An aboveground–belowground herbivore interaction in a woody perennial crop and its response to elevated atmospheric CO_2

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A thesis submitted for the degree of Doctor of Philosophy

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Thesis summary

Interactions between aboveground and root-feeding herbivores can be influenced by changes in plant traits, such as tissue chemistry and morphology. Environmental heterogeneity and perturbations also affect these plant-mediated interactions. Climate change is a multi-faceted phenomenon; increases in atmospheric carbon dioxide (CO_2) concentrations lead to increased global mean temperature and an associated higher frequency of extreme weather events. These factors can potentially perturb ecosystem function by altering both plant–herbivore and herbivore–herbivore interactions.

A detailed understanding of whether above–belowground interactions are affected by climate change remains lacking. In an attempt to fill knowledge gaps in this understudied area of ecology, this thesis investigates, through a series of glasshouse experiments, the effects of elevated CO_2 and other aspects of climate change, such as altered phenology, on the interspecific interaction between the aboveground large raspberry aphid (*Amphorophora idaei*) and the root feeding larvae of the vine weevil (*Otiorhynchus sulcatus*), mediated by the shared raspberry (*Rubus idaeus*) host-plant.

Under ambient climate conditions, reciprocal feeding facilitation was observed to occur between aphids and vine weevil larvae feeding on raspberry, with the presence of one increasing the abundance of the other herbivore, and *vice versa*. This occurred regardless of plant cultivar and order of herbivore arrival on the plant. It is likely that this facilitative relationship is driven by over-compensatory plant growth in response to herbivory. Although tougher, adult vine weevils show a feeding preference for leaves grown in elevated CO_2. Herbivory may be more influential than CO_2 in determining plant–herbivore interaction outcomes. Aphids affect plant intraspecific competition to a greater extent than elevated CO_2 by altering plant biomass of both infested and non-infested plants. In conclusion, this particular plant–herbivore system would seem to be relatively robust in the face of possible future CO_2 concentration scenarios.

Contents

List of figures

Chapter I

Chapter II

Chapter III

Chapter IV

Chapter V

Chapter VI

Chapter VIII

List of tables

Chapter II

Chapter III

Chapter V

Chapter VI

Chapter I

Above- and belowground herbivory, interactions and climate change: A Literature Review.

1.1 Introduction

How organisms interact within an environment influences ecosystem function (Wardle *et al*., 2004a; Tylianakis *et al*., 2008; Van der Putten *et al*., 2009; Bardgett and Wardle, 2010). The nature of these ecological interactions may be direct (Walling, 2000), for example by physical interference (Denno *et al*., 1995), or indirect, usually mediated via a third organism, such as plant-mediated effects between two spatially separated herbivores (Gange and Brown, 1989; Scheu *et al*., 1999; Bezemer *et al*., 2002; Bezemer *et al*., 2003; Van Dam *et al*., 2003; Wäckers and Bezemer, 2003; Bezemer *et al*., 2004; Van Dam *et al*., 2005). Increasing community complexity may alter the magnitude, and sometimes the direction, of the ecological interaction (Van der Putten *et al*., 2001; Wardle *et al*., 2004a; Bardgett and Wardle, 2010; Megías and Müller, 2010; Heil, 2011). These interactions can be fragile and environmental stresses have the potential to modify the efficiency and overall function at both an individual and ecosystem level (Kimball *et al*., 1993; Watt *et al*., 1995; Whittaker, 2001; Newman *et al*., 2003; Staley and Johnson, 2008).

Plants and their herbivores interact in a number of ways; these interactions may be beneficial (Poveda *et al*., 2010) or detrimental (Van Dam, 2009) at either or both trophic levels. Herbivory may directly reduce plant fitness and population size, but may also shape plant interspecific interactions and promote diversity (Walling, 2000). Herbivores may interact with other organisms in the same trophic level via the plant (Bardgett and Wardle, 2003; Poveda *et al*., 2005; Staley *et al*., 2007; Wurst and Van der Putten, 2007; Heil, 2011); thus herbivores feeding aboveground are able to interact with those feeding belowground, and *vice-versa*. The importance of herbivory in ecosystem function has long been recognised aboveground (Walling, 2000); but the important role of belowground herbivores is becoming apparent (Brown and Gange, 1990; Wardle *et al*., 2004a; Johnson *et al*., 2008; Bardgett and Wardle, 2010). While a plethora of studies explore interactions between plants and their herbivores (Walling, 2000), far fewer studies consider plant-mediated above–belowground herbivore interactions. Fewer still have investigated how the drivers of anthropogenic-derived climate change (e.g. elevated CO_2) may affect such above–belowground interactions. Above- and belowground interactions play an important role in ecosystems and communities, and there is potential for elevated atmospheric CO_2 levels to disrupt such interactions and, consequently, ecosystem processes (Bazzaz, 1990; Harrington *et al*., 1999; Whittaker, 2001; Walther *et al*., 2002; Staley and Johnson, 2008).

Environmental stress can originate from both natural (e.g. drought and flooding) and anthropogenic (e.g. intensified grazing pressure, pollution and climate change) causes. The latest Intergovernmental Panel on Climate Change (IPCC) scenario predictions suggest a potential increase of atmospheric CO_2 concentrations to 970 ppm by 2100, with a rise in mean global temperature of up to 5.8°C (IPCC, 2013). Some organisms respond idiosyncratically, which results in many interactions being species-specific, making feeding guild and niche generalisations difficult (Wurst and Van der Putten, 2007). How organisms respond and adapt to the impacts of these environmental stresses is of immediate relevance to conservation, biodiversity and crop security (Cammell and Knight, 1992; Rogers and Dahlman, 1993; Rogers *et al*., 1995; Gregory *et al*., 2009; Van der Putten *et al*., 2009).

1.2 Direct plant–herbivore interactions

The interactions between herbivores and their associated plant hosts have been well studied in a wide variety of ecosystems (Walling, 2000). While the exact nature of the relationship is often interaction-specific, some general trends emerge. High levels of herbivory are detrimental to a plant's reproductive success (e.g. aphids can reduce seed production by up to 60% (Moran and Whitham, 1990)), however, in some cases the plant may benefit from moderate herbivory (Van Dam *et al*., 2005).

On being grazed, plants often show compensatory growth, both above- (Walling, 2000) and belowground (Andersen, 1987), but this growth is dependent on the degree of herbivory experienced (McNaughton *et al*., 1998). This happens with both root and foliar herbivores; for example, root herbivory can increase plant nectar production (Wäckers and Bezemer, 2003), which, in turn, attracts pollinators beneficial to the plant (Poveda *et al*., 2005). Belowground herbivory can also stimulate growth of existing roots (Gange and Brown, 1989), whilst also initiating new root growth (Andersen, 1987), mainly in the form of fine, lateral roots. Both above- and belowground herbivores can induce changes in plant nutrient allocation and root exudation (Bardgett *et al*., 1998; Poveda *et al*., 2005; Johnson *et al*., 2009). For instance, aboveground herbivory by the grasshopper *Romalea guttata* increased the amount of carbon allocation to roots, whilst decreasing shoot allocation in maize, *Zea mays* (Holland *et al*., 1996). Plant nutrient allocation is altered not only by the herbivore itself, but also by the site of herbivory (Bezemer *et al*., 2003; Bezemer *et al*., 2004). Grazing of young shoots, for example, can elicit a greater nutrient allocation response over herbivory of more mature leaves (Bezemer *et al*., 2003). This response can be further altered when the plant is subjected to both, rather than one of, above- and belowground herbivory (Smith, 1977). Plant characteristics such as size, nutrient content and water concentration (Davidson *et al*., 1970; Staley *et al*., 2007) can all further influence the outcome of plant–herbivore interactions.

As well as showing compensatory growth in response to herbivory, plants may also modify their production of defence chemicals (Van der Putten *et al*., 2001; Van Tol *et al*., 2001; Kessler and Baldwin, 2002; Wäckers and Bezemer, 2003; Rasmann and Turlings, 2007; McCall and Fordyce, 2010; Rasmann *et al*., 2011; Van Dam and Heil, 2011). These chemicals may act as herbivore repellents (Van Dam, 2009) or attractants to recruit predators to the herbivore population (Rasmann and Turlings, 2007). Plants are also known to induce defence chemicals in areas not yet subjected to herbivory (Bezemer *et al*., 2004). Chemical defence compounds can, for example, upon herbivory, be induced systemically in undamaged plant tissue elsewhere (Erb *et* *al*., 2009; Hiltpold *et al*., 2011), or be translocated from the point of attack (Heil and Ton, 2008; Kaplan *et al*., 2008a). Feeding by a folivore, for example, may elicit an increase in terpenoid concentrations in roots as well as leaves (Wäckers and Bezemer, 2003; Bezemer *et al*., 2004). The Optimal Defence Theory suggests that, in general, a plant will invest the highest concentration of defence chemicals in tissues that are of greater energetic cost if removed; for example, young shoots (McCall and Fordyce, 2010). This, however, can be altered by herbivory with the plant concentrating terpenoid concentration in damaged tissue to prevent further damage (Bezemer *et al*., 2004). The exact nature and concentration of secondary chemicals appear to be dependent on both herbivore species identity and feeding location on the plant (Van Dam, 2009).

Plant responses may also be herbivore species dependent. Leaf-chewers, for example, generally elicit a greater plant defence chemical production than phloem-feeding insects such as aphids (Masters, 1995; Wardle *et al*., 2004b; Wurst and Van der Putten, 2007; Kaplan *et al*., 2009). This is probably a consequence of the greater mechanical damage caused by the chewer feeding strategy. Moreover, herbivore guilds are affected differentially by defensive chemicals. Sap-sucking insects, such as aphids, may circumvent many plant defences as there are fewer defence chemicals present in plant phloem than leaf tissue (Johnson, 2011). Consequently, aphids are generally affected less than leaf-chewing caterpillars (Walling, 2000), although there is some evidence to the contrary (Kaplan *et al*., 2009).

Compared to aboveground, relatively little is known about belowground herbivore–plant interactions (Johnson *et al*., 2012a). Belowground biota has suffered from the “out of sight, out of mind” attitude (Hunter, 2001). In many field studies, recognising the presence of a belowground herbivore is difficult unless there is a noticeable negative aboveground effect (e.g. wilting). Research over the past 20 years has begun to correct the dearth of information available on belowground interactions. This has led to a much better appreciation of the importance of the rhizosphere.

Root herbivores, particularly nematodes, play the role of both “hero” and “villain” (Johnson *et al*., 2008). They are frequently a major pest (Moorhouse *et al*., 1992), but also have been used successfully as biocontrol agents against weeds (Blossey and Hunt-Joshi, 2003). In fact, their application as a biocontrol agent has proved to be more effective than their aboveground herbivore equivalent (Blossey and Hunt-Joshi, 2003). In the field, soil biota abundance can vary markedly between relatively proximal plants. The spatial distribution of root-feeding invertebrates is often patchy, mainly as a result of the selective oviposition by the adults for host-plant suitability (Brown and Gange, 1990; Moorhouse *et al*., 1992). Given this patchy distribution, soil invertebrate larvae show a considerable degree of density dependent competition in terms of significantly reduced growth rates and increased mortality (Clark *et al*., 2012a). This competition can be moderated by plant palatability with less palatable plants supporting fewer, but heavier, larvae than more palatable plants (Clark *et al*., 2012a). Slower growing female larvae also suffer more under high larval densities, producing a skewed sex ratio (Gange and Brown, 1989).

Interactions between plants and soil biota can occur at an individual plant, population and community level (Masters *et al*., 2001; Scheu, 2001; Wäckers and Bezemer, 2003; Wardle *et al*., 2004b). There is, for example, evidence that aboveground herbivory stimulates nutrient cycling and plant productivity (Van der Putten *et al*., 2001; Bardgett and Wardle, 2003; Bardgett and Wardle, 2010) with defoliation eliciting increases in root exudation (Bardgett *et al*., 1998). This, in turn, increases soil biotic activity (Bardgett *et al*., 1998; Newington *et al*., 2004; Poveda *et al*., 2005), and ultimately nutrient availability to the plant (McNaughton *et al*., 1998; Wardle *et al*., 2004b). There is mixed evidence of how aboveground herbivory affects root productivity, with positive (Milchunas and Lauenroth, 1993), negative (Guitian and Bardgett, 2000) and neutral (McNaughton *et al*., 1998) effects being reported, implying there is no consistent belowground plant response to aboveground herbivory.

1.3 Indirect herbivore interactions

It was Brown and Gange (1990) who predicted that above- and belowground herbivores would interact indirectly as a consequence of sharing a common food source. They suggested that such an interaction would be founded around the fact that root-feeding causes water stress, and that stressed plants are more susceptible to herbivory (Brown and Gange, 1990). A review by Scheu (2001) highlighted the importance of the structure and activity of belowground biota when considering the aboveground food web, and identified belowground herbivores as potential drivers in aboveground processes with respect to both plant and herbivore communities.

Two hypotheses were originally postulated to describe the plant-mediated interaction between above- and belowground herbivores. The first is the 'plus-minus' response suggested by Masters *et al.* (1993), where aboveground herbivores benefit at the expense of root-feeders. This was later modified by Bezemer *et al*. (2002) as the Stress Response Hypothesis (Figure 1.1). The proposed mechanism is that root-herbivore damage impairs the uptake of sufficient water and nutrients. This induces a nutrient and water 'stress' on the plant which results in the plant foliage containing a higher concentration of dissolved carbohydrates and amino acids, improving the foliar nutritional quality for aboveground herbivores. The increase in abundance and/or feeding rate of foliage-feeding insects causes the plant to re-allocate nutrients for compensatory aboveground plant growth at the expense of root growth, thus reducing the abundance and quality of the root-feeders food source.

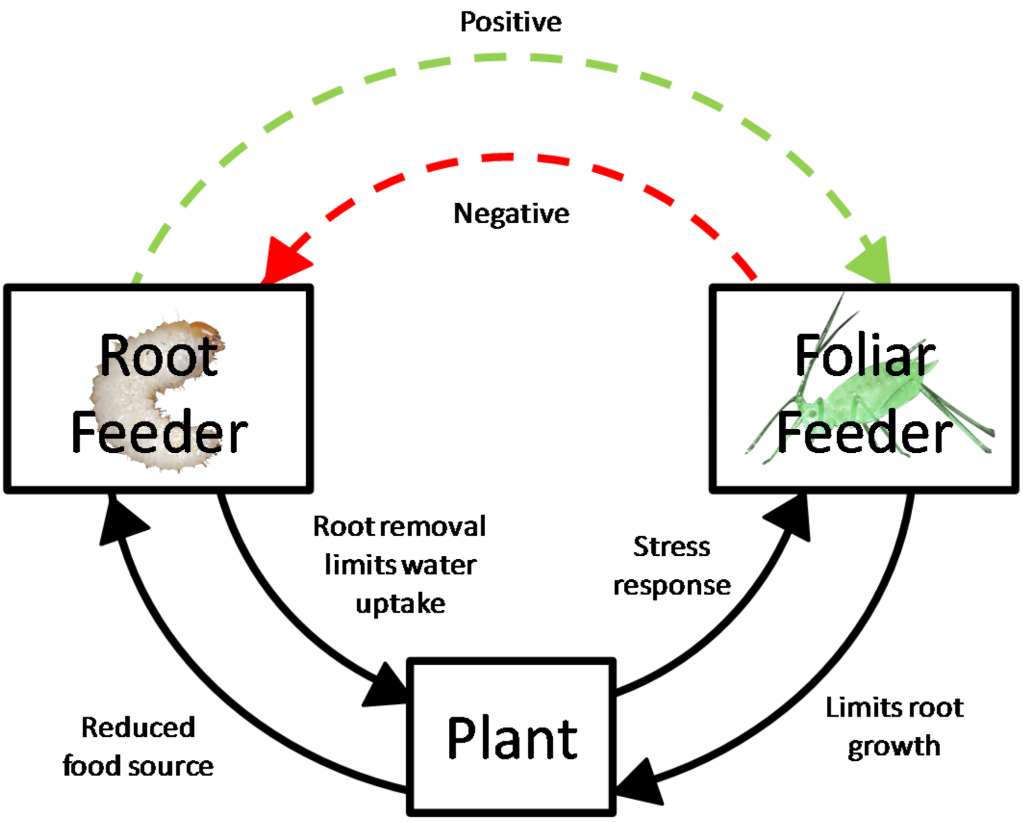

Figure 1.1: The Stress Response Hypothesis; solid lines represent a direct effect, dotted an indirect effect (adapted from original Figure 1 in Masters *et al.*, 1993).

The second hypothesis is the Defence Induction Hypothesis (Figure 1.2), also proposed by Bezemer *et al*. (2002). Belowground herbivory can stimulate a plant to produce defensive compounds that are translocated to other parts of the plant to prevent or retard further herbivory or, alternatively, synthesised *in situ* in the leaves. This results in a negative effect of root-feeders on folivores, and a null effect for the reciprocal interaction. Various published studies support both the Stress Response and Defence Induction Hypotheses (e.g. Gange and Brown, 1989; Soler *et al*., 2005). Although the two hypotheses suggest differing results of above–belowground interactions, they may not be mutually exclusive and can occur in the same study system (Johnson *et al*., 2008). It has also been noted that when a plant is attacked by both above- and belowground herbivores, the defence induction tends to be greater in the aboveground tissue (Bezemer *et al*., 2004; Kaplan *et al*., 2008b).

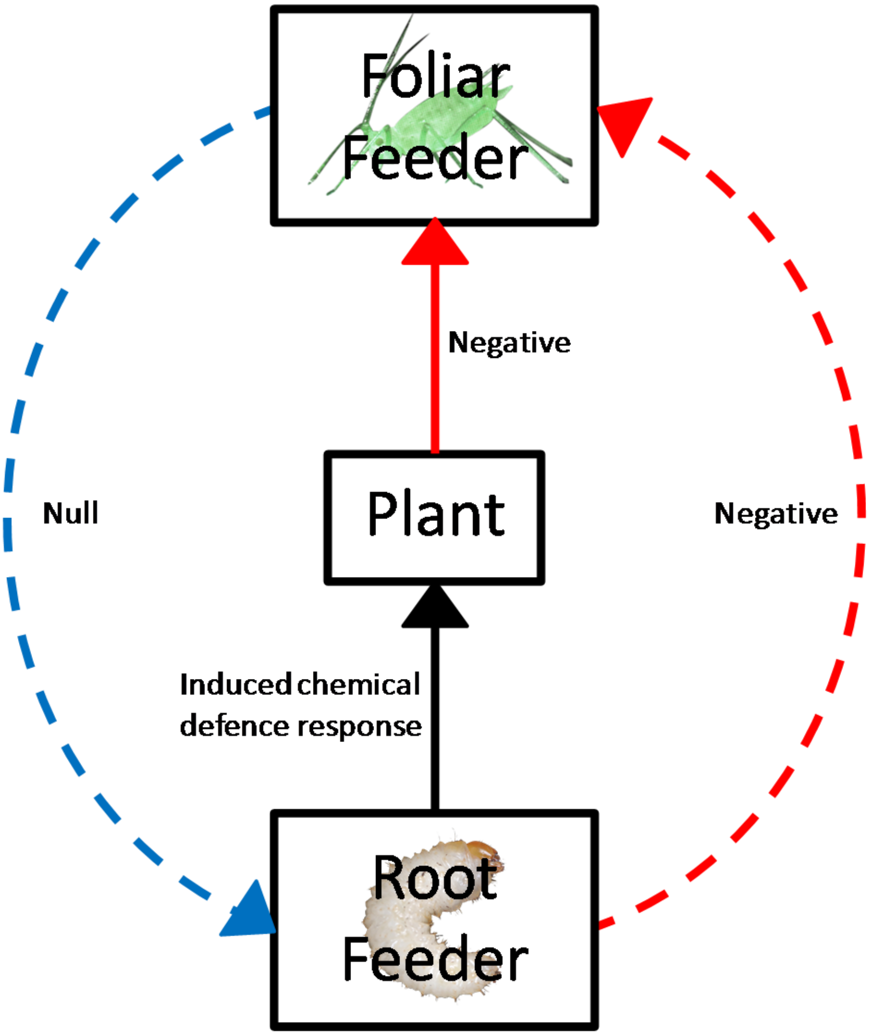

Figure 1.2: The Defence Induction Hypothesis; solid lines represent a direct effect, dotted an indirect effect.

Even with these two hypotheses, the consequences of plant-mediated interactions between the two herbivores cannot be entirely predicted and individual species within guilds react differently to various herbivore competitors (Johnson *et al.*, 2012a). The same interacting herbivore species, for instance, can react differently dependent on the shared host-plant species (Scheu *et al.*, 1999; Johnson *et al.*, 2008; Johnson *et al.*, 2009).

The majority of empirical studies to date have focussed on the effect an aboveground herbivore has on a belowground herbivore; few have investigated the reverse relationship (Johnson *et al*., 2012a). Emerging patterns in above–belowground interaction outcomes may also be a result of this different emphasis of various studies. Many studies, for example, have been carried out on root-chewing insects, interacting with either leaf-chewing or sap-sucking aboveground insects; other guilds have been largely ignored (e.g. stem borers). Above–belowground interactions are not just limited to insect herbivores. There are also a number of studies investigating the relationship between belowground herbivorous nematodes and aboveground insect herbivores, with both negative (Bezemer *et al*., 2005) and positive (Kaplan *et al*., 2008b) results reported.

A recent meta-analysis (Johnson *et al*., 2012a) determined that the sequence of herbivore arrival on the plant was the most important factor governing the consequence of above–belowground herbivore interactions. In general, belowground herbivores had a positive effect on aboveground herbivores, but only when arriving simultaneously on the plant. When root feeders arrived either before or after the aboveground herbivores, the effect was neutral. More specifically, belowground herbivores had no significant effects on aboveground herbivores in a number of performance parameters (e.g. relative growth rate, development time, mass or size gain, fecundity, abundance and offspring mass). Reciprocally, aboveground herbivores had a negative effect on belowground herbivore survival and a positive effect on population growth rate, whilst mass, abundance and fecundity all remained unaffected.

Johnson *et al*.'s meta-analysis also found insect order to be an important factor in determining above- and belowground herbivore interaction outcomes. For example, if the belowground herbivore was a Diptera, it had a negative effect on aboveground herbivores. Coleoptera belowground herbivores, on the other hand, had a positive effect on aboveground Homoptera, but no significant effect on Lepidoptera, Coleoptera, Diptera and Hymenoptera. Exploring the reciprocal interaction (e.g. aboveground herbivore effects on belowground Coleoptera), only aboveground

Coleoptera had a negative effect. All other taxonomic groups showed neutral effects. The outcomes of aboveground–belowground herbivore interactions also tended to be influenced by the "type" of study. Aboveground herbivores had a negative influence on belowground herbivores in a laboratory context, but there was no relationship in field studies. In contrast, aboveground herbivores remained unaffected by belowground herbivores, regardless of study type. The type of plant species mediating the interaction also influenced the interaction outcome. Aboveground herbivores negatively affected belowground herbivores when attacking an annual, but not a perennial, plant species. Plant type had no impact on the effect of belowground herbivores on aboveground herbivores.

In nature, different plant genotypes, and resulting diversification of phenotypes, can alter plant–herbivore interactions (Kumschick *et al.*, 2013). Similarly, crop plants often exist as multiple cultivars, each possessing individual characteristics that make them more suitable for certain environments/conditions than others. In raspberry (*Rubus idaei*), for example, cultivars vary in their susceptibility to aphid infestation. This resistance can arise from increased number of trichomes, thicker leaf cuticle or the possession of resistance genes (Knight *et al.*, 1959; Keep and Knight, 1967; Hall *et al.*, 2009; McMenemy *et al.*, 2009; Martin and Johnson, 2011). Host-plant susceptibility appears to play a large role determining the outcome of above- and belowground herbivore interactions (Moran and Whitham, 1990). When feeding on susceptible plants, for example, aboveground galling by the aphid *Hayhurstia atriplicis* reduced root-feeding aphid *Pemphigus betae* numbers by 91%. When feeding on a resistant plant, however, *H. atriplicis* populations were reduced and had no effect on *P. betae* populations (Moran and Whitham, 1990). This suggests that both above- and belowground plant defence mechanisms are inherently linked, as are the herbivores that are affected by these defensive compounds (Kaplan *et al.*, 2008a).

Relationships between above- and belowground biota are not restricted to interacting herbivores; adjacent and higher trophic levels may also be affected (Scheu, 2001; Van der Putten *et al*., 2001; Van Tol *et al*., 2001; Van Dam *et al*., 2003; Wardle *et al*., 2004b; Bezemer *et al*., 2005; Soler *et al*., 2005; Rasmann and Turlings, 2007; Soler *et al*., 2009; Megías and Müller, 2010; Heil, 2011). Belowground herbivory has been shown, through altered plant quality, not only to affect the oviposition behaviour of butterflies (Soler *et al*., 2010), but also various parasitoids attacking the herbivore (Masters *et al*., 2001; Soler *et al*., 2005; Rasmann and Turlings, 2007). Similarly, detritivores may also affect aboveground herbivore performance (Scheu *et al*., 1999; Bonkowski *et al*., 2001; Newington *et al*., 2004; Megías and Müller, 2010). For instance, in one study system detritivores decreased aphid and foliar herbivore abundance aboveground (Megías and Müller, 2010).

The importance of combining both above- and belowground elements has already been highlighted as a means of improving our understanding of multitrophic ecology and evolution (Van der Putten *et al*., 2001; Johnson *et al*., 2008; Soler *et al*., 2009), and the extent to which one trophic factor influences the other via the host-plant. As implied earlier, above–belowground interactions are affected by a range of anthropogenic factors. Of these, global climate change is considered a major player (Van der Putten *et al*., 2004; Staley and Johnson, 2008); the next section considers the effect of one of the causal factors of climate change, enhanced atmospheric carbon dioxide levels, on above–belowground interactions.

1.4 Effects of elevated CO_2 on plants and herbivore interactions

Since the Industrial Revolution, atmospheric CO_2 concentrations ([CO_2]) have risen by around 30% from 280 ppm to 400 ppm (IPCC, 2013). This has been attributed mainly to an increase in anthropogenic activity, notably increased fossil fuel consumption and deforestation, and, to a lesser extent, increased agriculture and land-use changes. The recent IPCC predictions suggest that global CO_2 concentrations will increase further from the current level of 400 ppm to between 540-970 ppm by 2100, depending on various emission scenarios (IPCC, 2013). These predictions are based on the carbon cycle data and climate drivers mentioned previously (e.g. fossil fuel usage). Based on these socio-economic “storylines”, a number of different climatic scenarios have been modelled. Conservative models (e.g. B2) are based on atmospheric CO_2 stabilising and continuing at current levels for the next century. Other models (e.g. A2) predict atmospheric CO_2 concentrations, based on an increasing rate of carbon cycling (IPCC, 2013). This section of the introduction addresses the empirical evidence for the effects of elevated atmospheric CO_2 on plants, and how it has been shown to affect the herbivores that feed upon them. It then continues to look at the effect this may have on herbivore–herbivore interactions, specifically above–belowground herbivore interactions.

1.4.1 Effects of elevated CO_2 on plants

Carbon dioxide is a major driving force in changing biotic activity (Fajer, 1989) and there are numerous reviews of the effects of CO_2 on plants (Bazzaz, 1990; Kimball *et al*., 1993; Rogers and Dahlman, 1993; Robinson *et al*., 2012). This section concentrates on the empirical evidence describing changes to plant chemistry and physiology that may affect herbivores and above–belowground species interactions. It is widely accepted that elevated CO_2 benefits plants, but there are major quantitative differences in effect size (Ainsworth and Long, 2005). Plants grown in elevated CO_2 environments tend to have a significantly larger biomass, with increased C:N ratios and decreased nitrogen concentrations in their tissues. Concentrations of tannins and other phenolic compounds are also enhanced (Stiling and Cornelissen, 2007). Carbon dioxide enrichment often increases plant productivity (Bazzaz, 1990), both below- and aboveground. Elevated CO_2 increases root growth and root exudation in a wide range of plant species including trees (Norby, 1994; Janssens *et al*., 1998), legumes (Johnson and McNicol, 2010) and grasses (Stulen and den Hertog, 1993). Increased photosynthesis aboveground increases the availability of nutrients which, in turn, allows greater exploratory growth of roots. Elevated CO_2 alters host-plant quality by decreasing leaf nitrogen concentration (through dilution, as there is more tissue but the same amount of nitrogen), whilst increasing both carbohydrate and phenolic concentrations (Robinson *et al*., 2012). The C:N ratio can also increase because less nitrogen is transferred to ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), an enzyme that plays a major part in carbon fixation (Newman *et al*., 2011). This occurs because the efficiency of RuBisCo to convert CO_2 to carbohydrates increases in a higher atmospheric CO_2 environment, therefore fewer RuBisCo molecules need to be synthesised (Jacob *et al*., 1995). RuBisCo constitutes a large component of plant nitrogen, therefore if there is less RuBisco, plant nitrogen will be lower, thus increasing plant C:N (Jacob *et al*., 1995). Plant water content remains unaltered by elevated CO_2 (Bezemer and Jones, 1998). Elevated CO_2 has been found to increase plant uptake of phosphates from the soil (Lambers, 1993), a consequence, at least in part, of elevated CO_2 increasing the number of mycorrhizae and rhizosphere bacteria (Lambers, 1993).

Although atmospheric CO_2 enrichment usually stimulates plant growth and yield (Rogers and Dahlman, 1993), the growth of both roots and shoots can be disproportionate (Rogers *et al*., 1995). The root to shoot ratio in crop plants has been shown to increase in 59.5%, remain unchanged in 3% and decrease in 37.5%, of studies under conditions of elevated CO_2. Plant growth and yield have typically increased more than 30% with a doubling of CO_2 concentration (Rogers *et al*., 1995). Several studies have suggested that under conditions of water-stress, the CO_2 growth stimulation is as large, or even larger, than under well-watered conditions (Stulen and den Hertog, 1993). The direct CO_2 effect will compensate, at least to a degree, for a hotter drier climate under predicted future conditions (Rogers and Dahlman, 1993; Rogers *et al.*, 1995).

Elevated CO_2 not only affects plant development aboveground, but also growth and morphology belowground. Enhanced atmospheric CO_2 leads to an increase in root production, length and biomass (Stulen and den Hertog, 1993). For example, seedlings of *Pinus echinata* grown in elevated CO_2 allocated proportionally more photosynthate to fine roots, produced larger fine root mass and had higher mycorrhizal density than plants grown in ambient CO_2 (Norby *et al.*, 1987). Elevated CO_2 can stimulate root growth and activity, and provide a positive feedback on plant growth (Norby, 1994), along with an increase in short-term root exudation (Norby *et al.*, 1987) and increased root respiration rates (Janssens *et al.*, 1998).

1.4.2 Effect of CO_2 on herbivore-plant interactions

While the predicted increase in atmospheric CO_2 may not directly affect insect herbivores, it is likely to affect them indirectly through alterations in host-plant fitness (Bazzaz, 1990). Insect herbivore responses to elevated CO_2 have shown both increases and decreases in abundance (Watt *et al.*, 1995; Bezemer *et al.*, 1999; Hughes and Bazzaz, 2001). Insect responses to elevated CO_2 are not general, and many plant–insect interactions may be unique and unpredictable. Some differences can, however, be explained, with insects responding positively to elevated CO_2 levels, until they become nitrogen–limited (Newman *et al.*, 2003).

As productivity of C_3 plants increases under elevated CO_2 conditions (Sudderth *et al.*, 2005), so does the intensity of feeding of their associated herbivores (Lincoln *et al.*, 1986). Nitrogen levels are often a limiting factor in ecosystems, restricting both plant growth and herbivore abundance (Bazzaz, 1990). Elevated CO_2 decreases foliar nitrogen concentrations and, as a result, herbivores tend to increase their consumption rate (Bazzaz, 1990).

Populations of some phloem-feeders may not be affected adversely by increased CO_2 concentrations (Hughes and Bazzaz, 2001; Gao *et al*., 2008; Sun and Ge, 2011); in other cases, they benefit (Awmack *et al*. 1997; Bezemer and Jones, 1998). Aphids, for example, may be able to compensate for changes in host-plant quality caused by elevated CO_2 by altering feeding behaviour or by synthesising amino acids via symbionts (Hughes and Bazzaz, 2001). There is little evidence that aphid herbivory, even at high levels, will substantially modify the response of plants to elevated CO_2 (Hughes and Bazzaz, 2001), for instance, by reducing plant biomass gains at elevated CO_2. Moreover, comparisons between different experimental systems (field versus laboratory) show that long-term aphid population responses to elevated CO_2 cannot be reliably predicted from detailed measurements on individual aphids (Bezemer *et al*., 1999).

Another way that herbivores might be affected by elevated CO_2 is increased development time and slower overall growth (Traw *et al*., 1996). This slower growth has, however, been predicted to reduce herbivore fitness because of an increased exposure time to potential predators (Bale *et al*., 2002; Thomson *et al*., 2010). Insect herbivores may also not be able to complete their seasonal development within a given time period (Bale *et al*., 2002). It may be the case that the predicted negative effects of elevated CO_2 on herbivores are mitigated by an increase in temperature that is also associated with climate change (Zvereva and Kozlov, 2006).

Stiling and Cornelissen (2007), in a meta-analysis, highlighted the general trends of the effect of elevated CO_2 on plant–herbivore interactions. Increased CO_2 significantly increases both relative plant consumption rates and total consumption by certain herbivores, for instance the beetle, *Phratora vitellinae* (Veteli *et al*., 2002); this is coupled with an extended herbivore development time, mainly due to an altered C:N ratio. Elevated CO_2 also significantly decreases herbivore abundance, relative growth rate, conversion efficiency and pupal weight (Stiling and Cornelissen, 2007). An increase in the accumulation of phenolic compounds in plants has also been predicted when they are grown in elevated CO_2 (Robinson *et al*., 2012). This occurs as a result of limited nutrients, rather than as a direct effect of elevated CO_2 (Lambers, 1993). Elevated CO_2 stimulates photosynthesis, and this increase in carbon production appears to dilute leaf protein concentrations (Lincoln, 1993). This may change the feeding behaviour of some insects under increased CO_2, but these changes vary considerably across species (Knepp *et al*., 2005). For example, the same herbivore can respond differently to two closely–related plant species under elevated CO_2; the relative growth rate of gypsy moth larvae (*Lymantria dispar*) feeding on yellow birch (*Betula allegheniensis*) grown in elevated CO_2 was lower than for those on grey birch (*B. populifolia*) (Traw *et al*., 1996). Like many studies, conflicting evidence has been identified and elevated CO_2 has also been shown to have no significant impact on herbivore fitness (Díaz *et al*., 1998).

Phenology, the timing of events in the natural environment, is susceptible to climate change. This has mainly been attributed to increased atmospheric temperature (a result of increased atmospheric CO_2) decreasing the number of degree days required to attain certain stages of development (Bale *et al*., 2002). This has been observed for a wide range of species across many taxa (Gordo and Sanz, 2005; Pearce-Higgins *et al*., 2005; Thackeray *et al*., 2010). Moreover, these changes in phenology have been observed to affect species' interactions, as climate change can have disproportionate effects on different trophic levels (Voigt *et al*., 2003). The effect of altered phenology can lead species interactions to become decoupled, affecting the organisms involved in a negative way (Hunter, 1990). In extreme cases, this decoupling may lead to an increased extinction risk (Singer and Parmesan, 2010). Some species interactions are affected by this alteration in synchrony, whereas others remain relatively unaffected with different trophic levels having reacted proportionally to the environmental regime shift (De Lucia *et al*., 2012). Organisms also have the potential to adapt to this change in phenology; for the winter moth (*Operophtera brumata)* (Van Asch *et al*., 2007) feeding on oak, although the moth phenology was altered by increased temperature, the synchrony between the herbivore and the oak remained intact (Buse and Good, 1996). It is not just increased temperature that can cause alterations in phenology. Increased CO_2 can bring forward flowering phenology with its associated effects on nectar production; this could potentially affect pollinators that rely on this resource as a food source (Erhardt and Rusterholz, 1997).

Changes in host-plant quality arising from CO_2-enrichment of the atmosphere tend to affect insect herbivores differently according to their mode of feeding (Robinson *et al*., 2012). Phloem-feeders and leaf-scrapers are the only two insect guilds to show a positive response to elevated CO_2, other feeding guilds show either a negative or null response (Robinson *et al*., 2012). Population sizes of these two guilds generally increased in elevated CO_2 and development time of phloem-feeders was reduced by 17% (Bezemer and Jones, 1998). In contrast, Stiling and Cornelissen (2007) concluded that no significant differences were observed among insect herbivore guilds in their response to elevated CO_2.

Compared with what is known of the effects of elevated CO_2 on crop physiology and yield, little is known of crop–pest interactions under predicted climate change scenarios (Gregory *et al*., 2009). The prevalence of some insect pests is predicted to change under conditions of climate change; with milder winters, pest species are expected to increase their range by colonising more northerly latitudes and higher altitudes (Cammell and Knight, 1992). Elevated CO_2 may accelerate the breakdown of crop resistance to insect pests by compromising expression of resistance genes. Martin and Johnson (2011) found that when investigating the effects of elevated CO_2 on raspberry, plant growth rates significantly increased regardless of cultivar. There is also some evidence suggesting that raspberry plants containing the A_1 gene, which confers some resistance to aphid herbivory, were more susceptible to aphid attack when grown in an elevated CO_2 environment. In these conditions, aphid population size doubled and individuals were 38% larger compared with those at ambient CO_2. Aphid performance on plants containing the A_1 gene grown at elevated CO_2 was therefore similar to that of aphids reared entirely on susceptible plants under either CO_2 treatment, suggesting resistance had been overcome at elevated CO_2. Aphids feeding on another cultivar possessing the more effective A_{10} resistance gene, however, showed no response to elevated CO_2, illustrating that different raspberry cultivars may fare differently under a future climate. Other studies have also illustrated that CO_2 does not alter plant resistance to herbivory (e.g. Lau and Tiffin, 2009). These latter authors found that plants grown in elevated CO_2 are generally less tolerant to herbivory and are unable to compensate for herbivory in terms of biomass loss compared to ambient plants.

Limited research has been conducted on the effects of elevated CO_2 on root herbivores and belowground processes. Soil organisms are, to a large extent, buffered by the physical nature of the soil environment and are therefore less susceptible to changes in climate than organisms aboveground (Staley and Johnson, 2008). As CO_2 concentrations are already high in the soil (up to 1000 ppm), mainly due to natural belowground processes, such as root respiration (Parry, 1992; Rogers *et al*., 1995; Staley and Johnson, 2008; Bardgett and Wardle, 2010), predicted changes in atmospheric CO_2 may not have such a strong effect on soil biota as on aboveground organisms. Elevated CO_2 has, however, been shown to increase root growth (Norby *et al*., 1987; Bazzaz, 1990; Rogers *et al*., 1995; Janssens *et al*., 1998), suggesting that elevated CO_2 would increase food resources for root herbivores. Specifically, the stimulation of lateral root growth by elevated CO_2 (Janssens *et al*., 1998), which are typically relatively fine structures may benefit smaller or early instar root-feeders. Other abiotic factors may have greater direct effects on soil invertebrates than elevated CO_2. Many invertebrates are, for instance, highly sensitive to desiccation (Zvereva and Kozlov, 2006; Staley *et al*., 2007), hence increased temperatures and other weather extremes arising from climate change may be more detrimental than elevated CO_2 itself. Increased temperatures have, for example been shown to increase mortality, decrease distribution and affect the phenology of belowground herbivores (Staley and Johnson, 2008).

Of the few studies that have explored the effects of elevated CO_2 on root feeders, one showed that populations of vine weevil larvae feeding on the roots of raspberries in elevated CO_2 decreased in number by approximately 33%, and their body mass by 23% (Johnson *et al*., 2010). This was attributed to a decrease in root biomass of 16% in elevated CO_2 conditions. The study also noted that weevils induced an increase of root phenolic concentrations at ambient CO_2, but a decrease at elevated CO_2, which also may explain the negative effect on weevil larvae observed.

Even fewer studies have investigated the consequences of climate change for above–belowground herbivore interactions; those that do, provide no evidence of a directed response. For example, the interaction between the root-feeding (*Pemphigus populitransversus*) and shoot-feeding (*Aphis fabae fabae*) aphids*,* on *Cardamine pratensis* was unaffected by CO_2 (Salt *et al*., 1996). Root herbivore populations were always smaller in the presence of an aboveground herbivore regardless of the CO_2 environment. A second study investigated the conspecific interaction between aboveground adults and belowground larvae of the clover root weevil (*Sitona lepidus*) (Johnson and McNicol, 2010). Elevated CO_2 increased leaf consumption (due to compensatory feeding) by adult weevils but resulted in lower rates of oviposition. Despite reduced rates of oviposition, larval survival was much greater at elevated than at ambient CO_2 levels, potentially due to increased nodulation (increased food source) of the host-plant (*Trifolium repens*) under elevated CO_2 conditions.

1.5 Herbivore–plant study system

Historically, plant–herbivore interactions have been studied in both field and laboratory environments. Each approach has its merits and downfalls. Laboratory microcosm experiments are a way of studying organisms in highly controlled environments, and therefore, although artificial, offer a number of advantages over field studies. Their easily replicable design makes them ideal for experiments investigating above–belowground interactions. Microcosms have been criticised as not being truly representative of the field. It is important to note that the objective of microcosm experiments is not necessarily to replicate “natural” conditions, but to identify interactions and mechanisms in a simplified and controlled manner (Lawton, 1998). The results from such work can then be tested under natural conditions to assess the reliability and relevance of the findings.

*1.5.1 Raspberry (*Rubus idaeus *L.)*

According to the Food and Agriculture Organisation of the United Nations, the United Kingdom is currently the sixth largest global producer of raspberries, making it an important crop for the UK economy. Over the past 20 years, raspberry production has almost doubled, but the area farmed for the crop has decreased by 66% (FAO, 2014). The fact that raspberry farms have become fewer in number but larger in area, may make them more susceptible to pest outbreaks which will consequently spread quicker throughout the crop. This emphasises the need for greater understanding of the potential impacts of pests on the crop under more natural conditions. Raspberry plants (Figure 1.3) also exist as a number of cultivars that possess resistance genes making some varieties less susceptible to herbivory than others (Hall *et al*., 2009). These cultivars have fared well at reducing the number of pest species, including the large raspberry aphid, which can successfully feed on them. The exact mechanism behind the resistance is still unknown, but it is thought to be associated with the thickness of leaf epicuticular wax (McMenemy *et al*., 2009).

Figure 1.3: Raspberry (*Rubus idaeus*) plant showing above- and belowground tissue.

*1.5.2 European Large Raspberry Aphid (***Amphorophora idaei*** Börner)*

As its name suggests, the large raspberry aphid (Figure 1.4) is an obligate feeder on raspberry including both the sexual and asexual stages of its lifecycle, with peak populations occurring between June and August (McMenemy *et al*., 2009). It is the most economically important pest in raspberries (McMenemy *et al*., 2009) and it can reduce plant vigour (Gordon *et al*., 1997). In general, aphids have a rapid rate of natural population increase and are highly sensitive to changes in host-plant quality. They are consequently ideally adapted to exploit changes in the environment brought about through climate change. Their high reproductive rates, short generation time and ability to colonise new plants or regions rapidly suggests that under favourable conditions their impact as a pest species could worsen (Dixon, 1973). Milder winters in temperate regions (a predicted outcome of climate change) may increase the survival rate of overwintering aphid adults and, in turn, increase the potential number of generations per year (Harrington *et al*., 2007). This higher generation turnover may lead to aphids overcoming pesticide and cultivar resistance faster (Cammell and Knight, 1992). Vine weevils have also been shown to compromise aphid resistance in some raspberry cultivars, increasing populations of *Amphorophora idaei* by 80% (McMenemy *et al*., 2009).

Figure 1.4: The European Large Raspberry Aphid (*Amphorophora idaei*).

*1.5.3 Vine weevil (*Otiorhynchus sulcatus *Fabricius)*

Many root feeders play an important role as agricultural pests. The polyphagous larvae of the vine weevil (*Otiorhynchus sulcatus*) (Figure 1.5) are a major pest species of a wide variety of plant roots and are widespread throughout temperate Europe. Adult vine weevils reproduce via parthenogenesis; meaning their offspring are therefore clonal. This makes them an ideal study species as this reduces genetic diversity and thus variation between individuals. Adults can also be maintained easily in laboratory conditions and individuals produce a large number of eggs over their lifetime (Moorhouse *et al*., 1992; Fisher and Bruck, 2004; Son and Lewis, 2005). Younger larvae tend to feed on the finer roots, with older larvae feeding on the larger, more membranous, roots closer to the base of the plant. High populations of larvae frequently destroy entire plants. This can be particularly damaging in arable fields where the close proximity and abundance of crop plants facilitates weevil spread (Moorhouse *et al*., 1992). Oviposition choice by adults is the main determinant of larval distribution in the soil, but individuals can move short distances to fresh plant roots if required (Barnett and Johnson, 2013). The CO_2 produced by the plant acts as an attractant to the larvae. Vine weevil larvae focus their feeding on rootlets and also on the cambium tissue of larger roots near the soil surface. The root-feeding larvae are active from autumn to early spring – when they emerge as adults to oviposit (Moorhouse *et al*., 1992). Although raspberry plants can withstand higher levels of root herbivory than many other crop species (Clark *et al*., 2012b), weevil larvae feeding may still cause stunted plant growth and reduced yield. There is also potential to breed weevil-resistant cultivars of raspberry. The larvae do not feed on blackberry or black raspberry, and their reproduction cycle has been shown to be affected when feeding on Glen Prosen and Malling Leo cultivars of raspberry (Hall *et al*., 2009). It is not known how this plant–herbivore interaction may be influenced by elevated CO_2.

Root-feeding insects can have a major impact on ecosystem functioning (Johnson *et al*., 2008). Through their effect on plant performance, belowground herbivory can have marked influence on the structure, composition and growth of plant (Andersen, 1987) and herbivore (Masters *et al*., 1993; Wardle *et al*., 2004a; Wardle *et al*., 2004b; Johnson *et al*., 2012a) communities aboveground. The plant–herbivore interaction above- and belowground can be affected by anthropogenic environmental change (Wolters *et al*., 2000; Antoninka *et al*., 2009; Johnson and McNicol, 2010; González-Megías and Menéndez, 2012; Van der Putten, 2012). Using simple species systems to understand the complexities of the interaction between spatially–separated herbivores and how this is affected by external stressors, including effects mediated through the plant, may make it possible to propose testable hypotheses to investigate wider consequences for ecosystems.

Figure 1.5: Soil-dwelling larva (left) and leaf chewing adult (right) of the vine weevil (*Otiorhynchus sulcatus*).

1.6 Thesis objectives and outline

The overall aim of this thesis is to investigate how aspects of climate change affects the interaction between the aboveground feeding large raspberry aphid (*Amphorophora idaei*) and the root-feeding larvae of the vine weevil (*Otiorhynchus sulcatus*), both of which feed on raspberry (*Rubus idaeus*). The first empirical chapter (**Chapter II**) investigates the general interaction between the two spatially-separated herbivores under ambient CO_2 conditions. As well as establishing the relationship between the aphids and vine weevil larvae, the study also explores whether the interaction changes as the herbivores feed on multiple cultivars of raspberry that vary in their resistance to herbivory. Specifically it sets out to test the hypothesis that reciprocal facilitation (identified by increased abundance for both herbivores in the presence of the other), occurs between an aboveground and belowground herbivore, mediated by a host-plant that varies in susceptibility to herbivory. An adaptation of this chapter has been published in *Biology Letters* (McKenzie, S. W., Vanbergen A. J., Hails, R. S., Jones, T. H. and Johnson S. N. (2013) Reciprocal feeding facilitation between above- and belowground herbivores. *Biology Letters* **9:** 20130341).

Climate is known to alter insect phenology and this, in turn, can have marked consequences on interacting organisms, particularly when it leads to asynchrony between species. Moreover, the order in which herbivores arrive on a plant has a major influence on interaction outcomes between competing herbivores. In **Chapter III** the experimental work progresses to investigate the effect of altering the timing of herbivore arrival on the plant. Specifically this study tests the hypothesis that aphid abundance and population growth rate would be greatest when herbivore treatments replicated natural phenology (i.e. weevil larvae chewing on roots before the onset of aphid feeding).

In **Chapter IV**, the effect of elevated atmospheric CO_2 on the interaction between the two herbivore species was investigated. Increasing atmospheric CO_2 concentrations are predicted to affect both plant productivity and chemistry. This has cascading effects on herbivores that consume the plant, and has the potential to alter the interaction between heterospecific herbivores feeding on the same host-plant. Given what is known about plant–herbivore interactions and elevated CO_2, this experiment aimed to test whether plants that are resistant to herbivores will become more susceptible under elevated CO_2. It was also hypothesised that this effect will be exacerbated when grazed by both herbivore species simultaneously.

Elevated CO_2 has also been shown to alter leaf palatability to herbivores. **Chapter V** focuses on adult vine weevils and their feeding preferences for leaves grown under ambient and elevated CO_2 conditions. Leaves were presented to vine weevils and the degree of herbivory assessed. It was hypothesised that leaves would have an increased C:N ratio and be tougher in elevated CO_2 than in ambient levels. Based on this it was predicted that vine weevils will consume more leaf tissue from plants grown in elevated CO_2 than in ambient, to compensate for their inferior nutritional quality. When given a choice, it was hypothesised that vine weevils would show a feeding preference for leaves grown in ambient conditions, over those grown in elevated CO_2; this again, was based on their inferior nutritional quality.

Chapter VI investigates the feeding preferences of root herbivores, in the presence and/or absence of aboveground aphids, and the consequential effects of herbivory for intraspecific plant competition. This chapter sets out to test the hypothesis that plants infested with aphids aboveground will be more susceptible to belowground herbivory, due to the feeding facilitation observed previously. Consequently, root mass will be reduced to a greater extent compared with plants without aphids. It was hypothesised that this insect–plant interaction would cause aphid-free plants to dominate aphid-infested plants. Finally it was hypothesised that plant compensatory growth under elevated CO_2 conditions will mitigate the effects of insect herbivory on plant competition. The importance of herbivores and above–belowground interactions as regulators of plant competition are highlighted.

Chapter VII provides a synthesis of current knowledge specifically on how climate change effects above–-belowground insect interactions. It reviews current literature on the effects of climate change on above–belowground interactions, and identifies research gaps for future experimentation. An adaptation of this chapter has been published in *Frontiers in Plant Science* (McKenzie, S. W., Hentley, W. T., Hails, R. S., Jones, T. H., Vanbergen, A. J., and Johnson, S. N. (2013) Global climate change and above- belowground insect herbivore interactions. *Frontiers in Plant Science* **4:** 412).

In the final chapter, **Chapter VIII**, the results of the empirical studies are discussed and placed in a wider ecosystem context. The chapter also attempts to explain the importance of investigating the effects of climate change on above–belowground interactions.

Chapter II

Reciprocal feeding facilitation between above- and belowground herbivores

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An adaptation of this chapter has been published in *Biology Letters*

McKenzie, S. W., Vanbergen A. J., Hails, R. S., Jones, T. H. and Johnson S. N. (2013) Reciprocal feeding facilitation between above- and belowground herbivores. *Biology Letters* **9:** 20130341.

2.1 Abstract

Interspecific interactions between insect herbivores predominantly involve asymmetric competition. In contrast, facilitation, whereby herbivory by one insect benefits another via induced plant susceptibility, is uncommon. Positive reciprocal interactions between insect herbivores are even rarer. Here, a novel case of reciprocal feeding facilitation between aboveground aphids (*Amphorophora idaei*) and root-feeding vine weevil larvae (*Otiorhynchus sulcatus*), attacking raspberry (*Rubus idaeus*) is revealed. Using two raspberry cultivars with varying resistance to these herbivores, it is further demonstrated that feeding facilitation occurred regardless of host-plant resistance. This positive reciprocal interaction operates via an, as yet, unreported mechanism. Specifically, the aphid induces compensatory growth, possibly as a prelude to greater resistance/tolerance, whereas the root herbivore causes the plant to abandon this strategy. Both herbivores may ultimately benefit from this facilitative interaction.

2.2 Introduction

Plant-mediated interactions between insect herbivores feature prominently in most terrestrial ecosystems (Denno *et al*., 1995). Above- and belowground insect herbivores attacking the same plant can affect each other's performance (Bardgett and Wardle, 2003; Johnson *et al*., 2008) via induced changes in plant architecture (Price *et al*., 2011), allocation of primary metabolites (Johnson *et al*., 2009) or chemical defences (Bezemer and Van Dam, 2005). Such plant-mediated herbivore interactions can also have consequences for higher trophic levels (Soler *et al*., 2005).

While competition dominates plant-mediated herbivore interactions (Denno *et al*., 1995; Bezemer and Van Dam, 2005), facilitation – whereby herbivory by one species benefits another – has been reported in only 11% of interspecifc interactions between aboveground herbivores (Denno *et al*., 1995). Such facilitative interactions, however, tend to be asymmetric, benefiting only a single species (Johnson *et al*., 2012a). There is even less evidence that reciprocal facilitation between herbivore species occurs frequently (Van Dam and Heil, 2011). This lack of evidence for reciprocal facilitation may have arisen because many above–belowground studies focus on aboveground herbivore performance, while belowground herbivore performance remains under-reported (Kaplan *et al*., 2008b).

In a microcosm experiment, the hypothesis that reciprocal facilitation, identified by increased insect abundance, would occur between an aboveground (large raspberry aphid *Amphorophora idaei*) and belowground (vine weevil *Otiorhynchus sulcatus*) herbivore, interacting via a host-plant (raspberry *Rubus idaeus* L.) that varies in susceptibility to herbivory was tested. This experiment mimics the natural phenological succession of these herbivore species on the plant in field situations. Weevils over-winter on plants in all life-stages (McMenemy *et al*., 2009), whereas aphids over-winter as eggs and do not feed on the plant until the growing season is underway (Clark *et al*., 2011); consequently weevil herbivory generally precedes aphid herbivory.

2.3 Materials and methods

Two raspberry cultivars varying in susceptibility to both experimental herbivores (Glen Ample: highly susceptible; Glen Clova: moderately susceptible) (McMenemy *et al*., 2009; Clark *et al*., 2011; Clark *et al*., 2012a) were challenged with *A. idaei* and *O. sulcatus*. Each replicate plant (grown from rootstock at James Hutton Institute (JHI), Dundee, UK) was established in a rhizotube (40 cm plastic cable trunking containing 2:1 compost: sand (see Clark *et al*., 2012a) that allowed access to roots and weevils. Insects were obtained from cultures at the JHI (McMenemy *et al*., 2009; Clark *et al*., 2011; Clark *et al*., 2012a).

The experiment ran in a climate- (day 20°C ± 2°C; night minimum 10°C ± 2°C) and photoperiod- (16:8 Light :Dark) controlled glasshouse for 10 weeks. Experimental treatments applied to 48 replicates of each raspberry cultivar comprised: a control (no insects), a single herbivore (‘weevil’ or ‘aphid’) or weevil and aphid together (‘combination’) (*N* = 12 each). Eight plants were randomly assigned to spatial blocks, each a full replicate of every insect treatment–cultivar combination (Week 0). In Week 4, replicates randomly assigned to ‘weevil’ and ‘combination’ treatments were inoculated with 20 weevil eggs, with eclosion occurring in Week 6. Three adult aphids were added to each ‘aphid’ and ‘combination’ replicate in Week 8. Plant height was recorded at Week 0 and again at Week 10. Insects were counted and plant biomass oven-dried (80° C for 24 hours) and weighed in Week 10.

Data were analysed using generalised linear mixed models (GLMM) with insect (aphid or weevil larvae) counts and plant biomass (above- or belowground dry weight), modelled with Poisson and Gaussian error distributions, respectively (PROC GLIMMIX, SAS Institute). Parameter estimation used Restricted Maximum Likelihood

(REML) for plant biomass and pseudo-likelihood for insect counts. Replicate plant nested within spatial block was specified as a random effect and, for aphid counts, an observation-level random component was included to account for overdispersion (Elston *et al.*, 2001). Cultivar (categorical) was fitted to all models. Models of weevil and aphid responses also included the abundance of the co-occurring herbivore (‘combination’ treatment only). Above- and belowground plant biomass were always fitted to models of insect abundance. Models of the above- or belowground plant biomass response did not, however, include the corresponding biomass measure as an explanatory term because they were strongly positively correlated ($P < 0.0001$, r = 0.89). Plant height at Week 0 was fitted to all models to account for initial between-replicate variation in growth. Models underwent forward stepwise selection until a minimum adequate model was obtained. Statistical significance of main effects are always reported, whereas two-way interactions are reported only where $P < 0.05$. Degrees of freedom were estimated using the Satterthwaite approximation.

Partial residual plots were constructed to show the influence of particular explanatory variables on response parameters accounting for other significant terms retained in the model. Gaussian and Poisson models used raw and standardised (residuals/fitted) values, respectively (Cook and Croos-Dabrera, 1998).

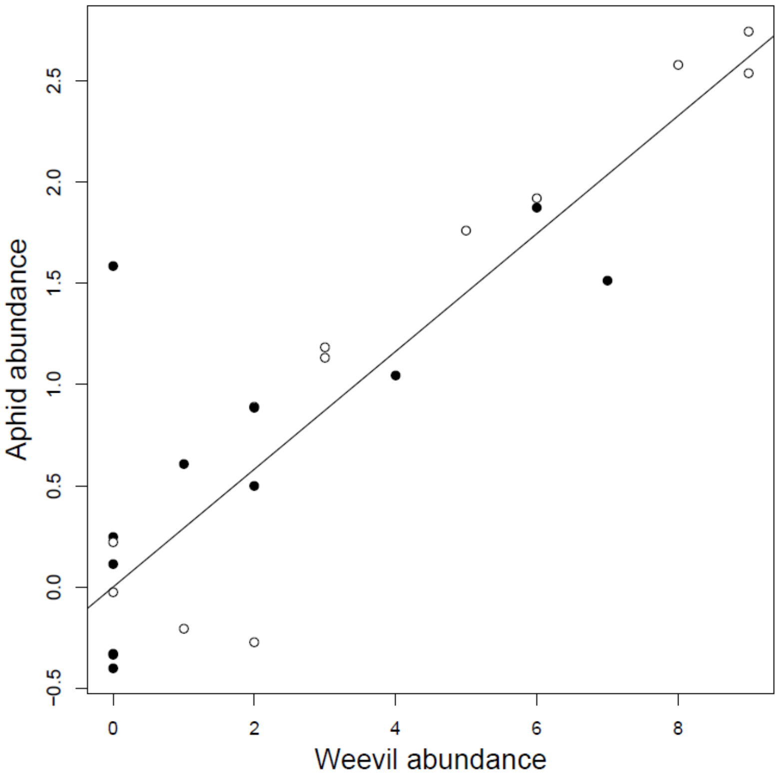
2.4 Results

Aphid and weevil abundance were positively correlated (Figure 2.1a & b; Table 2.1). This relationship suggests reciprocal feeding facilitation, although weevil abundance exerted a larger positive effect on aphid densities than *vice versa* (Figure 2.1a & b; Table 2.1). Aphid abundance also increased with aboveground plant biomass, indicating an effect of the plant resources (Table 2.1). While the positive effect of weevil abundance on aphid abundance occurred regardless of aboveground plant biomass, it was reduced when aboveground biomass was low (Figure 2.1c; Table 2.1:

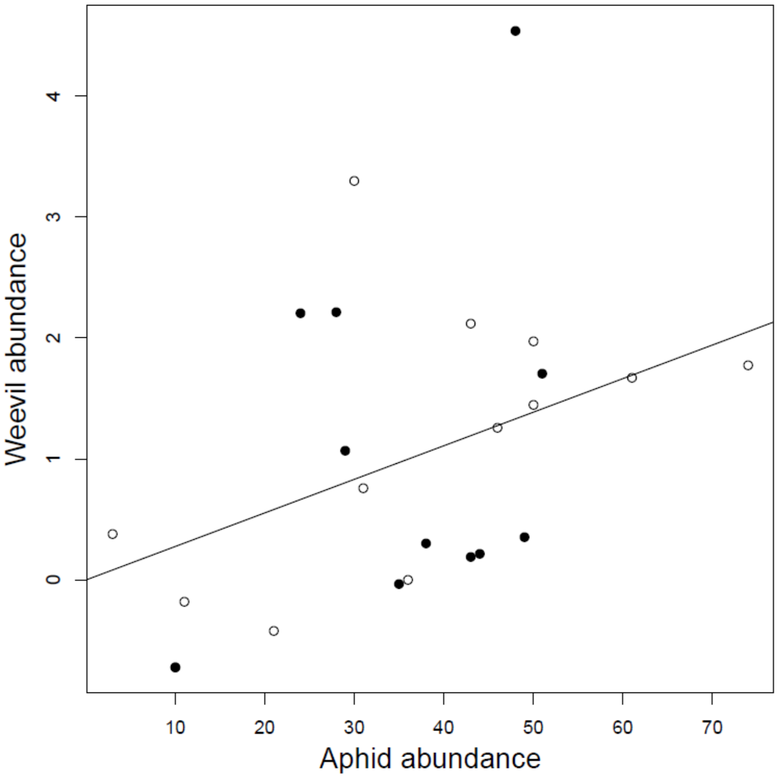
Aboveground dry weight*weevil abundance). Cultivar did not affect aphid abundance, but these herbivores were less abundant on the moderately susceptible (Glen Clova) plants (Table 2.1).

Aboveground and belowground biomass did not vary between cultivars (Table 2.1). The herbivore species affected aboveground plant biomass differently. Greater densities of aphids (Figure 2.2a; Table 2.1) and weevil larvae (Figure 2.2b; Table 2.1) increased and decreased aboveground biomass, respectively. Greater weevil abundance reduced root biomass, whereas aphids had no effect (Table 2.1). Initial plant height did not affect the abundance of aphids ($F_{1,16} = 0.17$, $P = 0.6857$), weevils ($F_{1,15} = 0.00$, $P = 0.9456$) or final plant biomass (aboveground $F_{1,20} = 0.07$, $P = 0.8006$; belowground $F_{1,45} = 0.04$, $P = 0.8512$).

Figure 2.1a)


Aphid abundance
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
0
2
4
6
8
Weevil abundance

b)


Weevil abundance
0
1
2
3
4
10
20
30
40
50
60
70
Aphid abundance

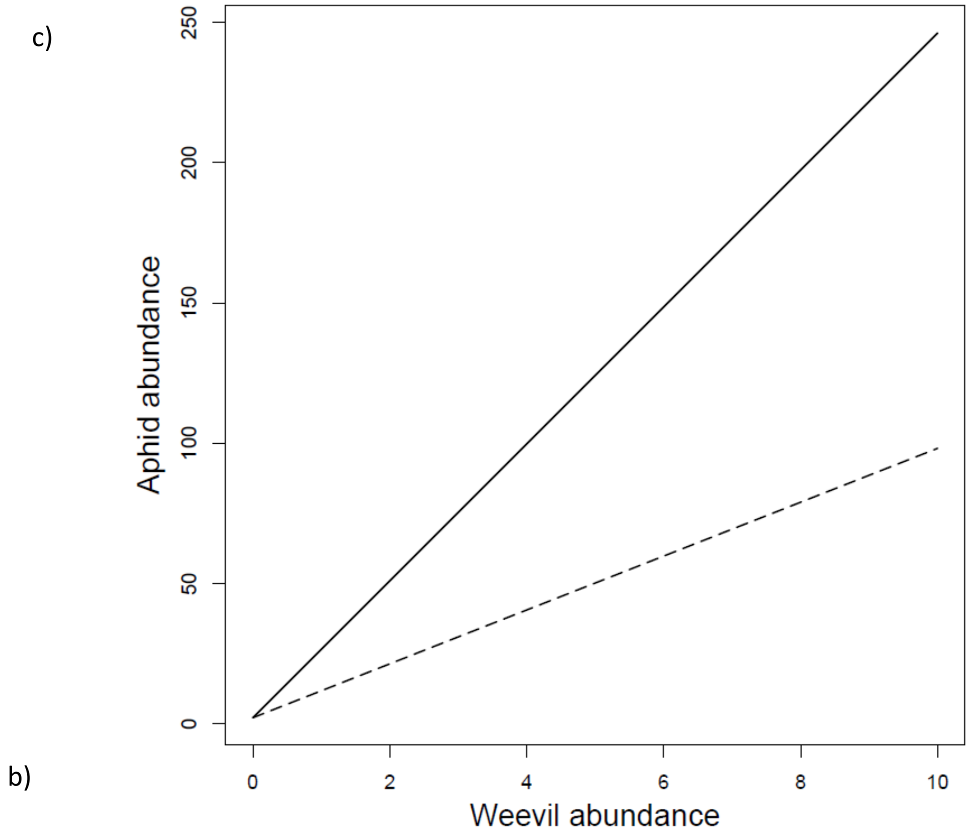


Figure 2.1: Partial residual plots on the linear predictor scale of the response of (a) aphid and (b) weevil abundance to the other herbivore concurrently feeding on a moderately (closed symbols) or highly (open symbols) susceptible cultivar in the 'combination' treatment only; (c) effect of weevil abundance on aphid abundance at modelled high (solid line) and low (dotted line) aboveground dry weight (predicted slopes (m) used when fixing aboveground dry weight at its highest and lowest value, respectively, with the modelled intercept (c) from the final model output. This equation was then applied to weevil abundance (x), giving a resultant aphid abundance value (y)).

Table 2.1: GLMM results summary for herbivore and plant response parameters. Bold type indicates parameters retained in the final model.

MPE = Multiple Parameter Estimates

Response Variable	Explanatory variables	Estimate	*F* (ndf,ddf)	*P*
Herbivore				
Aphid abundance	Cultivar	MPE	$1.78_{(1,16)}$	0.2021
Random effect estimate = 0.1766	**Weevil abundance**	**0.2910**	$\mathbf{13.79_{(1,16)}}$	**0.0021**
	Belowground dry weight	-0.03500	$0.04_{(1,14)}$	0.8513
	Aboveground dry weight	**0.5524**	$\mathbf{13.79_{(1,30)}}$	**0.0019**
	Aboveground dry weight * weevil abundance	**-0.1308**	$\mathbf{7.25_{(1,15)}}$	**0.0163**
Weevil larvae abundance	**Cultivar**	**MPE**	$\mathbf{10.53_{(1,20)}}$	**0.0041**
Random effect estimate = 0.4054	**Aphid abundance**	**0.02774**	$\mathbf{5.68_{(1,14)}}$	**0.0316**
	Belowground dry weight	-0.1991	$1.62_{(1,19)}$	0.2181
	Aboveground dry weight	-0.2310	$1.59_{(1,17)}$	0.2239
Plant				
Aboveground dry weight	Cultivar	MPE	$0.21_{(1,20)}$	0.6502
Random effect estimate = 0.5555	**Weevil abundance**	**-0.1676**	$\mathbf{4.70_{(1,21)}}$	**0.0417**
Residual variance = 0.5176	**Aphid abundance**	**0.03154**	$\mathbf{5.21_{(1,21)}}$	**0.0330**
Belowground dry weight	Cultivar	MPE	$5.01_{(1,45)}$	0.5165
Random effect estimate = 1.6410	**Weevil abundance**	**-0.1934**	$\mathbf{8.31_{(1,46)}}$	**0.0060**
Residual variance = 0.7008	Aphid abundance	0.03526	$4.18_{(1,21)}$	0.0537

Figure 2.2a)

b)

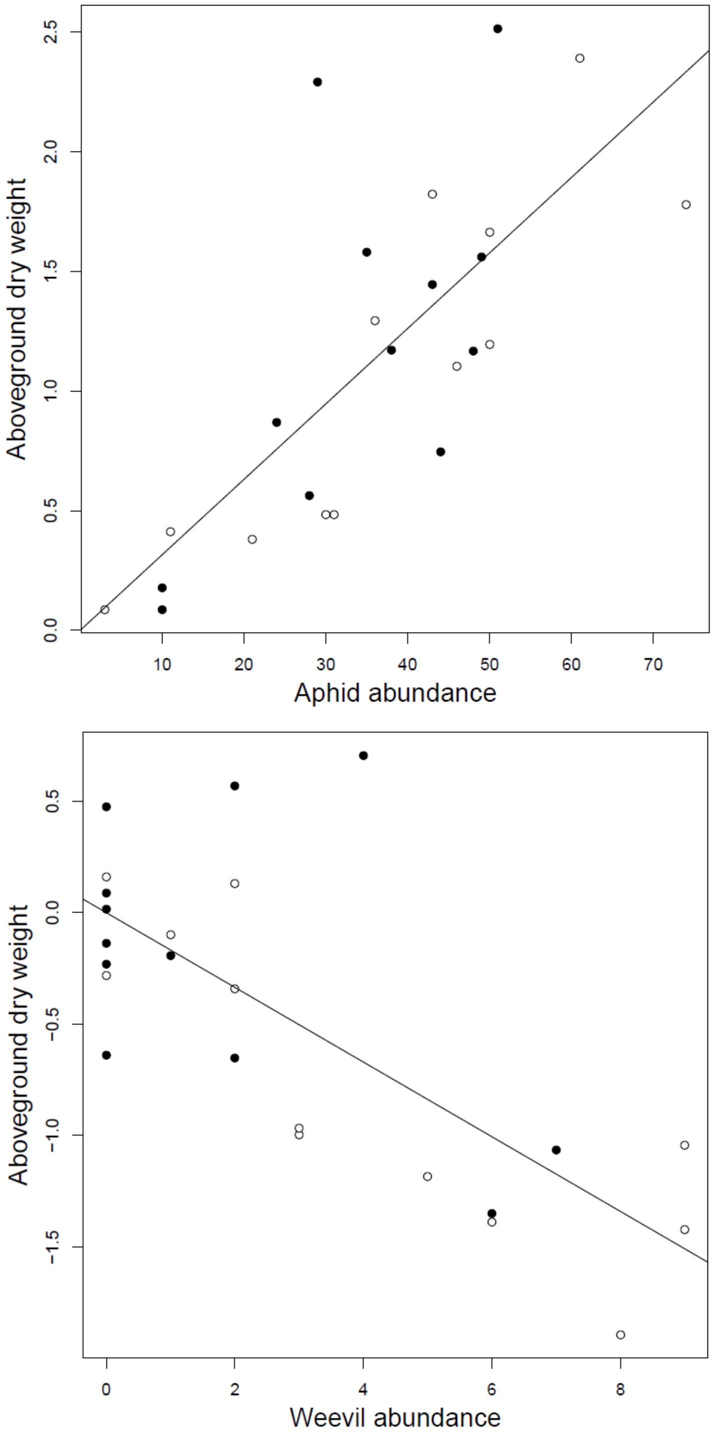


Figure 2.2: Partial residual plots on the linear predictor scale of the response of aboveground plant biomass to (a) aphid and (b) weevil abundance feeding on a moderately (closed symbols) or highly (open symbols) susceptible cultivar.

2.5 Discussion

This chapter provides compelling evidence for reciprocal feeding facilitation between root and shoot herbivores, a phenomenon that could be under-reported for above–belowground interactions (Kaplan *et al*., 2008a; Johnson *et al*., 2012a). This positive relationship between the abundances of the two herbivore species persisted despite variation in aboveground plant biomass. Although facilitative, there remained a degree of asymmetry in the interaction, with weevils exerting a much greater effect on aphid abundance than *vice versa*. While feeding facilitation has been found aboveground (e.g. Soler *et al*., 2012a), positive non-reciprocal effects of aboveground herbivores on belowground herbivores are generally scarce and only one other study (Johnson *et al*., 2009) has demonstrated reciprocal facilitation between above- and belowground herbivores. In that case, the abundance of wireworms (*Agriotes* spp.) and the aphid *Rhopalosiphum padi* feeding on barley (*Hordeum vulgare*) increased by 30% and 25%, respectively (Johnson *et al*., 2009). Over a longer time scale facilitative relationships may give way to competitive interactions (Denno *et al*., 1995), and sustained herbivory in this system may still eventually lead to competition between the two herbivores. Even a short term positive interaction may, however, impact greatly on the host-plant, because *A. idaei* is the principal vector of raspberry viruses (Clark *et al*., 2011). Therefore, the positive effects of root herbivory could have wider implications for plant pathogen transmission beyond the temporal conjunction of the herbivores.

This study suggests that root-feeding weevils reduced overall plant biomass, whereas sap-sucking aphids stimulated compensatory plant growth aboveground, suggesting that functional adaptations are key to shaping plant–herbivore interactions. Vine weevils have a large and direct impact on plant biomass by chewing and severing primary roots, compromising plant compensation by limiting water and nutrient uptake (Johnson *et al*., 2008). Stimulating plant compensatory growth is known to be an evolutionary strategy for tolerance or resistance to herbivory (McMenemy *et al*., 2009). The data suggests that aphid induction of plant compensation benefits the co-occurring, but spatially-separated, vine weevil. This concurs with other studies that showed plant growth improved *O. sulcatus* performance (Johnson *et al*., 2011; Clark *et al*., 2012b).

Induced susceptibility to aphid colonisation following root attack by beetles appears the most common above–belowground herbivore interaction (Johnson *et al*., 2012a). The potential for positive reciprocal interactions is, however, largely unknown, as few studies quantify both above- and belowground herbivore performance simultaneously (Johnson *et al*., 2012a). The sequence of herbivore arrival is often important in many above–belowground herbivore interactions (Kaplan *et al*., 2008a). Soler *et al*. (2013) suggested that inter-guild herbivore interactions are more likely than intra-guild interactions to result in positive outcomes, because the former triggers different phytohormonal pathways, potentially leading to signal cross-talk. For instance, root-feeding induces jasmonic acid which reduces the salycilic acid defence response to aphid herbivory (Soler *et al*., 2013). Other potential mechanisms are induction of ethylene or abscisic acid in leaves by root herbivory, which reduces plant resistance to aphids (Mantelin *et al*., 2009; Soler *et al*., 2013). Above–belowground interactions have a crucial role in multi-species interactions, and the reciprocal feeding facilitation between herbivores described here may be more prevalent than previously thought.

Chapter III

Sequence of herbivory does not alter facilitation between an above- and a belowground herbivore

3.1 Abstract

Early colonisation of a host-plant is one adaptive strategy enabling insect herbivores to avoid direct and indirect interspecific competition, and minimise the impact of plant secondary chemistry. Plant-mediated interactions between above- and belowground herbivores can be affected by the sequence in which herbivores colonise the plant. Climate change can modify insect development time and alter insect herbivore phenology. If this effect is disproportionate between above- and belowground herbivores, it may ultimately alter the herbivore colonisation sequence and interspecific trophic interactions. Using a microcosm approach I investigate how altering the colonisation sequence of a belowground herbivore (Vine weevil – *Otiorhynchus sulcatus*) affected the reciprocal facilitative interaction with an aboveground herbivore (Large raspberry aphid – *Amphorophora idaei*) on a shared host-plant (raspberry – *Rubus idaeus*). Two plant genotypes with high and moderate susceptibility to insect herbivory were used. Aphid population growth rate was 32% greater on the highly susceptible genotype and positively correlated with plant growth aboveground. Weevil and aphid abundance was unaffected by genotype, but was respectively negatively and positively related to aboveground plant mass. The reciprocal facilitative nature of this above–belowground herbivore interaction was shown by the positive relationship between weevil abundance and the abundance and population growth rates of aphids, with weevils increasing aphid abundance in a ratio of 1:2. Varying the sequence of belowground herbivore arrival, however, had no effect on the reciprocal facilitation between the two spatially-separated herbivores. This indicates a level of stability in plant-mediated interactions where reciprocal facilitation occurs between herbivores occupying different feeding guilds. Colonisation sequence of host-plants by herbivores may only be important when herbivores compete, occupy the same feeding guild or when chemical defensive mechanisms underpin the interaction.

3.2 Introduction

Climate change has the potential to alter the phenology of organisms across many taxa (Gordo and Sanz, 2005; Van Asch *et al.,* 2007; Thackeray *et al*., 2010). Invertebrate phenology has been shown to be sensitive to climatic variables (Staley and Johnson, 2008; De Lucia *et al*., 2012). For instance, milder winters or increased spring temperatures – a predicted outcome of climate change (Meehl *et al*., 2007) – may improve the overwintering success of many invertebrates, leading to earlier emergence and at greater densities (Bale *et al.,* 2002). Such climate-induced alterations of phenology may affect the temporal sequence in which different herbivore species colonise host-plants, potentially altering interspecific interactions and ultimately community dynamics (De Lucia *et al*., 2012).

Despite being spatially-separated, herbivores feeding on either above- or belowground plant tissue interact via plant morphological (e.g. compensatory growth) and chemical (e.g. induction) responses (Masters and Brown, 1992; Bezemer *et al.,* 2003; Heil, 2011). Belowground herbivory can have negative (e.g. Van Dam *et al*., 2005) and positive (e.g. Johnson *et al*., 2009) effects on aboveground herbivores. Similarly, aboveground herbivores can have negative (e.g. Bezemer *et al*., 2003) or neutral (e.g. Soler *et al*., 2007) effects on their belowground counterparts. Positive reciprocal interactions between belowground and aboveground herbivores, where both benefit from the interspecific interaction, although rarer, have also been reported (Johnson *et al*., 2009; Huang *et al*., 2013).

A recent meta-analysis identified the sequence of herbivore arrival on a host-plant as the most influential factor dictating plant-mediated above–belowground herbivore interactions (Johnson *et al*., 2012a). Early colonisation of a host-plant helps to avoid direct competition with other herbivores (Hunter, 1990; Blossey and Hunt-Joshi, 2003; Singer and Parmesan, 2010) and avoid induced plant defences (Poelman *et al.,* 2008). For example, an aboveground herbivore significantly reduced weight gain of a belowground herbivore on maize, but only when the folivore colonised the host-plant before the root feeder (Erb *et al*., 2011). Colonisation sequence has also been reported to affect uni-directional facilitative interactions (where a species directly or indirectly benefits another species, yet itself gains nothing from the interaction). For instance, the interaction outcome between two aboveground beetle species (*Acanthocinus aedilis* and *Rhagium inquisitor*) changed when timing of arrival was altered (Victorsson, 2012). When arriving simultaneously or arriving first on a plant, *A. aedilis* produced 161% and 84% more offspring, respectively, than when alone on the plant. In contrast, simultaneous colonisation caused decreased larval weight in *R. inquisitor*. When *R***.** *inquisitor* was first, however, the positive impacts on *A. aedilis* were not apparent and neutralism occurred, whereas *R. inquisitor* larval mass increased. This study concluded that arrival sequence was highly important as both herbivores fared better when arriving first and the facilitative interaction diminishes when time of arrival is altered.

In this study it was investigated how colonisation sequence affected the facilitative interaction between an aboveground aphid herbivore (*Amphorophora idaei*) and the root-feeding larvae of the vine weevil (*Otiorhynchus sulcatus*) on two genotypes of a shared host-plant (*Rubus idaeus*). These two genotypes varied in their susceptibility to insect herbivory: one genotype (Glen Ample) was highly susceptible while the other (Glen Clova) showed moderate susceptibility (Clark *et al*., 2011; McMenemy *et al*., 2009). The use to two cultivars tests the generality of the herbivore response to arrival time, as the cultivars affect the herbivores differently. Weevil larvae are naturally present in the soil from early summer until pupation in early spring the following year (Moorhouse *et al*., 1992). The feeding rate, development and eventual emergence of the weevil larvae are highly temperature-dependent (Son and Lewis, 2005). Mid-level Intergovernmental Panel on Climate Change (IPCC) scenarios (A1T and B2) for global mean temperatures between 2090–2099 predict an increase of 2.4°C, with a likely range of 1.4–3.8°C (Meehl *et al*., 2007). This temperature change could have major impacts on the life history and phenology of the weevil larvae. Aboveground, aphid phenology is generally expected to respond to climate change, with an average earlier first occurrence of eight days for aphids over the next 50 years (Harrington *et al*., 2007). As well as an alteration in the phenology of *A. idaei,* a specific temperature increase from 10°C to 15°C results in an increase in reproductive period but a reduction in their longevity and pre-reproductive period (Mitchell, 2007).

A microcosm experiment was conducted to test whether herbivore colonisation sequence altered a facilitative plant-mediated interaction between above- and belowground herbivore species. It was hypothesised that aphid abundance and population growth rate would be greatest when herbivore treatments replicated natural phenology (e.g. weevil larvae present on the plant before aphids). It was also hypothesised that the colonisation sequence would be of greater importance on the more resistant plant genotype which may only maintain resistance if natural phenological succession occurs.

3.3 Materials and methods

Rootstock for two raspberry genotypes (Glen Ample and Glen Clova) was grown at the James Hutton Institute (JHI) in Dundee under conditions of 18:6 Light : Dark and 18°C (±2°C) for 10 weeks. After this initial 10 week growing period, 45 individual plants from each of the two genotypes (90 plants in total) were planted into rhizotubes (plastic cable trunking of length 40 cm; Chapter II) and the plants' initial height recorded. The rhizotubes were randomly allocated to nine spatial blocks, each of which comprised a complete replicate of a genotype–herbivore treatment combination (see below).

Correlating IPCC predictions (Meehl *et al*., 2007) with the temperature-dependent data of weevil egg development (Son and Lewis, 2005) suggests a potential advancement of larval eclosion by two weeks (based on average Scottish summer mean temperature increasing from 12°C to 15°C). Conversely, a temperature decrease of 1°C, from 12°C to 11°C, could prolong egg development for a further two weeks. Thus, five colonisation sequence treatments were implemented with weevil larvae eclosion timed to occur: a) two weeks and b) one week before the addition of aphids; c) simultaneous to aphid addition; and d) one week and e) two weeks after aphid addition. These five herbivore arrival treatments, coupled with the two raspberry genotypes, resulted in each spatial block containing 10 individual plants.

Under experimental glasshouse conditions, vine weevil eggs take two weeks to eclose at a mean temperature of 18°C (Son and Lewis, 2005). Twenty eggs were consequently added to each replicate rhizotube two weeks prior to the anticipated larval hatching date. These eggs were obtained from adult vine weevils harvested from raspberry field plots at the JHI. Plants were inoculated with three adult aphids from cultures maintained at the JHI. All aphids were added in the same week to standardise the time aphid populations spent on the plant and subsequently counted weekly. Aphids were reared in controlled environment chambers at 18°C and fed on a raspberry cultivar not used in the experiment (Malling Jewel).

The experiment was harvested five weeks after aphid inoculation (Week 9) at which point the final aphid abundance was quantified. The aboveground plant biomass was then harvested, freeze-dried for 24 hours and then weighed. Vine weevil larvae were extracted from the soil over 24 hours using Tullgren funnels and then counted.

Data were analysed using generalised linear mixed effects (GLMM) models in SAS (version 9.2). The rate of aphid population increase per replicate was determined by a linear regression of aphid counts over time (five weeks). The slope (b) estimates from these linear equations (i.e. rate of aphid population increase per replicate) was fitted, following $\log_{10}$ transformation, as the response in a GLMM with a Gaussian distribution and identity link function. Aphid abundance at the final harvest (Week 9) was also $\log_{10}$ transformed and modelled with Gaussian distribution. The parameter “block” was fitted as a random effect to all models to account for the spatial layout of replicate plants in the glasshouse. Weevil colonisation sequence and plant genotype (see above) were fitted as categorical explanatory variables along with aboveground plant biomass as a covariate. Plant roots were exposed to different durations of root herbivory in each weevil colonisation sequence treatment; because of this confounding effect the changes in belowground biomass were not analysed.

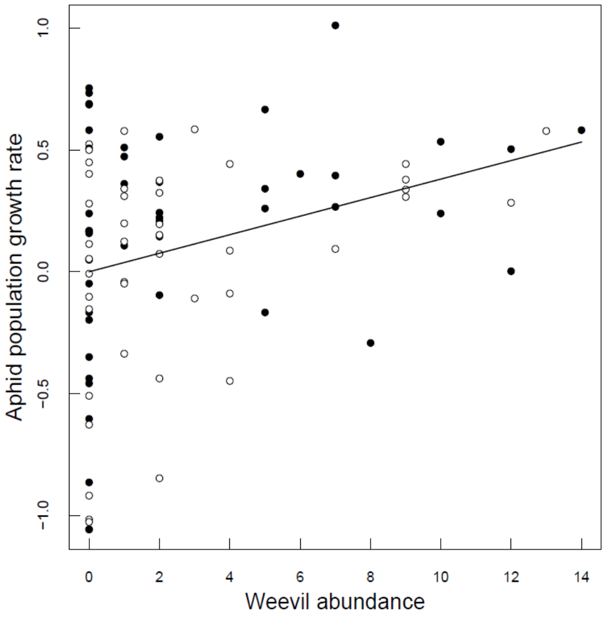
3.4 Results

Aphid population growth rate was positively related to weevil abundance (Figure 3.1a; Table 3.1) and aboveground plant biomass (Figure 3.1b; Table 3.1), as was final aphid population size (Table 3.1). Weevils increased aphid abundance in a ratio of 1:2. Aphid abundance across treatments at harvest ranged from 0 to 176 individuals, with a maximum population growth rate of 9.46 aphids per week. Mean aphid population growth rate was 1.87 aphids per week on the more susceptible genotype and 1.78 aphids per week on the less susceptible genotype. After adjusting for the influence of aboveground biomass and weevil abundance, the aphid population growth rate (slope of aphid densities over time = b) on the more susceptible plant genotype (least square mean, b = 0.90 ± 0.079) was statistically significantly greater than on the less susceptible genotype by 32% (b = 0.68 ± 0.079) (Table 3.1).

Table 3.1: GLMM results summary for herbivore response parameters. Bold type indicates parameters retained in the final model. MPE = Multiple Parameter Estimates.

Response variable	Explanatory variables	Estimate	$F_{(ndf,ddf)}$	P
Aphid abundance at harvest	Colonisation sequence	MPE	0.42 $_{(4,76)}$	0.7929
Random effect estimate = 0.1332	Genotype susceptibility	MPE	3.22 $_{(1,79)}$	0.0767
Residual variance = 0.1856	**Weevil abundance**	**0.04235**	**10.59** $_{(1,86)}$	**0.0016**
	Aboveground plant dry weight	**0.1635**	**5.94** $_{(1,83)}$	**0.0169**
Aphid population growth rate	Colonisation sequence	MPE	0.32 $_{(4,75)}$	0.8662
Random effect estimate = 0.01003	**Genotype susceptibility**	**MPE**	**4.29** $_{(1,80)}$	**0.0416**
Residual variance = 0.2	**Weevil abundance**	**0.03806**	**8.08** $_{(1,84)}$	**0.0056**
	Aboveground plant dry weight	**0.2359**	**9.00** $_{(1,83)}$	**0.0036**
Weevil larval abundance at harvest	Colonisation sequence	MPE	8.59 $_{(1,4)}$	0.2499
Random effect estimate = 0.3242	Genotype susceptibility	MPE	1.81 $_{(1,88)}$	0.1819
	Aphid abundance	**0.009788**	**26.09** $_{(1,88)}$	**<0.0001**
	Aboveground plant dry weight	**-0.2241**	**5.24** $_{(1,88)}$	**0.0245**

Figure 3.1a)



b)

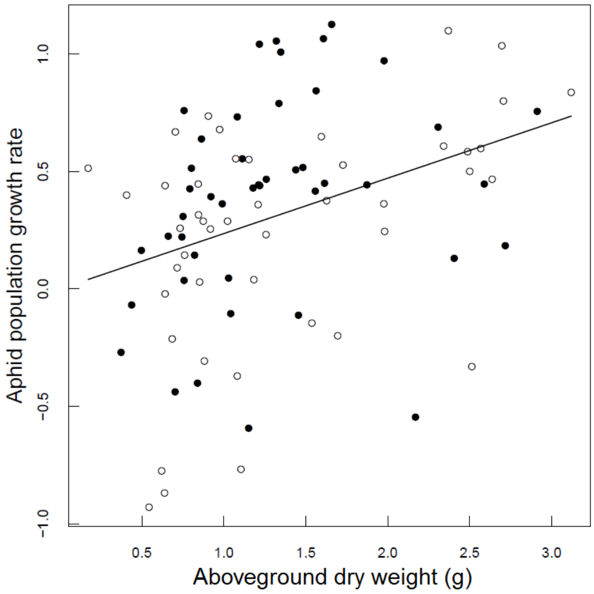


Figure 3.1: Partial residual plots on the linear predictor scale for aphid population growth rate (slope estimate derived from linear regression of aphid numbers per replicate against time) versus a) weevil abundance; and b) aboveground biomass (open symbols = highly susceptible genotype; closed symbols = moderately susceptible genotype). Data for aphid abundance and population growth rates were $\log_{10}$ transformed prior to analysis.

Sequence of weevil colonisation did not, however, have a significant effect on either final population size (Figure 3.2a; Table 3.1) or population growth rate (Figure 3.2b; Table 3.1) of the co-occurring aphid herbivore during the five week experiment.

Weevil abundance ranged from zero, where 100% mortality had occurred, to 14 individuals at the time of harvest across all colonisation sequence treatments. There were no differences in weevil abundance across colonisation sequence treatments (Table 3.1). Weevil abundance was, however, positively related to aphid abundance (Table 3.1). Weevil abundance was not affected by plant genotype (Table 3.1), but was negatively correlated with aboveground plant biomass (Figure 3.3; Table 3.1), which potentially reflected the negative impacts of root herbivory on plant growth (see below).

Figure 3.2a)

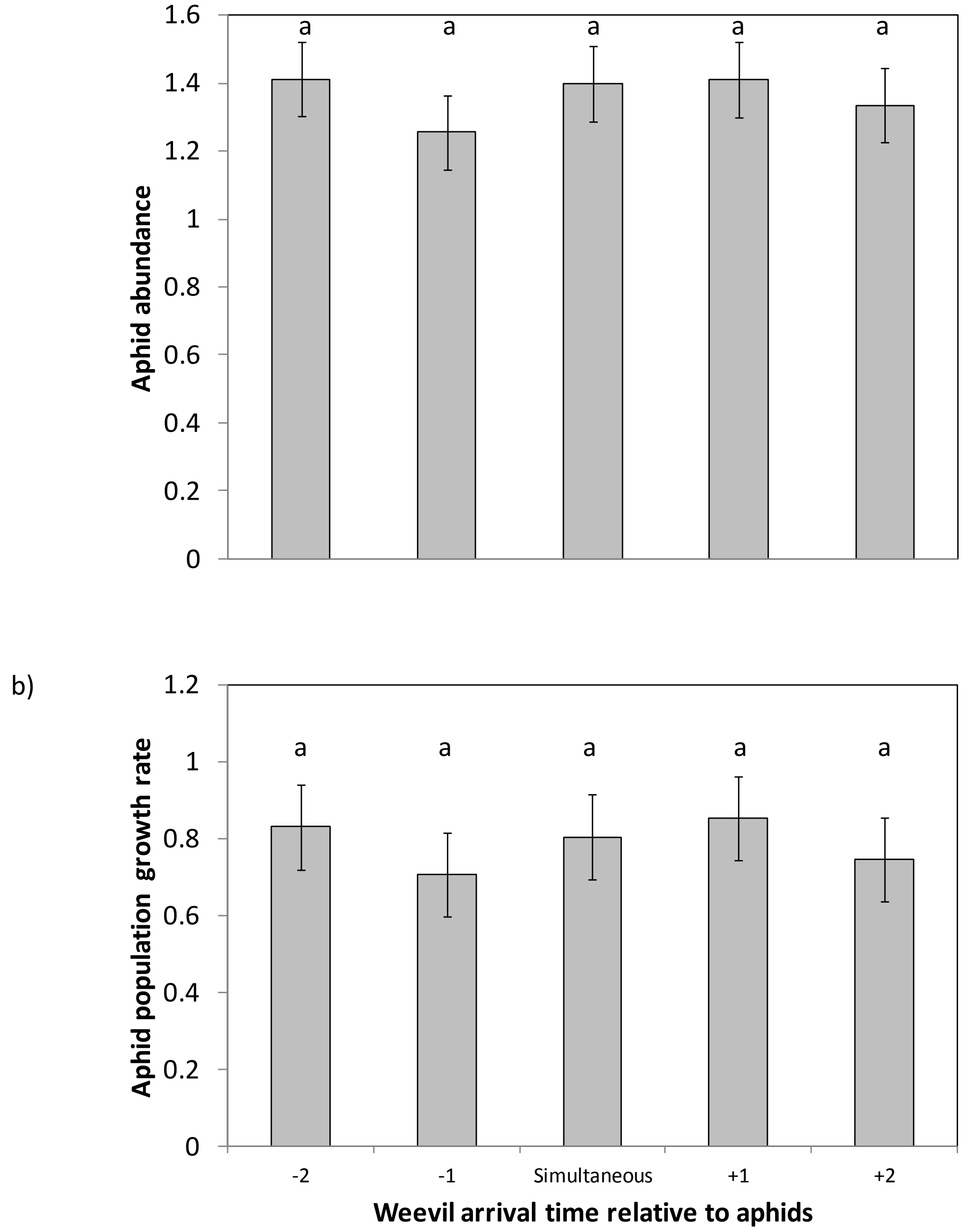


Figure 3.2: The non-significant effects of the timing of weevil colonisation on a) aphid abundance and b) aphid population growth rate (LS mean ± SE from final model). Colonisation sequence treatments were weevil larvae eclose: two weeks (-2) and one week (-1) before aphid inoculation; simultaneous with aphid inoculation; one week (+1) and two weeks (+2) after aphid inoculation. Data for aphid abundance and population growth rates were $\log_{10}$ transformed prior to analysis. Identical characters indicate non-significance ($P > 0.05$)

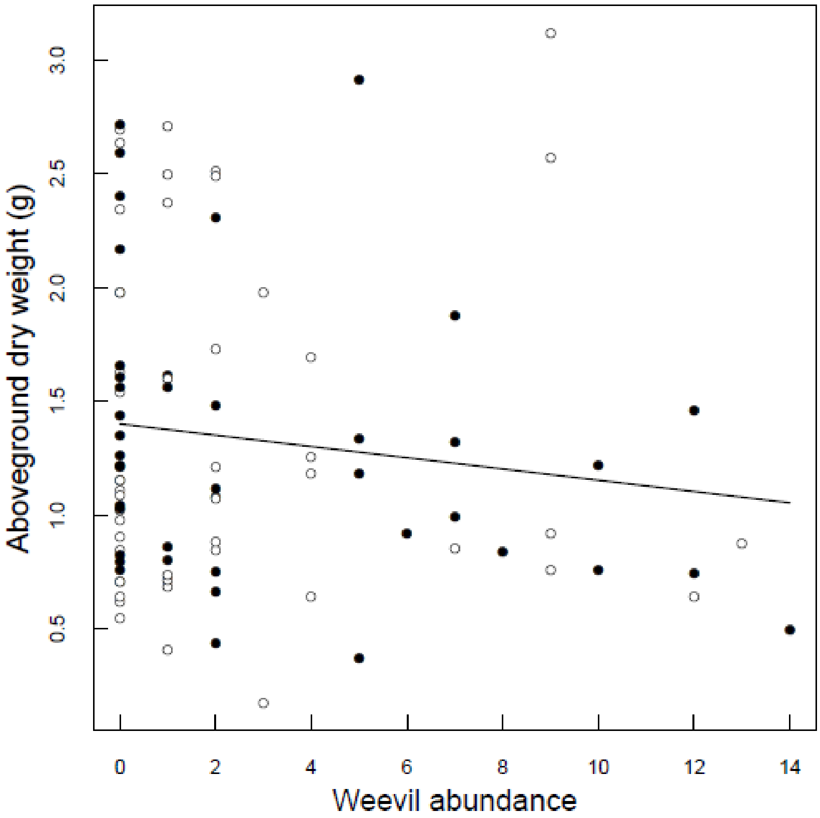


Figure 3.3: Plot for weevil abundance versus aboveground plant biomass (g) (closed symbols = highly susceptible genotype; open symbols = moderately susceptible genotype).

There was no significant difference in plant height between genotypes at the start of the experiment ($F_{1,88} = 0.19$, $p = 0.664$). When the experiment was harvested plant genotype had a significant effect on aboveground biomass ($F_{1,87} = 28.33$, $p = <0.0001$). The highly susceptible genotype (1.002g ± 0.88) had significantly less aboveground biomass than the moderately susceptible genotype (1.667g ± 0.88).

3.4 Discussion

In contrast to competitive herbivore interactions, positive interactions may be less sensitive to herbivore-induced changes to the plant environment, especially when they are reciprocal. In studies where facilitative interactions have been altered by the sequence of herbivore arrival (i.e. Victorsson, 2012) the facilitation was uni-directional (e.g. only one herbivore benefitted at a time). This contrasts with the current study involving a reciprocal feeding facilitation, whereby both herbivores benefitted simultaneously, which persisted despite variation in sequence of herbivory. This may be explained, to some extent, by the different feeding guilds investigated in the studies. Feeding guild, like colonisation sequence, is also known to be an important determinant in above–belowground herbivore interaction outcomes (Johnson *et al.*, 2012a) For example, jasmonic acid induction by root feeders interferes with and reduces the plant response to aphid attack (e.g. salycilic acid induction) (Soler *et al.*, 2013). Along with the type of interaction (antagonistic or facilitative), the induction of different phytohormonal pathways by different herbivore feeding guilds may therefore be another mechanism influencing interaction outcomes. Whether induction of different phytohormonal pathways underpins the stability of the reciprocal facilitation, regardless of herbivore arrival sequence, remains an untested hypothesis.

Reciprocal feeding facilitation between *O. sulcatus* and *A. idaei* (Chapter II) persists regardless of which herbivore colonises the host plant first. This differs from other studies which illustrate that herbivore colonisation sequence on a plant is an important factor governing interspecific herbivore interactions (Erb *et al.*, 2011; Johnson *et al.*, 2012a; Victorsson, 2012). In general, early colonising herbivores are at a competitive advantage over other herbivores arriving subsequently (Johnson *et al.*, 2012a). For example, an initial herbivore can induce plant chemical defences making it more difficult for subsequent herbivore competitors to become established on the plant (Viswanathan *et al.*, 2005). Above–belowground herbivore interactions are predominantly competitive (Johnson *et al*., 2012a), and therefore variation in colonisation sequence may produce plant-mediated effects that alter the interaction. For example, when the aboveground herbivore *Spodoptera frugiperda* arrived first on a plant it negatively affected colonisation and weight gain by the belowground herbivore *Diabrotica virgifera*. If, however, *S. frugiperda* arrived after *D. virgifera***,** no negative effects were found (Erb *et al*., 2011). This alteration in competitive outcomes was attributed to leaf-herbivore-induced increases in feeding-deterrent and/or toxic secondary metabolites.

Although some secondary metabolites (e.g. phenolic compounds) are known to have defensive properties against herbivores (Harborne, 1994), these are relatively ineffective in raspberry plants against either *O. sulcatus* (Clark *et al*., 2011) or *A. idaei* (McMenemy *et al*., 2012). Instead, raspberry tends to be more reliant on physical defences such as spines (Mitchell, 2007) and leaf waxes (Shepherd *et al*., 1999a). Herbivore colonisation sequence may, therefore, be less relevant in plant systems where physical resistance mechanisms dominate compared with those with inducible or constitutive chemical defences (e.g. Brassicas).

In Chapter II weevils performed better on the highly susceptible cultivar, but there was no difference in plant biomass between the two genotypes. In the present study it was found that weevils performed equally well on both genotypes. This may have occurred because aboveground biomass of the moderately susceptible genotype was significantly greater in this study which probably enhanced weevil abundance. This seems likely since aboveground biomass is tightly correlated with belowground biomass (Chapter II) which in turn is correlated with weevil performance (Johnson *et al*., 2011; Clark *et al*., 2011). Only the abundance of the weevils were measured, but more detailed information about which specific performance parameters were being affected might shed light on which plant traits are the most important.

In conclusion, the reciprocal herbivore facilitation reported here is robust to variation in the timing of herbivory. This has implications when considering the effect of climate change altering interactions between above- and belowground herbivores. For example, factors such as decreased development rate or increased fecundity, caused by increased atmospheric temperature, may be more decisive in facilitative above–belowground interactions than changes in herbivore colonisation sequence. Variation in the sequence of herbivory may, however, continue to be important in dictating competitive interactions or uni-directional facilitation between above–belowground herbivores.

Chapter IV

*Elevated atmospheric CO_2 stimulates feeding rates and induces host-plant preference by adult vine weevils (*Otiorhynchus sulcatus*)*

4.1 Abstract

While anthropogenic increases in atmospheric CO_2 have been predicted to stimulate plant productivity, the nutritional value of foliage to herbivores may be reduced, partly due to increased leaf carbon to nitrogen (C:N) ratio. To compensate for this deterioration in food quality, some herbivorous insects consume more plant tissue. Plants grown in elevated CO_2 may, however, also exhibit a lower specific leaf area (increased leaf toughness), which may restrict the herbivore's capacity to feed.

This study investigated the feeding rates (no-choice tests) and preference (choice tests) of vine weevils (*Otiorhynchus sulcatus*) feeding on raspberry (*Rubus idaeus*) grown in current (390 ppm) and elevated (550 ppm) levels of atmospheric CO_2 enrichment.

Weevil leaf consumption was six times greater under elevated CO_2 conditions compared to ambient CO_2 in no-choice tests. Furthermore, in the choice experiment weevils actively preferred and consumed disproportionately more (217%) leaf tissue from plants grown under elevated CO_2. This increased herbivory occurred despite raspberry leaves grown under elevated CO_2 being 11% tougher. Although marginally non-significant ($p=0.056$), the C:N ratio was nonetheless 20% higher in leaves grown in the elevated CO_2 environment.

The alteration of leaf nutritional quality (increased C:N ratio) stimulated herbivore compensatory feeding rates in the no-choice tests. This alteration of plant C:N content by atmospheric CO_2 enrichment was, however, insufficient to deter the herbivore from consuming leaves grown under elevated CO_2 in choice tests. This may result in chewing herbivores consuming a greater amount of foliage in a future higher CO_2 atmospheric environment.

4.2 Introduction

Atmospheric carbon dioxide (CO_2) concentrations are expected to increase to 550 ppm over the next 100 years (IPCC, 2013). While elevated CO_2 can directly increase primary productivity (Ainsworth and Long, 2005; Robinson *et al*., 2012), herbivores may be indirectly affected via changes in the nutritional quality or quantity of the plant resource (Dermody *et al*., 2008; Coviella and Trumble, 1999; Robinson *et al*., 2012; Watt *et al*., 1995). At current atmospheric CO_2 concentrations, the main enzyme involved in the assimilation of atmospheric CO_2 into carbohydrate (RuBisCo), is operating at below its optimum (De Lucia *et al*., 2012). Increased atmospheric CO_2 concentrations will, therefore, lead to an increased rate of photosynthesis, carbon fixation and plant biomass accumulation (Ainsworth and Long, 2005). This increased plant productivity may alter the nutritional quality of plant tissues for herbivores due to dilution of plant nitrogen content, already a limiting nutrient for many insect herbivores (Awmack *et al*., 1997). As the conversion of CO_2 to carbohydrates increases due to improved RuBisCo activity, less nitrogen will need to be invested in the enzyme to maintain high levels of photosynthesis, which usually acts as a substantial sink for leaf nitrogen (Jacob *et al*., 1995). This increased carbon to nitrogen ratio requires the overall amount of tissue consumed to be increased through herbivore compensatory feeding in order to attain the necessary amount of nitrogen required for insect growth and development (Awmack *et al*., 1997; Robinson *et al*., 2012). A consequence of atmospheric CO_2 enrichment may thus be reduced insect herbivore growth but greater herbivory rates (Coviella and Trumble, 1999). Such alteration of plant–herbivore interactions may nullify any enhanced primary productivity in future CO_2 environments.

In addition to primary chemistry, the morphology and physical traits of plants may also be affected by elevated CO_2 (Robinson *et al*., 2012), which can subsequently impact on herbivore performance (Lincoln *et al*., 1993 and references therein). Leaf toughness influences the ability of herbivores to consume, digest and extract nutrients from the plant tissue (Sanson *et al*., 2001; Peeters *et al*., 2007). Generally elevated atmospheric increases in CO_2 will increase leaf toughness (De Lucia *et al*., 2012; Zvereva and Kozlov, 2006; Robinson *et al*., 2012; Stiling and Cornelissen, 2007; Dury *et al*., 1998), by increasing cuticle thickness (Kinney *et al*., 1997), stimulating cuticular-wax production (Percy *et al*., 2002) or reducing the specific leaf area (area: mass; De Lucia *et al*., 2012). This increase in structural carbon also acts to further dilute leaf nitrogen content (De Lucia *et al*., 2012). Where elevated CO_2 enrichment has not affected leaf toughness, there is often more marked responses in chemical responses, for example, ellagitannin in maple, *Acer saccharum* (Kinney *et al*., 1997).

Relatively few studies have investigated the indirect effects of CO_2 on herbivore feeding rates mediated by changing leaf toughness. Dury *et al*. (1998) found that increased CO_2 reduced leaf toughness, but with no effect on the herbivores. In contrast, Johns *et al*. (2003) showed CO_2-induced increases in leaf toughness reduced herbivore consumption rates, although when herbivores were presented with a choice of leaves grown at ambient or elevated CO_2 levels no difference in consumption was observed. There remains a need to investigate further how elevated CO_2 affects leaf toughness and the subsequent interaction with phytophagous insects.

This study investigated how elevated CO_2 affected the feeding preference of the herbivorous vine weevil *Otiorhynchus sulcatus* (Fabricius), a generalist pest of the raspberry (*Rubus idaeus* L.) crops. It was hypothesised that plants grown under elevated atmospheric CO_2 would have greater C:N content and increased leaf toughness leading to herbivore compensatory feeding, and that given a choice the herbivore would select foliage grown under ambient CO_2 conditions.

4.3 Materials and methods

Raspberry rootstock (Glen Clova cultivar) obtained from the James Hutton Institute, Dundee, were grown in Levington M3 compost at 18°C (±2°C). Ten trays (30cm x 20cm) of rootstock were distributed between two chambers in ambient (390 ppm) or elevated (550 ppm) CO_2 conditions at the GroDome™ climate change research facility at the Centre for Ecology & Hydrology (CEH Wallingford). When at least 25 plants from each CO_2 treatment were at least 3cm (around 10 weeks) they were separated and planted into 3 litre pots containing a mixture of 2:1 (soil:sand). Plants were allowed to grow in these conditions for a further 8 weeks to produce sufficient leaves for the experiments.

Vine weevil eggs obtained from reproducing adults at JHI were inoculated into pots containing established raspberry plants and maintained at 18°C in controlled environment rooms. Eggs were allowed to hatch and weevil larvae to develop and feed on the raspberry roots until their emergence. Newly emerged adult weevils were then used in the experiments.

4.3.1 No-choice experiment

A single leaf was excised (the uppermost unfurled leaf on each plant) from 10 of the raspberry plants from each CO_2 treatment, respectively (n = 20). These leaves of similar size were weighed and leaf area calculated (Licor; LI-3100). Each leaf was placed individually into a culture cup (500 ml), with the stem submerged in water, together with an adult weevil of known weight. Cups containing leaves and weevil adults were then placed in the ambient CO_2 chamber. Weevils were allowed to feed on the leaves under conditions of 18:6 Light : Dark and 18°C (±2°C). After one week the leaves were removed and reweighed and the remaining leaf area measured.

Weevil weight gain (mg), leaf weight loss (mg) and leaf area consumption (cm^2) were calculated.

4.3.2 Choice experiment.

As before, a single leaf was excised (the uppermost unfurled leaf on each plant) from 20 of the raspberry plants from each CO_2 treatment, respectively. Two size and age matched excised leaves from each CO_2 treatment were then placed *together* and alongside one another in a culture cup (n = 20), along with an adult vine weevil (from cultures described previously). Weevils were allowed to feed on the leaves for one week under ambient conditions. As before, after one week the leaves were removed from the cups and reweighed and the remaining leaf area measured. Weevil weight gain (mg), leaf weight loss (mg) and leaf area consumption (cm^2) were calculated.

Specific leaf weight (SLW, $g^{-1}m^{-2}$) was calculated for each leaf as an indicator of leaf thickness. Leaf toughness was determined using a 1 cm diameter leaf disk cut from the experimental leaves at the end of the experiment (3 discs per leaf, avoiding the midrib) following the leaf tearing protocol of Graça and Zimmer (2005). This protocol allows the amount of force (Newtons/kg^{-1}) required to tear a leaf of known size to be calculated; this measurement of force is then used as a representative of leaf toughness (tougher leaves requiring a greater force to be torn). Individual leaf tissue was freeze-dried for 24 hours and ball-milled in a tissue lyser for five minutes to a fine powder. Chemical analysis of carbon and nitrogen concentrations of leaf and root tissue was undertaken at the James Hutton Institute, Dundee, using an Exeter Analytical Elemental Analyser (EAI, Coventry, UK). Using the carbon and nitrogen concentrations produced during the analysis, the C:N of the leaf tissue was then able to be calculated (see Johnson *et al*. (2011) for details).

4.3.3 Statistical analysis

Data were analysed in SAS (version 9.2). The no-choice experiment and leaf toughness were analysed using General Linear Models (GLM), total leaf area consumed and C:N data were log-transformed to meet model assumptions of normal distribution. The choice experiment was analysed using a general linear mixed model (GLMM) with “replicate” (n =20 pots) fitted as a repeated measure, to identify the fact that each pot contained one leaf from each CO_2 treatment. Response variables modelled were weevil weight, leaf area consumption, leaf C:N and leaf toughness with CO_2 treatment fitted as an explanatory variable in all models.

4.4 Results

Leaf consumption was greater when plants were grown in an elevated CO_2 environment (Figure 4.1) in both the no-choice ($F_{1,19} = 9.85$, $P = 0.0057$) and choice ($F_{1,38} = 10.71$, $P = 0.0023$) experiments, supporting the hypothesis that compensatory feeding takes place under elevated CO_2 (Robinson *et al*., 2012, and references therein).

This preference for leaves grown in high CO_2 persisted even when the insect had a choice, contrasting with an earlier study (Johns *et al*., 2003), and despite the increased toughness of leaf tissue grown in elevated CO_2 (Figure 4.2; $F_{1,38} = 10.14$, $P = 0.0029$). Leaf thickness (SLW), however, remained unaffected ($F_{1,19} = 3.16$, $P = 0.091$) as was weevil weight ($F_{1,19} = 0.02$, $P = 0.897$) by CO_2 treatment.

Leaves grown in an elevated CO_2 environment had a higher C:N ratio (Figure 4.3), although this was not a strong effect being marginally non-significant at the 95% significance level ($F_{1,19} = 4.17$, $P = 0.056$).

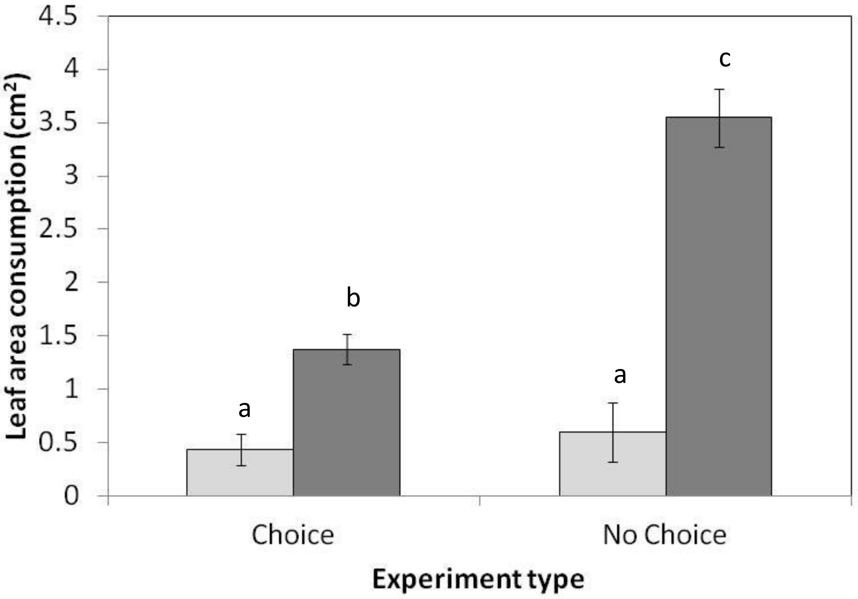


Figure 4.1: Mean (± SE) consumption (mg) of ambient (light bars) and elevated (dark bars) CO_2 leaves by vine weevils in a choice (n=10 per CO_2 treatment) and no-choice experiment (n=20 per CO_2 treatment). Differing characters indicate a significant difference between treatments ($P < 0.05$).

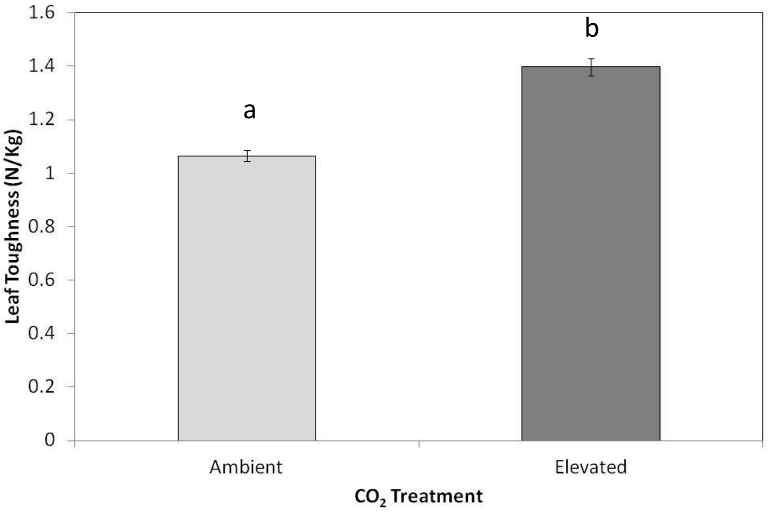


Figure 4.2: Variations in mean (±SE) leaf toughness (the force required to tear a 1 cm disc of leaf tissue; Newtons/kg^{-1}) between raspberry leaves grown under ambient (390 ppm) and elevated (550 ppm) CO_2 conditions (n=20 per CO_2 treatment). Differing characters indicate a significant difference between treatments ($P < 0.05$).

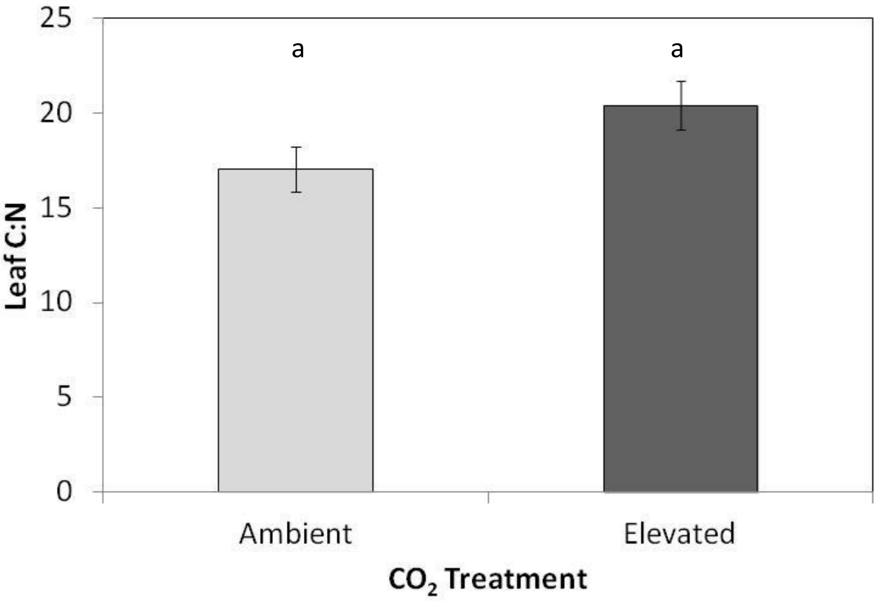


Figure 4.3: Non-significant variations in mean (±SE) leaf C:N ratio between raspberry leaves grown under ambient (390 ppm) and elevated (550 ppm) CO_2 conditions (n=12 per CO_2 treatment). Identical characters indicate no significant difference between treatments ($P > 0.05$).

4.5 Discussion

This study provides evidence that adult weevils consumed more leaf tissue from plants grown under elevated CO_2 conditions. This supports previous findings that herbivorous insects will need to increase plant consumption to compensate for the poorer nutritional quality (Robinson *et al.*, 2012). It was hypothesised that, when given a choice between leaves grown under ambient and elevated CO_2 conditions, weevils would exhibit a feeding preference for ambient leaves, again a consequence of them having a higher nutritional value. It was found, however, that the opposite was true, and weevils showed a preference for elevated CO_2 leaves, despite them also being tougher.

In support of previous findings (Stiling and Cornelissen, 2007; Robinson *et al.*, 2012) it was found that elevated CO_2 increased leaf toughness. This increased leaf toughness may be attributed to increases in leaf epicuticular wax which also increases under elevated CO_2 (Percy *et al*., 2002). It is known that raspberry plants also rely on leaf waxes (Shepherd *et al*., 1999a) as an anti-herbivore defence, while this is thought to be an adaptation to inhibit stylet insertion by phloem-feeders (Shepherd *et al*., 1999a), it may also affect leaf-chewers by increasing leaf toughness. In grass species increased leaf toughness has been shown to have costs to the herbivore such as increased mandibular wear, which consequently may prevent effective feeding and reduces individual fitness (Massey and Hartley, 2009). It remains to be tested whether mandibular wear would be an important factor in this plant–herbivore interaction.

The marginally non-significant increase in C:N ratio of leaves grown under elevated CO_2 conditions may have been sufficient to stimulate the compensatory feeding observed in the no-choice test, whilst being insufficient to induce a preference for nutritionally better quality leaves from the ambient CO_2 treatment in the choice tests. Vine weevils in particular, do not discriminate very well between plants of different nutritional qualities (Coyle *et al*., 2011; Clark *et al*., 2012b). This would not, however, explain the strong preference for foliage grown under elevated CO_2, as leaf consumption was predicted to have been reduced due to the increased toughness of leaves and a lower nutritional content (i.e. lower C:N). Compensatory feeding normally takes place because of higher C:N, but increased feeding observed in the present study could be attributed to a factor overriding reduced plant quality at elevated CO_2, such as feeding stimulants. Elevated CO_2 can alter production of chemicals such as fatty acids that act as host acceptance cues (Percy *et al*., 2002), which may also explain increases in herbivory. This has been shown to occur for the leaf-chewing forest tent caterpillar (*Malacosoma disstria*) feeding on quaking aspen (*Populus tremuloides*) leaves (Percy *et al*., 2002) and may have been sufficient to override weevil preferences for the seemingly inferior foliage in this current system. Generally, CO_2 has been shown to decrease nitrogen based secondary metabolites in plants significantly (Robinson *et al*., 2012). This includes the suppression of plant defences in the tissues and VOC production, both of which may deter herbivory (De

Lucia *et al*., 2012). In particular, if some VOCs are suppressed, it may increase a plant’s susceptibility to herbivory, potentially overriding any differences in plant C:N.

In this system there appears a currently unknown benefit to this herbivore in consuming a greater amount of leaf tissue in elevated CO_2 conditions. These early findings provide the basis of hypothesis testing for such differences in feeding behaviour of insects under elevated CO_2. Future experiments could investigate more specifically which chemicals are altered in the leaf tissue, which could explain the differences in feeding preferences. It would be beneficial to use whole plants, as their response may be different to excised leaves and is more realistic. More research needs to be done on isolating the effects of CO_2 on primary and secondary leaf chemistry and how these changes in chemistry may alter plant–herbivore interactions in a future climate.

Chapter V

The effect of elevated atmospheric CO_2 on above- and belowground herbivory: evidence for root herbivory mitigating impacts on roots but not shoots

5.1 Abstract

Anthropogenic increases in atmospheric carbon dioxide (CO_2) may indirectly affect insect herbivores, both above- and belowground, by altering plant chemistry, such as potential increased C:N ratio. This change in tissue C:N may also affect interactions between spatially separated herbivores. This chapter presents a microcosm experiment that investigated the effect of elevated CO_2 on the plant-mediated interaction between the large raspberry aphid feeding aboveground, and the larvae of the vine weevil feeding on the raspberry roots. Elevated atmospheric CO_2, as a main effect, had minimal effect on the plant responses measured, and also minimal effect on above- and belowground insect abundance. Belowground herbivory played a much larger role than elevated CO_2 in influencing root C:N (increase 7%) and biomass (decrease 30%). Elevated CO_2 did, however, mitigate the effect of belowground herbivores on roots via increased root growth. This may suggest that the negative effects of root herbivory on root growth observed at ambient CO_2 concentrations may be less in a future climate.

5.2 Introduction

Atmospheric carbon dioxide (CO_2) concentrations are expected to increase over the next 100 years from current levels of 390 ppm up to 550 ppm (Meehl *et al*., 2007). This is widely attributed to anthropogenic processes, particularly the burning of fossil fuels (IPCC, 2013). Elevated atmospheric CO_2 is known to have significant direct effects on plant productivity (Ainsworth and Long, 2005), which can affect the performance of herbivores (Coviella and Trumble, 1999; Robinson *et al*., 2012). Herbivores, for example, may be indirectly affected via changes in the quality (e.g. altered carbon and nitrogen content) of their plant food resource (Watt *et al*., 1995).

Interspecific interactions between organisms above- and belowground have a key role in ecosystem processes like herbivory and decomposition (Van der Putten *et al*., 2001; Van der Putten *et al*., 2009; Wardle *et al*., 2004a; Wardle *et al*., 2005). Climate change may alter or disrupt these interactions and processes (Schröter *et al*., 2004; Stevnbak *et al*., 2012). Elevated CO_2, for example, has the potential to affect interactions between above- and belowground herbivores by altering the nutritional quality of the shared host-plant (De Lucia *et al*., 2012; Robinson *et al*., 2012). Both root and shoot biomass has been shown to increase under elevated CO_2 (Robinson *et al*., 2012), but root herbivory at elevated CO_2 can reduce plant biomass to similar levels observed at ambient concentrations (Johnson and Riegler, 2013). The ratio of carbon to nitrogen (C:N) in leaves also often increases under elevated CO_2 (Robinson *et al*., 2012), but this has been shown to be modified by root herbivory (Johnson and Riegler, 2013).

There have been relatively few studies investigating the effects of climate change on above- and belowground herbivore interactions, with differing outcomes. One study investigated the effects of elevated CO_2 on the interaction between a root- (*Pemphigus populitransversus*) and a shoot- (*Aphis fabae fabae*) feeding aphid species, attacking lady's smock (*Cardamine pratensis*) (Salt *et al*., 1996). This study concluded that the interaction between the above- and belowground feeding aphid species was unaffected by CO_2 and that the root herbivore populations were always smaller in the presence of an aboveground herbivore. Another study investigated the interaction between aboveground adults and belowground larvae of the clover root weevil (*Sitona lepidus*) (Johnson and McNicol, 2010). It suggested that elevated CO_2 significantly increased leaf consumption and decreased oviposition by adult clover root weevils putting the weevils at a disadvantage. This was reported to be a response to reduced leaf quality (high C:N). Despite reduced oviposition, larval survival was higher at elevated than at ambient CO_2. It was suggested that this could be due to increased nodulation of the host-plant (*Trifolium repens*) under elevated CO_2 conditions (Johnson and McNicol, 2010), which provided the larvae with a larger food resource. Furthermore, a later study of the interaction between the larvae of the weevil *Sitona discoideus* and the pea aphid, *Acyrthosiphon pisum*, feeding on lucerne (*Medicago sativa*) found that root nodulation can be increased under elevated CO_2 conditions (Ryalls *et al*., 2013). These authors also found that weevil larvae herbivory caused over-compensatory root growth, increasing net root biomass by 31%. Under elevated CO_2 conditions larval development increased resulting in increased weevil emergence, whilst aphid colonisation aboveground was unaffected. Although non-significant, there was a 10% reduction in plant colonisation by aphids when weevil larvae were feeding belowground, suggesting a competitive interaction between the two spatially-separated herbivores.

Root herbivory has been hypothesised to modify the expected plant responses to elevated CO_2 (Johnson and Riegler, 2013): increased root herbivory can reduce plant photosynthetic ability with potential effects on herbivores feeding aboveground. Climate change therefore has the potential to disrupt above- and belowground trophic interactions among insect herbivore species and plants, with implications for higher trophic levels and ecosystem processes.

In this study the influence of elevated atmospheric CO_2 on two cultivars of a small, but high-value, crop plant (raspberry – *Rubus idaeus*), along with the plant-mediated interaction between an aboveground (large raspberry aphid – *Amphorophora idaei*) and belowground (vine weevil larvae – *Otiorhynchus sulcatus*) herbivore are investigated. In chapter II a facilitative relationship was found to exist between these two herbivores feeding on two cultivars of raspberry which differed in their susceptibility to herbivory. Both herbivores benefitted when feeding simultaneously on the plant. The following hypotheses were tested:

1) The root and leaf biomass and the carbon to nitrogen (C:N) ratio of the plants will be increased in response to elevated atmospheric CO_2;

2) The presence of weevil root herbivory will increase the plant tissue C:N ratio by restricting root uptake of nitrogen;

3) The greater amount of foliar biomass available under elevated CO_2 conditions will lead to greater aphid abundance;

4) The enhanced plant growth due to elevated CO_2 will be nullified by the presence of weevils due to increased root damage; and

5) The facilitative relationship between the above- and belowground herbivore will persist despite the challenge to the trophic system presented by elevated CO_2.

5.3 Materials and methods

5.3.1 Experimental design

A microcosm experiment was carried out (November 2011 – November 2012) involving four herbivore treatments (herbivore-free control, aphid only, weevil only and a combination of both herbivores) that were assigned to each cultivar (12 replicates of each). Each microcosm was a 50cm length of cable trunking as described in Chapter II. The two cultivars used in this study were Glen Ample and

Glen Clova. These cultivars were used because feeding facilitation between our study herbivores persisted, despite each cultivar having differing degrees of susceptibility (Chapter II). There were also two different CO_2 treatments: ambient (390 ppm ±50 ppm) and elevated (550 ppm ±50 ppm). These CO_2 regimes were based on IPCC atmospheric CO_2 predictions by 2100 (Meehl *et al.*, 2007). Each CO_2 treatment contained 12 replicates of each cultivar–herbivore treatment combination. To minimise pseudo-replication of CO_2 treatments the experiment was repeated three times. Individual plant replicates were randomly distributed within spatial blocks with each block comprising one complete cultivar–herbivore treatment combination.

Experiments were carried out in four controlled environment chambers (approximately 4×10 m^2) of the GroDome™ climate change research facility at the Centre for Ecology and Hydrology (CEH), Wallingford, U.K. A CO_2 sensor (GMW22; Vaisala, Finland) was in every chamber and connected to a controller unit (AL2-24MR-D micro-controller; Mitsubishi, Japan). If CO_2 levels fell below treatment level (390 and 550 ppm, respectively), CO_2 gas (BOC, U.K.) was injected for 1s, followed by a 30-s delay, repeating until the required concentration was reached. Plant were grown from rootstock obtained from JHI in a 2:1 compost (Levington M3) : sand mixture (The plants were grown in glasshouse chambers for 10-weeks under conditions of 16:8 hour (Light:Dark) with additional lighting provided by halide bulbs (400 W) when active radiation dropped below 400 $\mu mol\ s^{-1}m^{-2}$, and a controlled daytime temperature of 18°C (±2°C) and minimum night temperature of 10°C (±2°C). Weevil eggs collected from cultures maintained at 18°C were added to the soil of appropriate replicates in Week 4, hatching in Week 6 (as reported by Son and Lewis, 2005). Three adult large raspberry aphids were added to the upper-most unfurled leaf of the appropriate plants in Week 8. The colonisation sequence of weevils and aphids replicates natural phenology (McMenemy *et al.*, 2009; Moorhouse *et al.*, 1992).

5.3.2 Plant and insect sampling

After 10 weeks, the height and number of leaves on each plant were recorded and the aphid population sizes counted, and then removed. The aboveground plant material was then removed and snap-frozen in liquid nitrogen. Roots were carefully separated from the soil, washed and snap-frozen. The soil was then placed in Tullgren funnels for 24 hours to extract vine weevil larvae. The larvae collected were counted and stored in ethanol. After being snap-frozen the roots and shoots were freeze dried for a further 24 hours, after which the tissue was ball-milled to a fine powder for subsequent C:N analysis. Chemical analysis of carbon and nitrogen concentrations of leaf and root tissue was undertaken at Centre for Ecology and Hydrology, Lancaster, using an Exeter Analytical Elemental Analyser (EAI, Coventry, UK). The carbon and nitrogen content in organic and inorganic compounds can be determined by combustion of the weighed sample (typically 2–5 mg) that occurs in pure oxygen under static conditions. Various reagents ensure complete oxidation and removal of undesirable by-products and the gases are eventually passed through thermal conductivity cells where the output is converted to percentage carbon and nitrogen. Using these values for the carbon and nitrogen content of the leaves, it is then possible to calculate the C:N.

5.3.3 Data analysis

Statistical analysis of the experiment was carried out in SAS (version 9.3) using generalised linear mixed effects models (GLIMMIX). Response variables were plant biometrics (above- and belowground C:N and biomass), and aphid and weevil abundance (counts). Categorical explanatory variables included: ‘herbivore treatment’ (herbivore-free control, aphid only, weevil only, both herbivores), ‘Cultivar’ (Glen Ample or Glen Clova) and ‘CO_2 treatment’ (ambient and elevated). Above- and belowground plant dry weight and leaf and root C:N were fitted as continuous explanatory variables to insect models only.

All plant biometrics were modelled with Gaussian distribution and an identity link function. For plant biomass models the data were log transformed to meet the assumption that residuals were normally distributed with homogeneity of variance. All other response variables remained untransformed. Aphid and weevil counts were modelled with a Poisson distribution and a log link function.

Random effects in the GLIMMIX models were: ‘chamber’ nested within ‘run’ to account for different chambers used during the three experimental runs and ‘block’ which accounted for the randomised block design within each chamber. To account for the overdispersion of count data, ‘replicate’ was included as an additional random effect in insect models.

Models underwent forward stepwise selection until a minimum adequate model was obtained. Statistical significance of main effects are always reported, whereas two-way interactions are reported only where $P < 0.05$. Degrees of freedom were estimated using the Satterthwaite approximation. Partial residual plots were constructed to show the influence of particular explanatory variables on response parameters accounting for other significant terms retained in the model.

5.4 Results

5.4.1 Plant biomass

As a main effect elevated CO_2 did not affect shoot or root biomass (Table 5.1). As hypothesised, root herbivory reduced root biomass with both treatments that contained weevil larvae yielding significantly less root biomass than those without weevils (Figure 5.1a, Table 5.1). There was also a significant interaction between herbivore and CO_2 treatments affecting root biomass (Table 5.1). Under ambient conditions, weevils significantly reduced root biomass, but this effect dissipated under elevated CO_2, suggesting a mitigation of herbivory impacts on roots (Figure 5.1a).

Figure 5.1a)

b)

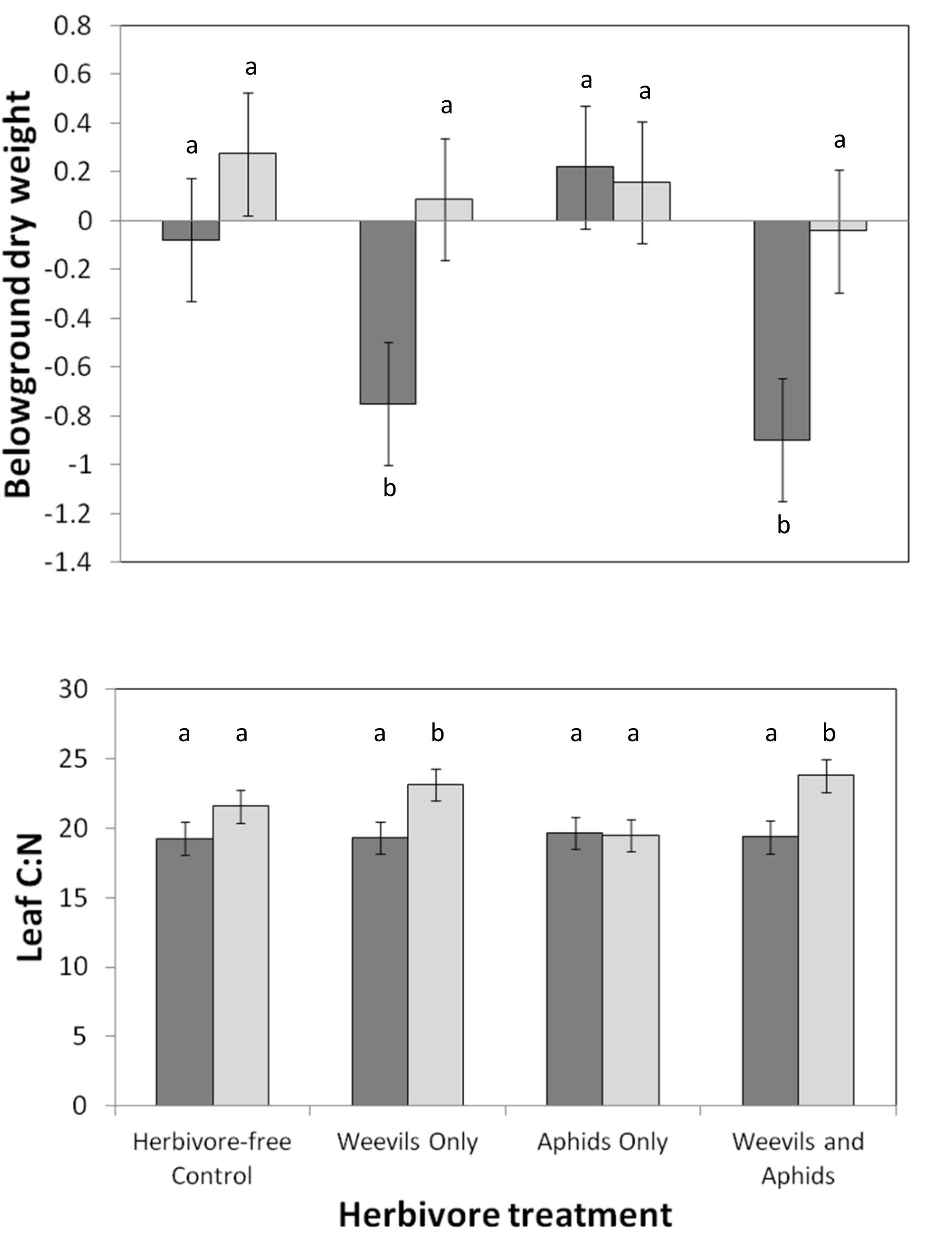


Figure 5.1: The interaction between CO_2 treatment (ambient = dark bars; elevated = light bars) and the four herbivore treatments and their effect on: a) belowground dry weight; and b) ratio of carbon to nitrogen (C:N) in leaf tissues (Least square means ±SE derived from GLMM). Differing characters indicate a significant difference between treatments ($P < 0.05$).

Table 5.1: GLMM results summary for plant response parameters. Bold type indicates parameters retained in the final model. “*” indicates interaction terms. MPE = multiple parameter estimates.

Plant response variable	**Explanatory variables**	**Estimate**	$F_{(\text{ndf, ddf})}$	***P***
Aboveground plant dry weight	Herbivore treatment	-0.02742	$0.91_{(3,172)}$	0.4378
	Cultivar	**-0.1309**	**$4.69_{(1,174)}$**	**0.0316**
	CO$_2$ treatment	-0.3363	$1.15_{(1,4)}$	0.3433
	Leaf C:N	**0.03025**	**$19.87_{(1,183)}$**	**<0.0001**
Belowground plant dry weight	**Herbivore treatment**	**0.3147**	**$10.22_{(3,171)}$**	**<0.0001**
	Cultivar	**0.1994**	**$4.03_{(1,170)}$**	**0.0462**
	CO$_2$ treatment	-0.8564	$2.53_{(1,4)}$	0.1884
	Root C:N	**0.07249**	**$23.67_{(1,180)}$**	**<0.0001**
	Herbivore treatment*CO$_2$ treatment	**0.5042**	**$5.14_{(3,171)}$**	**0.002**
Leaf C:N	Herbivore treatment	-2.1943	$2.13_{(3,175)}$	0.0976
	Aboveground plant dry weight	**2.663**	**$20.04_{(1,140)}$**	**<0.0001**
	Cultivar	0.7914	$1.61_{(1,175)}$	0.2056
	CO$_2$ treatment	-4.3942	$4.03_{(1,4)}$	0.1101
Root C:N	**Herbivore treatment**	**-4.6978**	**$15.79_{(3,174)}$**	**<0.0001**
	Belowground plant dry weight	**-0.04296**	**$18.69_{(1,172)}$**	**<0.0001**
	Cultivar	0.4126	$0.93_{(1,170)}$	0.3371
	CO$_2$ treatment	-0.5785	$0.2_{(1,4)}$	0.6815
	Belowground plant dry weight *Herbivore treatment	**2.2412**	**$7.68_{(3,173)}$**	**<0.0001**

Table 5.2: GLMM results summary for insect response parameters. Bold type indicates parameters retained in the final model. “*” indicates interaction terms. MPE = multiple parameter estimates.

Response variable	**Explanatory variables**	**Estimate**	***F*** $_{(ndf, ddf)}$	***P***
Herbivore responses				
Aphid abundance	Weevil abundance	0.3706	$0.83_{(1,31)}$	0.3706
Random effect estimate = 0.695	**Aboveground plant dry weight**	**0.1529**	$\mathbf{6.72_{(1,72)}}$	**0.0115**
	Belowground plant dry weight	0.1446	$0.77_{(1,68)}$	0.3842
	Cultivar	0.1844	$0.48_{(1,69)}$	0.4894
	CO_2 treatment	-1.5295	$2.79_{(1,8)}$	0.1322
	Leaf C:N	0.02029	$0.49_{(1,72)}$	0.4843
	Root C:N	0.0529	$1.76_{(1,72)}$	0.1883
	Aboveground plant dry weight *CO_2 treatment	**1.383**	$\mathbf{4.51_{(1,72)}}$	**0.0372**
Weevil abundance	Aphid abundance	0.007077	$3.73_{(1,27)}$	0.0639
Random effect estimate = 0.2071	Aboveground plant dry weight	-0.01534	$0.01_{(1, 63)}$	0.9205
	Belowground plant dry weight	-0.1096	$1.8_{(1,71)}$	0.1838
	Cultivar	0.1071	$0.63_{(1,68)}$	0.4311
	CO_2 treatment	-0.309	$0.55_{(1,4)}$	0.4996
	Leaf C:N	0.02575	$2.73_{(1,75)}$	0.1029
	Root C:N	-0.00635	$0.06_{(1,83)}$	0.8132

Aboveground dry weight was positively correlated with leaf C:N (Table 5.1), suggesting larger plants had a greater C:N ratio, potentially due to a greater amount of structural carbon present in those plants, or dilution effect of nitrogen with increasing biomass. Similarly, there was a positive relationship between root C:N ratio and root biomass (Table 5.1). Aboveground dry weight was also greatest in the less susceptible cultivar – Glen Clova (Table 5.1). The reverse was true for belowground dry weight, which showed the more susceptible cultivar (Glen Ample) to have significantly greater root biomass than the less susceptible cultivar (Table 5.1).

5.4.2 Plant C:N

As a main effect elevated CO_2 conditions had no effect on leaf C:N (Table 5.1; Figure 5.2). However, similar to the effect detected on root biomass, the C:N ratio of leaf tissue was affected by the interaction between herbivore and CO_2 treatments. Under elevated CO_2 conditions, leaf C:N was increased by the presence of weevils (Table 5.1; Figure 5.1b), whereas this effect disappeared under ambient CO_2 conditions (Figure 5.1b). There was no evidence that herbivore treatment affected the C:N ratio of leaf tissues (Table 5.1; Figure 5.3).

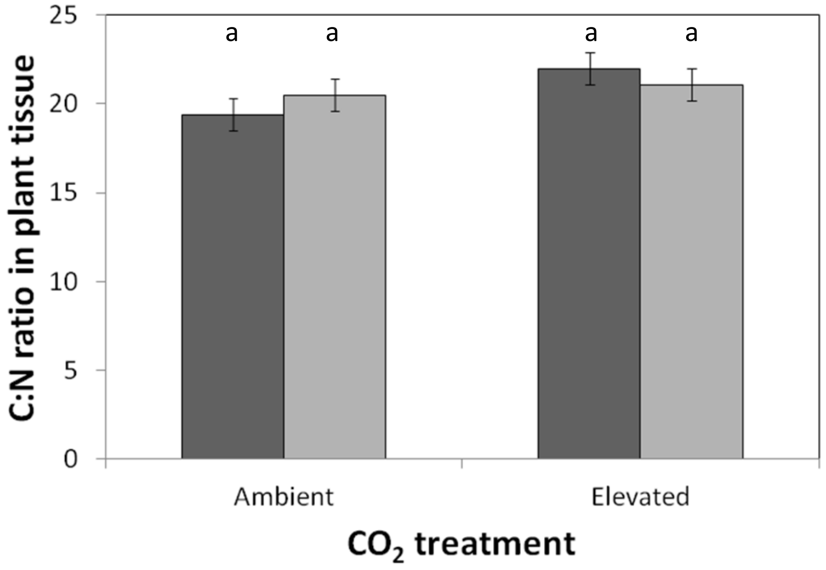


Figure 5.2: The non-significant effect of CO_2 treatment on the ratio of carbon to nitrogen (C:N) in leaf (dark bars) and root (light bars) (Least square means ±SE derived from GLMM). Identical characters indicate no significant difference between treatments ($P > 0.05$).

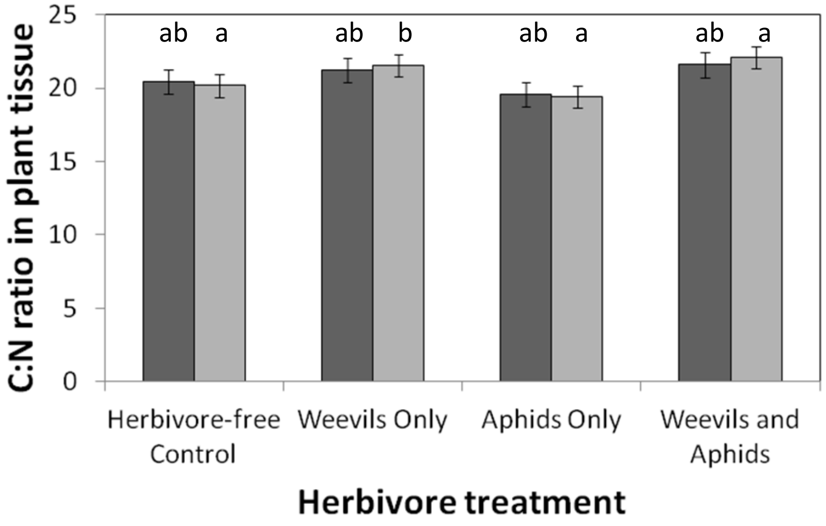


Figure 5.3: The effect of herbivore treatment on the ratio of carbon to nitrogen (C:N) in leaf (dark bars) and root (light bars) tissues (Least square means ±SE derived from GLMM). Differing characters indicate a significant difference between treatments ($P < 0.05$).

Root C:N was significantly higher in roots where weevil larvae were present in the soil (Table 5.1; Figure 5.3), suggesting that the plants may be unable to take up sufficient nitrogen due to root damage as suggested in Hypothesis 2. As a main effect belowground dry weight significantly decreased root C:N (Table 5.1). When accounting for the main effects of root biomass and herbivore treatment there was a significant interaction between the two variables that affected root C:N (Table 5.1). Increased root biomass was associated with an increased C:N ratio of root tissues (Figure 5.4a & b), but this was significantly reduced where weevil larvae were present (Figure 5.4c & d).

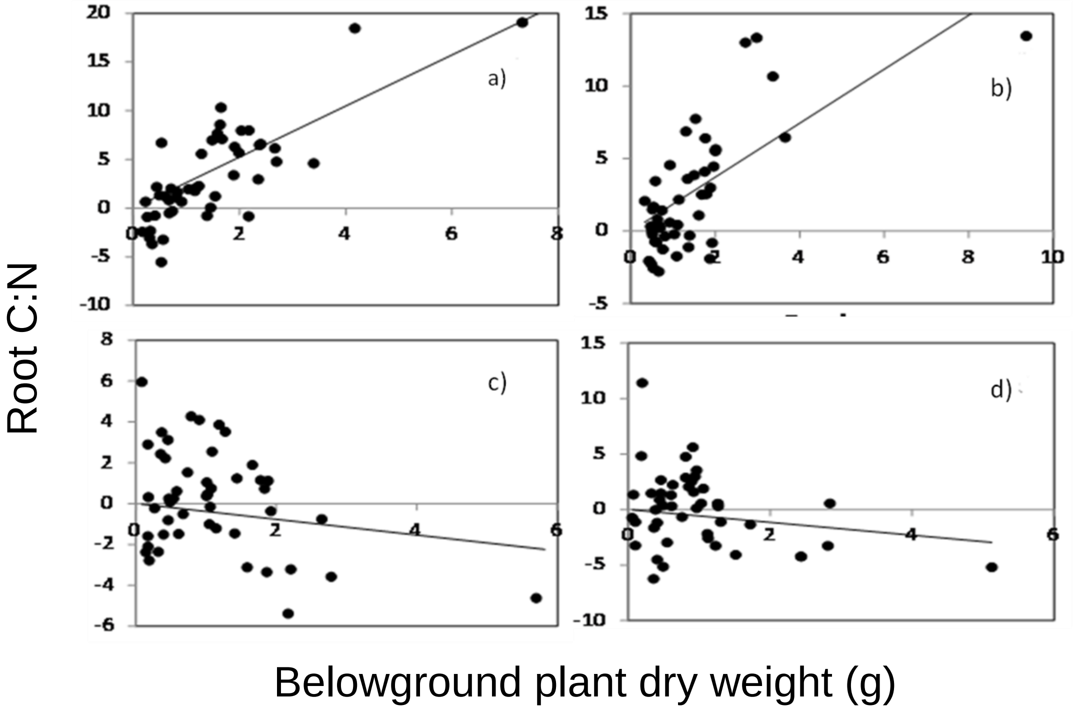


Figure 5.4: Partial residual plots on the linear predictor scale for the effect on root C:N of the interaction between the four herbivore treatments: a) herbivore-free control; b) aphid only; c) weevil only; and d) weevil and aphid and plant root dry weight (g).

5.4.3 Insect responses

Aphid abundance was positively related to aboveground plant dry weight (Table 5.2), but the interaction between aboveground dry weight and CO_2 treatment showed the relationship to be stronger under ambient conditions (Table 5.2; Figure 5.5). There were no differences in insect numbers between cultivars or CO_2 treatments (Table 5.2). There was no evidence to suggest that feeding facilitation occurred between the herbivores with aphid abundance having no effect on weevil larvae abundance (Table 5.2) and weevil larvae abundance having no effect on aphid abundance (Table 5.2).

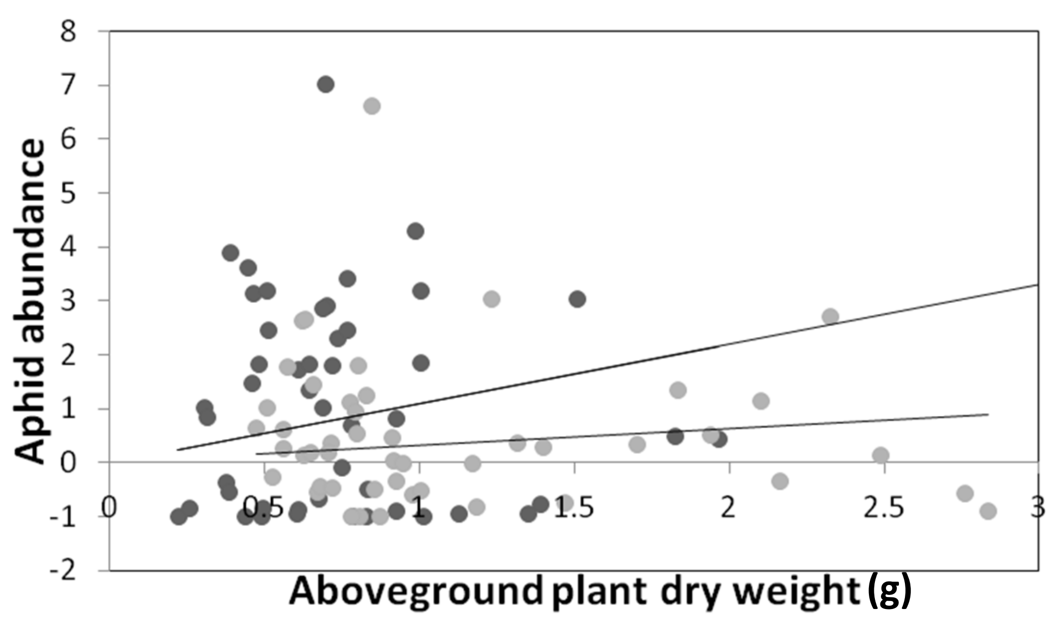


Figure 5.5: Partial residual plots on the linear predictor scale for effect on aphid abundance of the interaction between the two CO_2 treatments (ambient = dark dots; elevated = light dots) and aboveground plant dry weight (g).

5.5 Discussion

Contrary to Hypothesis 1, root and leaf biomass and the C:N ratio of the plants remained unaltered by elevated atmospheric CO_2 as a main effect. Having controlled for main effects, however, leaf C:N was higher in the elevated CO_2 weevil treatment, but there were no significant effects observed in the elevated CO_2 conditions. This could be attributed to the varying effect of elevated CO_2 on annual and perennial plants. Woody perennials, such as raspberry, tend to react less to elevated CO_2 than annual plants due to their capacity to store carbon and nitrogen, mainly in the plant stem. This investment in storage may restrict immediate plant growth but be advantageous in the long-term with increased growth and survival (Gerbauer *et al*., 1996).

It was then hypothesised (Hypothesis 2) that the presence of weevil larvae would increase the C:N ratio in the plant, given that root herbivory can restrict nitrogen uptake from the soil. It was found that the presence of weevil larvae in the soil increased root, but not shoot, C:N. This could be explained by the plant investing a greater volume of carbon to the roots for growth and therefore increasing the concentration of carbon in those tissues. That roots are being severed as a result of herbivory will also decrease the plants’ ability to take up nitrogen from the soil, therefore increasing the C:N ratio further. As a main affect, root C:N decreased with increasing belowground root biomass, showing that the larger the plant the more nitrogen it can take up. Presumably, greater root mass increased the nitrogen concentration of the roots, consequently reducing the C:N ratio. The interaction between herbivore treatment and belowground dry weight on root C:N suggested that increased belowground dry weight increased root C:N, but was significantly reduced by root herbivory. This interaction term accounts for the variability observed in the effects of herbivore treatment and belowground dry weight, and therefore illustrates that this positive relationship between root C:N and belowground dry weight is contingent on the type of herbivory experienced.

Hypothesis 3 predicted that aphid abundance would increase under elevated CO_2 conditions due to the greater amount of biomass available. In actuality, with no effect of elevated CO_2 on plant biomass, aphid abundance did not change. Aphid abundance increased with increasing aboveground dry weight, more so under ambient conditions. This finding is supported by the fact that plants grown under ambient conditions are of higher nutritional quality (Robinson *et al*., 2012). To date, aphid responses to elevated CO_2 have been idiosyncratic, with positive, negative and null responses observed (Bezemer *et al*., 1999; Newman *et al*., 2003; Sun and Ge, 2011). Although the meta-analysis by Robinson *et al*. (2012) showed that elevated CO_2 increased phloem feeder abundance significantly by 22%, this estimate includes non-aphid taxa and there are plenty of examples of aphids not responding to CO_2 (Sun and Ge, 2011). It has previously been shown that in the majority of cases (12 out of 19 studies) where aphid population response has been investigated there were no changes (Newman *et al*., 2003). This may be attributed to whether the plant showed a significant response to CO_2 or not. In this present study, we found that elevated CO_2 did not affect the plant (except when interacting with other variables), and therefore the effects did not cascade to the next trophic level of the aphids. Newman *et al*. (2003) also produce a model that goes some way to predict why this may be the case and why aphid responses to CO_2 in general are idiosyncratic. Their model shows that aphids may be nitrogen–limited; therefore any increase in leaf C (caused by increased atmospheric CO_2 synthesis) does not benefit them. Aphid nitrogen limitation is dependent on the soil quality (e.g. nitrogen content) and the aphid species–specific nitrogen requirements. Nitrogen limitation may have occurred in the current study and therefore could explain the lack of effect of CO_2 on this aphid species.

Hypothesis 4 predicted that enhanced plant growth caused by elevated CO_2 would be nullified by the presence of weevils due to increased root damage. As stated previously, as a main affect CO_2 did not alter plant growth. It was found that in ambient conditions weevil abundance negatively affected root biomass. However, after accounting for the main effects of CO_2 and herbivory, the interaction between these two variables provided a further relationship: increased CO_2 mitigated the negative effects of root herbivory on plant roots. The potential mechanism behind this may be that increased atmospheric CO_2 provides more carbon that the plant can synthesise for root re-growth, therefore lessening the net root loss. The net effect of root herbivory and elevated CO_2 was similar to that found by Johnson and Riegler (2013), in that elevated CO_2 and root herbivory together returned root biomass of eucalypts to levels similar to ambient, with no herbivory. In this earlier study, elevated CO_2 increased root biomass, which was subsequently reduced by herbivory. In the current study, the negative effect of root-herbivory seen under ambient CO_2 conditions was mitigated due to increased root production at elevated CO_2; however, the net effect remained the same.

The final hypothesis (Hypothesis 5) was that the facilitative relationship previously observed between the herbivores will persist despite being challenged by elevated CO_2. In this current study, however, a null interaction between the two herbivores was found. It is currently unknown why this would be the case. Different growing conditions and the fact that the experiment was carried out over three temporal runs could, however, go some way to explain the differences between the studies. The studies were also carried out in different locations, despite being climate controlled.

Herbivore treatment played a significant role in influencing plant biomass and C:N. This suggests that herbivory could potentially play a larger role in a future climate than the effect of elevated CO_2 alone on the plant. This is emphasised by the significant interactions between herbivore and CO_2 treatment in determining the outcome of leaf C:N and belowground biomass. Moreover, it emphasises the importance of belowground chewing herbivores as mediators in plant–soil interactions by amending plant C:N and how this plant–herbivore interaction may be altered by climate change. This study reveals the importance of biological interactions over atmospheric CO_2 enrichment. The study also emphasises the importance of considering interactions between herbivory and environmental change. In particular, belowground herbivory appears important when investigating the effects of climate change not only on the plant responses to elevated CO_2 but also potentially on other spatially separated herbivores.

Chapter VI

Aboveground herbivory, not elevated CO_2, causes over-compensatory growth of grazed plants and reduces neighbouring plant biomass

6.1 Abstract

Above- and belowground herbivory can indirectly influence intraspecific plant competition, and ultimately individual dominance. The hetero- or conspecific context of a plant individual may, through associational resistance or susceptibility, govern the regulatory effects of herbivores on plant populations. Anthropogenic enrichment of atmospheric CO_2 concentrations is expected to lead to greater primary productivity, which may also affect the impact of herbivory. In this chapter, the effects of above- and belowground herbivory, and an elevated CO_2 (550 ppm) climate on intraspecific plant competition were investigated. It was experimentally tested whether a belowground herbivore (vine weevil larvae) preferentially fed on a host-plant (raspberry) already attacked by an aboveground herbivore (large raspberry aphid). The impact this had on the productivity of plant conspecifics free of either above- or belowground herbivory, and moreover the role of elevated CO_2 in affecting the plant–herbivore interaction was also tested. The belowground herbivore showed no preference between aphid-infested and non-infested plants, by distributing themselves freely between the roots of both plants. Aphid-infested plants exhibited over-compensatory growth and were 64% larger than neighbouring aphid-free plants. Atmospheric CO_2 level did not affect either the plant mediated above- and belowground interaction or intraspecific plant competition This experiment shows the potential for above- and belowground herbivory to affect primary productivity, and potentially alter intraspecific plant competition, to a greater extent than elevated atmospheric CO_2 concentrations.

6.2 Introduction

Above-and belowground biota are intrinsically linked (Van der Putten *et al*., 2009) and aboveground herbivory can have a profound impact on belowground processes and *vice versa* (Bardgett *et al*., 1998; Bardgett and Wardle, 2003). Ultimately, the interactions between herbivores and the plant may affect intra- and interspecific competition both in the rhizosphere and aboveground (Van der Putten *et al*., 2001; Blossey and Hunt-Joshi, 2003). Moreover, root herbivory has been identified as being potentially more important than plant competition in determining plant community composition (Nötzold *et al*., 1997). Herbivores in the rhizosphere influence intra- and interspecific plant competition by damaging plant root biomass which retards nutrient uptake and primary production (Olff and Ritchie, 1998; Bonser and Reader, 1995; Steinger and Müller-Schärer, 1992). Variation in host-plant quality may lead to preferential grazing by root herbivores and this could result in some plants being at a competitive disadvantage (Olff and Ritchie, 1998; Rees and Brown, 1992).

Plants can compete directly for space, light and nutrients (Casper and Jackson, 1997; Pacala and Crawley, 1992; Weigelt and Jolliffe, 2003; Trinder *et al*., 2013), but they can also affect the levels of herbivory a competing plant experiences. Plant community composition can affect plant competition indirectly through the processes of associational resistance or susceptibility (Barbosa *et al*., 2009). The concentration of the host-plant resource in conspecific aggregations means that an individual plant may experience greater herbivore recruitment and damage than when it is surrounded by heterospecific plants (Kos *et al*., 2014). Associated susceptibility can also occur as a consequence of neighbouring heterospecific plants increasing the attractiveness of a particular plant species to insect herbivores (Barbosa *et al*., 2009; Baraza *et al*., 2006). For instance, cottonwood (*Populus angustifolia* × *P. fremontii*) plants growing under box elder (*Acer negundo*), received a greater amount of herbivory from cankerworm (*Alsophila pometaria*) than when growing around conspecifics or in the open (White and Whitham, 2000). Alternatively, growing among or near to conspecific plants can confer associated resistance on an individual plant. Consequently, the plant experiences reduced herbivory via the reduced ability of herbivores to locate the plant host, either chemically or visually (e.g. Hambäck *et al*., 2000; Barbosa *et al*., 2009). These processes governing patterns of herbivory may therefore contribute to the outcome of intra- and interspecific plant competition, with implications for plant performance and ultimately community structure (Ohgushi, 2005).

These neighbourhood effects can also be mediated belowground. Plants are able to detect hetero- and conspecifics via physical and chemical root interactions, and this can play a major role in plant competition belowground, which can have systemic effects on aboveground plant tissue, such as plant height or leaf area production (Chen *et al*., 2012). Insect herbivory aboveground can cause systemic induction of plant defence volatiles in heterospecific neighbouring plants, via shared mycorrhizal networks in the soil, enabling the plant to initiate defences prior to herbivore attack (Babikova *et al*., 2013). This may be modified by belowground herbivory, as severed roots and altered mycelia contact between neighbouring plants will reduce the plants' ability to produces volatiles (Babikova *et al*., 2013). This effect may be exacerbated if the herbivore shows a preference for one plant species or individual over another.

Most work to date looking at the role of herbivores in plant competition has related to the aboveground process (e.g. Pacala and Crawley 1992; Crawley, 1989; Schädler *et al*., 2007; Haag *et al*., 2004; Hambäck *et al*., 2013; White and Whitham, 2000), with only a few studies investigating the effects of belowground herbivores (e.g. Kos *et al*., 2014), due to the difficulty of studying the effect of belowground processes on wider ecosystem function (Brussaard, 1998). Investigating the effect of belowground herbivory on plants poses practical problems because it is difficult to assess real-time movement and herbivore damage within intact soil (Johnson *et al*., 2008). The relative importance of certain factors aboveground, such as plant defence or the conspecificity or heterospecificity of adjacent plants may also be relevant to belowground herbivore–plant interactions, but remain to be fully tested.

Previous studies on the effects of herbivory on plant competition have examined systems where the herbivore species were competing for the shared plant resource (e.g. Barbosa *et al*., 2009). The outcome of such indirect plant competition would, however, be expected to differ where positive interactions occurred between insect herbivores. Feeding facilitation between above- and belowground herbivore species has been reported recently (Huang *et al*., 2013), where attacks on the plant by each herbivore elicit changes to the host, resulting in a mutual increase in the abundance of both herbivores. Reciprocal feeding facilitation between the aboveground large raspberry aphid (*Amphorophora idaei*) and the belowground larvae of the vine weevil (*Otiorhynchus sulcatus*) feeding on raspberry (*Rubus idaeus*) has been reported in Chapter II. This positive relationship is potentially mediated through over-compensatory plant growth in response to herbivory. This would suggest it would benefit the vine weevil to choose to feed on plant individuals that are attacked by large raspberry aphids aboveground, with potential additional positive (over-compensatory growth) or negative (reduced biomass) effects for the plant. Furthermore, how the combination of above- and belowground herbivory affects consequential competition between plants in this system is yet to be tested empirically.

Global environmental changes such as increasing atmospheric CO_2 levels (IPCC, 2013), are expected to lead to enhanced primary productivity (Kimball *et al*., 1993) and also affect trophic interactions (Antoninka *et al.*, 2009; Chakraborty *et al.*, 2012; Harrington *et al.*, 1999; Sanders *et al.*, 2004; Schröter *et al.*, 2004; Stacey and Fellowes, 2002; Tylianakis *et al.*, 2008; Van der Putten *et al.*, 2004; Voigt *et al.*, 2003). Therefore, there is the potential that the impact of herbivores on plants may be mitigated by atmospheric CO_2 enrichment increasing plant growth (Johnson *et al*., 2011). Such changes in primary production are likely to affect direct and herbivore-mediated plant competition, potentially altering the role of higher trophic levels in shaping plant community structure (Antoninka *et al.*, 2009; Tylianakis *et al.*, 2008; Van der Putten *et al.*, 2004). This, however, is yet to be tested empirically.

This experiment investigated whether the presence of an aboveground herbivore (Large raspberry aphid) on raspberry makes the plant more susceptible to colonisation by a belowground herbivore (vine weevil larvae). It explores the effect this may have on the relative above- and belowground biomass and photosynthetic ability of the two conspecific plants. The study also sets out to investigate how elevated CO_2 affected the outcome of these plant–herbivore and plant–plant interactions, particularly if it affects the distribution of weevil larvae between aphid-infested and non-infested plants. Consequently, the following hypotheses were postulated:

1) Feeding facilitation between the herbivore species predicts that weevil larvae will preferentially select host-plants where an aboveground herbivore is present;

2) Aphids have been shown to stimulate over-compensatory growth in this study system, consequently aphid infested plants will attain greater biomass than aphid-free plants, and hence a competitive advantage; and

3) An elevated carbon dioxide environment will produce over-compensatory plant growth and increased rates of plant photosynthesis where herbivores are present, thereby reducing the overall impact of herbivory.

6.3 Materials and methods

6.3.1 Experimental design

A 9-week microcosm experiment was carried out at the Grodome climate change facility at the Centre for Ecology and Hydrology, Wallingford, from September 2013 to November 2013. Microcosms were randomly assigned to two CO_2 treatments: ambient (390 ppm) and elevated (550 ppm), and four herbivore treatments: herbivore-free control (A-W-); aphid only (A+W-); weevil only (A-W+); and both herbivores (A+W+) (Figure 6.1). The four herbivore treatments exist at the *pot* level, but within the pot there was also aphid treatment at the *plant* level. Where aphids were present in the pot (A+W- and A+W+), out of the two plants in the pot only one was inoculated with aphids (“focal”), the other plant was termed the “neighbouring” plant (Figure 6.1). The remaining herbivore treatments (A-W- and A-W+) are termed “absent” plants, as there were no aphids present in the pot, or on individual plants (Figure 6.1). The CO_2 treatments were divided across four chambers: two ambient and two elevated. There were 12 replicate pots of each herbivore–CO_2 treatment, giving a total number of 96 replicate pots and 192 experimental plants in 24 experimental blocks. The experimental chambers were maintained at 18°C (±2°C) and a 16:8 light:dark photoperiod.

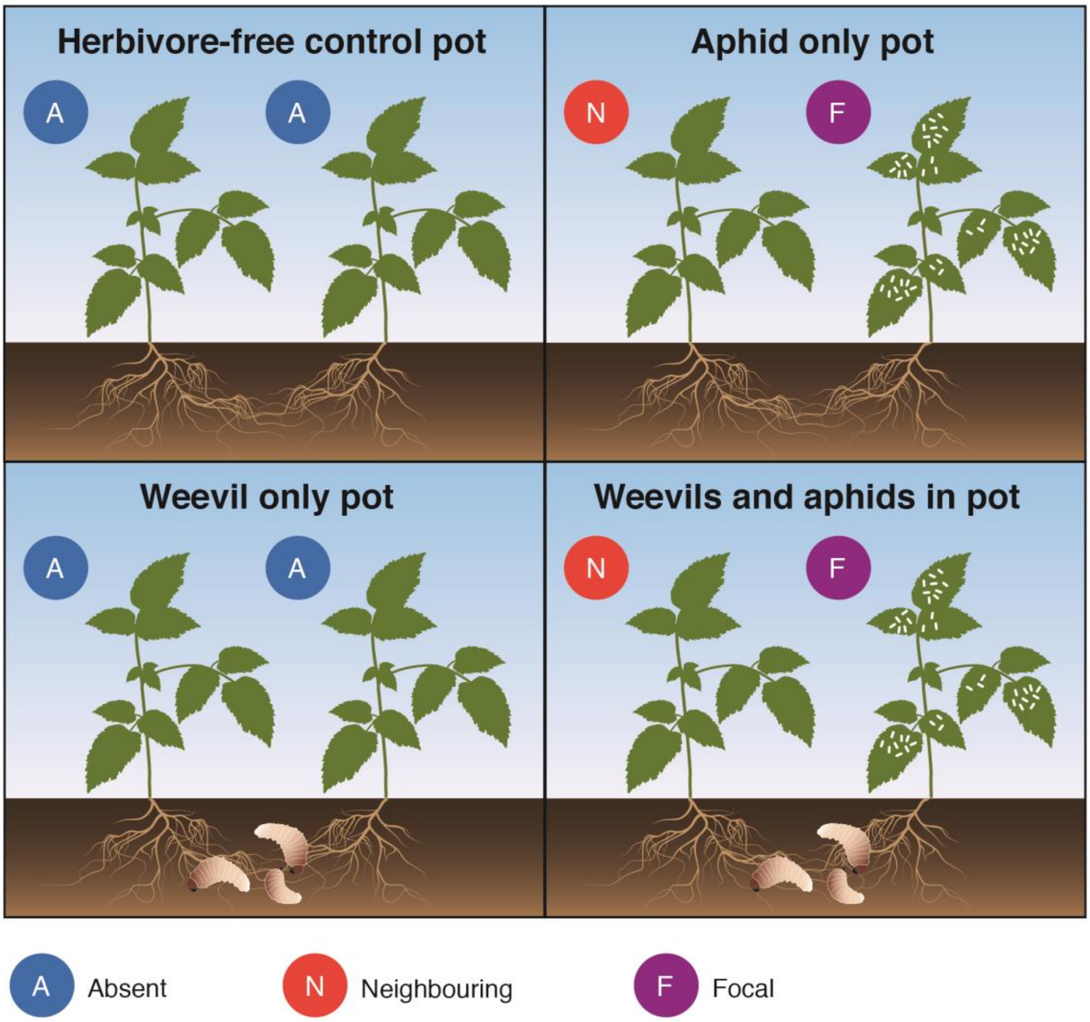


Figure 6.1: Experimental design showing the four herbivore treatments at pot level (herbivore-free control; aphid only; weevil only and; weevils and aphids), and the aphid treatments at plant level (absent, neighbouring and focal).

6.3.2 Experimental set-up

Two randomly selected raspberry plants (Glen Clova cultivar) were planted 10 cm apart in a 3 litre pot. This distance was sufficiently close to allow plant interaction within the pot during the experimental timeframe. Plants were grown from rootstock sourced from the James Hutton Institute (JHI) for 10 weeks until each plant was at least 5cm in height. Experimental pots were filled with a 2:1 mixture of compost (M3 Levington) : sand, which allowed roots to be subsequently teased apart and the weevil larvae to be removed with relative ease. Plant height and photosynthetic ability were measured to ensure there were no differences between plants from the experimental treatments at the outset. Plant photosynthetic ability was determined using a chlorophyll fluorometer which measured the fluorescence yield of the leaf tissue (PAM-2100, Walz, Germany).

Two weeks after planting the raspberry plants into the experimental pots, 20 vine weevil eggs were placed equidistant between the two plants of the appropriate treatments (A-W+ and A+W+). Vine weevil eggs were sourced from parthenogenic adults that had been cultured in a controlled temperature room at 18°C at the Centre for Ecology and Hydrology (CEH), Wallingford. Weevil larvae eclosion was timed to occur three weeks later (Week 5), based on temperature dependent development time in Son and Lewis (2005). Also, during Week 5, three adult large raspberry aphids were randomly added to one of the two plants (henceforth termed the “focal” plant) of the appropriate treatments (A+W- and A+W+); the other plant in the pot (the “neighbouring” plant) remained aphid-free (Figure 6.1). This ensured that aphids were already present on the plant as the weevils eclosed and commenced feeding on roots. Porous, plastic “Cryovac” bags were placed over all plants to prevent aphid migration between plant individuals and pots, but allow passage of atmospheric gases and moisture.

After a further four weeks (Week 9) the experiment was harvested. Based on previous experimental observations this was deemed sufficient time for vine weevil larvae to have exerted measurable herbivore damage on the roots, whilst avoiding the possibility of an aphid population crash. At harvest, plant photosynthetic ability was re-measured, and aphids were counted and removed from the plants. Plants and soil were carefully removed from the pot by hand and the identity of which plants the larvae were feeding on determined. The roots and larvae were then separated by hand, and larvae counted. Additionally, weevil larvae feeding damage was characterised by root mass; the lower the root mass, the greater the herbivore damage, as shown in previous chapters. All above- and belowground plant tissue was separated, freeze-dried and the dry mass weighed.

6.3.3 Statistical analysis

Data were analysed with general linear mixed effects models (proc mixed) carried out in SAS (version 9.3). Aphid and weevil abundance (counts) were modelled with a Poisson distribution and log link function. Chlorophyll fluorescence (Ft) and aboveground and belowground plant dry weight (g) were each modelled with a Gaussian distribution and identity link function, plant biomass data were log transformed prior to analysis to ensure that the model assumptions of normally distributed residuals with homogeneity of variance were met.

Available categorical explanatory variables included: above- (focal, neighbouring and absent) and below- (present and absent) ground herbivore treatments and CO_2 treatment (ambient or elevated). Above- and belowground dry-weight were also used as continuous explanatory variables in the insect and chlorophyll fluorescence (Ft) models. The identity of CO_2 chamber and experimental block were fitted as random effects in all models to account for spatial variability across the experiment. Additionally, replicate pot was fitted as a random effect in models of weevil and aphid abundance models to account for over-dispersion in the count data. Degrees of freedom were estimated with the Satterthwaite approximation method.

6.4 Results

Contrary to Hypothesis 1, there was no evidence of a facilitative relationship between the two herbivore species, as indicated by the lack of any difference in weevil distribution between plants according to aphid presence and the lack of an effect of weevil treatment on aphid abundance (Table 6.1). Aphid abundance at harvest ranged between 24 and 457 individuals, whilst weevil abundance varied from 0 to 11 per host-plant.

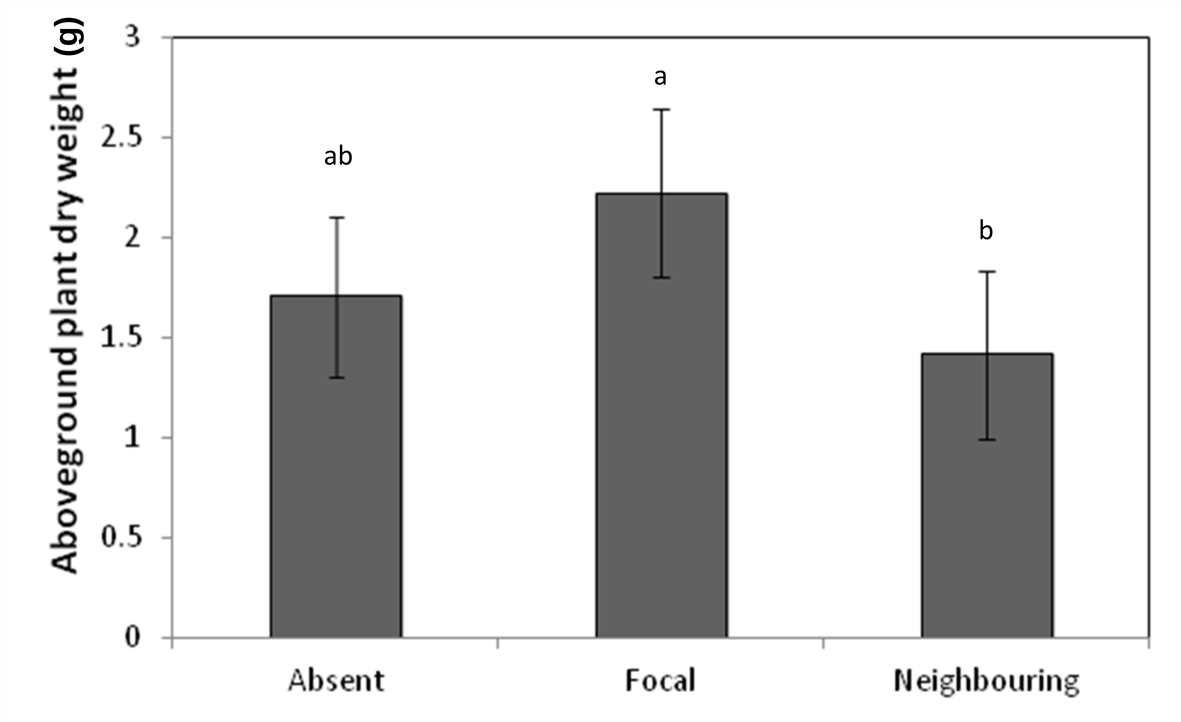


Figure 6.2: The effect of aphid treatments (absent, neighbouring and focal) on aboveground plant dry weight (g). Data are least square means ± SE from GLMM. Differing characters indicate a significant difference between treatments ($P < 0.05$).

In support of Hypothesis 2, focal plants had significantly greater biomass than the neighbouring aphid-free plant (Figure 6.2; Table 6.2), implying over-compensatory plant growth in response to herbivory. There were no statistically significant differences in aboveground dry weight between plants where aphids were absent from the pot (Figure 6.1 treatment A) compared with plants assigned to the focal or neighbouring aphid treatments (Figure 6.2; Table 6.2).

Above- and belowground biomass were positively associated with aphid (Figure 6.3; Table 6.2) and weevil larvae (Figure 6.4; Table 6.2) abundance, respectively. Plants on which weevils were present had significantly greater root-mass than plants where weevil larvae were absent (Table 6.2). The response of plant biomass above and belowground suggests that herbivory had stimulated over-compensatory plant growth. There was evidence suggestive of a positive feedback loop between the herbivores and their associated above- and belowground plant tissue.

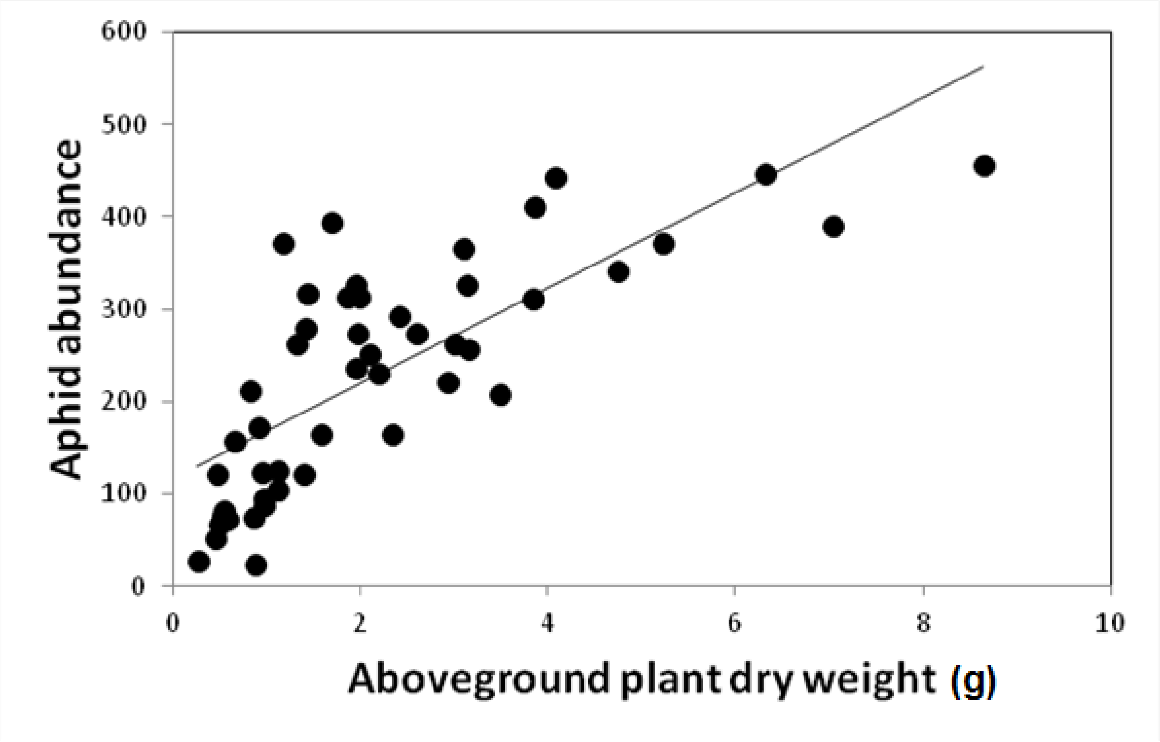


Figure 6.3: Plot showing the positive effect of aboveground dry plant weight (g) on aphid abundance.

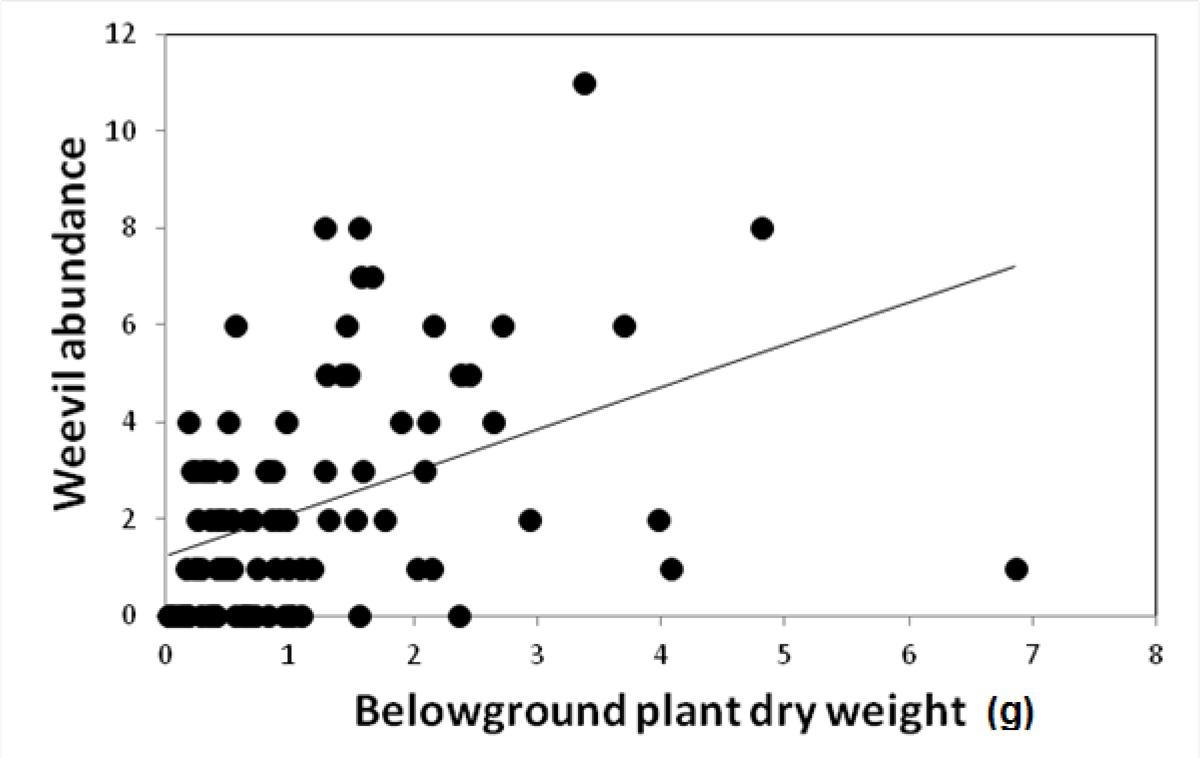


Figure 6.4: Plot showing the positive effect of belowground dry plant weight (g) on weevil larvae abundance.

Chlorophyll fluorescence, and therefore plant photosynthetic ability, was not significantly related to above- or belowground plant biomass or weevil or aphid abundance, although the latter relationship was a marginally non-significant negative effect (Table 6.2). Contrary to Hypothesis 3, elevated atmospheric CO_2 had no effect on above- or belowground plant biomass and aphid or weevil abundance (Table 6.2).

6.5 Discussion

This study found that “focal” plants had significantly greater biomass than the “neighbouring” plant, suggestive of plant over-compensatory growth in the presence of aphids. This may have increased shading which may have contributed to the tendency for the neighbouring conspecific plant to have lower aboveground biomass than either the aphid infested or control plants. The present study showed that a plant's proximity to another conspecific plant infested with an aboveground herbivore indirectly reduced its biomass. This study involved a perennial plant that has previously been shown to exhibit over-compensatory growth in response to herbivory (Chapter II). It is this additional growth following herbivory that may explain the aphid-inoculated plant dominance in the pot. Weevil larvae showed no preference between host-plants, regardless of the presence of aphids aboveground. Chapter II illustrated feeding facilitation where plants were grown in smaller rhizotubes. This current study, however, used larger 3-litre pots, producing larger plants with a greater amount of feeding material. This finding suggests that feeding facilitation may only occur when food resources are limiting. As no plant preference was shown by weevils, no differences in overall belowground plant biomass were observed. It has previously been shown that above- and belowground effects are disproportionate and neighbour effects on plant–herbivore interactions are predominantly aboveground (Kos *et al*., 2014). This has been reinforced in this study where aphid treatments had a greater significant effect on plant biomass of neighbouring plants than belowground herbivory. A previous meta-analysis showed that intra- and interspecific root and shoot competition is less important for crop species compared to wild-type plants, and herbs opposed to grasses (Kiær *et al*., 2013). These conclusions contrast with the current study that used a perennial crop plant, showing the important role of herbivory in influencing plant root and shoot competition.

Aboveground herbivory in maize has been shown to induce a systemic chemical response belowground that increased levels of *p*-coumaroyltyramine in root exudates, subsequently metabolised by belowground herbivores (*Spodoptera littoralis*) leading to increased larval growth (Marti *et al*., 2013). In this maize system, it is possible that belowground herbivores use *p*-coumaroyltyramine in root exudates to locate plant roots that are also being attacked by an aboveground herbivore, with a concomitant improvement in the soil herbivores performance. In the current study, despite previous evidence for a positive herbivore interaction, there were no differences in weevil distribution between plants experiencing different levels of aphid herbivory. One potential explanation is that a lack of chemical induction following aphid colonisation meant that aphid infested plants may not be detectable by the vine weevil larvae in the soil via chemical cues.

Although the aggregation of soil dwelling larvae, in general, is mostly dictated by the oviposition preference of the adults (Johnson *et al*., 2006), this may not drive their acceptance or rejection of the host-plant. The lack of weevil spatial aggregation depending on aboveground herbivore distribution may also be explained by the physical structure of the soil matrix limiting larval movement between plants (Barnett and Johnson, 2013). The neighbour effect may be less of an important issue belowground given the smaller reliance on chemical signals to herbivores and associated natural enemies. The substrate may, therefore, play a more important role in determining the movement of root-herbivores over a chemical cue to plants with aboveground herbivores (Barnett and Johnson, 2013).

The lack of an effect of CO_2 enrichment on primary productivity and on the above–belowground interaction is consistent with the findings from the previous chapters, suggesting that this particular plant–insect trophic interaction is not likely to be affected by future changes in atmospheric CO_2 concentration. Many patterns have been previously observed by multiple studies with regard to the effects of elevated CO_2 on plant–herbivore interactions, such as increased biomass, C:N ratio and tannin concentration seen in plants, and reduced abundance, increased plant consumption rates and development time in herbivores (Robinson *et al*., 2012; Stiling and Cornelissen, 2007; Zavala *et al*., 2013). The lack of a CO_2 enrichment effect could, however, also be due to the low degrees of freedom resulting from the limited number of environmental chambers that could be employed in this study, hence the lack of an detectable effect may be due to low statistical power. The lack of elevated

CO_2 effect on the plant may also be a result of experimental length – a longer-term experiment may have detected a plant and/or herbivore response to increased atmospheric CO_2 change. Aphids may have been unresponsive to increased elevated CO_2 due to nitrogen being limited, which is often cited as the major factor governing aphid population growth (Newman *et al*., 2003), and herbivores in general (Mattson, 1980).

This experiment illustrated the key role of herbivory, both above- and belowground, in regulating plant biomass and the potential to shape intraspecific competition. This may ultimately affect the individual dominance within the plant population. In this trophic system, herbivory may play a greater role than CO_2 in determining primary productivity and potentially even plant dominance. Larger scale field experiments would help to understand the extent to which above- and belowground herbivory influences plant competition and community structure.

Chapter VII

Global climate change and above–belowground insect herbivore interactions

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An adaptation of this chapter has been published in *Frontiers in Plant Science*

McKenzie, S. W., Hentley, W. T., Hails, R. S., Jones, T. H., Vanbergen, A. J., and Johnson, S. N. (2013) Global climate change and above- belowground insect herbivore interactions. *Frontiers in Plant Science* **4:** 412.

7.1 Abstract

Predicted changes to the Earth's climate are likely to affect above–belowground interactions. Our understanding is limited, however, by past focus on two-species aboveground interactions mostly ignoring belowground influences. Despite their importance to ecosystem processes, there remains a dearth of empirical evidence showing how climate change will affect above–belowground interactions. The responses of above- and belowground organisms to climate change are likely to differ given the fundamentally different niches they inhabit. Yet there are few studies that address the biological and ecological reactions of belowground herbivores to environmental conditions in current and future climates. Even fewer studies investigate the consequences of climate change for above–belowground interactions between herbivores and other organisms; those that do provide no evidence of a directed response. This chapter highlights the importance of considering the belowground fauna when making predictions on the effects of climate change on plant-mediated interspecific interactions.

7.2 Introduction

Trophic interactions are likely to be crucial in shaping net effects of global climate change on ecosystems (e.g. Harrington *et al*., 1999; Tylianakis *et al*., 2008). Modified interactions between trophic groups (e.g. spatial or phenological decoupling of herbivore and predator populations) could have far reaching consequences across a range of natural and managed ecosystems with implications for food security (Gregory *et al*., 2009). In particular, the plant-mediated interactions between above- and belowground herbivores (Blossey and Hunt-Joshi, 2003; Johnson *et al.,* 2012a) may be important in the structuring of herbivore and multi-trophic communities (Bardgett and Wardle, 2010; Johnson *et al*., 2013; Megías and Müller, 2010; Soler *et al*., 2012a). Surprisingly, investigating the potential impacts of climate change on above–belowground interactions, has received little attention (Schroter *et al*., 2004). Given that root and shoot herbivores affect plants in dramatically different ways, but also interact with each other (Meyer *et al*., 2009), the conclusions drawn from studies of climate change impacts limited to only aboveground herbivores may be misleading.

This chapter uses empirical examples to illustrate how belowground herbivores influence aboveground plant–insect interactions. It draws on studies concerning above–belowground interactions as well as studies showing how climate change can alter soil herbivore communities. Finally, it considers the few examples that exist where above–belowground interactions have been studied under climate change scenarios to show how such plant-mediated interactions are, or may be, modified. Thus, this chapter will highlight the potential for previous predictions of climate change impacts on plant–insect relationships to have been incomplete or inaccurate, because of lack of consideration of belowground interactions.

7.3 Above–belowground interactions in the current climate

Studies of plant-mediated interactions between spatially-separated herbivores have revealed contrasting ecological patterns (Van Dam and Heil, 2011) that have evolved and built upon two major hypotheses: the Stress Response Hypothesis (Masters *et al.*, 1993; Bezemer *et al*., 2004) and the Defence Induction Hypothesis (Bezemer *et al.,* 2002). The Stress Response Hypothesis suggests root herbivory impairs the plant's capacity for water and nutrient uptake, which can lead to the accumulation of nitrogen compounds in foliage (White, 1984) to increase palatability to aboveground herbivores. In contrast, the Defence Induction Hypothesis, suggests that belowground herbivores will induce a systemic increase in plant-defence chemicals, making it more difficult for herbivore colonisation to occur aboveground (Bezemer and Van Dam, 2005; Kaplan *et al*., 2008b). These plant-mediated mechanisms arise through a complex path of communication between root and shoot tissues involving primary (e.g. Johnson *et al*., 2009) and secondary (Bezemer and Van Dam, 2005) chemicals. The nature and mode of signalling between roots and leaves is a rapidly expanding area of research (Rasmann and Agrawal, 2008). Some hypotheses suggest that interactions between phytohormonal pathways regulate interspecific herbivore interactions (Soler *et al*., 2013). Different feeding guilds elicit different phytohormonal pathways. For example, jasmonic acid (induced by root-chewers) reduces a plant's salycilic acid defence response against aphids (Soler *et al*., 2013). Given that above- and belowground herbivores can systemically alter the defensive phenotype of plants, future models of plant defence allocation would benefit greatly from a systemic-plant approach (Rasmann *et al*., 2009).

The consequences of interactions between spatially-segregated organisms are more far-reaching than simple pair-wise herbivore–herbivore interactions, with effects cascading across species networks spanning trophic levels and the above- and belowground sub-systems (Scheu, 2001; Wardle *et al*., 2004a). The effects of root herbivory can, for instance, affect tertiary trophic levels. Root herbivores such as the cabbage root fly (*Delia radicum*) have been observed to affect, via the host-plant, an aboveground herbivore (*Pieris brassicae*), its parasitoid (*Cotesia glomerata)* and hyper-parasitoid (*Lysibia nana*) (Soler *et al*., 2005). In this instance, *D. radicum* increased the development time of *P. brassicae* and *C. glomerata*, and the body size of both parasitoid and hyper-parasitoid were reduced. These effects were attributed to an alteration in the blend of phytotoxins (glucosinolates) emitted post-herbivory. Conversely, aboveground herbivory can have a negative effect on belowground herbivores and associated natural enemies (Jones and Finch, 1987; Soler *et al*., 2007). For instance, the presence of butterfly larvae (*P. brassicae*) reduced the abundance of the belowground herbivore (*D. radicum*) and its parasitoid (*Trybliographa rapae*) by up to 50% and decreased the body size of emerging parasitoid and root herbivore adults (Soler *et al*., 2007). If these broader interactions between organisms inhabiting the plant rhizosphere and canopy are typical, they could scale-up to play important roles in governing ecosystem function.

7.4 Climate change and belowground herbivores

Many studies and comprehensive reviews address the effects of global climate change on aboveground insect herbivores (e.g. Bale *et al*., 2002; Cornelissen, 2011), whereas there are substantially fewer studies of the impacts on belowground organisms (Staley and Johnson, 2008). Soil fauna are, at least to some extent, buffered from the direct impacts of climate change (Bale *et al*., 2002). Carbon dioxide concentrations are already high within the soil due to root respiration and microbial processes (Haimi *et al*., 2005), and therefore soil fauna are less likely to be affected by increased atmospheric CO_2 directly. Soil fauna may, however, be affected indirectly by increased growth of root resources caused by increased atmospheric CO_2 (Norby, 1994). While higher soil temperature may also increase root growth, temperature increase may directly affect soil herbivore development and insect phenology (Van Asch *et al*., 2007). Reduced soil moisture, potentially a consequence of increased temperature, can also impact many soil insect life-history traits, such as survival and abundance (Pacchioli and Hower, 2004). Predicted increases in climatic extremes under a future climate (e.g. increased flooding and drought events) may also drown or desiccate soil biota and herbivores, thus reducing their prevalence in the soil (Parmesan *et al.*, 2000).

Soil-dwelling insect herbivores feed on the roots and therefore have very different effects on plant traits than their aboveground counterparts. These effects may alter the predicted consequences of global climate change on shoot herbivores (Robinson *et al.*, 2012; Zavala *et al.*, 2013). For instance, most plants increase biomass accumulation and rates of photosynthesis in response to elevated CO_2 (Ainsworth and Long, 2005); this depends on plants maximising water and nitrogen use efficiency. To facilitate this, many plants increase their root:shoot biomass ratio in response to elevated CO_2, but this may be compromised by root herbivores, which remove root mass, therefore impairing water and nutrient uptake (Johnson and Murray, 2008). A recent meta-analysis by Zvereva and Kozlov (2012) showed that root herbivores reduced rates of photosynthesis in host-plants; this contrasts with many aboveground herbivores that actually stimulate it (Thomson *et al.*, 2003). Empirical evidence also suggests that root herbivory can effectively reverse the effects of elevated CO_2 on eucalypt chemistry (e.g. increased foliar C:N ratio) and biomass, potentially altering the outcomes for aboveground herbivores (S. N. Johnson, *pers. obs*.).

7.5 Climate change and above-belowground interactions: empirical evidence

To date, there are only two peer-reviewed published examples describing how an elevated CO_2 environment affects the interaction between above- and belowground herbivores. The first focused on the interaction between the root-feeding (*Pemphigus populitransversus*) and shoot-feeding (*Aphis fabae fabae*) aphids*,* on

Cardamine pratensis (Salt *et al.*, 1996). The study concluded the interaction between these spatially-separated aphids was unaffected by CO_2, because root herbivore populations were always smaller in the presence of an aboveground herbivore regardless of the CO_2 environment. The second study investigated the conspecific interaction between aboveground adults and belowground larvae of the clover root weevil (*Sitona lepidus*) (Johnson and McNicol, 2010). Elevated CO_2 increased leaf consumption by adult weevils but resulted in lower rates of oviposition. These patterns were interpreted by the authors to be a compensatory feeding response to reduced leaf nitrogen and lower reproductive output due to inadequate nutrition. Despite reduced rates of oviposition, larval survival was much greater at elevated than at ambient CO_2 -levels potentially due to increased nodulation (increased food source) of the host-plant (*Trifolium repens*) under elevated CO_2 conditions (Johnson and McNicol, 2010).

Enrichment with CO_2 is not only expected to increase plant biomass both above- and belowground, but also to reduce plant tissue quality through increases in the C:N ratio and secondary metabolite concentrations (Bezemer and Jones, 1998). Compensatory feeding by phytophagous insects in an elevated CO_2 environment may thus increase exposure to defensive chemicals present in plant tissue. This is likely, however, to be contingent on plant taxonomic identity, as concentrations of defensive chemicals may increase (e.g. glucosinolates in *Arabidopsis thaliana* (Bidart-Bouzat *et al.*, 2005)), or remain unchanged (e.g. tannins in *Quercus myrtifolia* (Rossi *et al.*, 2004)) in response to CO_2 enrichment.

Temperature changes may alter above–belowground interactions either by affecting invertebrate phenology directly (Gordo and Sanz, 2005; Harrington *et al.*, 2007) or indirectly through changes in the plant (Bale *et al.*, 2002; Harrington *et al.*, 1999; Singer and Parmesan, 2010), although this remains to be tested empirically. A predicted increase in global mean temperatures may also result in an increased water stress response in plants (Huberty and Denno, 2004), making them more susceptible to herbivory both above- and belowground.

Summer drought is another factor associated with climate change that has been shown to influence above–belowground interactions. Typically, root-chewing *Agriotes* sp. larvae reduced the abundance and performance of leaf-mining *Stephensia brunnichella* larvae and its associated parasitoid (Staley *et al*., 2007). This effect was, however, negated under drought conditions. Changes to summer rainfall may, therefore, reduce the occurrence or alter the outcome of plant-mediated interactions between insect herbivores.

Above–belowground interactions may also be influenced by variation in soil moisture. Experimentally elevated rainfall increased the suppression of an outbreak of the herbivorous moth larvae *Hepialus californicus* by an entomopathogenic nematode (*Heterorhabditis marelatus*), thereby indirectly protecting the host-plant – bush lupine (*Lupinus arboreus*) (Preisser and Strong, 2004). Thus climate change, by altering patterns of precipitation, has the potential to modify herbivore–natural enemy interactions to reduce herbivore pressure.

Few studies have integrated the multiple abiotic factors associated with climate change (i.e. water supply, temperature, CO_2 etc.) to investigate their combined effects on above–belowground interactions. One such study (Stevnbak *et al.*, 2012) manipulated CO_2 concentration, air and soil temperature and precipitation to show that soil microbial biomass was altered by aboveground herbivory (*Chorthippus brunneus*). The combination of multiple climate change treatments with aboveground herbivory increased microbivorous protist abundance in the soil, emphasising the importance of considering climate change in above–belowground interactions.

7.6 The future of above–belowground interactions and climate change research

Johnson *et al*. (2012a) conducted a meta-analysis on two-species above–belowground herbivore interactions. Although restricted by not including other trophic groups, the meta-analysis did identify several factors that determine the outcomes of interactions between spatially-separated herbivores. From these outcomes it is possible to develop hypotheses of how specific interactions are likely to be affected by climate change. The chronological sequence in which herbivores fed on shared plants was a major determinant of interaction outcome. In particular, aboveground herbivores negatively affected belowground herbivores when they fed first, but not when feeding synchronously or following belowground herbivores. Conversely, belowground herbivores typically had positive effects on aboveground herbivores only when synchronously feeding, otherwise they had a negative impact. Many of the data on aboveground species are from aphids; from these data it is known that elevated CO_2 and temperature results in earlier and longer seasonal occurrences of many pest species, including aphids (Harrington *et al.*, 2007). Therefore in the future it might be reasonable to expect that some aphids may initiate feeding on the plant prior to belowground herbivores. Under such circumstances, aphids may negatively affect the belowground herbivore while remaining unaffected themselves, the reverse of the interaction under current conditions. Likewise, if drought conditions delayed root herbivore development this change could become even more pronounced.

Feeding guild identity (e.g. chewers, suckers, gallers) can affect the outcome of above–belowground interactions. Johnson *et al*. (2012a) showed that the effects on aboveground herbivores depended on belowground herbivore guild. Individual feeding guilds and trophic levels respond differently to climate change (Voigt *et al*., 2003), but how this translates into changes in above–belowground trophic interactions remains unexplored. The increased level of defence compounds in plant tissue, predicted to occur under climate change scenarios (Robinson *et al*., 2012), are likely to have a disproportionate effect between (a) herbivores feeding above- or belowground: defence compounds may be concentrated in either leaf or root tissue, and (b) different feeding guilds: chewing insects are more susceptible to defensive compounds than phloem-feeders. There is, however, a strong bias in the literature, with certain herbivore guilds and orders (e.g. Lepidoptera) having been represented disproportionately within empirical studies (Robinson *et al*., 2012). Conclusions extrapolated regarding general herbivore-responses to climate change should, therefore, be treated with appropriate caution.

There are few long-term above–belowground interaction studies. Some Arctic long-term manipulative field studies (e.g. Ruess *et al*., 1999) that illustrate the effects of climate warming on soil fauna provide essential information on legacy effects in natural ecosystems. These indicate that above–belowground interactions may be separated temporally (Kostenko *et al*., 2012) as well as spatially. Long-term field experiments may also yield different results to laboratory experiments conducted over a smaller timescale (Johnson *et al*., 2012a).

7.7 Conclusions and research agenda

Our understanding of how individual species respond to climate change has increased dramatically over the past 25 years. We have a relatively well-informed understanding of how aboveground herbivores may react to different aspects of climate change (e.g. Bale *et al.,* 2002) but our knowledge of belowground species responses remains lacking. Johnson and Murray (2008) illustrate how this area of research is a “hot topic” for multidisciplinary research while others (Soler *et al*., 2005; Van Dam and Heil, 2011) underline the importance of a more integrated understanding of climate change impacts on ecosystems that incorporates above- and belowground trophic linkages.

Based on current knowledge of above–belowground interactions it is possible to formulate hypotheses that could be tested empirically in future research. For example:

1. Root herbivory is likely to change fundamentally plant responses to an elevated CO_2 environment, since root function usually underpins the plants ability to respond to environmental changes. It could be hypothesised that inclusion of root herbivores will reverse the effects of elevated CO_2 on certain aboveground herbivores, particularly those negatively affected by higher C:N ratios (e.g. leaf-miners).
2. Plant functional identity may shape how above–belowground interactions respond to climate change. For instance, plants with C_3 and C_4 photosynthetic pathways will respond differently to climate change, and notably elevated CO_2 (Barbehenn *et al.*, 2004a). In particular, C_3 plants potentially show a greater decline in nutritional quality than C_4 plants, which are often inherently less favourable hosts to insect herbivores (see the C_3-C_4 hypothesis of Scheirs *et al.*, 2001). This might lead to compensatory feeding on C_3, but not C_4, plants in future climates (Barbehann *et al.*, 2004b). It could be hypothesised that above–belowground interactions are likely to be more affected on C_3 than C_4 plants.
3. Belowground herbivory induces a water stress on the plant, similar to drought. Experiments investigating drought effects on aboveground plant–herbivore interactions may, therefore, be analogous to above–belowground herbivore interactions generally. It could, therefore, be hypothesised that the combination of a drought treatment and a belowground herbivore may have additive negative effects on the plant and consequently on aboveground herbivores (through increased susceptibility to herbivory).

Increasing trophic complexity in empirical climate change research will strengthen the ability to make more accurate predictions of trophic interactions in future environments (Robinson *et al.*, 2012). Making predictions based on simple plant–herbivore interactions compared to wider communities may be misleading and interaction outcomes may be altered with the inclusion of higher trophic levels. As seen aboveground, climate change may not directly affect the abundance of a herbivore; however, if the abundance or impact of an associated antagonist is reduced then climate change may increase herbivore abundance indirectly. Disrupted phenological synchrony between predator and prey (Hance *et al*., 2007) may be one mechanism, another may be a reduction in plant production of chemical attractants (synomones) that recruit natural enemies, which then regulate herbivore numbers (Yuan *et al*., 2009). Alternatively, climate change may benefit the prey and antagonist equally, with any increase in herbivore abundance merely supporting greater numbers of natural enemies and thus leading to no net change in populations (e.g. Chen *et al*., 2005). An integrated approach considering trophic interactions as an integral part of an ecosystem comprising above- and belowground components will provide a more accurate estimation of climate change impacts. For example, a positive effect of root herbivores on folivores at higher temperatures may, if climate change positively affected antagonist efficacy (e.g. Bezemer *et al*., 1998; Hance *et al*., 2007), be cancelled-out with the inclusion of an above- or belowground antagonist. For the most part this remains to be tested empirically. Moreover, with more empirical data it may be possible that – as has been observed with other areas of climate change research (Robinson *et al*., 2012) – apparent idiosyncratic outcomes of climate change impacts on plant–herbivore interactions give way to reveal generalities. Trends have become apparent in some aspects of insect herbivory in elevated CO_2 (Zavala *et al*., 2013); for example, phloem feeders increase in abundance under elevated CO_2, whereas leaf-miners decrease (Robinson *et al*., 2012). Alternatively, further research may simply reveal a lack of general responses of above–belowground interactions to climate change. For instance, despite the large body of research on aphid–plant interactions under climate change, aphid responses to CO_2 enrichment still appear to be highly species-specific (see Sun and Ge, 2011 and references therein). The challenge for ecologists therefore is to utilise current knowledge of individual species responses to climate change and develop our understanding into general hypotheses for functional guilds, networks of species and ecosystem processes.

Chapter VIII

General Discussion

The overall aim of this thesis was to investigate how certain aspects of climate change (e.g. elevated atmospheric CO_2 and altered phenology) affected the interaction between the aboveground large raspberry aphid (*Amphorophora idaei*) and the root-feeding larvae of the vine weevil (*Otiorhynchus sulcatus*), both of which feed on raspberry (*Rubus idaeus*). Under ambient atmospheric CO_2 conditions a reciprocal facilitative relationship existed between aphids and weevil larvae, where both species had a positive effect on the other's abundance, mediated through feeding behaviour (Chapter II). In this thesis, the reciprocal relationship was not altered by the chronology of herbivore arrival (Chapter III), which departs from the main finding of a recent meta-analysis (Johnson *et al*., 2012a). For this raspberry-aphid-weevil system, it would therefore seem that changes in chronology of herbivore colonisation of host-plants as a result of future climate changes may not affect the facilitation between these spatially-separated herbivores.

In general, elevated atmospheric CO_2 increases leaf toughness (Robinson *et al*. 2012), and this was supported by this thesis study (Chapter IV). This increased leaf toughness did not, however, prevent vine weevil adults from increasing leaf consumption rates compared with the ambient CO_2 situation. Moreover, when given a choice adult weevils preferentially fed on foliage from plants grown under elevated CO_2 conditions. Belowground herbivory generally has a negative effect on root biomass (Andersen, 1987); elevated CO_2 may mitigate this negative effect by increasing plant growth (Chapter V). In this raspberry-aphid-weevil system, however, biotic plant–herbivore interactions seem to play a larger role in dictating insect abundance and plant biomass than the effects of elevated CO_2. The effects of herbivory on plant biomass and C:N were far greater than those of increased CO_2 concentrations. Finally, the presence of an aboveground herbivore, at least in this raspberry–aphid system, appears to have the potential to affect surrounding conspecific plants through the consequences of herbivore-induced over-compensatory growth (Chapter VI).

Reciprocal feeding facilitation was observed in two studies (Chapters II and III); the abundance of both herbivores increased in the presence of the other. Examples of this phenomenon are rare in nature as most interspecific insect interactions are competitive with one species having a negative effect on the abundance of the other (Denno *et al*., 1995). Of the numerous reasons why facilitative relationships may occur (Denno *et al*., 1995; Johnson *et al*., 2009; Price *et al*., 2011; Soler *et al*., 2012b), in this study system, the potential driving force is most likely to be the over-compensatory growth aboveground caused by increased herbivory (Chapter II).

One study that illustrates a uni-directional facilitative relationship between conspecifics, where aboveground herbivory benefits belowground herbivores but with no belowground effect on the aboveground herbivore, was between adults and larvae of the flea beetle (*Bikasha collaris*) feeding on Chinese tallow (*Triadica sebifera*) (Huang *et al*., 2013). This conspecific interaction was attributed to changes in plant chemistry. When feeding alone on the plant, belowground herbivores caused the plant to produce defensive tannins. The presence of aboveground herbivory by adult flea beetles, however, prevented the plant producing root tannins, which subsequently caused larval survival to double. Above- and belowground herbivory in tandem, however, increased foliar tannins which subsequently decreased adult survival. These alterations in plant chemistry show that facilitation in this instance was caused by the suppression of root defence compounds.

Evidence for reciprocal above–belowground interspecific interactions between herbivores exists, with root-feeding wireworms (*Agriotes* spp.) increasing foliar-feeding aphid (*Rhopalosiphum padi*) numbers by 30% and aphids increasing wireworm mass by 25% on barley (*Hordeum vulgare*) (Johnson *et al*., 2009). The increase in wireworm mass was attributed to the increases in root mineral concentrations (especially root sulphur) induced by aphid feeding. Wireworms also reinforced the aphid-induced increases in root sulphur concentrations by 35% after short exposure, but reduced aphid-induced increases by 10% after longer exposure, suggesting herbivore-induced changes in root mineral concentrations maybe a dynamic process and change over time. In another system, although not reciprocal, vine weevils increased field populations of *Cryptomyzus galeopsidis* aphids by 700% (Johnson *et al*., 2013). This positive effect of weevils on aphids was attributed to the increase in the proportion of foliar essential amino acids by 4% which was driven by belowground herbivory. Examining specific plant nutrients and plant defence chemistry could be critical to understanding conditional outcomes of insect–plant interactions. Evidence suggests there may be numerous mechanisms (e.g. change in phytohormones, amino acids or tannins) that underpin facilitative relationships between herbivores, but these mechanisms may change over time, as longer exposure to herbivory alters plant chemistry further (Karban and Myers, 1989).

Reciprocal interspecific interactions have also been observed between aboveground herbivore species. Soler *et al*. (2012b), for example, found that the reciprocal interaction between the aphid *Brevicoryne brassicae* and *Pieris brassicae* caterpillars feeding on *Brassica oleracea* may be due to the disruption of phytohormones. Jasmonic acid (a phytohormone involved in the chemical defence against chewing herbivores) was reduced 10-fold by aphid feeding, which benefited the caterpillars by increasing their rate of development and individual size. Levels of plant glucosinolates and the carbon:nitrogen ratio remained unaffected by either aphid or caterpillar presence, suggesting these factors did not underpin this facilitative relationship.

Facilitative relationships may give way to competitive interactions over time (Denno *et al*., 1995; Price *et al*., 2011); the length of time over which an experiment is carried out may, therefore, be a significant determinant of whether facilitative relationships are observed. The experiments showing facilitative relationships referred to above took place over 27 (Huang *et al*., 2013), 10 and 20 (Johnson *et al*., 2009), and 6 (Soler *et al*., 2012b) days. In the present study, with aphid and vine weevil larva interacting (Chapters II and III), the experiment ran for 14 days. All are relatively short-term (less than a month), therefore, facilitation may have been observed because of the relatively short nature of the study (Price *et al*., 2011).

That feeding facilitation occurred between vine weevils and aphids in some studies (Chapters II and III) and not in others (Chapters V and VI) raises interesting questions. Why did facilitation not occur in these instances? It is likely that prevailing conditions played an important role in determining the outcome. As soil volume was equal in two of the studies giving a different result (Chapters II and V) it is unlikely that this was the causal reason. The lengths of the experiments and growing conditions were also the same (mean day temperature 18°C ±2°C and a 16:8 light:dark regime), despite being carried out in glasshouses in different locations. Variation in the presence or absence of facilitation between aphid and weevil cannot be explained by differential and systematic differences in insect abundance or aboveground plant biomass because a similar range of population sizes and biomasses were found within experiments. A possible explanation is suggested by belowground differences among experiments. Facilitation was observed in the first study (Chapter II) when shoot:root ratio was 0.8:1. This ratio was largely driven by high mean belowground biomass (2.15 g), compared to a shoot:root ratio of 1:1 and a mean root biomass of 0.8 g in the study reported in Chapter V. Unfortunately root biomass was not measured in the second study (Chapter III), the weevil larvae having been feeding for variable periods on the roots but in the third experiment (Chapter IV) where facilitation was again not observed plants had a relatively low root biomass (0.8 g), with a shoot to root ratio of 2.4:1. This implies that feeding facilitation may be contingent on root biomass, only occuring when root biomass is high and the ratio of shoot to root biomass is less than 1:1.

Another reason why feeding facilitation may have occurred in only chapters II and III may be down to the compost used during the experiments. Chapters II and III used a specific blend of peat-perlite mixture containing 17N : 10P : 15K obtained from Wiliam Sinclair Horticulture Ltd. All following experiments used Levington M3 compost with a nutrient balance of 233N : 104P : 339K. It may be these differences in the soil nutrients (nitrogen, phosphorous and potassium) that affect the associated nutrients in the plant tissue, which in turn may affect the herbivores. The Levington M3 compost has a much higher ratio of potassium compared to the other two nutrients in the compost. If there are low potassium levels in the soil in chapters II and III, it may cause the plant to be potassium deficient. Previous studies have shown that soybean aphids (*Aphis glycines*) feeding on soybean (*Glycine max*) performed better on K-deficient plants with regard to net reproductive rate (Myers *et al*., 2005) and abundance (Walter & Di Fonzo, 2007), than on non-deficient soybean. It may therefore, be potassium levels of the soil and plant that drive the facilitative relationship between the above- and belowground herbivores. This suggests that nutrient content of both the plant and soil may play an important role in determining inter-specific herbivore interactions.

More generally, feeding above-and belowground facilitation may only occur between certain herbivore guilds, insect body size classes, or insect developmental stages. Mechanical damage by chewers is far more acute and can cause much more severe plant damage than sap feeding insects (Andersen, 1987). Moreover, the damage caused by two chewing species of interacting insects may be too severe for the plants to tolerate; therefore it is likely that facilitation may be more likely to occur in interactions involving phloem feeding insects that cause less mechanical damage to the plant. Possibly, facilitative relationships between above- and belowground herbivores may only occur in early root-herbivore instars (Chapter II). Smaller larvae graze on the finer roots, which may only cause limited damage to a plant, but as the larvae develop they will feed on increasingly larger roots and will consequently be more detrimental to the plant (Moorhouse *et al*., 1992).

Furthermore, interspecific plant–herbivore interactions are known to be dynamic processes and may change over time (Ohgushi, 2005).

In addition, moderate herbivory can cause over-compensatory plant growth (Chapters II, III and VI). It was suggested in Chapter II positive feedback between aphid and weevil via induced over-compensatory plant growth was the driver of the feeding facilitation. Therefore facilitative interactions may also be contingent on plants showing over-compensatory growth.

It is also a possibility that the occurrence of herbivore facilitation is governed by plant defence strategy. Plants that rely on chemical defence may host more competitive interactions between herbivores – especially if plants rely on induced defence where the time of arrival of a herbivore species on the plant would be of greater importance (Viswanathan *et al*., 2005; Erb *et al*., 2011). Plants that defend themselves physically may lend themselves to facilitative herbivore interactions as shown in Chapters II and III. Soler *et al*. (2012b), however, suggest that signal crosstalk (e.g. between jasmonic and salicylic acid pathways) could occur in chemically defended plants resulting in a disruption of chemical defence, ultimately benefiting both herbivores of various feeding guilds.

Although this is the first study to investigate in detail the combined interaction between vine weevil larvae and aphids on raspberry, other studies investigating each herbivore provide supporting evidence. For example, other studies have shown that the large raspberry aphid promotes raspberry growth by 18–37% (Johnson *et al*., 2012b). In addition, vine weevils have been shown to promote large raspberry aphid populations by 80% (McMenemy *et al*., 2009), although the effects on vine weevil populations were not recorded.

The sequence in which insect herbivores colonise host-plants is considered to be the most important determinant of the outcome of above–belowground species interactions (Johnson *et al*., 2012a). Belowground herbivory has a positive effect on aboveground herbivores, but only when they arrival simultaneously on the plant. When belowground herbivores arrive either before or after aboveground herbivores, the net effect is neutral. Conversely, aboveground herbivores only affect belowground herbivores negatively when they colonise the plant first (Johnson *et al*., 2012a). When they arrive before or at the same time as belowground herbivores there is no interaction outcome. The facilitative relationship observed in the studies reported in this thesis persisted, however, regardless of the sequence of herbivore arrival on the host-plant (Chapter III).

The lack of herbivore arrival time effect on interaction outcomes (Chapter III) may be a result of the persistence of the reciprocal facilitative relationship previously observed (Chapter II). Studies that have shown that herbivore arrival time was an important factor in determining interspecific herbivore interaction outcomes often involved competitive interactions (Viswanathan *et al*., 2005; Erb *et al*., 2011). In that situation herbivores arriving first have a competitive advantage over those arriving subsequently, due to induction of plant defence compounds (Erb *et al*., 2011). Arriving first avoids any induced plant defences and also direct competition with other herbivores for space on the plant (Viswanathan *et al*., 2005; Erb *et al*., 2011; Victorsson, 2012). In this system, raspberry plants are less reliant on chemical defence, so this avoidance of induced chemical defence is not likely to be a major factor governing the performance of herbivores.

It would be beneficial to assess what factors govern the impact of herbivore arrival time on the plant. Chapter III suggested that time of herbivore arrival may only be an issue where herbivores are competing for the plant food resource. Further research on a wide range of herbivore–herbivore interactions, specifically above–belowground interactions, would help shed light on whether the nature of herbivore interaction (competitive versus facilitative) governs the importance of herbivore arrival on the plant.

Attempts to identify and generalise the responses of insect herbivores to elevated atmospheric CO_2 have met with varying degrees of success (Bezemer *et al*., 1999; Newman *et al*., 2003; Zvereva and Kozlov, 2006; Stiling and Cornelissen, 2007; Cornelissen, 2011; Newman *et al*., 2011; Sun and Ge, 2011; Robinson *et al*., 2012; Zavala *et al*., 2013). Increased CO_2 has an indirect effect on the herbivores mediated through changes in host-plant, e.g. increased leaf C:N. Insect responses to elevated CO_2 are highly idiosyncratic (Newman *et al*., 2011); no consistent effects are reported for either folivores or Coleoptera (Robinson *et al*., 2012), whilst Homoptera and phloem feeders generally increase in abundance (Robinson *et al*., 2012). In this present study, the large raspberry aphid did not respond to elevated CO_2 in terms of population abundance. This was not a typical response because host-plant specialists such as this aphid species are reported generally to show a negative response to changes in plants induced by increased atmospheric CO_2 levels (Robinson *et al*., 2012).

The effects of elevated CO_2 recorded in this present study, on both raspberry and its associated herbivores, appear at variance with the generalisations that have been drawn from other studies. For example, both above- and belowground plant biomass tends to increase under conditions of elevated CO_2 (Robinson *et al*., 2012); this was not found in the present study. Generally, plant tissues also have an increased carbon to nitrogen ratio when grown in elevated CO_2 (Bazzaz, 1990; Kimball *et al*., 1993; Ainsworth and Long, 2005). This has frequently been used to explain levels of increased plant consumption by herbivores (Lincoln *et al*., 1986). There can be a tendency for an increase in C:N in response to increased CO_2 in raspberry plants in some studies (Martin and Johnson, 2011) but this is never a strong response, and indeed the response is absent in this thesis (Chapters IV & V). This, as previously discussed in another context above, could be due to the short duration of the experiment being insufficient to detect such differences in a woody perennial plant species. Increasing experimental duration, might allow such chemical constituent changes to be recorded. Elevated CO_2 led to an increase in leaf tissue toughness (caused by increased epicuticular wax (Percy *et al*., 2002)), a generalisation recorded in various other studies (Robinson *et al*., 2012). The unchanged C:N ratio may also be caused by root herbivory causing nitrogen to be reallocated away from the damaged roots and concentrated more in the leaf tissue (Newingham *et al*., 2007).

Results from this thesis add to the body of literature that illustrates plants can benefit from herbivory through over-compensatory growth (Matches, 1992; Alward and Joern, 1993). This growth effect may be dependent on the level of herbivory received, as slight or moderate herbivory, both above- and belowground, can encourage plant growth (McNaughton, 1983), whereas prolonged or acute herbivory can be detrimental to the plant. There are examples in the literature that illustrate over-compensatory growth upon herbivory both above- (Trumble *et al*., 1993) and below- (McNaughton, 1983) ground. For instance, another weevil species, the lucerne weevil (*Sitona discoideus*), has also been shown to induce over-compensatory growth in lucerne (*Medicago sativa*), increasing root biomass by 31% (Ryalls *et al*., 2013).

Much work has been undertaken showing how aboveground herbivores can alter plant competition and community structure (Crawley, 1989; Pacala and Crawley, 1992; Haag *et al*., 2004; Schädler *et al*., 2007). There are few empirical studies that assess how the interaction between above- and belowground herbivores may affect plant competition (e.g. Kos *et al*., 2014), so the work done in chapter VI adds to the small body of data on this subject. The ability of herbivores to alter community composition, by reducing plant biomass allowing other plants to thrive in the environment has been well documented above- (Bonser and Reader, 1995; Olff and Ritchie, 1998) and below- (Brown and Gange, 1990; Van der Putten *et al*., 2001; Blossey and Hunt-Joshi, 2003; Wardle *et al*., 2004a; Bardgett and Wardle, 2010) ground. The majority of studies show that plants subjected to herbivory are at a competitive disadvantage compared to surrounding, herbivore-free plants. In this present study of the interaction between raspberry–aphid–weevil, aphids played a greater role in determining the outcome of plant competition than both belowground herbivory and elevated CO_2 (Chapter VI). It was hypothesised that plants subjected to herbivory would be at a disadvantage compared to neighbouring plants due to increased biomass removal. Upon herbivory, however, the plant showed plant over-compensatory growth – an effect consistently observed throughout the majority of the experiments in this thesis. This caused the herbivore inoculated plant to be significantly larger than the neighbouring plant. This illustrates above–belowground herbivory may have consequences for altering plant competition in the wider ecosystem, but this remains to be tested empirically.

8.1 Experimental critique

As previously discussed (Chapter I), a microcosm approach provides the researcher with many advantages (Lawton, 1998); highly replicable, they primarily allow detailed observation of population changes whilst controlling for numerous environmental factors. They have proved an ideal conduit for investigating above- and belowground interactions in this study system. Using rhizotubes (Chapters II, III and IV) made roots accessible and allowed weevil extraction. The restrictive environment of the tubes did not, however, allow plant roots to grow typically retarding growth and the uptake of soil nutrients and additional water, and this may have altered the herbivore–plant interaction. Larger pots that allow sufficient room for plant growth may be a better approach to studying above–belowground herbivore interactions (Körner, 1995).

The majority of the studies presented in this thesis were relatively short-term (9–12 weeks). It is suspected that observed plant–herbivore interactions will exhibit temporal dynamics and that the over-compensatory growth seen may give way to severe plant damage if herbivory was prolonged. The facilitative relationship could therefore become competitive over time with potentially greater plant damage arising (e.g. Price *et al.*, 2011). Longer-term studies also allow sufficient time to study a greater number of plant (e.g. fruit productivity) and aphid biometrics (e.g. developmental rate and fecundity). Restriction to insect abundance and growth rates may have limited the studies' abilities to identify potential climate change effects. There may also be long-term consequences of feeding on elevated CO_2 plants by insect herbivores, not detected in these experiments. While there may, for example, be no short-term effect of tougher leaves on adult vine weevils (Chapter IV), a longer term effect may be increased mandibular wear. For example, there are examples in the literature that show the silica present in grasses that acts as a physical defence against herbivores can cause long-term damage to insect mandibles (Massey *et al*., 2006; Massey and Hartley, 2009). Short-term exposure of *Spodoptera exempta* to silica-rich diets caused increased mandible wear. This damage increased with the duration of exposure with late instar affected greater than early instar larvae, reducing feeding efficiency and growth rates further (Massey and Hartley, 2009).

8.2 Future directions and study

The studies reported in this thesis mainly address patterns in insect abundance and some aspects of plant primary productivity. A more mechanistic approach to isolate the specific pathways and plant compounds that govern these interactions is the obvious next step. Insect interactions can be governed by the defensive pathways and/or changes in plant chemistry that are affected as a consequence of feeding. Raspberry plants are more reliant on physical than chemical defence (Hall *et al*., 2009). Although secondary compounds are not the primary mode of defence for raspberry plants investigating the effects of plant phenolics, present in raspberry, and how they may affect the above–belowground interaction is an obvious avenue of study. Increased phenols in raspberry tissues have been shown to affect vine weevil larvae mass (Clark *et al*., 2011; Johnson *et al*., 2011) and the number of weevils per plant (Johnson *et al*., 2011) positively. Elevated CO_2, however, reduced the positive effect of raspberry phenol concentration on weevil abundance (Johnson *et al*., 2011).

Aphid resistance in raspberry is believed to be underpinned by epicuticular wax thickness preventing effective aphid stylet insertion (Shepherd *et al*., 1999a; Shepherd *et al*., 1999b). Leaf chemistry may underpin the effects of elevated CO_2 on leaf toughness (Chapter IV); leaf wax chemistry and toughness, in particular, play an important role in aboveground herbivory (Raupp, 1985; Shepherd and Griffiths, 2006). Investigating and potentially identifying the chemical mechanisms involved may give better predictions of the effects of elevated CO_2 on a wide range of plant–herbivore interactions.

Soluble nitrogen and amino acids may play a role in determining the outcome of above–belowground interactions (Gange and Brown, 1989; Johnson *et al*., 2009). Aphids are often nitrogen limited and it may be these compounds that prevent aphid populations increasing under conditions of elevated CO_2 (Watt *et al*., 1995). Determining changes to plant amino acid compositions and concentrations which could potentially be altered by root herbivory would extend insights into this observation.

Natural ecosystems are far more complex than simple plant–herbivore interactions and involve multi-species interacting within multiple trophic levels (Figure 8.1). These trophic interactions include pollinators (Poveda *et al*., 2003), detritivores (Newington *et al*., 2004; Megías and Müller, 2010) and soil microbes (Wardle *et al*. 2004b; Pieterse and Dicke, 2007), all of which have received relatively little attention within a climate change above–belowground context. By progressively increasing microcosm studies’ complexity it is possible to create a more accurate representation of how organisms might be interacting in the natural world.

Increasing trophic complexity may modify some of the interactions observed in simple plant–herbivore, herbivore–herbivore interactions. For example, where weevils have been shown to increase field populations of aphids this was followed by an increase in the abundance of aphid natural enemies (Johnson *et al*., 2013). Additionally, where facilitation has been found between aboveground herbivores, their natural enemies also increased (Soler *et al.*, 2012b). Again, in this latter situation, natural enemies act to moderate the herbivore interspecific interaction and trends, and the simple plant–herbivore relationships observed under experimental conditions may not be occurring in the wider ecosystem.

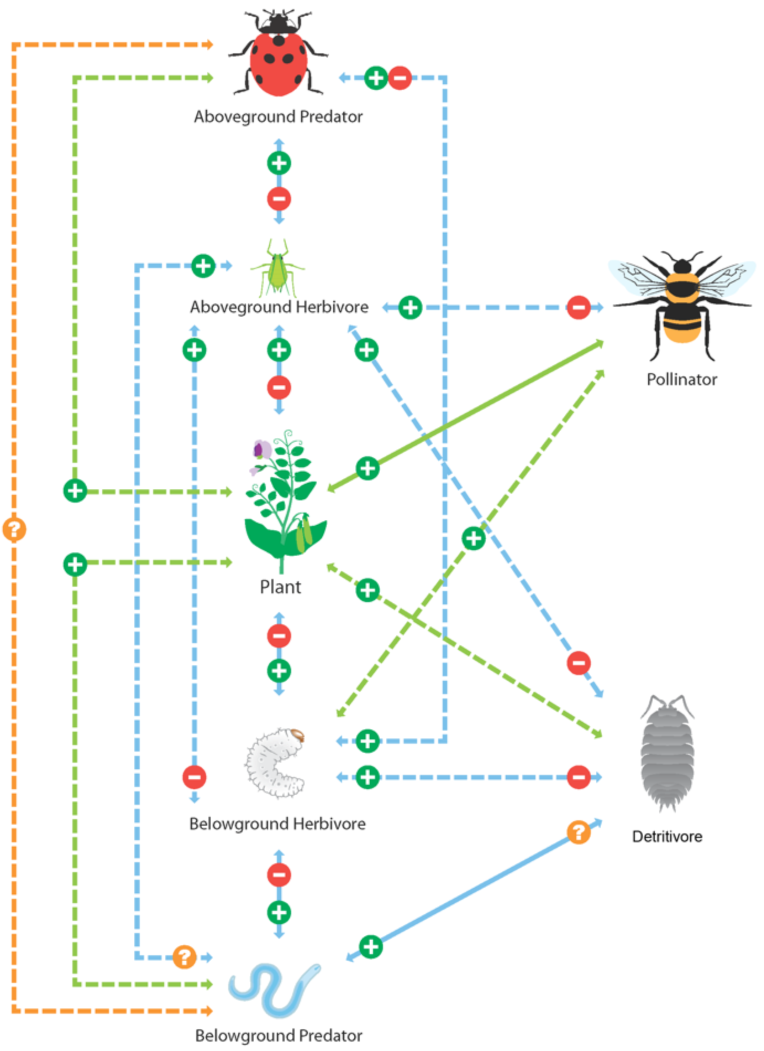

Figure 8.1: Conceptual model illustrating trophic interactions between above- and belowground organisms based on current knowledge (solid line represents direct interaction; dotted line represents indirect interaction; “+” represents overall positive effects; “-” represents overall negative effects; “?” represents unexplored interaction).

The large raspberry aphid is known to be a vector for at least four raspberry disease viruses, including the black raspberry necrosis virus and the raspberry leaf mottle virus (McMenemy *et al*., 2009; McMenemy *et al*., 2012). Infected raspberry plants are initially more attractive to aphids than uninfected plants; however, aphid development took three days longer on these infected plants (McMenemy *et al*., 2012). These two viruses both caused a two-fold increase in soluble amino acids, mainly glutamate, which may underpin the negative effect on aphid development. Root-herbivory is known to reduce the amount of root mass and therefore also restrict the amount of nitrogen the plant can take up from the soil (Brussaard, 1998). The composition and concentrations of plant nitrogen-based amino acids may therefore be affected by belowground herbivory, with cascading effects to aphids and the plant viruses. Exploring this avenue would incorporate another level of complexity, that of plant disease transmission, into the system being studied.

Different components of climate change (e.g. CO_2, temperature, precipitation) do not act on organisms in isolation, but rather in combination as they are intimately linked in actual ecosystems (Mikkelsen *et al*., 2008; Stevnbak *et al*., 2012; Scherber *et al*., 2013). Therefore, differences that are revealed when investigating a single climatic factor may not be the same when a combination of abiotic factors are studied (Valkama *et al*., 2007). These different abiotic treatment combinations can affect the plant both physically and chemically (Veteli *et al*., 2002), and herbivores that feed on them (Zvereva and Kozlov, 2006). For example, a study investigated the combined effects of temperature and CO_2 on the leaf-miner, *Dialectica scalariella*, feeding on Paterson's Curse, (*Echium plantagineum*) (Johns and Hughes, 2002). This study showed larval development time was reduced at the high temperature, but increased at elevated CO_2. This resulted in the larval development time being reduced by around 14 days in the elevated CO_2/high temperature treatment, compared to the ambient CO_2/low temperature treatment. These effects also occur belowground. For example, a study showed increased temperature had a negative effect on lucerne weevil emergence for soil-living larvae (*Sitona discoideus*) on lucerne (*Medicago sativa*). However, elevated CO_2 increased emergence from 0 to 5% (Ryalls *et al.*, 2013).

Longer term studies may illuminate the effects that above–belowground herbivory may have on wider ecosystem functions, such as nutrient cycling. The severing of roots by belowground herbivores provides a greater amount of necro-mass that can be broken down by decomposers including insects, fungi and bacteria. These organisms are important in ecosystem functioning (Bardgett and Wardle, 2010), therefore any changes in their abundance and activity could have cascading effects across the plant–soil food web though altered trophic and mutualistic interactions. Whilst the benefits of carrying out work in controlled environment conditions have been discussed, the next stage would be to conduct associated experiments under more natural conditions in the field. Free-Air CO_2 Enrichment (FACE) facilities provide an opportune, yet expensive, way to investigate the effects of elevated CO_2 on plant–herbivore and herbivore–herbivore interactions (Ainsworth and Long, 2005) although this approach also poses a set of challenges (e.g. replication issues), albeit different to glasshouse experiments. While there is undoubtedly a trade-off between the control of abiotic and biotic conditions in field and glasshouse experiments, field experiments do allow the involvement of more variation in background factors (e.g. temperature). Glasshouse and chamber experiments operate at set conditions, whereas field experiments are perhaps a better representation of what interactions may be occurring in ecosystems. Field experiments would be particularly optimal conditions to test hypotheses surrounding the effects on above–belowground herbivory on plant community structure.

8.3 Concluding remarks

This thesis provides novel evidence of reciprocal feeding facilitation between above- and belowground herbivores – an interaction seemingly robust to changes in the chronology of herbivore species colonisation of the host-plant. This interaction, however, is more likely to occur when root biomass is high, and the ratio of shoot to root biomass is less than 1:1. This study finds that the negative effects of root herbivory on plant biomass may be mitigated by increased CO_2 and adult weevils show a feeding preference for leaves grown in elevated CO_2 despite an increase in leaf toughness. This study also shows that herbivory causes over-compensatory plant growth. The findings of this thesis raise some important questions on understanding patterns and relationships between herbivorous insects feeding above- and belowground, as well as effects they have on the host-plant and *vice-versa*. The mechanisms behind the interactions have not yet been investigated. Some important pathways and mechanisms have been identified as important factors influencing above–belowground interactions. For example, crosstalk in plant phytohormones underpins a facilitative relationship between two herbivores (Soler *et al*., 2012b); whilst abscisic acid regulates the interaction between western corn rootworm (*Diabrotica virgifera virgifera*) and *Spodoptera littoralis* aboveground (Erb *et al*., 2009). These mechanisms can occur systematically throughout the plant showing that leaf and root tissue are tightly linked.

Little empirical work has been conducted on the wider ecosystem effects of above- and belowground herbivory, although much has been hypothesised. Many current challenges still remain in studying belowground herbivory as it is, by its nature, difficult to observe. Greater still are the challenges facing ecologists in quantifying the effects of belowground herbivory on the wider ecosystem. The importance of above- and belowground interactions in determining host-plant performance and also that of the wider plant community may be greater than currently acknowledged. It would add greatly to current understanding of above–belowground interactions to investigate what effects both specialist and generalist herbivores have on such factors as plant competition as they may play a larger role than currently acknowledged in influencing plant communities.

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