme climate events, which are predicted to increase in frequency under most climate change models (Planton *et al*., 2008).

There were several order level increases in abundance over the course of the sampling which are interesting to consider in relation to environmental conditions but do not seem to be influenced by eCO_2. For example, there was an increase in winter moth sampled during December 2017, which preceded the mass defoliation event during May/June 2018. It is possible that the increase in nutrient flow into the leaf litter/soil caused by this event drove the increase in Collembola recorded during the following months. The original increase in

winter moth abundance may have been part of the cyclical population dynamics of this species (Ims *et al*., 2004), although temperature during key stages of the life cycle is known to affect populations (Visser *et al*., 2001). This highlights the potential role of climatic variables on arthropod abundance. Chapter 2 began to explore this over the first 12 months of the experiment, corroborating the role of temperature in influencing arthropod abundance. Analysis of longer-term datasets of arthropod abundance and meteorological variables at FACE sites could help to further develop our understanding of the relationship between climatic variables and arthropod populations under future climate scenarios. Given the complexities of these relationships, it may be useful to identify a single key, model species, such as the winter moth, to focus on in this regard.

The lack of an observed response in arthropod abundance to eCO_2 over a 3-year period is by no means an indication that there weren't population level impacts on this group (or that they will not occur in the longer term). There are three potential ways in which the arthropod community responses to eCO_2 may remain undetected by the present studies (temporal, spatial and taxonomic). The first mechanism is temporal, whereby eCO_2 is driving change in the system but these responses are sufficiently small or slow to remain undetected by measuring arthropod abundance over relatively short timescales. If changes in ecosystem function are operating at longer timescales, then it would require further long-term monitoring in order to detect changes in arthropod abundance as populations respond. The second potential cause of the response of arthropods to eCO_2 being masked is spatial. The scale over which the experiment operates, and the highly mobile nature of many of the invertebrates being studied, makes it impossible to exclude the possibility that many of the species sampled are utilising both control and eCO_2 arrays. This is more of an issue for certain

taxa, such as flying insects. The abundance of some groups may change under eCO_2, but movement between sampling points may dampen the ability to measure it. As such, measuring the impact of eCO_2 on highly sessile taxa, such as leaf miners, or isolated individuals, such as within clip cages, may be more informative. The third possible mechanism is that responses to eCO_2 may have been species specific, as has been found to be the case in other systems (Hillstrom *et al*., 2014). Such responses may remain undetected at order/family level, although they would need to occur in a relatively balanced and opposing manner between different species of the same order in order to cancel out. It may be possible to detect potentially 'hidden' species level responses by identifying samples to a more precise taxonomic level. It would take considerable effort to identify huge numbers of samples to species level, especially given the abundance of groups for which microscopic examination of the genitalia is the only way to determine species morphologically. This issue may be sidestepped by identifying samples parataxonomically to 'morphospecies', however this approach is prone to error (Krell, 2004). Alternatively, identifying key model species/networks to investigate in greater detail may be a useful method. In such scenarios, species should be easy to identify, abundant and representative of their functional group.

7.06 General conclusions

The findings in this thesis represent an initial but important contribution to our understanding of the impact of eCO_2 on arthropods in a mature temperate woodland and some of the key ecological processes they underpin. The responses represent subtle, small scale changes, but over ecological scales and/or longer timescales the impact of each response could multiply up to drive major shifts in ecosystem functioning and community reorganisation. Furthermore, in

complex ecosystems many species have multiple interactions with other species, which can lead to amplification of minor alterations in function along complex pathways and cascades. In this instance, eCO_2 represents just one aspect of climate change, with factors such as increasing temperature, disrupted precipitation and increase frequency of extreme events potentially also interacting with eCO_2. Whilst there is some evidence that increasing temperature tends to counteract the effects of eCO_2 (Zvereva and Kozlov, 2006), the extent of this is unclear and testing of the impacts of other factors, such as drought or elevated concentrations of Nitric Oxide, remains limited.

The core sampling programme has provided a characterisation of the arthropod community at the BIFoR FACE site and highlighted the greater level of biodiversity within this mature system than other previous forest FACE experiments. Furthermore, the composition of the invertebrate fauna in the temperate woodland is markedly different to that of previous studies (e.g. Facey *et al*., 2016) For example, within the arthropods some groups were far more abundant (Sciomyzidae, Chironomidae, Syrphidae), whereas others were surprisingly scarce (Formicidae). The response of herbivores to eCO_2 was found to be trophic group or even species specific, corroborating the findings of previous studies and confirming that herbivory is impacted by eCO_2 similarly in a mature, complex system. The limited previous empirical testing of the impact of eCO_2 on pollination directly meant that the bluebell experiment was highly novel, and the findings have implications for how this subject should be explored in the future. The small-scale impacts of eCO_2 on these key ecosystem processes may be the beginnings of profound ecosystem change. Whilst these changes are not yet significant enough to manifest as broad changes in arthropod communities, long-term monitoring should continue, preferably with fine scale analysis to detect changes as they

occur. If the composition or structure of arthropod communities were to change, particularly that of insects driving key processes such as herbivores and pollinators, this would have significant implications for ecosystem composition and function, potentially resulting in feedback that alters process such as nutrient cycling and carbon sequestration. In this way the impact of eCO_2 on insects may result in them becoming key drivers of change in woodland systems under climate change.

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APPENDIX I
BIFoR FACE site invertebrate species list

Order	Family	Species	Common name	Date first recorded
Araneae	Agelenidae	*Tegenaria sp.*	A house spider	
Araneae	Araneidae	*Araneus diadematus*	Garden spider	
Araneae	Lycosidae	*Pardosa sp.*	A wolf spider	
Araneae	Philodromidae	*Philodromus dispar*	A running crab spider	15/05/2019
Araneae	Pholcidae	*Pholcus phalangioides*	Daddy long-legs spider	
Araneae	Tetragnathidae	*Metellina mengei*	A long-jawed orb weaver	07/05/2020
Araneae	Tetragnathidae	*Tetragnatha sp.*	A long-jawed orbweb spider	
Araneae	Theridiidae	*Paidiscura pallens*	A comb-footed spider	
Araneae	Thomisidae	*Diaea dorsata*	A crab spider	07/05/2019
Coleoptera	Attelabidae	*Apoderus coryli*	Hazel leaf-roller	12/07/2019
Coleoptera	Buprestidae	*Agrilus angustulus*	A jewel beetle	27/06/2017
Coleoptera	Buprestidae	*Agrilus biguttatus*	Oak jewel beetle	
Coleoptera	Cantharidae	*Cantharis decipiens*	A soldier beetle	30/04/2019
Coleoptera	Cantharidae	*Cantharis nigricans*	A soldier beetle	22/05/2018
Coleoptera	Cantharidae	*Cantharis sp.*	A soldier beetle	23/05/2017
Coleoptera	Cantharidae	*Malthinus sp.*	A soldier beetle	03/07/2019
Coleoptera	Cantharidae	*Rhagonycha fulva*	Common Red Soldier Beetle	13/07/2017
Coleoptera	Carabidae	*Abax parallelepipedus*	A ground beetle	21/02/2017
Coleoptera	Carabidae	*Carabus problematicus*	A ground beetle	27/06/2017
Coleoptera	Carabidae	*Cychrus caraboides*	Snail Hunter	24/05/2017
Coleoptera	Carabidae	*Nebria brevicollis*	A ground beetle	20/10/2016
Coleoptera	Carabidae	*Notiophilus biguttatus*	A ground beetle	20/12/2017
Coleoptera	Carabidae	*Poecilus cupreus*	A ground beetle	30/08/2017
Coleoptera	Carabidae	*Pterostichus madidus*	Black Clock	18/04/2018
Coleoptera	Carabidae	*Pterostichus melanarius*	A ground beetle	
Coleoptera	Cerambycidae	*Alosterna tabacicolor*	A longhorn beetle	04/06/2018
Coleoptera	Cerambycidae	*Anaglyptus mysticus*	A longhorn beetle	27/07/2018
Coleoptera	Cerambycidae	*Grammoptera ruficornis*	A longhorn beetle	04/06/2018
Coleoptera	Cerambycidae	*Leiopus nebulosus agg.*	Black-clouded longhorn beetle	30/05/2019
Coleoptera	Cerambycidae	*Phymatodes testaceus*	Tanbark borer	18/06/2019
Coleoptera	Cerambycidae	*Rhagium mordax*	Black-spotted longhorn	18/06/2019
Coleoptera	Cerambycidae	*Stenocorus meridianus*	Variable longhorn	18/06/2019
Coleoptera	Chrysomelidae	*Bruchus rufimanus*	Broad-bean weevil	29/03/2019
Coleoptera	Chrysomelidae	*Chrysolina sp.*	A leaf beetle	17/05/2017
Coleoptera	Chrysomelidae	*Gastrophysa viridula*	Green Dock Beetle	23/05/2017
Coleoptera	Coccinellidae	*Anatis ocellata*	Eyed Ladybird	24/05/2017
Coleoptera	Coccinellidae	*Calvia quattuordecimguttata*	Cream-spot ladybird	01/05/2019
Coleoptera	Coccinellidae	*Coccinella septempunctata*	7-spot Ladybird	05/04/2017

Coleoptera	Coccinellidae	*Halyzia sedecimguttata*	Orange Ladybird	28/03/2017
Coleoptera	Coccinellidae	*Harmonia axyridis*	Harlequin Ladybird	13/07/2017
Coleoptera	Coccinellidae	*Propylea quattuordecimpunctata*	14-spot Ladybird	30/04/2019
Coleoptera	Coccinellidae	*Tytthaspis sedecimpunctata*	16-spot Ladybird	11/01/2019
Coleoptera	Curculionidae	*Cionus tuberculosus*	A weevil	18/06/2019
Coleoptera	Curculionidae	*Curculio nucum*	Nut Weevil	24/05/2017
Coleoptera	Curculionidae	*Phyllobius argentatus*	Green Nettle Weevil	20/04/2017
Coleoptera	Curculionidae	*Strophosoma melanogrammum*	A weevil	26/09/2017
Coleoptera	Elateridae	*Agriotes sp.*	A click beetle	11/05/2018
Coleoptera	Elateridae	*Athous sp.*	A click beetle	11/05/2018
Coleoptera	Helophoridae	*Helophorus sp.*	A water-scavenging beetle	29/03/2019
Coleoptera	Histeridae	*Margarinotus striola*	A hister beetle	
Coleoptera	Hydrophilidae	*Sphaeridium scarabaeoides*	A water-scavenging beetle	18/04/2019
Coleoptera	Lucanidae	*Dorcus parallelipipedus*	Lesser stag beetle	
Coleoptera	Lucanidae	*Sinodendron cylindricum*	Rhinoceros Beetle	16/06/2018
Coleoptera	Lymexylidae	*Lymexylon navale*	Ship timber beetle	28/03/2018
Coleoptera	Malachiidae	*Malachius bipustulatus*	Malachite Beetle	24/05/2017
Coleoptera	Pyrochroidae	*Pyrochroa coccinea*	Black-headed Cardinal Beetle	23/05/2018
Coleoptera	Pyrochroidae	*Pyrochroa serraticornis*	Common Cardinal Beetle	22/05/2018
Coleoptera	Scarabaeidae	*Aphodius rufipes*	A dung beetle	28/09/2018
Coleoptera	Scarabaeidae	*Melolontha melolontha*	Common Cockchafer	06/06/2018
Coleoptera	Silphidae	*Dendroxena quadrimaculata*	4-spotted carrion beetle	17/05/2019
Coleoptera	Silphidae	*Nicrophorus humator*	Black Sexton Beetle	11/04/2017
Coleoptera	Silphidae	*Nicrophorus investigator*	A carrion beetle	27/06/2017
Coleoptera	Silphidae	*Nicrophorus vespilloides*	A carrion beetle	11/04/2017
Coleoptera	Silphidae	*Oiceoptoma thoracicum*	Red-Breasted Carrion Beetle	13/07/2017
Coleoptera	Silphidae	*Silpha atrata*	Black Snail Beetle	11/04/2017
Coleoptera	Staphylinidae	*Lathrobium sp.*	A rove beetle	21/09/2017
Coleoptera	Staphylinidae	*Ocypus olens*	Devil's Coach-horse	25/10/2017
Dermaptera	Forficulidae	*Forficula auricularia*	Common Earwig	11/04/2017
Diptera	Agromyzidae	*Phytomyza ilicis*	Holly Leaf Gall Fly	18/04/2018
Diptera	Agromyzidae	*Phytomyza lappae*	A leaf-mining fly	20/07/2018
Diptera	Asilidae	*Dioctria linearis*	Small Yellow-legged Robber Fly	26/06/2019
Diptera	Bibionidae	*Bibio marci*	St Marks Fly	11/04/2017
Diptera	Bombyliidae	*Bombylius major*	Bee Fly	29/03/2019
Diptera	Calliphoridae	*Lucilia sp.*	A greenbottle fly	22/05/2018
Diptera	Dolichopodidae	*Dolichopus wablbergi*	A long-legged fly	
Diptera	Empidae	*Empis livida*	A dagger fly	21/05/2019
Diptera	Empidae	*Empis tessellata*	A dagger fly	24/05/2017
Diptera	Rhagionidae	*Rhagio lineola*	Common snipefly	13/07/2017
Diptera	Rhagionidae	*Rhagio scolopaceus*	Downlooker Snipefly	08/06/2018

Diptera	Scathophagidae	*Scathophaga stercoraria*	Yellow dung fly	13/07/2017
Diptera	Stratiomyidae	*Sargus iridatus*	Iridescent centurion	26/07/2017
Diptera	Syrphidae	*Baccha elongata*	Gossamer hoverfly	08/06/2017
Diptera	Syrphidae	*Cheilosia illustrata*	Bumblebee Cheilosia	13/07/2017
Diptera	Syrphidae	*Cheilosia variabilis*	Figwort cheilosia	07/05/2019
Diptera	Syrphidae	*Chrysogaster solstitialis*	Dark-winged Chrysogaster	04/06/2018
Diptera	Syrphidae	*Dasysyrphus venustus sensu lato*	Broad-barred dasysyrphus	24/05/2017
Diptera	Syrphidae	*Episyrphus balteatus*	Marmalade Hoverfly	20/03/2017
Diptera	Syrphidae	*Eristalis pertinax*	Tapered Dronefly	10/05/2018
Diptera	Syrphidae	*Eristalis tenax*	Common dronefly	10/05/2018
Diptera	Syrphidae	*Eupeodes corollae*	Migrant Hoverfly	21/05/2019
Diptera	Syrphidae	*Helophilus hybridus*	Marsh tiger hoverfly	10/05/2018
Diptera	Syrphidae	*Helophilus pendulus*	Tiger hoverfly	10/05/2018
Diptera	Syrphidae	*Leucozona lucorum*	Blotch-winged hoverfly	03/05/2017
Diptera	Syrphidae	*Melanostoma scalare*	Slender Melanostoma	
Diptera	Syrphidae	*Merodon equestris*	Greater Bulb-Fly	21/06/2017
Diptera	Syrphidae	*Myathropa florea*	Batman hoverfly	21/06/2017
Diptera	Syrphidae	*Platycheirus albimanus*	A hoverfly	03/05/2018
Diptera	Syrphidae	*Platycheirus sp.*	A hoverfly	
Diptera	Syrphidae	*Rhingia campestris*	Common snout hoverfly	03/05/2017
Diptera	Syrphidae	*Rhingia rostrata*	Grey-backed snout hoverfly	21/05/2019
Diptera	Syrphidae	*Sphaerophoria scripta*	Long hoverfly	15/08/2019
Diptera	Syrphidae	*Syrphus ribesii*	Humming syrphus	11/04/2017
Diptera	Syrphidae	*Volucella pellucens*	Large pied hoverfly	26/06/2019
Diptera	Syrphidae	*Xylota sylvarum*	Golden tailed hoverfly	13/07/2017
Diptera	Tabanidae	*Haematopota pluvialis*	Notch-horned Cleg	26/07/2017
Diptera	Tabanidae	*Hybomitra distinguenda*	Bright Horsefly	13/07/2017
Diptera	Tachinidae	*Tachina fera*	A tachinid	24/05/2017
Diptera	Tipulidae	*Ctenophora pectinicornis*	A cranefly	24/05/2017
Diptera	Tipulidae	*Tipula vittata*	A cranefly	26/04/2018
Diptera	Xylophagidae	*Xylophagus ater*	Common awl-fly	24/05/2017
Glomerida	Glomeridae	*Glomeris marginata*	Pill Millipede	20/03/2017
Hemiptera	Acanthosomatidae	*Acanthosoma haemorrhoidale*	Hawthorn Shieldbug	21/12/2017
Hemiptera	Acanthosomatidae	*Elasmucha grisea*	Parent bug	18/04/2019
Hemiptera	Anthocoridae	*Anthocoris nemorum*	Common Flower Bug	20/12/2017
Hemiptera	Aphididae	*Brachycaudus lychnidis*	Campion aphid	26/04/2019
Hemiptera	Aphididae	*Drepanosiphum platanoidis*	Common sycamore aphid	20/04/2017
Hemiptera	Aphididae	*Periphyllus acericola*	Sycamore periphyllus aphid	22/05/2018
Hemiptera	Aphididae	*Periphyllus testudinaceus*	Common periphyllus aphid	21/05/2019
Hemiptera	Aphididae	*Tuberculatus annulatus*	Common oak aphid	
Hemiptera	Aphrophoridae	*Philaenus spumarius*	Common spittlebug	18/05/2017
Hemiptera	Berytidae	*Metatropis rufescens*	A stiltbug	30/04/2019
Hemiptera	Cercopidae	*Cercopis vulnerata*	Red-and-black froghopper	18/06/2019

Hemiptera	Cicadellidae	*Empoasca vitis*	A leafhopper	29/03/2019
Hemiptera	Coreidae	*Coreus marginatus*	Dock Bug	27/09/2018
Hemiptera	Miridae	*Calocoris alpestris*	A mirid	23/05/2017
Hemiptera	Miridae	*Closterotomus norwegicus*	Potato capsid	
Hemiptera	Miridae	*Grypocoris stysi*	A mirid	13/07/2017
Hemiptera	Miridae	*Harpocera thoracica*	A mirid	23/05/2017
Hemiptera	Miridae	*Rhabdomiris striatellus*	A mirid	23/05/2017
Hemiptera	Pentatomidae	*Dolycoris baccarum*	Hairy Shieldbug	27/09/2018
Hemiptera	Pentatomidae	*Palomena prasina*	Green Shieldbug	27/09/2018
Hemiptera	Pentatomidae	*Pentatoma rufipes*	Forest Bug	31/08/2017
Hemiptera	Pentatomidae	*Troilus luridus*	Bronze Shieldbug	27/09/2018
Hemiptera	Pentatomidae	*Zicrona caerulea*	Blue Shieldbug	21/03/2018
Hymenoptera	Andrenidae	*Andrena bicolor*	Gwynne's Mining Bee	29/03/2019
Hymenoptera	Andrenidae	*Andrena chrysosceles*	Hawthorn mining bee	04/06/2018
Hymenoptera	Andrenidae	*Andrena cineraria*	Grey Mining Bee	21/06/2017
Hymenoptera	Andrenidae	*Andrena clarkella*	Clarke's Mining Bee	29/03/2019
Hymenoptera	Andrenidae	*Andrena flavipes*	Yellow Legged Mining Bee	24/05/2017
Hymenoptera	Andrenidae	*Andrena fulva*	Tawny Mining Bee	26/04/2018
Hymenoptera	Andrenidae	*Andrena haemorrhoa*	Early Mining Bee	24/05/2017
Hymenoptera	Andrenidae	*Andrena helvola*	Coppice Mining Bee	10/05/2018
Hymenoptera	Andrenidae	*Andrena nitida*	Grey-patched Mining Bee	11/05/2018
Hymenoptera	Andrenidae	*Andrena scotica*	Chocolate mining bee	17/04/2019
Hymenoptera	Andrenidae	*Andrena subopaca*	Impunctate Mini-miner	03/05/2018
Hymenoptera	Apidae	*Apis mellifera*	Honeybee	31/05/2019
Hymenoptera	Apidae	*Bombus hortorum*	Small Garden Bumblebee	17/05/2017
Hymenoptera	Apidae	*Bombus hypnorum*	Tree Bumblebee	18/04/2018
Hymenoptera	Apidae	*Bombus lapidarius*	Large Red-tailed Bumblebee	10/05/2018
Hymenoptera	Apidae	*Bombus lucorum/terrestris*	White/Buff-tailed Bumblebee workers	20/03/2017
Hymenoptera	Apidae	*Bombus pascuorum*	Common Carder Bee	21/04/2017
Hymenoptera	Apidae	*Bombus pratorum*	Early Bumblebee	20/04/2017
Hymenoptera	Apidae	*Bombus sylvestris*	Forest Cuckoo Bumblebee	10/05/2018
Hymenoptera	Apidae	*Bombus terrestris*	Buff-tailed Bumblebee	18/04/2018
Hymenoptera	Apidae	*Bombus vestalis*	Vestal Cuckoo Bee	10/05/2018
Hymenoptera	Apidae	*Nomada flava*	Flavous Nomad Bee	10/05/2018
Hymenoptera	Apidae	*Nomada leucophthalma*	Early Nomad Bee	29/03/2019
Hymenoptera	Apidae	*Nomada panzeri*	Panzer's nomad bee	30/05/2019
Hymenoptera	Chrysididae	*Chrysis ignita agg.*	A ruby-tailed wasp	30/05/2018
Hymenoptera	Crabonidae	*Crossocerus sp.*	A digger wasp	
Hymenoptera	Crabronidae	*Nysson spinosus*	Large sprurred digger wasp	04/06/2018
Hymenoptera	Crabronidae	*Pemphredon lugubris*	Mournful Wasp	21/06/2017
Hymenoptera	Cynipidae	*Andricus fecundator*	Artichoke Gall	07/03/2017
Hymenoptera	Cynipidae	*Andricus quercuscalicis*	Knopper Gall	03/11/2017
Hymenoptera	Cynipidae	*Neuroterus quercusbaccarum*	Common Spangle Gall	03/11/2017

Hymenoptera	Cynipidae	*Andricus kollari*	Oak marble gall wasp	19/03/2019
Hymenoptera	Cynipidae	*Biorhiza pallida*	Oak apple gall wasp	
Hymenoptera	Cynipidae	*Neuroterus numismalis*	Silk button gall wasp	
Hymenoptera	Embolemidae	*Embolemus ruddii*	An Embolemid wasp	28/11/2017
Hymenoptera	Formicidae	*Myrmica ruginodis*	An ant	29/05/2018
Hymenoptera	Ichneumonidae	*Diphyus quadripunctorius*	An ichneumon wasp	25/07/2017
Hymenoptera	Ichneumonidae	*Gelis sp.*	An ichneumon wasp	
Hymenoptera	Ichneumonidae	*Ophion costatus*	An ichneumon wasp	07/10/2020
Hymenoptera	Tenthredinidae	*Apethymus filiformis*	A sawfly	07/10/2020
Hymenoptera	Tenthredinidae	*Dolerus sp.*	A sawfly	26/03/2019
Hymenoptera	Tenthredinidae	*Nematus pavidus*	A sawfly	27/09/2018
Hymenoptera	Tenthredinidae	*Periclista lineolata*	A sawfly	10/05/2018
Hymenoptera	Tenthredinidae	*Tenthredo mesomela/mioceras*	A sawfly	21/05/2019
Hymenoptera	Tenthredinidae	*Tenthredo scrophulariae*	Figwort sawfly	04/06/2018
Hymenoptera	Torymidae	*Torymus varians*	Apple seed chalcid	
Hymenoptera	Vespidae	*Symmorphus gracilis*	A tube-nesting wasp	30/05/2018
Hymenoptera	Vespidae	*Vespa crabro*	Hornet	24/05/2017
Hymenoptera	Vespidae	*Vespula vulgaris*	Common Wasp	20/03/2017
Isopoda	Oniscidae	*Oniscus asellus*	Common Shiny Woodlouse	18/04/2018
Isopoda	Philosciidae	*Philoscia muscorum*	Common Striped Woodlouse	26/04/2019
Isopoda	Trichoniscidae	*Trichoniscus pusillus*	Common pygmy woodlouse	18/04/2018
Lepidoptera	Adelidae	*Adela reaumurella*	Green Long-horn	20/04/2017
Lepidoptera	Adelidae	*Nemophora degeerella*	Yellow-barred Long-horn	14/06/2018
Lepidoptera	Crambidae	*Anania hortulata*	Small Magpie	06/06/2018
Lepidoptera	Crambidae	*Anania lancealis*	Long-winged pearl	26/06/2020
Lepidoptera	Crambidae	*Eudonia angustea*	Narrow-winged Grey	07/10/2020
Lepidoptera	Crambidae	*Pleuroptya ruralis*	Mother of Pearl	29/06/2018
Lepidoptera	Crambidae	*Scoparia ambigualis*	Common Grey	06/06/2018
Lepidoptera	Crambidae	*Udea olivalis*	Olive pearl	26/06/2020
Lepidoptera	Drepanidae	*Falcaria lacertinaria*	Scalloped hook-tip	07/05/2020
Lepidoptera	Drepanidae	*Habrosyne pyritoides*	Buff Arches	29/06/2018
Lepidoptera	Drepanidae	*Polyploca ridens*	Frosted green	17/04/2019
Lepidoptera	Drepanidae	*Tethea ocularis*	Figure of eighty	26/06/2020
Lepidoptera	Drepanidae	*Thyatira batis*	Peach Blossom	29/06/2018
Lepidoptera	Erebidae	*Callimorpha dominula*	Scarlet tiger	26/06/2020
Lepidoptera	Erebidae	*Calliteara pudibunda*	Pale Tussock	06/06/2018
Lepidoptera	Erebidae	*Diaphora mendica*	Muslin Moth	03/05/2017
Lepidoptera	Erebidae	*Eilema lurideola*	Common Footman	22/06/2018
Lepidoptera	Erebidae	*Eilema sororcula*	Orange footman	07/05/2020
Lepidoptera	Erebidae	*Euproctis similis*	Yellow-tail	10/05/2018
Lepidoptera	Erebidae	*Herminia tarsipennalis*	The Fan-foot	29/06/2018
Lepidoptera	Erebidae	*Hypena proboscidalis*	The Snout	29/06/2018
Lepidoptera	Erebidae	*Laspeyria flexula*	Beautiful Hook-tip	22/06/2018
Lepidoptera	Erebidae	*Orgyia antiqua*	Vapourer moth	

Lepidoptera	Erebidae	*Rivula sericealis*	Straw Dot	06/06/2018
Lepidoptera	Erebidae	*Spilosoma lubricipeda*	White Ermine	06/06/2018
Lepidoptera	Erebidae	*Spilosoma lutea*	Buff Ermine	06/06/2018
Lepidoptera	Erebidae	*Tyria jacobaeae*	Cinnabar	18/06/2018
Lepidoptera	Geometridae	*Agriopis marginaria*	Dotted Border	21/03/2018
Lepidoptera	Geometridae	*Alcis repandata*	Mottled Beauty	29/06/2018
Lepidoptera	Geometridae	*Alsophila aescularia*	March Moth	21/03/2018
Lepidoptera	Geometridae	*Anticlea derivata*	Streamer	26/04/2018
Lepidoptera	Geometridae	*Archiearis parthenias*	Orange Underwing	21/03/2018
Lepidoptera	Geometridae	*Biston betularia*	Peppered moth	03/07/2019
Lepidoptera	Geometridae	*Biston strataria*	Oak Beauty	03/05/2017
Lepidoptera	Geometridae	*Cabera pusaria*	Common white wave	26/06/2020
Lepidoptera	Geometridae	*Campaea margaritaria*	Light Emerald	06/06/2018
Lepidoptera	Geometridae	*Colotois pennaria*	Feathered Thorn	28/09/2018
Lepidoptera	Geometridae	*Comibaena bajularia*	Blotched Emerald	29/06/2018
Lepidoptera	Geometridae	*Cyclophora linearia*	Clay triple lines	26/06/2020
Lepidoptera	Geometridae	*Dysstroma truncata*	Common Marbled Carpet	28/09/2018
Lepidoptera	Geometridae	*Ecliptopera silaceata*	Small Phoenix	31/08/2017
Lepidoptera	Geometridae	*Ectropis crepuscularia*	Engrailed/Small Engrailed	06/06/2018
Lepidoptera	Geometridae	*Erannis defoliaria*	Mottled Umber	07/10/2020
Lepidoptera	Geometridae	*Eupithecia abbreviata*	Brindled Pug	26/04/2018
Lepidoptera	Geometridae	*Eupithecia pulchellata*	Foxglove Pug	29/06/2018
Lepidoptera	Geometridae	*Gandaritis pyraliata*	Barred Straw	29/06/2018
Lepidoptera	Geometridae	*Geometra papilionaria*	Large Emerald	29/06/2018
Lepidoptera	Geometridae	*Hemithea aestivaria*	Common Emerald	29/06/2018
Lepidoptera	Geometridae	*Hydriomena furcata*	July Highflyer	29/06/2018
Lepidoptera	Geometridae	*Hylaea fasciaria*	Barred red	03/07/2019
Lepidoptera	Geometridae	*Idaea aversata*	Riband Wave	22/06/2018
Lepidoptera	Geometridae	*Idaea biselata*	Small Fan-footed Wave	29/06/2018
Lepidoptera	Geometridae	*Idaea dimidiata*	Single-dotted Wave	03/07/2019
Lepidoptera	Geometridae	*Lomaspilis marginata*	Clouded Border	06/06/2018
Lepidoptera	Geometridae	*Lomographa temerata*	Clouded Silver	28/05/2020
Lepidoptera	Geometridae	*Lycia hirtaria*	Brindled Beauty	21/03/2018
Lepidoptera	Geometridae	*Operophtera brumata*	Winter Moth	21/12/2017
Lepidoptera	Geometridae	*Opisthograptis luteolata*	Brimstone Moth	31/08/2017
Lepidoptera	Geometridae	*Ourapteryx sambucaria*	Swallow-tailed moth	26/06/2020
Lepidoptera	Geometridae	*Peribatodes rhomboidaria*	Willow Beauty	22/06/2018
Lepidoptera	Geometridae	*Petrophora chlorosata*	Brown Silver-line	28/05/2020
Lepidoptera	Geometridae	*Plagodis dolabraia*	Scorched wing	07/05/2020
Lepidoptera	Geometridae	*Thera britannica*	Spruce Carpet	07/10/2020
Lepidoptera	Geometridae	*Thera obeliscata*	Grey Pine Carpet	28/09/2018
Lepidoptera	Geometridae	*Timandra comae*	Blood-vein	28/05/2020
Lepidoptera	Geometridae	*Xanthorhoe ferrugata*	Dark-barred Twin-spot Carpet	07/05/2020
Lepidoptera	Geometridae	*Xanthorhoe montanata*	Silver ground carpet	26/06/2020

Lepidoptera	Gracillariidae	*Caloptilia sp.*	NA	26/06/2020
Lepidoptera	Gracillariidae	*Cameraria ohridella*	Horse-chestnut leaf-miner	
Lepidoptera	Gracillariidae	*Parornix devoniella*	Hazel Slender	03/11/2017
Lepidoptera	Gracillariidae	*Phyllonorycter coryli*	Nut Leaf Blister Moth	03/11/2017
Lepidoptera	Gracillariidae	*Phyllonorycter heegeriella*	Pale oak midget	07/10/2020
Lepidoptera	Gracillariidae	*Phyllonorycter nicellii*	Red Hazel Midget	03/11/2017
Lepidoptera	Gracillariidae	*Phyllonorycter quercifoliella*	Common Oak midget	03/11/2017
Lepidoptera	Gracillariidae	*Phyllonorycter spp.*	A leaf-miner	03/11/2017
Lepidoptera	Hepialidae	*Korscheltellus lupulina*	Common Swift	06/06/2018
Lepidoptera	Hesperiidae	*Ochlodes sylvanus*	Large Skipper	04/06/2018
Lepidoptera	Lasiocampidae	*Euthrix potatoria*	The Drinker	29/06/2018
Lepidoptera	Lasiocampidae	*Poecilocampa populi*	December Moth	18/11/2016
Lepidoptera	Nepticulidae	*Ectoedemia albifasciella*	White-banded Pigmy	03/11/2017
Lepidoptera	Nepticulidae	*Ectoedemia subbimaculella*	Spotted Black Pigmy	03/11/2017
Lepidoptera	Nepticulidae	*Stigmella aurella*	Golden Pigmy	18/04/2018
Lepidoptera	Nepticulidae	*Stigmella microtheriella*	Nut-tree Pigmy	03/11/2017
Lepidoptera	Nepticulidae	*Stigmella spp.*	A leaf-miner	03/11/2017
Lepidoptera	Noctuidae	*Agrochola macilenta*	Yellow-line Quaker	28/09/2018
Lepidoptera	Noctuidae	*Agrotis exclamationis*	Heart & Dart	29/06/2018
Lepidoptera	Noctuidae	*Allophyes oxyacanthae*	Green-brindled Crescent	28/09/2018
Lepidoptera	Noctuidae	*Anaplectoides prasina*	Green Arches	29/06/2018
Lepidoptera	Noctuidae	*Anorthoa munda*	Twin-spotted quaker	17/04/2019
Lepidoptera	Noctuidae	*Apamea crenata*	Clouded-bordered Brindle	28/05/2020
Lepidoptera	Noctuidae	*Apamea epomidion*	Clouded brindle	03/07/2019
Lepidoptera	Noctuidae	*Apamea monoglypha*	Dark Arches	29/06/2018
Lepidoptera	Noctuidae	*Asteroscopus sphinx*	Sprawler	18/11/2016
Lepidoptera	Noctuidae	*Autographa gamma*	Silver Y	06/06/2018
Lepidoptera	Noctuidae	*Autographa pulchrina*	Beautiful Golden Y	22/06/2018
Lepidoptera	Noctuidae	*Brachylomia viminalis*	Minor Shoulder-knot	29/06/2018
Lepidoptera	Noctuidae	*Caradrina morpheus*	Mottled Rustic	06/06/2018
Lepidoptera	Noctuidae	*Conistra vaccinii*	Chestnut	25/10/2017
Lepidoptera	Noctuidae	*Cosmia trapezina*	Dun-bar	29/06/2018
Lepidoptera	Noctuidae	*Deltote pygarga*	Marbled White Spot	29/06/2018
Lepidoptera	Noctuidae	*Diarsia mendica*	Ingrailed Clay	06/06/2018
Lepidoptera	Noctuidae	*Diloba caeruleocephala*	Figure of Eight	28/09/2018
Lepidoptera	Noctuidae	*Dryobotodes eremita*	Brindled Green	07/10/2020
Lepidoptera	Noctuidae	*Eugnorisma glareosa*	Autumnal Rustic	28/09/2018
Lepidoptera	Noctuidae	*Euplexia lucipara*	Small Angle Shades	22/06/2018
Lepidoptera	Noctuidae	*Eupsilia transversa*	Satellite	02/11/2017
Lepidoptera	Noctuidae	*Griposia aprilina*	Merveille du Jour	28/09/2018
Lepidoptera	Noctuidae	*Hoplodrina blanda*	The Rustic	22/06/2018
Lepidoptera	Noctuidae	*Hoplodrina octogenaria*	The Uncertain	29/06/2018
Lepidoptera	Noctuidae	*Hydraecia micacea*	Rosy Rustic	28/09/2018

Lepidoptera	Noctuidae	*Lacanobia oleracea*	Bright-line brown-eye	03/07/2019
Lepidoptera	Noctuidae	*Lacanobia thalassina*	Pale-shouldered Brocade	06/06/2018
Lepidoptera	Noctuidae	*Mesoligia furuncula*	Cloaked Minor	29/06/2018
Lepidoptera	Noctuidae	*Mythimna ferrago*	Clay	26/06/2020
Lepidoptera	Noctuidae	*Mythimna impura*	Smoky wainscot	03/07/2019
Lepidoptera	Noctuidae	*Mythimna pallens*	Common wainscot	29/05/2018
Lepidoptera	Noctuidae	*Noctua fimbriata*	Broad-bordered Yellow underwing	26/06/2020
Lepidoptera	Noctuidae	*Noctua pronuba*	Large Yellow Underwing	13/07/2017
Lepidoptera	Noctuidae	*Ochropleura plecta*	Flame Shoulder	03/05/2017
Lepidoptera	Noctuidae	*Oligia latruncula*	Tawny marbled minor	26/06/2020
Lepidoptera	Noctuidae	*Oligia strigilis*	Marbled Minor	22/06/2018
Lepidoptera	Noctuidae	*Oligia strigilis agg.*	Marbled Minor agg.	29/06/2018
Lepidoptera	Noctuidae	*Orthosia cerasi*	Common Quaker	26/04/2018
Lepidoptera	Noctuidae	*Orthosia gothica*	Hebrew Character	03/05/2017
Lepidoptera	Noctuidae	*Orthosia incerta*	Clouded Drab	26/04/2018
Lepidoptera	Noctuidae	*Panolis flammea*	Pine Beauty	03/05/2017
Lepidoptera	Noctuidae	*Phlogophora meticulosa*	Angle Shades	31/08/2017
Lepidoptera	Noctuidae	*Polia nebulosa*	Grey arches	03/07/2019
Lepidoptera	Noctuidae	*Rusina ferruginea*	Brown rustic	03/07/2019
Lepidoptera	Noctuidae	*Tiliacea aurago*	Barred Sallow	28/09/2018
Lepidoptera	Noctuidae	*Xanthia togata*	Pink-barred Sallow	28/09/2018
Lepidoptera	Noctuidae	*Xestia c-nigrum*	Setaceous Hebrew Character	31/08/2017
Lepidoptera	Noctuidae	*Xestia triangulum*	Double-square Spot	29/06/2018
Lepidoptera	Noctuidae	*Xestia xanthographa*	Square-spot Rustic	31/08/2017
Lepidoptera	Notodontidae	*Drymonia ruficornis*	Lunar Marbled Brown	03/05/2017
Lepidoptera	Notodontidae	*Phalera bucephala*	Buff-tip	22/06/2018
Lepidoptera	Notodontidae	*Pheosia gnoma*	Lesser swallow prominent	07/05/2020
Lepidoptera	Notodontidae	*Pheosia tremula*	Swallow Prominent	03/05/2017
Lepidoptera	Notodontidae	*Pterostoma palpina*	Pale Prominent	28/05/2020
Lepidoptera	Notodontidae	*Ptilodon capucina*	Coxcomb Prominent	22/06/2018
Lepidoptera	Nymphalidae	*Aglais urticae*	Small Tortoiseshell	05/09/2017
Lepidoptera	Nymphalidae	*Pararge aegeria*	Speckled Wood	11/04/2017
Lepidoptera	Nymphalidae	*Vanessa atalanta*	Red Admiral	13/07/2017
Lepidoptera	Nymphalidae	*Aglais io*	Peacock	28/03/2019
Lepidoptera	Nymphalidae	*Aphantopus hyperantus*	Ringlet	03/07/2019
Lepidoptera	Nymphalidae	*Argynnis paphia*	Silver-washed fritillary	20/07/2018
Lepidoptera	Nymphalidae	*Maniola jurtina*	Meadow brown	27/06/2018
Lepidoptera	Nymphalidae	*Polygonia c-album*	Comma butterfly	09/07/2018
Lepidoptera	Pieridae	*Anthocharis cardamines*	Orange-tip	03/05/2017
Lepidoptera	Pieridae	*Gonepteryx rhamni*	Brimstone	03/05/2017
Lepidoptera	Pieridae	*Pieris brassicae*	Large White	13/07/2017
Lepidoptera	Pieridae	*Pieris napi*	Green-veined White	13/07/2017
Lepidoptera	Pieridae	*Pieris rapae*	Small white	
Lepidoptera	Pyralidae	*Aphomia sociella*	Bee Moth	29/06/2018

Lepidoptera	Sphingidae	*Laothoe populi*	Poplar hawk moth	03/07/2019
Lepidoptera	Tischeriidae	*Tischeria ekebladella*	Oak Carl	03/11/2017
Lepidoptera	Tortricidae	*Acleris rhombana*	Rhomboid Tortrix	26/06/2020
Lepidoptera	Tortricidae	*Aleimma loeflingiana*	Yellow Oak Button	22/06/2018
Lepidoptera	Tortricidae	*Ancylis geminana*	Festooned Roller	06/06/2018
Lepidoptera	Tortricidae	*Apotomis turbidana*	White-shouldered Marble	22/06/2018
Lepidoptera	Tortricidae	*Archips crataegana*	Brown Oak Tortrix	29/06/2018
Lepidoptera	Tortricidae	*Archips podana*	Large Fruit-tree Tortrix	22/06/2018
Lepidoptera	Tortricidae	*Archips xylosteana*	Variegated Golden Tortrix	26/06/2020
Lepidoptera	Tortricidae	*Epinotia sp.*	NA	26/06/2020
Lepidoptera	Tortricidae	*Gypsonoma sp.*	NA	26/06/2020
Lepidoptera	Tortricidae	*Hedya nubiferana*	Marbled Orchard Tortrix	29/06/2018
Lepidoptera	Tortricidae	*Notocelia uddmanniana*	Bramble Shoot Moth	22/06/2018
Lepidoptera	Tortricidae	*Pandemis cerasana*	Barred Fruit-tree Tortrix	22/06/2018
Lepidoptera	Tortricidae	*Pandemis cinnamomeana*	White-faced tortrix	26/06/2020
Lepidoptera	Tortricidae	*Spilonota ocellana*	Bud moth	26/06/2020
Lepidoptera	Tortricidae	*Tortrix viridana*	Green Oak Tortrix	06/06/2018
Lepidoptera	Yponomeutidae	*Swammerdamia sp.*	NA	26/06/2020
Lepidoptera	Yponomeutidae	*Yponomeuta sp.*	An ermine moth	26/06/2020
Mecoptera	Panorpidae	*Panorpa communis*	A scorpionfly	
Mecoptera	Panorpidae	*Panorpa germanica*	A scorpionfly	27/04/2017
Megaloptera	Sialidae	*Sialis sp.*	Indet. Alder Fly	23/05/2017
Neuroptera	Chrysopidae	*Chrysoperla carnea*	A green lacewing	26/03/2019
Neuroptera	Hemerobiidae	*Hemerobius sp.*	A brown lacewing	16/04/2019
Odonata	Aeshnidae	*Aeshna cyanea*	Southern hawker	29/07/2016
Odonata	Coenagrionidae	*Coenagrion puella*	Azure damselfly	21/05/2019
Orthoptera	Acrididae	*Chorthippus albomarginatus*	Lesser marsh grasshopper	24/07/2018
Orthoptera	Acrididae	*Chorthippus brunneus*	Common field grasshopper	24/07/2018
Orthoptera	Tetrigidae	*Tetrix subulata*	Slender groundhopper	17/04/2019
Orthoptera	Tettigoniidae	*Meconema thalassinum*	Oak Bush-cricket	03/07/2018
Polydesmida	Polydesmidae	*Polydesmus sp.*	Flat-backed millipede	20/03/2017
Pseudoscorpiones	Chernetidae	*Lamprochernes nodosus*	Knotty shining claw	13/06/2018
Pseudoscorpiones	Chernetidae	*Pselaphochernes dubius*	Small chernes	
Pseudoscorpiones	Neobisiidae	*Neobisium carcinoides*	Moss neobisid	20/03/2017
Psocoptera	Ectopsocidae	*Ectopsocus briggsi*	A barklouse	29/03/2019
Pulmonata	Limacidae	*Limax maximus*	Great Grey Slug	19/09/2017
Siphonaptera	Hystrichopsyllidae	*Typhloceras poppei*	Wood mouse flea	

APPENDIX II
Embolemus ruddii recorded from a pitfall trap in Staffordshire

This article was published in the BWARS newsletter as: Crowley, L., 2018. Embolemus ruddii recorded from a pitfall trap in Staffordshire. *BWARS Newsletter*, 2018(1), pp.13-14.

On the 28th November 2017, a single female of the rarely seen Embolemine wasp, *Embolemus ruddii* Westwood, 1883, (Hymenoptera: Embolemidae) (Figure 1), was found in a pitfall trap at the Birmingham Institute for Forest Research Free-air CO_2 enrichment facility in Staffordshire (SJ 798 226).

The pitfall had been deployed on the 21st November 2017 and left for a sampling period of 1 week. It comprised a typical pitfall trap design, with a 570ml plastic cup sunk into the group so that the lip was flush with the soil surface. The individual trap is part of a network of 18 such traps across the site, which were installed in February 2017.

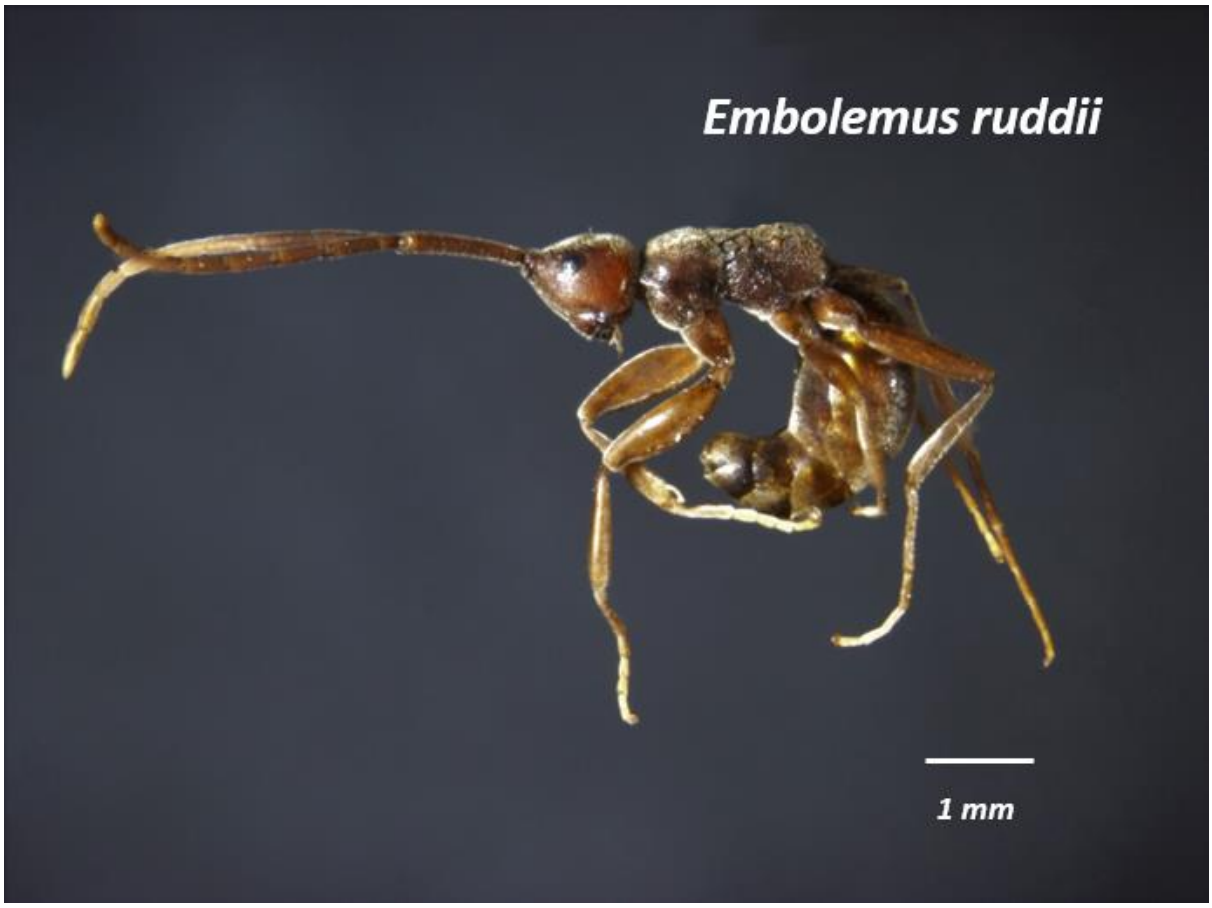

Figure 1 – The Embolemus ruddii specimen sampled.

The site is characterised as a typical mature semi-natural oak woodland, with ~150-year-old English oaks, *Quercus robur*, as the primary species, and an understory of coppiced common hazel, *Corylus avellane*. There are also a small number of other tree species throughout the woodland, including sycamore, *Acer pseudoplatanus*, and hawthorn, *Cretaegus monogyna*.

Embolemidae Foerster, 1856 (Hymenoptera: Chrysidoidea) contains 33 species worldwide, with 9 species in the genus *Emboleus* (Van Achterberg and Van Kats, 2000). The family is distinctive and easily recognised, in part due to the triangular shape of the head in lateral view, with the antennae projecting from the apex (Bladock, 2010).

E. ruddii is the most common species of Embolemidae found in Europe (Varrone and Olmi, 2010) and the only one to occur in Britain (Edwards, 1997). This species is very infrequently recorded, with only 20 previous British records since 1912, the most recent one from 1997 (Figure 2).

Females are apterous and have previously been collected in or near nests of ants and moles, suggesting some degree of a subterranean lifestyle. Interestingly, there is significant mole activity nearby at the BIFoR site. Whilst the specific host is unknown, the species has been found to parasitise tree-root feeding Cixiidae in Europe (Varrone and Olmi, 2010), also hinting at a subterranean nature.

The few pre-existing records of females range from May to September (BWARS), and they are believed to overwinter as adults (Burn, 1997). The occurrence of this individual in late November and in a pitfall trap suggests that it may have been looking for a suitable overwintering site, which given the subterranean associations, is likely to be in the soil or under bark.

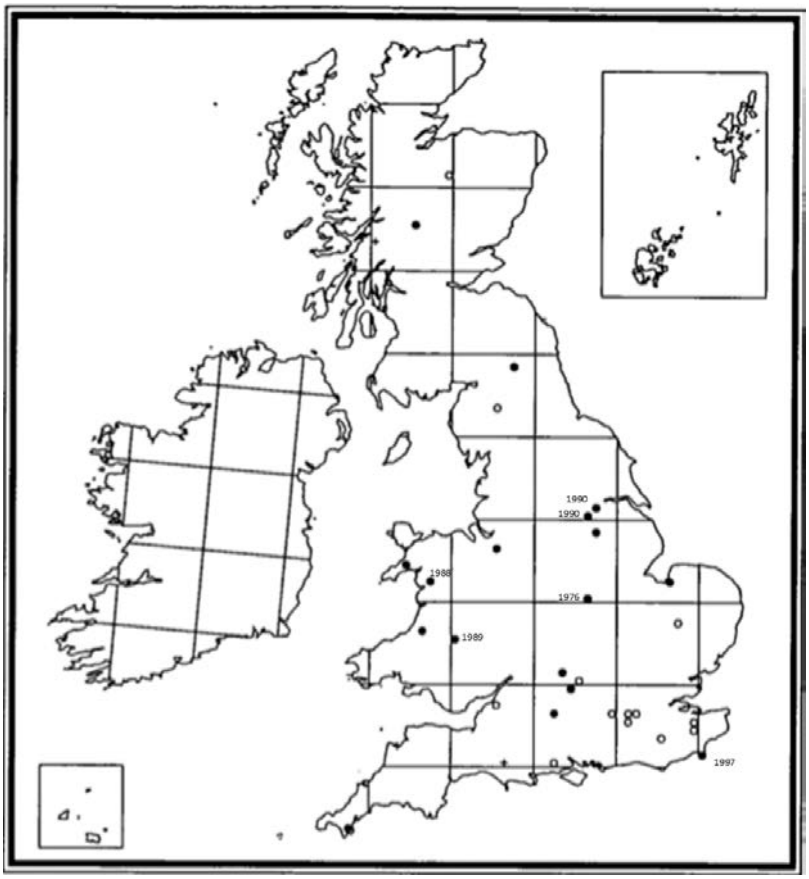

Figure 2 – An atlas of the occurrence of previous UK E. ruddii records. Some of the more recent records are annotated with the date of the record.

References

Baldock, D.W., 2010. Key to families of Aculeate Hymenoptera. In: *Wasps of Surrey*. Surrey Wildlife Trust.

Burn, J. T., 1997. http://www.bwars.com/index.php?q=wasp/embolemidae/embolemus-ruddii [accessed December 2017]

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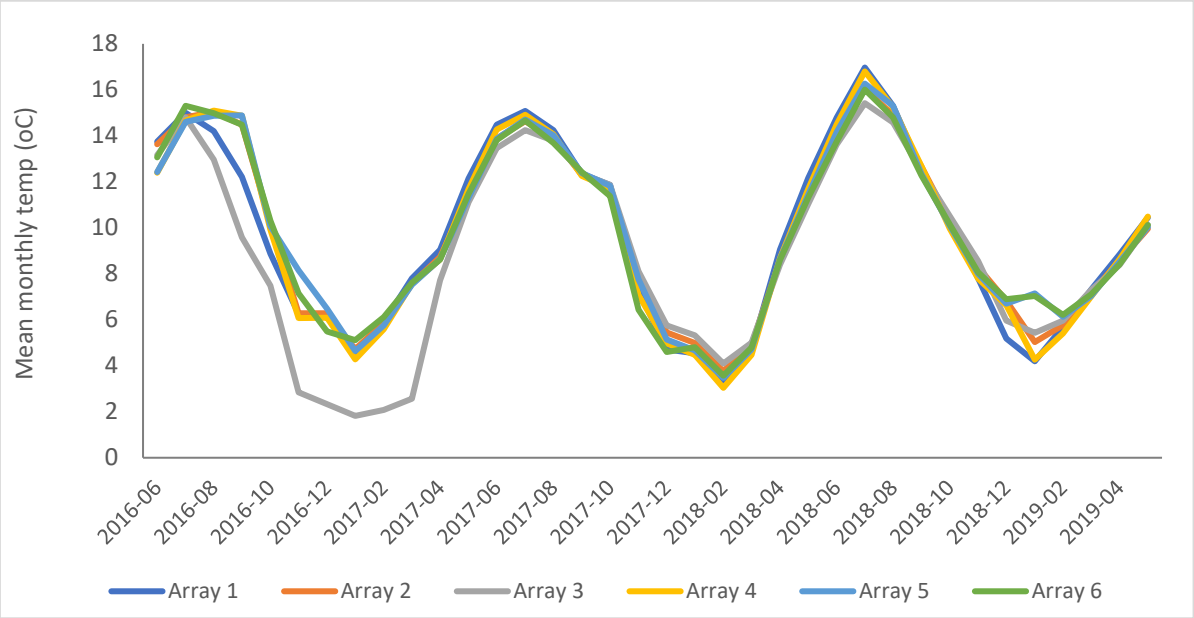
Varrone, R. and Olmi, M., 2010. First record of host of Embolemus ruddii Westwood (Hymenoptera Embolemidae). *Frustula entomologica*, 33, pp.91-95

APPENDIX III
Chapter 5 supplementary material

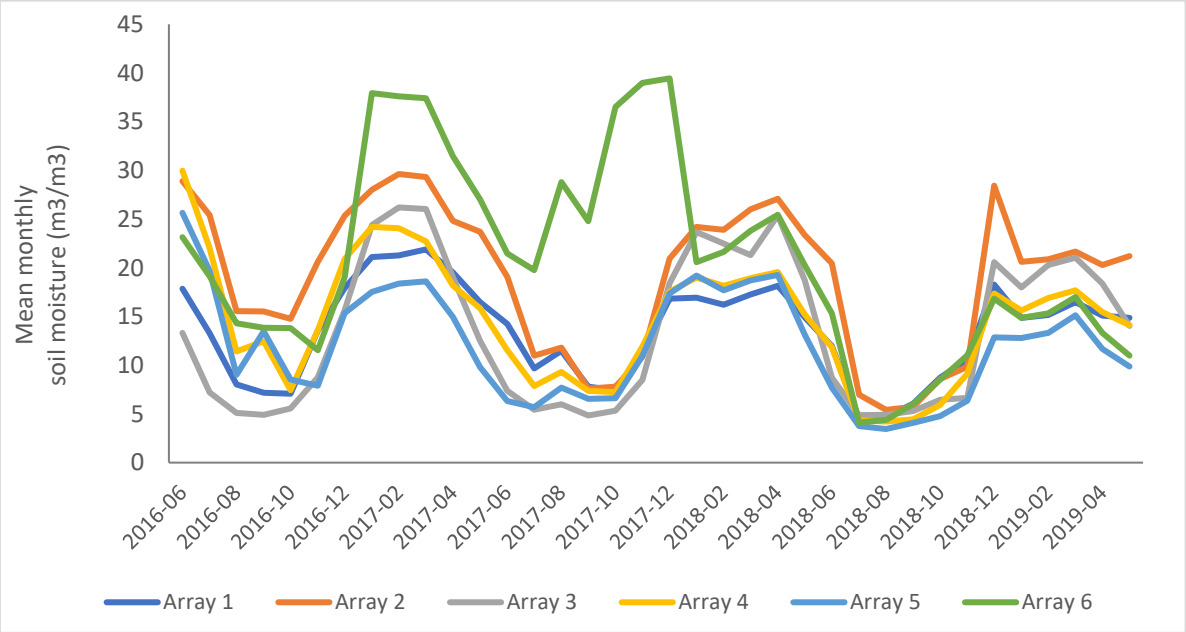
SM1 – Systematic review articles by topic

Topic	Articles
Flowering phenology	Bae and Sicher 2004; Bhattacharya et al. 1985; Bidart-Bouzat et al. 2004; Bloor et al. 2010; Bunce 2015; Carter et al. 1997; Case et al. 1998; Cleland et al. 2006; Curtis et al. 1994; Erhardt et al. 2005; Fabian et al. 2019; Farnsworth & Bazzaz 1995; Frick et al. 1994; Garbutt & Bazzaz 1984; Garbutt et al. 1990; Garruña-Hernández et al. 2012; He & Bazzaz 2003; He et al. 2005; Heinemann et al. 2006; Hicklenton & Jolliffe 1980; Hoover et al. 2012; Hovenden et al. 2009; Hovenden et al. 2008; Jablonski 1997; Johnston & Reekie 2008; Kinugasa et al. 2003; Kumar et al. 2014; Lake & Hughes 1999; Lee 2011; Leishman et al. 1999; McConnaughay et al. 1993; Musil et al. 1999; Osborne et al. 1997; Padhan et al. 2020; Posner 1971; Potvin & Strain 1985; Rämö et al. 2007; Rämö et al. 2006; Rathcke 1992; Reekie & Bazzaz 1991; Reekie et al. 1994; Reekie et al. 1997; Rogers et al. 2006; Rusterholz & Erhardt 1998; Seneweera et al. 1994; Sharma et al. 2020; Song et al. 2009; Van Der Kooij & De Kok 1996; Vanaja et al. 2015; Wagner et al. 2001; Wand et al. 1996; Ward & Strain 1997; Wolfe-Bellin et al. 2006; Woodin et al. 1992; Zhang & Lechowicz 1995.
Pollen	Albertine et al., 2014; El Kelish et al, 2014; Kim et al., 2018; Kobayasi et al., 2019; LaDeau and Clark 2006; Lake and Hughes, 1999; López-Cubillos and Hughes, 2016; Marshall et al., 2010; Prasad et al., 2011; Rogers et al, 2006; Silva et al., 2015; Singer et al., 2005; Wayne et al., 2002; Ziska and Caulfield, 2000; Ziska et al, 2016;
Nectar	Dag and Eisikowitch, 2000; Erhardt et al., 2005; Fabian et al., 2019; Hoover et al., 2012; Huber, 1956; Lake and Hughes, 1999; López-Cubillos and Hughes, 2016; Osborne et al., 1997; Rathcke, 1992; Rusterholz and Erhardt, 1998;
Pollination	Dag and Eisikowitch, 2000; Glenny et al., 2018; Hoover et al., 2012;

SM2 – Mean monthly soil temperature for 3 years proceeding observed flowering period



SM3 – Mean monthly soil moisture (m3/m3) for each array for 3 years proceeding observed flowering period



SM4 - Residuals of ANOVA for treatment against First flowering day of year, mid flowering day of year and flowering duration

Analysis of Variance Table					
Response: DOY_first					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treat	1	60.167	60.167	3.967	0.1172
Residuals	4	60.667	15.167		

Analysis of Variance Table					
Response: DOY_mid					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treat	1	60.167	60.167	12.893	0.02295*
Residuals	4	18.667	4.667		

Analysis of Variance Table					
Response: DOY_duration					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treat	1	0.167	0.1667	0.0091	0.9286
Residuals	4	73.333	18.3333		

SM5 – Correlation matrix of flowering time and environmental variables

	DOY_first	DOY_duration	DOY_median	Temp	Moist	LUX
DOY_first	1	-0.6738088	0.9409185	-0.3620968	0.7116293	0.675191
DOY_duration	-0.6738088	1	-0.3875461	0.4885743	-0.5887001	-0.4963
DOY_median	0.9409185	-0.3875461	1	-0.2287862	0.5860794	0.585017
Temp	-0.3620968	0.4885743	-0.2287862	1	-0.3770705	-0.46252
Moist	0.7116293	-0.5887001	0.5860794	-0.3770705	1	0.978308
LUX	0.6751914	-0.4962997	0.5850172	-0.4625195	0.9783081	1

SM6 – All species/species group recorded visiting bluebells during surveys

Order	Family	Species/Species group	Percent of total visits
Diptera	Syrphidae	Platycheirus spp.	35
Hymenoptera	Apidae	Bombus pratorum	17
Diptera	Syrphidae	Rhingia campestris	15
Diptera	Syrphidae	Syrphus spp.	10
Hymenoptera	Apidae	Bombus lapidarius	8
Diptera	Syrphidae	Melanostoma scalare	8
Hymenoptera	Ichneumonidae	Ichneumonidae spp.	8
Coleoptera	Elateridae	Athous sp.	8
Diptera	Bibionidae	Bibio sp.	6
Hymenoptera	Apidae	Bombus pascourum	4
Diptera	Syrphidae	Baccha elongata	4
Diptera	Syrphidae	Lecozon luecorum	4
Hymenoptera	Apidae	Bombus terrestris	2
Coleoptera	Cantharidae	Cantharis decipiens	2
Coleoptera	Coccinellidae	Tytthaspis sedecimpunctata	2
Mecoptera	Panorpidae	Panorpa sp.	2
Lepidoptera	Pieridae	Pieris rapae	2